9. **Anlagen - Der Habilitationsschrift zugrunde liegende Publikationen**

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Hypothermia related changes in electrocortical activity at stepwise increase of intracranial pressure in piglets

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With 4 figures and 2 tables

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Summary

Recent experimental studies have demonstrated that mild hypothermia can be effective in the control of intracranial hypertension. However, investigations to analyze the effects of hypothermia on changes in brain oxygen metabolism and electrocortical activity caused by increased intracranial pressure (ICP) are lacking. We examined the effects of mild hypothermia on electrocorticogram (ECoG) in combination with measurement of regional cerebral blood flow (CBF) and estimation of brain oxygen metabolism during stepwise increase of ICP. For this purpose thirteen female piglets (14 days old, 4-5 kg b.w.) were anaesthetized and mechanically ventilated. An epidural balloon was gradually inflated in order to increase ICP to 25 mmHg, 55 mmHg and 45 mmHg every 30 minutes at adjusted mean arterial blood pressures (MAP). This procedure resulted in gradual cerebral perfusion pressure (CPP) reduction of about 70%, 50%, and 30% of baseline [baseline CPP: normothermia (NT) 80 ± 3 mmHg; hypothermia (HT) 84 ± 3 mmHg]. Control animals were maintained in a normothermic state (38.6 ± 0.2 °C). HT animals were surface cooled and maintained at 31.9 ± 0.1 °C. ECoG, regional CBF, cerebral oxygen delivery (cDO2) and the cerebral metabolic rate of oxygen (CMRO2) were estimated during the normothermic period, after hypothermic stabilization, and after the gradual CPP reductions. The baseline ECoG showed the typical delta-dominated frequency pattern for isoflurane anaesthesia. At the hypothermic level, a frequency shift was seen from delta activity towards the higher frequencies (theta- and alpha activity) and the total spectral power was significantly reduced (56 ± 17% from baseline, p < 0.05). the cortical CBF decreased markedly to 67 ± 10% (p < 0.05), whereas the medulla oblongata blood flow increased slightly. During controlled increase of ICP by regional mass expansion from epidural balloon inflation, we found at mild and moderate stages of ICP increase (25 and 35 mmHg) only minimal changes in the ECoG in hypothermic animals compared to the hypothermic baseline, whereas the ECoG in normothermic animals showed a marked decrease in frequency, amplitude and total spectral power. We conclude that mild hypothermia produces an arousal-like ECoG activity with marked frequency shift to alpha activity and a change from high to low voltage activity. Furthermore, the hypothermic brain showed a preserved neuronal function at moderate stages of ICP. Obviously, hypothermia improves the functional tolerance of the brain to impaired oxygen supply.

Introduction

Traumatic head injury is the leading cause of death in children and young adults (WARD 1996). Obviously, children’s brains show respond differently to injuries than adult’s brains. The incidence of posttraumatic brain swelling is twice as high in the pediatric population as in the adult (ALDRICH et al. 1992), and is the common cause of an ICP. An initially increased ICP shortly after trauma is of considerable prognostic value and may correlate with adverse outcomes (BAREILAY et al. 1988; CIRICILLO et al. 1992).

Until now, the pathophysiology of brain swelling with increase of ICP following an acute space occupying lesion is not clearly understood. Factors such as duration and extent of cerebral compression accompanied with metabolic disturbance appears to be important in inducing brain swelling (MISHINA et al. 1994). Most dangerous consequence of ICP increase is the reduction of CPP as the driving force of CBF. This is accomplished by meta-
bolic disturbances, if the CPP falls below the autoregulatory threshold of CBF.

Depending on the extent of the metabolic disturbances the neuronal electrical activity becomes depressed, up to electrical silence, although the cells remain metabolically viable, at least for some period of time (Newlon 1996). The patterns of electroencephalography (EEG) change may vary according to the type and severity of the damage, but typically, the patterns progress from an initial loss of high-frequency activity to an increase in relatively synchronized delta-activity, and finally, a decrease in the amplitude of all activity (Rampil 1994).

Mild hyperthermia has been reported as a therapeutic tool for protecting brain tissue in ischemic and traumatic brain injury (Busto et al. 1989; Clifton et al. 1991, Pomeranz et al. 1993). The efficiency of hyperthermia lies in reducing the CMRO₂. The hypothermic decline in cerebral metabolism has been more closely linked to temperature effects on physicochemical processes than cerebral function, as represented by the EEG (Lafferty et al. 1978; Nemoto et al. 1996). The most common findings using EEG recording is, that cooling to 33 °C may produce cerebral stimulatory effects as reflected by arousal phenomena as well as small shifts in EEG frequencies to theta- and beta-activities (Blair 1965, Fitzgibbon et al. 1984). However, other investigations have shown that cooling induces progressive reduction of both amplitude and frequency of EEG (Mizrahi et al. 1989; Glaria et al. 1990). Additionally, the combined effects of hyperthermia and anesthetics have to be taken into account (Kochis 1995).

Up to now there have not been experimental or clinical investigations of changes in electrocorticographic activity under conditions of hyperthermia during compromised brain oxygen delivery caused by increased ICP.

Therefore, in the recent study we investigated the effects of mild hyperthermia on the ECoG patterns during stepwise reduction of brain oxygen metabolism due to increased ICP. We hypothesized that in contrast to normothermic conditions, mild hyperthermia may cause a distinct temporal preservation of the functional metabolism, which is indicated by special ECoG patterns during gradually decreased cerebral perfusion.

Local anaesthesia was infiltrated into the skin prior incision. A catheter was inserted into the left external jugular vein for injection of drugs and fluids. Another catheter was inserted via the femoral artery into the abdominal aorta for recording blood pressure and for withdrawal of reference samples for calculating CBF with colored microsphere (CMS) technique (CBF measurement see below at CMS technique). Further catheters were inserted into the left ventricle via the right common carotid artery and into the superior sagittal sinus in order to obtain brain venous blood samples.

Two burr holes were made into the left parietal skull for inserting a fiberoptic catheter into the subcortical white matter for ICP measurements (Camino Laboratories, San Diego, U.S.A) and a Clark-type PO₂ electrode together with a thermocouple catheter (Licox PO₂ Monitor, GMS mbH, Kiel-Mielkendorf, Germany) into the parietal cortex.

An oval-shaped burr hole (10 mm to 3 mm) was gently drilled through the right parietal skull parallel to the sagittal suture. An epidural latex balloon catheter was placed in the epidural space of the right parietal region. The burr holes were sealed with bone wax and covered with dental acrylic in order to fix in place the probes and epidural balloon catheter throughout the experiment (fig.1).

**Hemodynamic and blood gas monitoring:** Arterial, left ventricular and central venous catheters were connected with pressure transducers (P23Db, Statham Instruments, Puerto Rico). Electrocardiogram (ECG) recordings were made from standard limb leads using stainless steel needle electrodes. Physiological parameters were recorded on a multi-channel polygraph (MT95K2, Astro-Med, USA). Body temperature was measured by a rectal thermoprobe inserted about 10 cm and was maintained throughout the general instrumentation at 38 ± 0.3 °C using a water-flow pad connected to a heating-cooling thermostat and a feedback controlled heating lamp.

Blood pH, PCO₂, and PO₂ were determined using an ABL50 blood gas analyzer (Radiometer, Copenhagen, Denmark). Blood hemoglobin and arterial oxygen saturation were measured by an OSM2 hemoximeter (Radiometer, Copenhagen, Denmark), corrected to the body temperature at the time of sampling. The blood oxygen content was calculated as the product of blood oxygen saturation (%), oxy-

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**Methods**

**General Instrumentation:** All surgical and experimental procedures were approved by the ethical committee of the local authorities.

Thirteen piglets (14 days old, 4–5 kg b.w.) of female sex were anesthetized initially with ketamine hydrochloride (50 mg/kg b.w., i.m.), and anesthesia was maintained with isoflurane (0.8 Vol.%.) in a nitrous oxide / oxygen mixture (70 % / 30 %). An endotracheal tube (5.5 Ch) was inserted through a tracheotomy. After immobilization with pancuronium bromide (0.2 mg/kg b.w./h, i.v.), the animals were artificially ventilated (Servo Ventilator 900C, Siemens-Elema, Solna, Sweden).

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![Fig. 1. Preparation of the skull – epidural balloon and monitoring device in place; ICP = intracranial pressure; ECoG = electrocorticogram.](image-url)
gen capacity of hemoglobin (1.39 mlO2/g hemoglobin) and hemoglobin concentration (g/ml) plus dissolved O2 and expressed in μMol/min · 100g. Cerebral O2 delivery was calculated as the product of CBF times the arterial O2 content. The CMRO2 was calculated as arteriovenous oxygen content difference times CBF.

CPP was calculated as the difference between MAP and ICP.

**CMS technique for CBF measurement:** Cardiac output (CO) and regional CBF were measured by means of the reference sample color-labeled microsphere technique (Ko-WALLIK et al. 1991). Application and methodical considerations were presented and discussed in detail elsewhere (WALTER et al. 1997).

Briefly, in random sequence a known amount (~ 3 × 10^6 per injection) of colored polystyrene microspheres (diameter: 15.5 ± 0.33 μm) in 0.01 % Tween 80, surface coated with one of five dyes (white, yellow, red, violet, blue; Dye-Trak, Triton Technology, San Diego, U.S.A.) was thoroughly vortexed and sonicated and immediately injected in less than 20 seconds into the left ventricle. The injection line was then flushed with 2 ml saline. A blood sample was withdrawn from the descending aorta, as the reference sample beginning 15 seconds before the microsphere injection and continuing for 2 minutes with a rate of 1.5 ml/min (syringe pump SP210iW, World Precision Instruments, Sarasota, U.S.A.). The microsphere injection did not alter arterial blood pressure (ABP). After completion of the experiment the brains were removed and sectioned to determine blood flow to the following areas: brain stem (medulla oblongata, pons, midbrain), cerebellum, hippocampus, caudate nucleus, thalamus, white matter (corpus callosum and periventricular), hemispheres (gray matter pooled from frontal, parietal, temporal, and occipital lobes). Than all tissue (between 0.15 and 2.5 g) and blood samples were digested (4N KOH with 4 % Tween 80) for a minimum of four hours at 60 °C. The microspheres were retained by filtering each digested sample through an 8-μm pore PE-membrane filter (Fa. Costar, Bodenheim, Germany).

The filtration membrane was rinsed with 1% Tween 80 and subsequently with 70% ethanol. CMS were quantified by their dye content. The photometric absorption of each dye solution was measured by a diode-array UV/visible spectrophotometer (Model 7500, Beckman Instruments, Fullerton, U.S.A., wave length range, 300-800 nm, with a 2-nm optical bandwidth). Calculations were performed using the MISS software (Triton Technology, San Diego, U.S.A.). The number of microspheres was calculated using the specific absorbance value of the different dyes (provided by the manufacturer). Absolute flows to tissues measured by CMS were calculated by the formula: flow_tissue = number of microspheres_tissue / (flow_reference / number of microspheres_reference). Flows are expressed in milliliters per minute per 100g tissue by normalizing for tissue weight.

Gradual CPP reduction controlled by an external Proportional-Integral-Differential (PID)-Controller: A detailed description of this procedure involving an external blood pressure control-loop to adjust MAP to an given set-point (like the baseline ABP value, as used in this application) has been given elsewhere (BAUER et al. 1997). Briefly, this special control-loop consists of a remote infusion/withdrawal pump, which is controlled by a PID-controller running at a PC which is serially connected. ABP is controlled by changing the blood volume by arterial blood infusion or withdrawal, respectively. The PID-controller was designed so that relevant active and passive physiological properties of the cardiovascular system are simplified and embodied in the PID-controller box. In order to get a vanishing control-error of the control-loop, an integral behavior in the controller was realized by means of the remote infusion/withdrawal pump which allowed the variation of the blood volume. Variation of volume per unit time was proportional to the difference between the measured MAP and the given set-point. The resulting effect on ABP represented an integral relation. Additionally, there was a static nonlinear transition in this technical controller calculated using Boyle’s law (p · V = const.). A sufficient proportionality factor was found by trial and error. The use of this equipment allowed changing CPP as the difference of MAP minus ICP with good precision and stability and without any possible influences of reactive MAP changes due to stepwise ICP increase.

**ECoG recording:** Unipolar ECoG recordings were performed using four screw electrodes, which were drilled bila- terally into the skull above the somatosensory cortex (reference electrode on nasion). Electrode position was just posterior to the coronar suture and 5 and 10 mm lateral to the sagittal suture, respectively. ECoG signals were amplified, filtered (time constant was 0.1 second, cut off frequency was 1000 Hz), fed into a PC using a 16 channel A/D board (DT2821F, Data Translation, Marlboro, U.S.A.) and stored on the hard disk for off-line data analysis (sample rates were 512 Hz). ECoG was quantified for three minutes at each experimental period using the Fast Fourier Transformation (FFT). The spectral analysis consisted of calculating the spectral band power (total power: 1–30 Hz; delta band: 1–4 Hz; theta 5–8 Hz; alpha 9–12 Hz; beta 13–30Hz) and the delta ratio ([alpha band + beta band]/[delta band]).

**Experimental protocol:** After the surgical preparation had been completed, the piglets were allowed to rest for approximately 45 minutes. Then control values (control 1) were recorded and randomly chosen animals, HT group (n = 7), were surface cooled by crushed ice packs and cooled water flow pads maintained a body temperature (rectal temperature) of 31.9°C ± 0.3°C throughout the experiment. NT animals (n = 6) referred as control group to observe the effects of moderate hypothermia (control 2). At this stage the external ABP controller was established in order to adjust MAP at baseline ABP values. Then, a second series of values were obtained followed by gradual decrease of CPP by stepwise elevation of the ICP, beginning at 25 mmHg followed by 35 mmHg and 45 mmHg due to gradual epidural balloon inflation at a time of about 30 minutes each, and stabilization of the MAP by the external blood pressure control loop. Using this procedure steady state periods of reduced CPP of about 70 %, 50 %, and 30 % of baseline values were produced. Repeated measurement of all variables were recorded at the 25th minute of every steady state period. At the end of the experiments, the piglets were killed with KCl and the brains were removed for processing.

**Statistical analysis:** Data are reported as means ± SEM. The control variables were compared between groups with unpaired t tests. Two-way analysis of variance (ANOVA)
Table 1. Data of cerebral and systemic metabolism and hemodynamics during normothermia, hypothermia (32 °C) and stepwise increase of ICP.

<table>
<thead>
<tr>
<th></th>
<th>control 1</th>
<th>control 2</th>
<th>25 mmHg</th>
<th>35 mmHg</th>
<th>45 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR [b/min]</strong></td>
<td>NT 246 ± 15</td>
<td>244 ± 11</td>
<td>267 ± 14</td>
<td>234 ± 15</td>
<td>205 ± 12</td>
</tr>
<tr>
<td></td>
<td>HT 233 ± 14</td>
<td></td>
<td>176 ± 8*</td>
<td>164 ± 9*</td>
<td>158 ± 11*</td>
</tr>
<tr>
<td><strong>MAP [mmHg]</strong></td>
<td>NT 85 ± 4</td>
<td>84 ± 2</td>
<td>80 ± 3</td>
<td>76 ± 3*</td>
<td>71 ± 2*</td>
</tr>
<tr>
<td></td>
<td>HT 88 ± 3</td>
<td></td>
<td>82 ± 3</td>
<td>77 ± 4*</td>
<td>69 ± 6*</td>
</tr>
<tr>
<td><strong>Cl [ml/min*kg]</strong></td>
<td>NT 224 ± 16</td>
<td>225 ± 39</td>
<td>124 ± 22 $</td>
<td>184 ± 33</td>
<td>205 ± 24</td>
</tr>
<tr>
<td></td>
<td>HT 213 ± 33</td>
<td></td>
<td>204 ± 35</td>
<td>125 ± 22 $</td>
<td>187 ± 30</td>
</tr>
<tr>
<td><strong>ICP [mmHg]</strong></td>
<td>NT 7 ± 1</td>
<td>6 ± 1</td>
<td>25 ± 1*</td>
<td>34 ± 1*</td>
<td>47 ± 1*</td>
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<tr>
<td></td>
<td>HT 7 ± 1</td>
<td></td>
<td>6 ± 1</td>
<td>25 ± 1</td>
<td>36 ± 1*</td>
</tr>
<tr>
<td><strong>CPP [mmHg]</strong></td>
<td>NT 80 ± 3</td>
<td>79 ± 3</td>
<td>60 ± 2*</td>
<td>46 ± 2*</td>
<td>30 ± 5*</td>
</tr>
<tr>
<td></td>
<td>HT 84 ± 3</td>
<td></td>
<td>80 ± 4</td>
<td>59 ± 4*</td>
<td>44 ± 4*</td>
</tr>
<tr>
<td><strong>AVDO₂ [mmol/ml]</strong></td>
<td>NT 3.0 ± 0.2</td>
<td>2.8 ± 0.2</td>
<td>2.7 ± 0.3</td>
<td>3.2 ± 0.3</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>HT 3.0 ± 0.1</td>
<td>2.1 ± 0.2*$</td>
<td>2.0 ± 0.3*$</td>
<td>2.2 ± 0.1*$</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td><strong>CMRO₂ [µmol/min*100g]</strong></td>
<td>NT 179.7 ± 22.0</td>
<td>149.0 ± 8.4</td>
<td>154.9 ± 17.3</td>
<td>101. ± 10.6</td>
<td>25.1 ± 9.6*</td>
</tr>
<tr>
<td></td>
<td>HT 184.3 ± 32.0</td>
<td>92.3 ± 16.0*$</td>
<td>103.2 ± 14.6*$</td>
<td>80.2 ± 5.0*$</td>
<td>36.4 ± 9.4*</td>
</tr>
<tr>
<td><strong>gCBF [ml/min*100g]</strong></td>
<td>NT 66.8 ± 6.7</td>
<td>62.7 ± 4.7</td>
<td>73.7 ± 9.6</td>
<td>45.4 ± 5.6*</td>
<td>27.5 ± 10.9*</td>
</tr>
<tr>
<td></td>
<td>HT 69.0 ± 9.7</td>
<td>49.7 ± 4.8*</td>
<td>68.3 ± 9.0</td>
<td>51.0 ± 3.7*</td>
<td>19.5 ± 4.8*</td>
</tr>
<tr>
<td><strong>tpO₂ [mmHg]</strong></td>
<td>NT 22.7 ± 3.3</td>
<td>20.3 ± 3.0</td>
<td>15.1 ± 5.0</td>
<td>11.5 ± 4.9*</td>
<td>3.3 ± 1.4*</td>
</tr>
<tr>
<td></td>
<td>HT 22.7 ± 2.5</td>
<td>29.7 ± 3.4*$</td>
<td>21.2 ± 4.0$</td>
<td>15.5 ± 4.4</td>
<td>3.3 ± 0.7*</td>
</tr>
<tr>
<td><strong>paO₂ [mmHg]</strong></td>
<td>NT 147.7 ± 8.9</td>
<td>150 ± 8.9</td>
<td>145.8 ± 9.5</td>
<td>149.6 ± 9.1</td>
<td>146.4 ± 13.2</td>
</tr>
<tr>
<td></td>
<td>HT 135.5 ± 3.9</td>
<td>142.2 ± 8.4</td>
<td>128.4 ± 9.3</td>
<td>125.7 ± 8.7</td>
<td>131.4 ± 5.7</td>
</tr>
<tr>
<td><strong>paCO₂ [mmHg]</strong></td>
<td>NT 38.0 ± 0.4</td>
<td>40.2 ± 1.4</td>
<td>39.1 ± 0.5</td>
<td>38.7 ± 0.7</td>
<td>39.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>HT 37.3 ± 1.0</td>
<td>38.8 ± 1.9</td>
<td>39.0 ± 1.8</td>
<td>38.7 ± 1.3</td>
<td>38.2 ± 0.9</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>NT 7.42 ± 0.01</td>
<td>7.40 ± 0.02</td>
<td>7.39 ± 0.01</td>
<td>7.38 ± 0.02</td>
<td>7.35 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>HT 7.44 ± 0.02</td>
<td>7.38 ± 0.03</td>
<td>7.41 ± 0.02</td>
<td>7.40 ± 0.02</td>
<td>7.39 ± 0.02</td>
</tr>
<tr>
<td><strong>rectal temperature [°C]</strong></td>
<td>NT 38.2 ± 0.3</td>
<td>38.2 ± 0.2</td>
<td>38.2 ± 0.1</td>
<td>38.3 ± 0.1</td>
<td>37.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>HT 38.0 ± 0.2</td>
<td>31.7 ± 0.2*$</td>
<td>31.8 ± 0.2*$</td>
<td>31.8 ± 0.2*$</td>
<td>31.8 ± 0.1*$</td>
</tr>
<tr>
<td><strong>brain temperature [°C]</strong></td>
<td>NT 38.6 ± 0.2</td>
<td>38.3 ± 0.3</td>
<td>38.2 ± 0.3</td>
<td>38.2 ± 0.3</td>
<td>37.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>HT 38.4 ± 0.3</td>
<td>31.9 ± 0.1*$</td>
<td>31.7 ± 0.3*$</td>
<td>31.9 ± 0.1*$</td>
<td>31.0 ± 0.4*$</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; NT = 6 animals; HT = 7 animals; control 1: experimental stage of normothermia in both groups; control 2: HT animals were cooled to 32 °C, this temperature was maintained throughout the experimental procedure; 25 mmHg, 35 mmHg and 45 mmHg: the ICP-levels with associated CPP reduction to about 70%, 50% and 30% of baseline values (control 1).

* = significant differences to control 1 (p < 0.05);
$ = significant differences between NT and HT (p < 0.05)

with repeated measures was used to determine the effects of CPP alteration and group-CPPI interaction within each variable. Subsequently, one-way ANOVA with repeated measures was performed within each group. Post hoc comparisons were made with paired t-tests using Bonferroni correction for multiple use. Between groups comparisons were made with unpaired t-tests using Bonferroni correction for multiple use. All statistical tests were done using the statistical package SPSS for Windows Release 6.0 (SPSS Inc., Chicago, U.S.A.). Differences were considered significant when p < 0.05.

Results

The data of cerebral and systemic hemodynamics as well as the metabolic data are shown in Table 1. In the HT group after the baseline values had been recorded (control 1), the body temperature was reduced to 31.9 ± 0.3 °C by surface cooling and was maintained throughout the experiment. But, at the ICP-level of 45 mmHg the brain temperature decreased slightly more to 31.0 ± 0.4 °C.

Arterial blood gases (paO₂; paCO₂) and arterial pH were similar in the NT and HT group throughout the experimental procedure.

The cardiac index (CI) was unchanged during hypothermia, although the heart rate (HR) was strongly reduced by about 25% (p < 0.05). During ICP increase to 25 mmHg the CI was significantly diminished to 56 ± 17% (NT) and 58 ± 18% (HT) from baseline (p < 0.05), whereas at the higher ICP-levels (35 and 45 mmHg) the CI recovered almost completely.

MAP was virtually constant throughout the experiment (81 ± 11 mmHg), even though the MAP was reduced at the higher ICP-levels (35 and 45 mmHg, p < 0.05). The
Table 2. Frequency bands, total spectral power and delta ratio from ECoG - relative changes during hypothermia and stepwise increase of ICP in percent from normothermic baseline (control 1). The frequency band activity indicates percent from total spectral power at the experimental stage as 100%.

<table>
<thead>
<tr>
<th>delta band</th>
<th>normothermic animals (n = 6)</th>
<th>hypothermic animals (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control 1</td>
<td>68 ± 25</td>
<td>69 ± 20</td>
</tr>
<tr>
<td>control 2</td>
<td>72 ± 25</td>
<td>48 ± 22$</td>
</tr>
<tr>
<td>25 mmHg</td>
<td>72 ± 24</td>
<td>60 ± 24</td>
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<tr>
<td>35 mmHg</td>
<td>81 ± 27</td>
<td>70 ± 20</td>
</tr>
<tr>
<td>45 mmHg</td>
<td>85 ± 42</td>
<td>78 ± 48</td>
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<tr>
<td>alpha band</td>
<td>normothermic animals (n = 6)</td>
<td>hypothermic animals (n = 7)</td>
</tr>
<tr>
<td>control 1</td>
<td>5 ± 12</td>
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<tr>
<td>control 2</td>
<td>5 ± 20</td>
<td>14 ± 21$</td>
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<td>25 mmHg</td>
<td>6 ± 35</td>
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<td>45 mmHg</td>
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<td>4 ± 54</td>
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<td>control 1</td>
<td>100 ± 17</td>
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<td>control 2</td>
<td>98 ± 24</td>
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<td>35 mmHg</td>
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<td>45 mmHg</td>
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<td>15 ± 26</td>
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<td>15 ± 28</td>
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<td>45 mmHg</td>
<td>9 ± 40</td>
<td>13 ± 49</td>
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<tr>
<td>beta band</td>
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<td>hypothermic animals (n = 7)</td>
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<tr>
<td>control 1</td>
<td>13 ± 30</td>
<td>9 ± 19</td>
</tr>
<tr>
<td>control 2</td>
<td>10 ± 30</td>
<td>7 ± 22</td>
</tr>
<tr>
<td>25 mmHg</td>
<td>8 ± 37</td>
<td>6 ± 14</td>
</tr>
<tr>
<td>35 mmHg</td>
<td>4 ± 51</td>
<td>5 ± 33</td>
</tr>
<tr>
<td>45 mmHg</td>
<td>3 ± 315</td>
<td>5 ± 47</td>
</tr>
<tr>
<td>delta ratio</td>
<td>normothermic animals (n = 6)</td>
<td>hypothermic animals (n = 7)</td>
</tr>
<tr>
<td>control 1</td>
<td>100 ± 55</td>
<td>100 ± 24</td>
</tr>
<tr>
<td>control 2</td>
<td>102 ± 25</td>
<td>225 ± 25$</td>
</tr>
<tr>
<td>25 mmHg</td>
<td>62 ± 45$</td>
<td>105 ± 28$</td>
</tr>
<tr>
<td>35 mmHg</td>
<td>40 ± 69</td>
<td>36 ± 34$</td>
</tr>
<tr>
<td>45 mmHg</td>
<td>19 ± 26$</td>
<td>35 ± 25$</td>
</tr>
</tbody>
</table>

Values are mean ± SEM

$ = $ significant differences to control 1 (p < 0.05);
$S = $ significant differences between NT and HT (p < 0.05)

CPP was maintained at 60, 45 and 30 mmHg using the PID-controller and showed no differences between the normothermic and hypothermic group.

The baseline ECoG exhibited the typical delta-dominated frequency pattern for isoflurane anaesthesia. At the hypothermic level (control 2), the amplitude of ECoG activity was markedly diminished, indicated by the total spectral power reduction of 44 ± 17 % (p < 0.05), compared with baseline (table 2). Moreover, a frequency shift was seen towards the higher frequencies (theta- and alpha-band) (fig. 2). These hypothermia induced ECoG changes were associated with a reduction of the global CBF (72 ± 13 % from the baseline, p < 0.05). However, the regional CBF differed considerably between the brain regions at 32 °C (control 2), the cortical CBF decreased markedly to 67 ± 10 % (p < 0.05), whereas the regional CBF in the medulla oblongata increased slightly (114 ± 15 % from the baseline). The regional CBF of the thalamus and of the midbrain did not change significantly (fig. 3).

At this stage (control 2) the band power distribution of the ECoG power was markedly changed, compared with baseline values. The delta activity was reduced to 48 ± 22 % (p < 0.05), whereas the alpha activity increased to about three times as high and the theta activity was about twice as high as baseline (table 2, fig. 2). These changes in the band power distribution were also indicated by the increase of the delta ratio (225 ± 25 % from the baseline, p < 0.05).

Fig. 2. Frequency distribution of neuronal electrical activity in TN and HT animals during stepwise increase of ICP.
Fig. 3. Regional CBF in different brain regions during normothermia, hypothermia and stepwise increase of ICP. * = significant differences to control (p < 0.05); $ = significant differences between NT and HT (p < 0.05).

In the normothermic animals an increase of ICP to 25 mmHg with a following CPP reduction to about 70 % from baseline led to a frequency lowering of ECoG and an increase of delta activity, indicated by a reduced delta ratio (62 ± 45 %, p < 0.05) (table 2, fig. 2) and a slightly increased global CBF. The rise in the CBF was seen in all brain regions in both groups. The strongest increase of regional CBF was found in the medulla oblongata (NT: 166 ± 24 %, HT: 145 ± 26 %, p < 0.05) and in the thalamus (NT: 151 ± 45 %, HT: 153 ± 53 %, p < 0.05). An increase of midbrain blood flow occurred only in the NT group (158 ± 26 %, p < 0.05).

The ICP increase to 35 mmHg which was accompanied by a CPP reduction to about 50 % induced a marked decrease in global CBF to 68 ± 8 % in the NT and 74 ± 5 % in the HT group (p < 0.05), respectively. Global CBF diminishing was associated to a marked reduction of the delta amplitude in the ECoG of the NT group (fig. 4) and an increase of the delta band activity to 81 ± 27 %. In contrast, hypothermic animals exhibited ECoG patterns similar to those for control 2 stage (32 °C). Cortical CBF decreased significantly in both groups (NT: 58 ± 16 %; HT: 58 ± 10 %, p < 0.05). However, medulla oblongata blood flow remained elevated in both groups, nevertheless the medulla oblongata blood flow differed significantly between the normothermic (105 ± 18 %) and hypothermic (136 ± 14 %) animals (p < 0.05). Similar to the regional CBF in the medulla oblongata, differences occurred in the thalamus between the normothermic and the hypothermic group, still these differences were not significant.

The last stage of ICP increase (45 mmHg) caused a CCP reduction to about 30 ± 3 % from baseline. The resulting decrease of global CBF differed between the two groups (NT: 41 ± 16 %; HT: 28 ± 7 %, p < 0.05), but were comparable with reference to hypothermic baseline CBF (control 2) (NT: 41 ± 16 %; HT: 39 ± 10 %). Similar to the decrease of the global CBF the cortical blood flow decreased considerably in both groups, but was more pronounced in hypothermic (18 ± 6 %) than in normothermic animals (31 ± 13 %, p < 0.05). In contrast, medulla oblongata blood flow was only reduced by about 30 % of baseline in both groups.

In hypothermic animals a further ECoG frequency lowering, amplitude reduction (fig. 4) and maintained residual total spectral power were observed (table 2). In contrast, the ECoG activity in the normothermic group was almost completely suppressed and burst suppressions were sporadically observed in two animals (fig. 4).

Discussion

In the present study we determined the effects of hypothermia on the neuronal electrical activity in juvenile piglets during gradual increase of the ICP with consecutive CPP reduction using epidural brain compression. The major ECoG changes in the anaesthetized juvenile piglets at mild hypothermia (32 °C) were an arousal-like ECoG activity with a marked frequency shift from delta wave dominated activity to alpha wave activity and a concomitant reduction in total spectral power. Our results are similar to those of previous studies showing that mild hypothermia levels may produce cerebral stimulatory effects as reflected by a beginning depression of amplitude, hyperresponsive reflexes and arousal phenomena in patients with depressed sensory (BLAIR 1965). These findings confirm observations by Fay (FAY 1945, 1959), who described central stimulatory effects in adult humans during cooling to 32 °C. Additionally, in humans and mammals due to mild hypothermia also unchanged or marginally altered electrocortical activity with small shifts in EEG frequencies to an increase of theta and beta activity have been described (FITZGIBON et al. 1984; MICHENFELDER et al. 1991).

The frequency shift of the ECoG by hypothermia seems to be an electrical phenomenon, that is obviously related by the regional CBF and the oxidative metabolism. This suggestion is in agreement with findings obtained from regional CBF assessments of behavioral state
changes, where spontaneous changes from high to low voltage patterns of the ECoG were accompanied by an increase of regional CBF to areas of the brain corresponding to the arborization of the reticular formation (Jensen et al. 1986).

Commonly, an increased ECoG alpha activity is a sign for an improved cerebral activity, and is associated with an increase of CBF and cerebral oxidative metabolism (Sulg et al. 1981; Bischoff 1994).

We found, however, at hypothermia the global CBF and CMRO2 remained suppressed, whereas the ECoG alpha activity increased. In our study we investigated the ECoG changes in relation to regional CBF measurements at hypothermia. The discovery of differences in the regional CBF, which occurred under hypothermia may help explain the increased ECoG alpha activity. At hypothermia cortical blood flow was pronouncedly reduced compared with midbrain blood flow. Normally, midbrain's reticular formation modulates the frequency of the ECoG (Zschocke 1995). It is well known that the electrocortical activity, especially the alpha activity, is remarkably influenced by the neuronal activity of the midbrain, which is able to induce and keep rhythmic activity (Jones et al. 1983). Especially, the midbrain's reticular system modulates thalamic synchronization and desynchronization (Schmidt 1990). The blood flow in these brain regions was not found to be influenced by hypothermia. Above all, a reduction of the global CBF by hypothermia is caused by changes of the cortical blood flow. This cortical blood flow reduction is a possible cause of the reduction of total spectral power and electrocortical high-voltage activity at mild hypothermia. Furthermore, the reticular formation has diffuse thalamocortical projections that when activated, cause desynchronization of high-voltage activity to low voltage activity (Ingvar and Söderberg 1958). Presumably, the
frequency shift may be caused by the imbalance of inhibitory and stimulatory influences from the midbrain and from the thalamic frequency modulating fibres to the cortex, whose own electrical activity is suppressed by blood flow reduction at mild hypothermia. This relative stimulation evokes a partial desynchronization of the afferent reticular arousal system (BAUER et al. 1997). Another reason for these discrepancies in the results of the preserved high frequencies in the ECoG in this study and previous studies is probably due to differences in brain maturation. The other studies cited were done on adult dogs and we used juvenile piglets (STEEN et al. 1983; MICHENFELDER and MILDE 1991).

During controlled reduction of CPP by regional mass expansion due to epidural balloon inflation we found at mild and moderate stages only minimal changes in the ECoG in hypothermic animals compared to the hypothermic baseline (control 2), whereas the ECoG in normothermic animals showed a marked decrease in frequency, amplitude and total spectral power. These findings are consistent with classical pathophysiological EEG patterns observed in acute traumatic coma (IBRAHIM and ELIAN 1974; NEWLON 1996). The higher the proportion of EEG that is attributable to delta, the more likely is a poor prognosis. However, this EEG pattern in itself is not a particularly useful discriminator of outcome, because in mild and moderate traumatic head injuries the EEG pattern normalizes quickly (RUMPL 1993).

In our study, moderate brain compression (ICP 35 mmHg) caused a more pronounced affect on brain function in normothermic animals as compared with hypothermic animals. At 35 mmHg ICP differences in global CBF change between the two groups were notable. In HT animals the CBF did not change from the baseline (25% less than normothermic baseline) with moderate ICP increase. In normothermia the CBF was reduced by about 32% from baseline with moderate ICP increase. Despite the fact that the HT animals at hypothermic baseline (control 2) and the NT animals at ICP 35 mmHg have comparable CBF and CMRO2 values, there was a markedly different neuronal electrical activity. The preserved high frequency activity of HT animals is an indication of a maintenance of energy production adequate for the functional requirements of the brain. Failure of brain energy production occurs at a level of CBF somewhat lower than that causing loss of neuronal function (LOWING and GLEBE 1994). Obviously, the burst suppressions and the ECoG silence at the highest level of ICP increase to 45 mmHg, in NT animals, are caused by exceeding the threshold of neuronal and metabolic failure. In contrast, in HT animals, a residual activity of brain function is preserved.

The suppression of the cerebral metabolism at hypothermia of about 25% in CBF and 57% in CMRO2 in our study corresponds with findings in newborn piglets and amounted to nearly fifty percent more than was reported for adult animal brains (RUPP and SEVERINGHAUS 1986; NEMOTO et al. 1990).

With the use of anaesthetics the phenomenon of the frequency shift to theta and alpha-band at hypothermia could be influenced. With the use of a minimum alveolar concentration (MAC) of 1 MAC of isoflurane there is commonly a shift to slow frequency and high amplitude ECoG (JANTZEN 1992), which we have seen at control 1. Furthermore, hypothermia normally necessitates a MAC reduction of isoflurane (SATAS et al. 1995). Accordingly, with the use of 1 MAC isoflurane, we expected a further lowering of frequency and reduction of amplitude of the ECoG with hypothermia. ECoG frequency shifts to higher frequencies with hypothermia, as seen in our study, are not explainable by influences of anaesthetics. Obviously, the influence of hypothermia on neuronal electrical activity seems to be independent from basic anaesthetics.

In summary, the present data showed in juvenile piglets with isoflurane anaesthesia during mild hypothermia an arousal-like ECoG activity with marked frequency shift to theta and alpha activity and a change from high to low-voltage activity, indicated by a reduced total spectral power in the ECoG. Furthermore, the hypothermic brain showed a preserved neuronal function at moderate stages of impaired cerebral perfusion. Obviously, hypothermia improves the functional tolerance of the brain to reduced oxygen supply. This confirms the well-known effect of mild hypothermia on a decrease of cerebral oxidative metabolism.

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References
BUSTO R, GLOBUS MY, DIETRICH WD: Effect of mild hypothermia on ischemia-induced release of neurotrans-
A Piglet Model for Evaluation of Cerebral Blood Flow and Brain Oxidative Metabolism during Gradual Cerebral Perfusion Pressure Decrease

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**Key Words**
 Epidural balloon inflation · Colored microspheres · Cerebral metabolic rate of oxygen · Piglets

**Abstract**
A piglet model was developed to study the effect of epidural volume expansion on cerebral perfusion pressure (CPP) by stepwise elevating intracranial pressure (ICP). Mean arterial blood pressure (ABP) was strictly maintained using an extracorporeal ABP controller. Two-week-old piglets ($n = 10$) were studied by surgically placing an epidural balloon over the right parietal region and gradually increasing the inflation to increase ICP to 25, 35 and 45 mm Hg, maintaining each pressure level for 30 min. Regional cerebral blood flow was measured using the colored microsphere technique, and cerebral oxygen delivery and cerebral metabolic rate of oxygen were calculated at baseline conditions and after reaching ICP levels of 25, 35 and 45 mm Hg. The results showed that this model of epidural volume expansion reproducibly reduces CPP to 70, 50 and 33% of baseline CPP values with elevation of ICP, and that the physiological variables remained stable throughout each increase in ICP. We conclude that the model simulates the effects of an acute intracranial focal mass expansion and is well suited for the evaluation of different therapeutical strategies for increased ICP in newborns and infants.

**Introduction**
Intracranial hypertension (defined as a persistent elevation of intracranial pressure, ICP, over 20 mm Hg) is a common complication following severe traumatic brain injuries in infants and young children and is closely correlated to their outcome. Recently, it was reported that approximately 60\% of severely head-injured patients develop increased ICP [1], and the incidence of brain swelling following traumatic brain injuries in pediatric patients is twice that of adults [2, 3]. Moreover, a considerable number of patients suffer uncontrolled intracranial hypertension that progresses to cerebral nonperfusion and death. Much of the study to date has been in adults and older children, with little attention or investigation as to the effects of increased ICP and its cerebrovascular and brain metabolic effect in the developing brain [4]. The aim of the present study was to develop an animal model which allows sequential measures of regional cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMRO$_2$) during the gradual reduction of cerebral perfusion pressure (CPP) by stepwise epidural balloon inflation. Through a better understanding of the physiologic changes in the brain with decreasing perfusion, strategies could be developed in the future to reduce the detrimental effects of intracranial hypertension on cerebral metabolism.
Material and Methods

The committee of the Thuringian State government for animal research approved this protocol. The animals were managed in accordance with the guidelines of the American Physiological Society.

Surgical Preparation

Ten piglets of mixed German domestic breed (14 days old, body weight 4,490 ± 611 g) were initially sedated with ketamine hydrochloride (50 mg/kg b.w.) and then anesthetized with 1.5% isoflurane in 70% nitrous oxide and 30% oxygen. A central venous catheter was introduced through the left external jugular vein and was used for administration of drugs and for volume substitution (lactated Ringer's solution; 5 mL/h). An endotracheal tube was inserted by means of a tracheotomy. After immobilization with pancuronium bromide (0.2 mg/kg b.w./h i.v.), the animals were artificially ventilated (Servo Ventilator 900C; Siemens-Elema, Solna, Sweden). Anesthesia was maintained throughout the experiment with 0.8% isoflurane. Polyethylene catheters (inner diameter 1.5 mm) were advanced through the femoral arteries into the abdominal aorta in order to record arterial blood pressure (ABP) and withdraw reference samples for the colored microsphere technique. A further polyethylene catheter (inner diameter 0.3 mm) was inserted into the superior sagittal sinus and advanced to the confluence sinuum in order to obtain venous blood samples of the brain. The heart (left ventricle) was cannulated retrogradely via the right common carotid artery with a polyurethane catheter (inner diameter 0.5 mm). Arterial, left ventricular and central venous catheters were connected with pressure transducers (P23Db, Statham Instruments Inc., Hato Rey, Puerto Rico). Body temperature was monitored by a rectal thermoprobe and maintained throughout the experiment at 38 ± 0.3°C using a heating-cooling thermostat and a feedback-controlled heating lamp. Physiological parameters were recorded on a multichannel polygraph (MT95K2®; Astro-Med Inc., West Warwick, R.I., USA).

After a midline skin incision and scalp retraction, a craniostomy was performed in the left frontal bone using a high-speed air drill, and an ICP monitor was implanted into the subcortical white matter for ICP measurements (Camino Laboratories, San Diego, Calif., USA). A right-sided craniostomy (10 to 3 mm) was also drilled into the frontal bone, 4 mm lateral of midline and parallel to the sagittal suture; the dura was left intact. An epidural latex balloon attached to a catheter was placed in the epidural space of the right frontoparietal region. The burr holes were sealed with bone wax and covered with dental acrylic to keep the probes and the epidural balloon catheter in place throughout the experiment.

Experimental Protocol

After the surgical preparation had been completed, the piglets were allowed to recover for approximately 60 min. After baseline values of physiological parameters including CBF had been obtained, the external ABP controller was established to maintain the ABP at those baseline values. Following the 30-min period at controlled ABP, a second measure was performed (control 2). Then, the volume of the epidural balloon was increased so as to gradually decrease the effective CPP. This was calculated as the difference between mean ABP and ICP. A stepwise elevation of the ICP was achieved increasing the pressure to 15, 25, 35 and 45 mm Hg for 30 min at each level. The external blood pressure control loop was able to maintain a stable ABP throughout the experimental protocol. A detailed description of the procedure of mean ABP adjustment at an externally given set point (baseline ABP value, as used in this application) using an external blood pressure control loop has been given elsewhere [5]. In brief, ABP (controlled quantity) was controlled by a proportional differential integral controller running on a PC, by means of changing the blood volume by arterial blood infusion or withdrawal, respectively. An infusion/withdrawal pump was the controlling element. Since there are several nonlinear and nonstationary properties in the controlled physiological system, the parameters of the external technical controller were searched for by trial and error. Stable control of ABP was reached by means of the integrating property of the controller in particular. The integration time constant of Ki = 6.94 mm Hg·min⁻¹ was found to be appropriate during all states investigated.

Physiologic Data Measurements, CBF Measures and Analysis

Blood pH, pCO₂, and pO₂ were determined with aABL50® Blood Gas Analyzer (Radiometer, Copenhagen, Denmark). Blood hemoglobin and arterial oxygen saturation were determined using a Hemoxymer OSM2® (Radiometer).

Regional CBF was measured by means of a reference sample obtained through color-labeled microsphere technique [6, 7]. Briefly, in random sequence, a known amount (~1 × 10⁶ per injection) of colored polystyrene microspheres (diameter 15.5 ± 0.33 μm) in 0.01% Tween 80, surface coated with one of five dyes (white, yellow, red, violet, blue; Dye-Trak®; Triton Technology, San Diego, Calif., USA) was thoroughly vortexed and sonicated and immediately injected within 20 s into the left ventricle via the right carotid catheter and then flushed with 2 ml saline. A blood sample was withdrawn from the descending aorta as the reference sample [8], beginning 15 s before the microsphere injection and continuing for 2 min with a rate of 1.5 ml/min (syringe pump SP210W; World Precision Instruments Inc., Sarasota, Fla., USA). The microsphere injection did not alter ABP.

At the end of the experiment, the piglet was killed with KCl, and the brain was removed for processing. The brain was sectioned to determine blood flow to the following areas: brain stem (medulla, pons, midbrain), cerebellum, hippocampus, caudate nucleus, thalamus, white matter (corpus callosum and periventricular), hemispheres (gray matter pooled from frontal, parietal, temporal and occipital lobes). After sectioning, reference blood samples and tissue samples between 0.15 and 2.5 g were covered with an appropriate volume (approximately 3 ml/g) of digestive solution (4 N KOH with 4% Tween 80 in deionized water). All tissue and blood samples were digested for a minimum of 4 h at 60°C. In order to retain the microspheres, each digested sample was then filtered under vacuum suction through a PE membrane filter with 8-μm pores (Fa. Costar, Badenheim, Germany). The filtration membrane was gently rinsed with 1% Tween 80 and subsequently with 70% ethanol. Colored microspheres were quantified by their dye content. The membranes containing the microspheres on their surface were carefully folded and put into a conically shaped glass vial. The dye was recovered from the microspheres by adding 120 μl of dimethylformamide (DMFA) and subsequent vortexing of the vial in order to moisten the membrane completely. The photometric absorption of each dye solution was measured by a diode array UV/visible spectrophotometer (Model 7500; Beckman Instruments, Fullerton, Calif., USA; wavelength range 300–800 nm, with a 2-nm optical bandwidth). Calculations were performed using the MISS® software (Triton Technology). In a manner similar to that of the overlap correction in counting radioactive microspheres, the composite spectrum of each dye solution was...
resolved into the spectra of the individual constituents and corrected for spill over and background absorption. The amount of dye in a given sample was adjusted by appropriate dilution with DMFA to achieve absorbance values of no more than 1.3 AU (absorbance unit, 1 AU = -lg10% light transmission/100%) to ensure the linearity between absorbance and dye concentration according to the Lambert-Beer law. Samples with absorbances higher than 1.3 AU were further diluted with DMFA and analyzed again. The number of microspheres was calculated using the specific absorbance value of the different dyes (provided by the manufacturer). Absolute flows to tissues measured by colored microspheres were calculated by the formula:

\[
\text{flow}_{\text{tissue}} = \text{number of microspheres}_{\text{tissue}} \times (\text{flow}_{\text{reference}}/\text{number of microspheres}_{\text{reference}})
\]

flows are expressed in milliliters per minute per 100 g tissue by normalizing for tissue weight.

Assuming the oxygen capacity of hemoglobin to be 1.39 ml O2/g hemoglobin in piglets [9], blood O2 content was calculated as equal to g hemoglobin/ml·1.39 ml O2/g hemoglobin·%O2 saturation and expressed in μmol/min·100 g. Dissolved oxygen was added by calculation, using the measured pO2 and the temperature-corrected solubility coefficient of oxygen. Because the sagittal sinus drains the cerebral cortex, cerebral white matter and some deep gray structures (basal ganglia, hippocampus) [10], blood flow measured to the cerebrum included these structures (global CBF). CMRO2 was obtained by multiplying global CBF by the difference in cerebral arteriovenous O2 content. Cerebral O2 delivery was calculated as the product of global CBF times the arterial O2 content. Effective CPP was calculated as the difference between mean ABP and ICP. Total blood volume was assumed to be 12% of body weight [11].

**Statistical Analysis**

Data are reported as means ± SD. Data were subjected to analysis of variance for a repeated-measures design followed by the Tukey test to compare the means of parameters obtained during baseline conditions with those obtained after ABP adjustment and gradual ICP increase. Comparison of blood flow between the corresponding regions of the brain ipsi- and contralateral to the inflated epidural balloon was performed by two-way repeated-measures analysis of variance. Differences were considered significant when p < 0.05.

**Results**

Stable stages of gradually reduced CPP to 69 ± 1, 51 ± 3 and 32 ± 2% of baseline values (86 ± 7 mm Hg = 100%) could be achieved with stepwise increases in ICP to 25, 35 and 45 mm Hg, respectively (fig. 1 and table 1), by gradual epidural balloon inflation and by maintaining a constant ABP. ABP was adjusted by appropriate blood volume withdrawal/infusion (baseline: 84 ± 10 mm Hg; control 2: 87 ± 16 mm Hg; ICP 25 mm Hg: 80 ± 8 mm Hg, ICP 35 mm Hg: 76 ± 7 mm Hg, ICP 45 mm Hg: 71 ± 6 mm Hg) to ensure possible cardiovascular response effect of CPP due to the increased ICP. There was a considerable individual difference in cardiovascular ICP response as indicated by the time period and by the marked variance in the amount of changed blood volume at different experimental stages (table 1). The largest blood withdrawal was necessary at an increase in ICP to 25 mm Hg. In contrast to the earlier stages of ICP increase where blood withdrawal occurred, at the last stage of ICP increase, a complete reinfusion, partly by additional infusion of normal saline, was necessary in order to stabilize the ABP (table 1). However, at this level, a small but significant decrease in blood pressure occurred (p < 0.05).

Heart rate, arterial blood gases and acid-base balance, during baseline conditions and gradual ICP increase are shown in table 2. Heart rate, arterial blood gases and acid-base balance were widely unchanged throughout the earlier stages of the experimental procedure. During the last stage of CPP reduction, a small decrease in heart rate of 85 ± 15% occurred (p < 0.05).

Cerebral oxygen delivery and CMRO2 were widely maintained up to an increase in ICP to 25 ± 2 mm Hg which corresponded to a CPP reduction of 69 ± 1% of baseline values. This was caused by a slight increase in blood flow to the cerebrum by about 16%. A further increase in ICP to 36 ± 3 mm Hg with a concomitant
Table 1. Summarized presentation of the duration of the different experimental stages, and of the behavior of ABP, ICP and CPP during the whole period of every stage and during the periods of microsphere measurement and of blood infusion/withdrawal in order to adjust ABP at baseline values using an external ABP controller

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Control 2</th>
<th>ICP 25 mm Hg</th>
<th>ICP 35 mm Hg</th>
<th>ICP 45 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage duration, min</td>
<td>29±2</td>
<td>62±14</td>
<td>30±2</td>
<td>29±1</td>
<td>30±2</td>
</tr>
<tr>
<td>ABP during whole stage, mm Hg</td>
<td>93±7</td>
<td>90±6</td>
<td>86±4</td>
<td>82±2</td>
<td>78±2*</td>
</tr>
<tr>
<td>ABP during microsphere measurement, mm Hg</td>
<td>93±8</td>
<td>91±7</td>
<td>86±7</td>
<td>82±2</td>
<td>77±3*</td>
</tr>
<tr>
<td>ICP during whole stage, mm Hg</td>
<td>6±2</td>
<td>6±3</td>
<td>26±3*</td>
<td>37±3*</td>
<td>47±2*</td>
</tr>
<tr>
<td>ICP during microsphere measurement, mm Hg</td>
<td>7±2</td>
<td>6±2</td>
<td>25±2*</td>
<td>36±3*</td>
<td>45±2*</td>
</tr>
<tr>
<td>CPP during whole stage, mm Hg</td>
<td>86±7</td>
<td>84±7</td>
<td>59±4*</td>
<td>45±3*</td>
<td>31±3*</td>
</tr>
<tr>
<td>CPP during microsphere measurement, mm Hg</td>
<td>86±7</td>
<td>85±8</td>
<td>59±4*</td>
<td>44±4*</td>
<td>28±3*</td>
</tr>
<tr>
<td>Blood infusion/withdrawal, % of the calculated blood volume</td>
<td>-</td>
<td>-4.1±5.2</td>
<td>-8.8±8.3</td>
<td>-5.0±7.1</td>
<td>5.8±2.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. n = 10 animals with exception of stage ICP 45 mm Hg, because at this stage, in 2 animals an acute uncontrollable decrease in ABP occurred that the epidural balloon had to be deflated prematurely.

* p < 0.05, significant differences between baseline values and values obtained at different stages of the experimental procedure.

Table 2. Physiological parameters during periods of unchanged ICP and during gradual epidural balloon inflation at adjusted ABP by extracorporeal control

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Control 2</th>
<th>ICP 25 mm Hg</th>
<th>ICP 35 mm Hg</th>
<th>ICP 45 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, min⁻¹</td>
<td>240±45</td>
<td>245±31</td>
<td>259±42</td>
<td>226±45</td>
<td>200±34*</td>
</tr>
<tr>
<td>Arterial pO₂, mm Hg</td>
<td>154±28</td>
<td>154±28</td>
<td>151±28</td>
<td>154±27</td>
<td>152±33</td>
</tr>
<tr>
<td>Arterial pCO₂, mm Hg</td>
<td>38±1</td>
<td>40±4</td>
<td>39±2</td>
<td>39±2</td>
<td>39±2</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.42±0.03</td>
<td>7.40±0.05</td>
<td>7.40±0.04</td>
<td>7.39±0.05</td>
<td>7.36±0.09*</td>
</tr>
<tr>
<td>Arterial O₂ content, mmol⁻¹</td>
<td>6.6±0.8</td>
<td>6.7±0.8</td>
<td>6.2±0.7*</td>
<td>5.8±0.6*</td>
<td>5.6±0.7*</td>
</tr>
<tr>
<td>Arterial base excess, mmol⁻¹</td>
<td>0.1±2.3</td>
<td>0.5±2.3</td>
<td>-1.1±2.6</td>
<td>-1.6±3.4</td>
<td>-1.5±3.6</td>
</tr>
<tr>
<td>Arterial glucose content, mmol⁻¹</td>
<td>7.6±1.0</td>
<td>7.4±1.0</td>
<td>9.1±3.2</td>
<td>8.9±2.1</td>
<td>9.1±1.1*</td>
</tr>
<tr>
<td>Arterial lactate content, mmol⁻¹</td>
<td>1.4±0.2</td>
<td>1.3±0.1</td>
<td>1.9±0.9</td>
<td>2.0±1.0</td>
<td>2.5±1.3*</td>
</tr>
</tbody>
</table>

Values are means ± SD. n = 10 animals with exception of stage ICP 45 mm Hg, because at this stage, in 2 animals an acute uncontrollable decrease in ABP occurred so that the epidural balloon had to be deflated prematurely.

* p < 0.05, significant differences between baseline values and values obtained at different stages of the experimental procedure.

CPP reduction of 51 ± 3% led to an significant reduction of global CBF (72 ± 29% of baseline value), cerebral O₂ delivery (55 ± 31% of baseline value) and CMRO₂ (63 ± 30% of baseline value; table 3, p < 0.05). The last stage of ICP increase (45 ± 2 mm Hg) resulted in a severe CBF reduction (24 ± 18% of baseline value) with a more pronounced reduction of cerebral O₂ delivery (15 ± 13% of baseline value) and CMRO₂ (16 ± 15% of baseline value; table 3, p < 0.05). Moreover, some regional CBF showed a different behavior: infratentorial brain structures, thalamus and hippocampus tended to increase CBF during mild ICP elevation (fig. 2), but CBF was significantly reduced at the highest level of ICP increase (p < 0.05). Furthermore, CBF to regions ipsilateral to the inflated epidural balloon was similar to that of contralateral brain regions (fig. 2). This was also true for most cortical brain regions with exception of the frontal cortex and the rostral part of the parietal cortex. Here, brain structures which were attached to the inflated epidural balloon showed a significant blood flow reduction already at mild ICP.

Effect of Gradual CPP Decrease on CMRO₂ in Piglets

Fig. 2. Regional CBF as CPP was decreased (values are means ± SD). Black columns indicate the site contralateral and open columns the site ipsilateral to the epidural balloon. A–E indicate the different experimental stages (A = baseline, B = control 2, C = ICP 25 mm Hg, D = ICP 35 mm Hg, E = ICP 45 mm Hg). *p < 0.05, significant differences between baseline values and values obtained at different stages of the experimental procedure.

Table 3. Global CBF, cerebral oxygen delivery and cerebral oxygen consumption during periods of unchanged ICP and during gradual epidural balloon inflation at adjusted ABP by extracorporeal control

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Control 2</th>
<th>ICP 25 mm Hg</th>
<th>ICP 35 mm Hg</th>
<th>ICP 45 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global CBF, ml/min^-1^-100 g^-1</td>
<td>58 ± 21</td>
<td>54 ± 16</td>
<td>60 ± 29</td>
<td>34 ± 15*</td>
<td>12 ± 14*</td>
</tr>
<tr>
<td>Cerebral oxygen delivery, μmol/min^-1^-100 g^-1</td>
<td>397 ± 176</td>
<td>365 ± 125</td>
<td>372 ± 191</td>
<td>201 ± 99*</td>
<td>68 ± 73*</td>
</tr>
<tr>
<td>Cerebral oxygen consumption, μmol/min^-1^-100 g^-1</td>
<td>172 ± 67</td>
<td>146 ± 26</td>
<td>144 ± 58</td>
<td>104 ± 34*</td>
<td>43 ± 56*</td>
</tr>
</tbody>
</table>

Values are means ± SD. n = 10 animals with exception of stage ICP 45 mm Hg, because at this stage, in 2 animals an acute uncontrollable decrease in ABP occurred so that the epidural balloon had to be deflated prematurely.

*p < 0.05, significant differences between baseline values and values obtained at different stages of the experimental procedure.
increase ($p < 0.05$). This side difference existed also at the next stage of ICP increase, but disappeared during the strongest ICP increase (fig. 2).

**Discussion**

In the present study, an experimental model was designed to create a reproducible gradual decrease in CPP to levels considered to be increasingly harmful to the immature brain. This model of an epidural balloon expansion has not been previously described in immature animals. We purposely maintained a stable period of reduced CPP in order to study the effects on regional brain hemodynamics and brain oxidative metabolism.

**Methodology**

The method of raising ICP by means of an inflated epidural balloon has been widely used in adult animals [12–14] but not in immature animals. Moreover, in order to use this model for studying the relationship between alterations in ICP secondary to epidural volume expansion and cerebral perfusion, one has to be aware that early alteration of systemic circulation may occur (e.g. ABP increase [15]). The experimental variability of ABP was removed by external control to guarantee that the resultant changes in CPP were the prerequisite determinant of CBF regulation. The gradual ICP increase induced by stepwise epidural balloon inflation is regularly attended by a sustained increase in the ABP, as one component of the so-called ‘Cushing response’ (CR) [16]. CR is defined as a triad of an elevation in ABP, bradycardia and respiratory irregularities due to brain impairment [17]. The initial response is a graded rise in ABP [16]; irregularities in breathing may not occur until the brain stem has been injured. Controversies still exist concerning the underlying mechanisms for these responses, but may include ICP [13, 15, 16, 18], reduced blood volume [19], impaired cerebral blood supply [20, 21], brain stem distortion [22, 23] or localized pressure on certain areas of the brain stem [24]. Obviously, different mechanisms mentioned may play a role at different stages of brain impairment. Gradual ICP elevation induces an ABP increase prior to alterations in CBF as a result of decreasing CPP [15], as was observed in this experimental paradigm. In order to control for the stepwise steady-state changes of CPP in piglets, an external blood pressure controller was used to adjust mean ABP to maintain it at baseline values. Moreover, in this experimental application, the amount of withdrawn/infused blood volume necessary at the different ICPs indicates the intended cardiovascular regulatory responses. Indeed, lowering of the CPP led to the need to gradually exsanguinate the piglet to compensate for the ABP increase. This was true during conditions under which no relevant changes in brain oxygen metabolism occurred. Later, at the ICP of 45 mm Hg, which significantly reduced but did not eliminate brain oxygen delivery (e.g. 15 ± 13% of baseline value), maintenance of baseline ABP levels required massive reinfusion of the previously withdrawn blood volume as well as further volume expansion. This experimental procedure of gradual ICP increase by stepwise epidural balloon inflation and controlling for ABP also gradually reduces CPP and can be achieved with sufficient cardiovascular stability.

In order to estimate the usefulness of this experimental model in regard to the effects of ICP on forebrain oxygen metabolism, the regional deformation of the underlying cortex must be considered. Regional CBF measurements were reduced focally in the cortical regions which were in direct contact to the inflated epidural balloon (i.e. frontal lobe and the rostral part of the parietal lobe of the right hemisphere). During the initial mild balloon inflation (ICP = 25 mm Hg), a larger reduction of regional CBF was found in the ipsilateral hemisphere compared to the corresponding areas in the opposite hemisphere (fig. 2). This effect became more pronounced during moderate ICP increase, with the effect extended to other regions rostral to the tentorium. At the highest level of ICP, a homogenous decrement in CBF in both hemispheres indicates a fairly diffuse supratentorial spread of ICP and decrement in CPP. The effect of this artificial change of compartmental assignment with reference to ICP was monitored from the site opposite to the inflated balloon in order to verify effects which are representative for most parts of the brain outside the direct local effects of mass expansion.

To study a diffuse ICP increase, a comparable approach might be achieved by an intracranial fluid infusion (i.e. intraventricular or subdural infusion). Previously, this approach has induced a rapid cerebral circulatory arrest in newborn pigs [25]. Likely, it is necessary to similarly control ABP to define the effect of CPP in this model.

**CBF Estimation**

Regional CBF was measured using multiple colored microspheres. This technique in newborn piglets has previously been validated in our laboratory [7] for use in organ blood flow. In this study, a correlation between flow rates determined simultaneously by colored microspheres...

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*Pediatr Neurosurg* 1999;30:62–69
and radioactive-labeled microsphere technique was found even for organs with low perfusion. The quality of microsphere detection is a result of the whole procedure of colored microsphere quantification. In this study, the detection error of falsely detected missing microspheres as a valid indicator of the precision of microsphere detection, found an error of approximately 1-10% [7], which was comparable with studies using radioactive-labeled microspheres [26, 27]. Another procedure to assess the quality of colored microsphere detection by spectrophotometry and matrix inversion showed a coefficient of variation between 1.28 and 3.63% [6]. The amount of falsely detected missing microspheres is of particular importance in pathophysiological conditions where regional trickle-flow or no-flow conditions can appear. Therefore, we assume that even during the low-perfusion states, a reliable estimation of regional blood flow was performed because more than 400 microspheres were found in all tissue samples which were measured. The colored microsphere method for multiple quantitative blood flow estimation was used because this method appears to be a valid alternative for regional blood flow measurement in newborn piglets, and all disadvantages (e.g. economic, legal and health considerations) arising from radioactive labeling are avoided. Regional CBF can also be measured by quantitative autoradiography using radiolabeled io-quantipyrine [28]. Despite an improved spatial resolution, this procedure appears to be inadequate in this experimental design because only one measurement per experiment can be achieved using quantitative autoradiography.

**Effects on CBF and Oxidative Metabolism**

Our data show that in juvenile piglets, a CPP reduction of 30% is fully compensated for by increasing CBF and stable oxidative metabolism. A further decrease in CPP up to 50% of baseline values leads to a moderate CBF reduction and a restriction in cerebral oxygen delivery and consumption. Under these conditions, oxygen metabolism of large parts of the forebrain was not disturbed. However, it has to be considered that the estimation of CMRO₂ was based on blood sampling from the sagittal sinus and confluence sinus. Therefore, regional differences in oxygen consumption cannot be differentiated because of mixed venous blood obtained from the confluence sinus from both hemispheres. Regional CBF, though, gives an estimate of regional oxygen availability. As a result, the data of regional CBF enable us to estimate the CMRO₂ values which likely are representative for approximately 88% of the brain, calculated as the ratio between forebrain weight and whole brain in the piglets used in this study.

Cerebral oxidative metabolism was measured under slight general anesthesia. We used a combination narcosis with a considerably low level of the narcotic component (0.8% isoflurane) which corresponds under normothermia to a MAC of about 0.34 [29]. However, the resulting effects on oxidative brain metabolism are assumed to be relevant, because earlier studies in adult dogs [30] and cats [31] have shown that an increase in isoflurane MAC from zero to 0.5 resulted in a decrease in CMRO₂ of about 30%. A further doubling of isoflurane MAC induced a further CMRO₂ reduction of 18%. Therefore, there is a nonlinear relation between isoflurane MAC increase and oxidative brain metabolism deprivation with an increased metabolic susceptibility in low MAC values. A comparable reduction in oxidative brain metabolism is likely and in line with published values of cerebral O₂ uptake in non-anesthetized piglets [32], which were about 30% higher as found in this study. Mild CPP reduction at 30% of baseline values was completely compensated for by blood flow and oxygen uptake of the cerebrum. This finding corresponds to the autoregulatory threshold in the piglets [33]. Further CPP reduction surpasses the autoregulatory threshold and hence induced a concomitant decrease in blood flow to the cerebrum combined with a similar reduction in cerebral oxygen delivery and cerebral oxygen uptake.

**Outlook**

This experimental model has previously been used in adult animals to study the conditions which provoke a rebound of ICP after decompression of the intracranial mass lesion [34]. It was found that this is a threshold phenomenon which depends upon the CPP during compression and the duration of the compression. Release of the compression resulted in a marked cerebral hyperperfusion which generalized to the supratentorial but not the infratentorial structures [34]. Diffuse brain swelling with fatal outcome after balloon decompression was found in a canine outcome model [35]. In this study, an increased ICP of 62 mm Hg was induced by epidural balloon inflation for 90 min, followed by intensive care for 96 h. Our study gives an experimental basis to design an outcome model in order to investigate effects of decompression after temporal epidural balloon inflation which will mimic a clinical situation of epi- or subdural hematoma evacuation which is frequent in infants and young children after nonaccidental head injury [36, 37].
In summary, the present results show that the experimental design used here provides an approach to study pathophysiological conditions comparable with situations during very severe traumatic brain lesions with excellent stability of steady-state stages in order to obtain regional brain functions.

Acknowledgment

The authors thank Mrs. U. Jäger, Mrs. I. Witte and Mr. L. Wunder for skilful technical assistance and Dr. P.D. Adelson (Pittsburgh, Pa.) for his collegial review of the manuscript. This work was supported by BMBF 01ZZ9602.

References

Effect of mild hypothermia on cerebral oxygen uptake during gradual cerebral perfusion pressure decrease in piglets

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Objective: To study the effect of mild hypothermia on cerebral oxygen metabolism and brain function in piglets during reduced cerebral blood flow because of gradual reduction of the effective cerebral perfusion pressure (CPP).

Design: Comparison of two randomized treatment groups: normothermic group (NT; n = 7) and hypothermic group (HT; n = 7).

Setting: Work was conducted in the research laboratory of the Institute for Pathophysiology, Friedrich Schiller University, Jena, Germany.

Subjects: Fourteen piglets (14 days old) of mixed German domestic breed.

Intervention: Animals were anesthetized and mechanically ventilated. An epidural balloon was gradually inflated to increase intracranial pressure to 25 mm Hg, 35 mm Hg, and 45 mm Hg every 30 mins at adjusted mean arterial blood pressures. After determination of baseline CPP (NT, 79 ± 14 mm Hg; HT, 84 ± 9 mm Hg), CPP was reduced to ~70%, 50%, and 30% of baseline (NT, 38.1 ± 0.5°C; HT, 31.7 ± 0.5°C).

Measurements and Main Results: Every 25 mins after the gradual CPP reductions. Mild hypothermia induced a reduction of the cerebral metabolic rate of oxygen (CMRO₂) to 50% ± 15% of baseline values (baseline values, 352 ± 99 μmol·100 g⁻¹·min⁻¹) (p < .05). Moreover, the electrocorticogram was altered to a pattern of reduced delta activity (p < .05) but unchanged higher frequency activity. The cerebral oxygen balance in HT animals remained improved until CPP reduction to 50%, indicated by a reduced cerebral arteriovenous difference of oxygen but elevated brain tissue PO₂ (p < .05). Further CPP reduction gave rise to a strong CMRO₂ reduction (NT, 19 ± 21%; HT, 15 ± 15%; p < .05). However, the high-frequency band of electrocorticogram was less reduced in hypothermic animals (p < .05).

Conclusions: Mild whole body hypothermia improves cerebral oxygen balance by reducing of brain energy demand in juvenile piglets. The improvement of brain oxygen availability continues during a mild to moderate CPP decrease. A loss of the difference in CMRO₂ between the hypothermic and normothermic piglets together with the fact that brain electrical activity was less suppressed under hypothermia during severe cerebral blood flow reduction indicates that hypothermic protection may involve some other mechanisms than reduction of brain oxidative metabolism. (Crit Care Med 2000; 28:1128–1135)

Keywords: cerebral blood flow; cerebral oxygen consumption; intracranial pressure; epidural balloon inflation; extracorporeal arterial blood pressure controller; piglets; hypothermia; colored microspheres; neurologic emergencies; brain

For infants and young children, traumatic injury is the leading cause of death and mortality is greatly increased in the presence of brain injury (1). Increased intracranial pressure (ICP) is a common complication in infants and young children suffering from severe traumatic brain injuries and is closely correlated with an adverse outcome. It was recently reported that ~60% of severe head-injury patients develop an increased ICP, mostly as a result of brain swelling (2). The incidence of brain swelling in pediatric patients was twice as high as in adults (3, 4). This concurs with recent findings in experimental studies, in which immature rats were discovered to develop cerebral edema as a main cause of brain swelling more rapidly than mature rats (5). Recently, it was shown that the cerebrovascular response to traumatic brain injury differs in immature and more mature animals. Cerebral arterioles constricted to a greater extent in newborn pigs than juvenile ones after fluid percussion induced traumatic brain injury, and the resulting decrease in cerebral blood flow (CBF) was prolonged (6). However, experimental or clinical studies on the effects of mild to moderate hypothermia on cerebral oxygen metabolism in the immature brain during compromised brain oxygen delivery because of increased ICP have not as yet been performed.

We hypothesized that mild hypothermia improves cerebral oxygen balance during stages of decreased cerebral perfusion. To test this hypothesis, we examined the effects of mild hypothermia on cerebral hemodynamics and brain oxygen metabolism in 2-yr-old piglets after a stepwise reduction in cerebral perfusion pressure, induced by gradual inflation of an epidural balloon. An experimental procedure was used that allows sequential estimation of regional CBF and cerebral metabolic rate of oxygen (CMRO₂). This was realized using an external closed-loop controller of arterial blood pressure (ABP) to avoid ICP-related ABP alterations.
MATERIALS AND METHODS
This protocol was approved by the committee of the Thuringian State government for animal research. The animals were managed in accordance with the guidelines of the American Physiologic Society.

Subjects. Fourteen piglets of mixed German domestic breeds (14 days old; body weight, 4517 ± 536 g) were used in the study.

Surgical Procedures. Piglets were initially sedated with ketamine hydrochloride (50 mg/kg body weight) and then anesthetized with 1.5% isoflurane in 70% nitrous oxide and 30% oxygen. A central venous catheter was introduced through the left external jugular vein and was used for the administration of drugs and for volume substitution (lactated Ringer's solution, 5 mL/kg of body weight/hr). An endotracheal tube was inserted by means of a tracheotomy. After immobilization with pancuronium bromide (0.2 mg/kg body weight/hr, iv), the animals were artificially ventilated (Servo Ventilator 900C, Siemens-Elema, Solna, Sweden). Anesthesia was maintained throughout the experiment with 0.8% isoflurane. Polyethylene catheters (inner diameter, 1.5 mm) were advanced through the femoral arteries into the abdominal aorta to record ABP and to withdraw reference samples for the colored microsphere technique. A further polyethylene catheter (inner diameter, 0.3 mm) was inserted into the superior sagittal sinus and advanced to the confluence sinusum to obtain brain venous blood samples. The left cardiac ventricle was cannulated retrogradely via the right common carotid artery with a polyurethane catheter (inner diameter, 0.5 mm). Arterial, left ventricular, and central venous catheters were connected with pressure transducers (P23Db, Statham Instruments, Hato Rey, PR). Body temperature was monitored by a rectal thermoprobe advanced for 10 cm and was maintained throughout the general instrumentation at 38 ± 0.5°C using a water blanket connected to a heating-cooling thermostat and a feedback controlled heating lamp. Physiologic variables were recorded on a multichannel polygraph (MT95K2, Astro-Med, West-Warwick, RI).

Unipolar electrocorticogram (ECoG) recording was performed using screw electrodes. The electrode position was just posterior to the coronal suture, 10-mm lateral to the sagittal suture of the left parietal bone. The reference electrode was placed on the nasion.

Two holes were drilled into the left frontal bone, and a fiberoptic catheter was implanted into the suboccipital white matter for ICP measurements (Camino Laboratories, San Diego, CA). A Clark-type PO2 electrode (7, 8) together with a thermocouple catheter, serving as a temperature probe (LICOX PO2 monitor, GMS mbH, Kiel-Mitteorden, Germany), was implanted 3–5 mm into the parietal cortex. Measurements of brain tissue PO2 were corrected to 37°C. On the right side, an oval-shaped burr hole (10–3 mm) was gently drilled into the frontal bone at a distance of 4 mm and parallel to the sagittal suture so that the dura mater was entirely intact. An epidural latex balloon attached to a catheter was placed in the epidural space of the right frontoparietal region. The burr holes were sealed with bone wax and covered with dental acrylic to fix the probes and the epidural balloon catheter in place.

Experimental Protocol. After the surgical preparation had been completed, the piglets underwent no further interventions for 60 mins. After control values had been obtained, randomly chosen animals (n = i) were surface cooled by crushed ice packs and cooled water through the pad to a body temperature of 31.7 ± 0.6°C. At this stage, the external ABP controller was established to adjust the ABP at baseline. A second series of values was obtained ("hypothermia"), followed by a gradual decrease of the effective cerebral perfusion pressure (CPP), which was calculated as the difference between mean ABP and ICP by stepwise elevation of the ICP in both groups, beginning at 25 mm Hg and followed by 35 mm Hg and 45 mm Hg, achieved by gradual epidural balloon inflation for ~30 mins each and stabilization of the mean ABP by the external blood pressure control loop. By using this procedure, stepwise reductions of CPP of ~70%, 50%, and 30% of baseline were produced. Considerable stability of the CPP at each stage could be reached (Table 1). Repeated measurements of all variables were recorded at the 25th min of every steady-state period.

Physiologic Measurements. Cardiac output and regional CBF were measured by means of the reference sample color-labeled microsphere technique (9). Application in piglets and methodical considerations have been presented and discussed in detail elsewhere (10).

Prior to hypothermia, a 500-mL bolus of a 0.9% saline solution was administered to each animal. After the induction of hypothermia, the temperature in the microsphere injection site was maintained at 38–39°C. A 0.5% glucose solution containing 3% saline was slowly infused at a rate of 100 mL/h. At the end of each experiment, the piglet was killed with KCl and the brain was removed for processing. The brain was sectioned into 26 tissue samples to determine blood flow to different brain regions. After sectioning, reference blood samples and tissue samples between 0.15 and 2.5 g were covered with digestive solution (4N KOH with 4% Tween 80 in deionized water). To retain the microspheres, each digested sample was then filtered under vacuum suction through an 8-μm pore polyester membrane filter. Colored microspheres were quantified by their dye.

Table 1. Duration of different experimental stages and the behavior of cerebral perfusion pressure (CPP) during the whole period of every stage (normothermic baseline conditions, baseline hypothermia and gradual decrease of the CPP by gradual epidural balloon inflation) and during the periods of microsphere measurement

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Hypothermia</th>
<th>CPP-70%</th>
<th>CPP-50%</th>
<th>CPP-30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (mins)</td>
<td>30 ± 1</td>
<td>89 ± 5</td>
<td>30 ± 2</td>
<td>29 ± 1</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Normothermic</td>
<td>32 ± 3</td>
<td>96 ± 12</td>
<td>30 ± 2</td>
<td>30 ± 2</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>Hypothermic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPP during we stage (mm Hg)</td>
<td>87 ± 4</td>
<td>84 ± 4 (96)</td>
<td>60 ± 3 (69)</td>
<td>44 ± 3 (51)</td>
<td>39 ± 4 (35)</td>
</tr>
<tr>
<td>Normothermic</td>
<td>85 ± 5</td>
<td>82 ± 4 (96)</td>
<td>59 ± 4 (69)</td>
<td>44 ± 4 (51)</td>
<td>26 ± 5 (31)</td>
</tr>
<tr>
<td>Hypothermic</td>
<td>87 ± 3</td>
<td>84 ± 3 (96)</td>
<td>60 ± 3 (69)</td>
<td>44 ± 2 (50)</td>
<td>27 ± 2 (32)</td>
</tr>
<tr>
<td>CPP during microsphere measurement (mm Hg)</td>
<td>86 ± 4</td>
<td>81 ± 3 (93)</td>
<td>59 ± 3 (69)</td>
<td>44 ± 3 (51)</td>
<td>25 ± 3 (29)</td>
</tr>
</tbody>
</table>

Values are mean ± se; parentheses enclose per centage of mean CPP changes in relation to mean baseline values. n = seven animals in each group with the exception of stage CPP-30% of the normothermic group, because at this stage in one animal, an acute uncontrollable decrease of arterial blood pressure occurred so that the epidural balloon had to be deflated prematurely. "Hypothermia" indicates experimental stage of baseline 2 (normothermic group) and of that stage in which body temperature was lowered at ~32°C (hypothermic group); "CPP-70%," "CPP-50%," and "CPP-30%" indicate the lowering of CPP to ~70%, 50%, and 30% of the baseline values.
Table 2. Physiologic variables during normothermic baseline conditions, baseline 2/hypothermia, and gradual decrease of the cerebral perfusion pressure (CPP)

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Hypothermia</th>
<th>CPP-70%</th>
<th>CPP-50%</th>
<th>CPP-30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>234 ± 45</td>
<td>233 ± 27</td>
<td>255 ± 43</td>
<td>221 ± 36</td>
<td>194 ± 47</td>
</tr>
<tr>
<td>Hypothermic</td>
<td>236 ± 37</td>
<td>176 ± 16ab</td>
<td>176 ± 20ab</td>
<td>164 ± 25ab</td>
<td>158 ± 27ab</td>
</tr>
<tr>
<td>Cardiac output (mL/min/kg)</td>
<td>231 ± 53</td>
<td>213 ± 131</td>
<td>121 ± 57b</td>
<td>191 ± 91</td>
<td>222 ± 48</td>
</tr>
<tr>
<td>Normothermic</td>
<td>213 ± 96</td>
<td>205 ± 102</td>
<td>118 ± 61b</td>
<td>182 ± 87</td>
<td>220 ± 125</td>
</tr>
<tr>
<td>Hypothermic</td>
<td>144 ± 29</td>
<td>146 ± 29</td>
<td>140 ± 30</td>
<td>146 ± 30</td>
<td>143 ± 35</td>
</tr>
<tr>
<td>Arterial P O₂ (mm Hg)</td>
<td>135 ± 11</td>
<td>142 ± 22</td>
<td>128 ± 23</td>
<td>126 ± 23</td>
<td>131 ± 14</td>
</tr>
<tr>
<td>Normothermic</td>
<td>38 ± 1</td>
<td>40 ± 5</td>
<td>39 ± 1</td>
<td>39 ± 2</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>Hypothermic</td>
<td>37 ± 3</td>
<td>39 ± 5</td>
<td>39 ± 4</td>
<td>39 ± 3</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.42 ± 0.04</td>
<td>7.40 ± 0.06</td>
<td>7.38 ± 0.04</td>
<td>7.38 ± 0.04</td>
<td>7.38 ± 0.08</td>
</tr>
<tr>
<td>Normothermic</td>
<td>7.44 ± 0.06</td>
<td>7.38 ± 0.07</td>
<td>7.41 ± 0.06</td>
<td>7.40 ± 0.05</td>
<td>7.39 ± 0.05</td>
</tr>
<tr>
<td>Hypothermic</td>
<td>6.6 ± 0.7</td>
<td>6.7 ± 0.6</td>
<td>6.3 ± 0.6</td>
<td>5.9 ± 0.6</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>Arterial oxygen content (µmol/mL)</td>
<td>6.2 ± 0.7</td>
<td>6.5 ± 0.8</td>
<td>6.4 ± 0.6</td>
<td>6.1 ± 0.9</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Normal temperature (°C)</td>
<td>38.2 ± 0.8</td>
<td>38.1 ± 0.5</td>
<td>38.2 ± 0.4</td>
<td>38.2 ± 0.3</td>
<td>37.9 ± 0.4</td>
</tr>
<tr>
<td>Normothermic</td>
<td>38.0 ± 0.5</td>
<td>31.7 ± 0.6ab</td>
<td>31.8 ± 0.6ab</td>
<td>31.8 ± 0.6ab</td>
<td>31.8 ± 0.4ab</td>
</tr>
<tr>
<td>Hypothermic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = seven animals in each group with the exception of stage CPP-30% of the normothermic group, because at this stage in one animal, an acute uncontrollable decrease of arterial blood pressure occurred so that the epidural balloon had to be deflated prematurely. "Hypothermia" indicates experimental stage of baseline 2 (normothermic group) and of that stage in which body temperature was lowered at ~32°C (hypothermic group); "CPP-70%", "CPP-50%", and "CPP-30%" indicate the lowering of CPP to ~70%, 50%, and 30% of the baseline values.

Significant differences between normothermic and the hypothermic animals; *significant differences between baseline values and values obtained at different stages of experimental procedure; p < .05.

content. The dye was recovered from the microspheres by adding 150 µL of dimethylformamide. The photometric absorption of each dye solution was measured by a diode-array UV/visible spectrophotometer (model 7500, Beckman Instruments, Fullerton, CA). Calculations were performed using the MISS software (Triton Technolog, San Diego, CA). The number of microspheres was calculated using the specific absorbance value of the different dyes. All reference and tissue samples contained ~400 microspheres.

Heart rate, ABP, ICP, cerebral perfusion pressure, arterial and brain venous pH, P O₂, and P O₂ oxygen saturation, and hemoglobin values were measured at each study period. Blood pH, P O₂, and P O₂ were measured with a blood gas analyzer (model ABL50, Radiometer, Copenhagen, Denmark), and blood hemoglobin and oxygen saturation were measured using a hemoximeter (model OSM2, Radiometer) and corrected to the body temperature of the animal at the time of sampling, ABP, saggital sinus pressure, and ICP were continuously monitored.

**Calculated Values and Data Analysis.** Absolute flows to tissues measured by colored microspheres were calculated by the formula: flow tissuelmin = number of microspheres tissuelnum of microspheres referenced × (flow reference/number of microspheres referenced). Flows are expressed in ml/min/100 g of tissue by normalizing for tissue weight.

Assuming the oxygen capacity of hemoglobin to be 1.39 mL of oxygen/g of hemoglobin in piglets (12), blood oxygen content was calculated as equal to g of hemoglobin/mL.1.39 mL oxygen/g of hemoglobin %-oxygen saturation and expressed in µmol/min/100 g. Dissolved oxygen was added by calculation, using the measured P O₂ and the temperature-corrected solubility coefficient of oxygen. Because the sagittal sinus drains the cerebral cortex, cerebral white matter, and some deep gray structures (basal ganglia, hippocampus) (13), blood flow measured to the cerebrum included these structures. CMRO₂ was obtained by multiplying blood flow to the cerebrum by the cerebral arteriovenous oxygen content difference (Cv-aVO₂). Total blood volume was assumed to be 12% of body weight (14).

ECOG signals were amplified, filtered (time constant was 0.1 s); cut off frequency was 1000 Hz, fed into a PC using a 16-channel A/D board (DT2321F, Data Translation, Marlboro, MA), and stored on a hard disk for off-line data analysis (sample rate was 512 Hz). ECOG was quantified for 3 mins at each experimental period using Fast Fourier Transformation. Spectral band power was calculated for different frequency bands (delta band, 1.5–4 Hz; high-frequency band, 4–30 Hz) and normalized with the baseline spectral power.

A detailed description of the procedure of mean ABP adjustment at an externally given set point (baseline ABP value, as used in this application) using an external blood pressure control loop has been previously published (15, 16). In brief, ABP was controlled by a proportional-integral-differential-controller running on a personal computer, by means of changing the blood volume by arterial blood infusion or withdrawal, respectively. An infusion/withdrawal pump was the controlling element.

**Statistical Analysis.** If not otherwise indicated, data are reported as mean ± SD. Control variables were compared between groups with unpaired Student's t-tests. Two-way analysis of variance with repeated measures was used to determine the effects of CPP alteration and group CPP interaction within each variable. Subsequently, one-way analysis of variance with repeated measures was performed within each group. Post hoc comparisons were made with paired Student's t-tests using Bonferroni correction for multiple use. Comparisons between groups were made with unpaired Student's t-tests using Bonferroni correction for multiple use. Differences were considered significant when p < .05.

**RESULTS**

The lowering of rectal temperature to 31.8 ± 0.1°C, which was done by surface
cooling, required 95 ± 24 mins to accomplish and was maintained at this level of mild hypothermia (Table 2). The arterial blood gases and pH were similar throughout the experiment and not affected by mild hypothermia, because temperature-corrected values were used. Heart rate was strongly reduced by hypothermia by ~26% (p < .05). During baseline conditions and the period of hypothermia alone, brain temperature was higher than rectal temperature in normothermic as well as in hypothermic animals by 0.2–0.5°C (p < .05). However, in both groups during the period of decreased CPP, brain-to-body difference of temperature was first diminished, and at the lowest CPP level, the brain became cooler by 0.4–0.8°C (p < .05).

Gradual CPP reduction because of stepwise ICP increase (monitored contralateral to the gradually inflated epidural balloon) was determined reliably by means of epidural balloon inflation during feedback-controlled ABP stabilization (Fig. 1). ABP was maintained within small ranges throughout the experiment (81 ± 11 mm Hg), although slightly reduced ABP were found at the last two levels of reduced CPP (p < .05). No differences were found between normothermic and hypothermic groups in ABP, CPP, and ICP before and during mild hypothermia and during gradually reduced levels of the CPP. In contrast to the earlier stages of CPP lowering in which blood withdrawal occurred, at the last stage of CPP lowering, a complete transfusion, partly by additional infusion of normal saline, was necessary to stabilize ABP (Table 4).

Mild hypothermia led to a significantly reduced CMRO₂ to 56 ± 15% of baseline values (p < .05; Fig. 2). This was accompanied by a smaller reduction of CBF to 74 ± 20% of baseline values. The changes in CBF are entirely attributable to higher arterial PCO₂. With the exception of the medulla oblongata, thalamus, and basal cortex, the other brain regions showed a marked blood flow reduction (Table 3). Consequently, C(a-va)O₂ decreased by 31 ± 19% and brain tissue PO₂ increased by 31 ± 12% (p < .05). Moreover, the pattern of electrocortical activity changed considerably from a delta wave-dominated pattern during normothermia to a pattern of unchanged higher frequency activity but strong reduced delta activity (p < .05; Fig. 3).

Gradual CPP reduction to 70% of baseline values did not change the high-

Figure 1. Brain temperature, intracranial pressure (ICP), mean arterial blood pressure (MAP), and cerebral perfusion pressure (CPP) in normothermic and hypothermic pigs at different stages of CPP decrease. Values are mean ± SEM; n = seven animals in each group, with the exception of stage CPP-30% of the normothermic group, because at this stage in one animal an acute uncontrollable decrease of arterial blood pressure occurred so that the epidural balloon had to be deflated prematurely. Filled columns, normothermic group; open columns, hypothermic group; "hypothermia" indicates experimental stages of baseline 2 (normothermic group) and of that stage in which body temperature was lowered to ~32°C (hypothermic group). *Significant differences between the normothermic and hypothermic animals; §significant differences between baseline values and values obtained at different stages of the experimental procedure; p < .05.

Figure 2. Cerebral metabolic rate of oxygen (CMRO₂) (upper left), cerebral blood flow (CBF) (lower left), cerebral arteriovenous difference of oxygen content (C(a-va)O₂) (upper right), and brain tissue PO₂ (lower right) in normothermic and hypothermic pigs at different stages of cerebral perfusion pressure (CPP) decrease. Filled columns, normothermic group; open columns, hypothermic group; "hypothermia" indicates experimental stage of baseline 2 (normothermic group) and of that stage in which body temperature was lowered to ~32°C (hypothermic group). *Significant differences between the normothermic and hypothermic animals; §significant differences between baseline values and values obtained at different stages of the experimental procedure; p < .05.
frequency band of ECoG activity and CMRO₂ further in hypothermic animals, despite a recovery of CBF (Fig. 2). As shown in Table 4, during this first stage of gradual CPP lowered by stepwise ICP elevation, blood withdrawal was necessary in almost all animals to maintain ABP with the strongest compensatory blood withdrawal at stage CPP-70%. This was associated with a significant reduction of cardiac output (Table 2). Moreover, a redistribution of regional CBF was induced that significantly favored medulla oblongata, cerebellum, and the thalamus in normothermic animals (Table 3). Epidural balloon inflation led to a certain blood flow reduction ipsilateral to the inflated epidural balloon, which was most prominent in the adjacent frontal lobe with a reduction of 38% in normothermic (NT) animals and 30% in hypothermic (HT) ones (Table 3). Brain tissue Po₂ reached baseline values, but C(a-V)o₂ remained at a similarly low level to before baseline in HT animals (p < .05; Fig. 3). Similarly mild CPP reduction because of ICP increase had no significant effects on brain oxidative variables in NT animals, but ECoG activity was significantly reduced (p < .05).

Further CPP reduction to an amount of ~50% of baseline values led to a CBF decrease to 64 ± 34% (NT group) and to 66 ± 25% (HT group) of baseline values (p < .05). This was predominantly caused by significantly reduced regional blood flows of cortical regions ipsilateral to the inflated epidural balloon, with the strongest reduction in the frontal lobe in which 48% of that flow in the contralateral frontal lobe was measured in both groups (Table 3). Moreover, the higher frequency band of ECoG was significantly reduced (p < .05) in HT animals, despite further unchanged CMRO₂.

CPP reduction to ~30% of the baseline values lead to a further considerable reduction of CBF (NT, 35 ± 26%; HT, 20 ± 14%) in both groups investigated (p < .05). At this stage, the reduction of CMRO₂ (NT, 19 ± 21%; HT, 15 ± 15%) and reduction of brain tissue Po₂ (NT, 10 ± 18%; HT, 9 ± 10%) were more pronounced in both groups. At this stage of ICP increase because of epidural balloon inflation over the right frontal lobe, a similar regional blood flow reduction occurred in all supratentorial brain regions (Table 3). Moreover, ECoG was further reduced (p < .05). Despite almost equalized C(a-V)o₂ between both groups studied, the high-frequency band of ECoG was significantly less reduced in HT animals (p < .05). This was confirmed with results of visual inspection of ECoG, in which an ischemic suppression of ECoG up to isoelectricity (four animals)
or burst suppression pattern (three animals) was only found in NT animals.

**DISCUSSION**

The data show that an improved cerebral oxygen balance by means of a marked reduction of cerebral oxygen demand results from mild hypothermia, even at stages of mild to moderate CPP restriction. The improved cerebral oxygen balance is entirely attributable to increased arterial Pco₂. The reduction in demand was shown by a reduced C(a-v)O₂ and an improved or maintained level of brain tissue P0₂. Preserved EEG activity of the high-frequency band indicates an almost unchanged brain functional state. However, marked CPP reduction to −30% of baseline with a concomitant reduced cerebral oxygen availability generally abolished these differences in CMRO₂ between the NT and HT groups, although brain electrical activity was less suppressed.

Mild hypothermia confers a marked protective effect on histopathologic outcome after experimental brain trauma (17), attenuating neurochemical sequelae of cerebral oxygen lack and improving the behavioral outcome (18). Possible ex-
planations for hyperthermia-related improvement include the following: a) progressive reduction in cerebral metabolic rate for oxygen consumption; b) alterations in ion homeostasis (including calcium and potassium fluxes); c) increased membrane stability (including the blood-brain barrier); d) altered enzyme function (e.g., phospholipase, xanthine oxidase, nitric oxide synthase activity); e) alterations in neurotransmitter release and reuptake (e.g., glutamate); and f) changes in free radical production or scavenging (for review see References 19 and 20). Hyperthermia has been evaluated as a therapeutic procedure for hastening neurologic recovery and improving the outcome in adult patients with severe traumatic brain injury (21).

The present data show a reduction in cerebral oxygen consumption caused by mild hyperthermia by ~10%\(^{\circ}\)C. This corresponds with findings in newborn piglets (22) and amounted to nearly 50% more than was reported for adult brains (23, 24). However, in contrast to the results of Busija and Leffler (22) that were obtained during mild hypocapnia, obviously because of "o-stat" conditions, the corresponding blood flow reduction we observed was less pronounced.

The effect of the increased level of anesthesia because of hyperthermia by the improved solubility of the anesthetics used here on CMRO\(_2\) appears to be small. A reduction of brain and body temperature by 6\(^\circ\)C at an unchanged inhalation content of 0.8% isoflurane resulted in a macroalveolar concentration increase from 0.34 to 0.45 (25). The reason that CBF did not decrease by the same percentage as CMRO\(_2\) at 32\(^\circ\)C appears to be because of the kind of blood gas management used. We corrected blood gases and pH measurements under HT conditions to the body temperature of the animal at the time of sampling ("pH-stat"). This pH-stat management was chosen because recent reports suggest that there are mechanisms in effect during HT brain ischemia that could contribute to an improved cerebral outcome with pH-stat relative to more alkaline strategies (26–28). However, further studies are necessary to evaluate the importance of acid-base management on the relationship between cerebral oxygen delivery and demand under hyperthermia and during compromised brain perfusion.

Previous studies have shown that hyperthermia-induced suppression of the cerebral oxygen demand reflects influences on oxygen-consumining processes that are different from those suppressed by barbiturate-induced electroencephalogram suppression (24, 29, 30). Moreover, Nemoto and coworkers (31) also showed that the barbiturate-nonsuppressible CMRO\(_2\) component of the basal CMRO\(_2\) was much more temperature sensitive than the barbiturate-suppressible CMRO\(_2\) component. Our study clearly showed that the EEG pattern was changed by mild hyperthermia from a delta wave-suppressed EEG to a delta wave-dominated EEG to a delta wave-suppressed EEG pattern with unchanged spectral power of the higher frequency band. Because a higher frequency pattern is associated with maintained cortical synaptic activity and signal transfer (32), we assume that at least a considerable amount of hyperthermia-related CMRO\(_2\) suppression was caused by the non-EEG-associated CMRO\(_2\) suppression.

The manipulation of ICP was managed by regional mass expansion because of gradual inflation of an epidural balloon. Local effects were obviously restricted to the cortical regions that were in direct contact with the inflated epidural balloon (i.e., frontal lobe and the rostral part of the parietal lobe of the right hemisphere), as was shown by respective changes in regional CBF. The effects of this artificial intracranial volume occupation with reference to ICP, brain tissue P\(_{O_2}\), and ECoG were monitored from sites opposite the inflated balloon. Therefore, these effects are assumed to be representative for most parts of the brain outside the direct local effects of mass expansion.

Compared with the effects of mild hyperthermia, we found that mild alterations of cerebral oxygen delivery because of gradual CPP reduction in NT animals predominantly induced a reduction of spontaneous ECoG activity, i.e., the functional CMRO\(_2\) component was selectively compromised. This occurred in NT animals, even if the overall oxygen delivery and CMRO\(_2\) were not reduced. The only finding that corresponded with changed ECoG activity in NT animals was an increased regional CBF to the lower brain stem and the thalamus in concordance with reduced cardiac output because of the self-controlled stabilization of ABP to prevent Cushing response-like ABP increase (33). The CBF increase to these brain regions may reflect a functional activation that could result in a desynchronization of cortical high-voltage delta activity because of diffuse thalamocortical projections of reticular formation (34, 35). However, a specific arousal-like pattern of an increased activity of high frequency components was found only in HT animals during hyperthermia and mild CPP reduction with a strong increase of alpha activity.

In summary, the present results confirm the well-known effect of mild hypo-
thermia on a decrease of cerebral oxidative metabolism. This improves the cerebral oxygen balance. However, if brain oxygen delivery was further reduced by CBF decrease induced by a gradual CPP reduction, then cerebral oxidative metabolism was obviously determined by a greater reduced oxygen delivery in brain tissue. The previously existing CMRO₂ differences between normothermia and hypothermia ceased, although brain electrical activity was less suppressed in HT animals.

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REFERENCES

Abstract  Severe traumatic brain injury (TBI) often leads to a bad outcome with considerable neurological deficits. Secondary brain injuries due to a rise of intracranial pressure (ICP) and global hypoxia-ischemia are critical and may be reduced in extent by mild hypothermia. A porcine animal model was used to study the effect of severe TBI, induced by fluid percussion (FP; 3.5±0.3 atm) in combination with a secondary insult, i.e., temporary blood loss with hypovolemic hypotension. Six-week-old juvenile pigs were subjected to this kind of severe TBI; one group was then submitted to moderate hypothermia at 32°C for 6 h, starting 1 h after brain injury. Animals were killed after 24 h. TBI and hypothermia-associated alterations in the brains were investigated by immunohistochemistry with antibodies against microtubule-associated protein 2 (MAP-2) and β-amyloid precursor protein (βAPP). In addition, DNA fragmentation was investigated by the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) method. Seven of the 13 normothermic TBI animals developed a secondary increase in ICP (TBI-NT-ICP) after an interval of several hours. None of the animals in the hypothermic trauma (TBI-HT) group exhibited a secondary ICP increase, indicating a protective effect of the treatment. TBI-HT animals showed significantly higher levels of MAP-2 immunoreactivity, lower levels of βAPP immunoreactivity and less DNA fragmentation than the TBI-NT-ICP animals. Differences between the TBI-HT group and normothermic animals without an ICP increase (TBI-NT) were less marked. A considerable decrease in MAP-2 outside the site of TBI-FP administration was seen only in the TBI-NT-ICP animals. MAP-2 immunohistochemistry was thus a reliable marker of diffuse brain damage. Axonal injury was present in all TBI groups, indicating its special significance in neurotrauma. Thus, severe TBI caused by FP, combined with temporary blood loss, consistently produced traumatic axonal injury and focal brain damage. Mild hypothermia was able to prevent a secondary increase in ICP and its sequelae of diffuse hypoxic-ischemic brain injury. However, hypothermia did not afford protection from traumatic axonal injury.

Keywords  Trauma · Fluid percussion brain injury · Pig · Hypothermia · Immunohistochemistry

Introduction

Traumatic brain injury (TBI) leads to immediate primary and delayed secondary changes [1]. Initially, there is biomechanical interaction of neurons and their processes, glial cells and blood vessels as a direct consequence of mechanical energy transfer to brain tissue. The secondary changes are much more heterogeneous and are of clinical importance due to their strong correlation to bad outcome [2]. They include biochemical and pathophysiological processes, such as decreased blood pressure, altered blood flow and brain edema, and morphological alterations, especially diffuse axonal injury (DAI).

Numerous studies have evaluated the pathomorphological and behavioral sequelae of mild to moderate TBI induced by fluid percussion (FP). For the most part, lissencephalic rats and mice have been used to study therapeutic strategies for neuroprotection after TBI [3]. Despite making valuable contributions to the understanding of the complex pathogenesis of TBI, these models have some fundamental restrictions. Studies of the biomechanical properties of the brain tissue require an animal with a large gyrencephalic brain and substantial white matter domains. Pigs fulfill these criteria [4].
Lateral FP trauma is a well-established experimental model that reproduces clinically relevant features of neurotrauma [5, 6, 7], such as alterations in intracranial pressure (ICP) [3] and cerebral blood flow [8], disruption of the blood-brain barrier [9, 10], and focal and global alterations in cerebral metabolism [11]. Importantly, the lateral FP injury device is able to produce mild, moderate or severe levels of brain damage according to the magnitude of the impact [3]. However, up to now, a procedure which is able to produce a secondary ICP increase with critically altered cerebral perfusion pressure (CPP) has not been reported.

Hypothermia has been evaluated as a therapeutic procedure to hasten neurological recovery and may have improved the outcome of patients with severe traumatic brain injury [12, 13, 14]. Hypothermia is effective in preventing secondary brain damage, especially through reducing cerebral ischemia [15]. Furthermore, it reduces the ICP, and cerebral blood flow (CBF), and reduces formation of brain edema. In addition, oxygen consumption and release of excitatory neurotransmitters is diminished [16, 17]. Although post-traumatic hypothermia has been widely studied [18, 19, 20, 21, 22, 23, 24, 25], its influence on different types of brain damage is not fully understood. To date, hypothermia-associated protection has been evaluated mostly in relation to the preservation of neurons [18, 21, 23, 24, 26], and little consideration has been given to other changes, such as secondary brain damage and axonal injury.

The purpose of the present study was to determine the extent of brain damage in severe TBI induced by FP combined with temporary blood loss in juvenile pigs with and without postinjury mild hypothermia (of 32°C). The brain damage of juvenile pigs after normo- and hypothermia was assessed with immunomorphological markers, allowing analysis of both neurons and axons. Antibodies against microtubule-associated protein 2 (MAP-2), β-amyloid precursor protein (βAPP) as a marker for axonal injury, were used. In addition the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) method for DNA fragmentation was used.

**Materials and methods**

General instrumentation and measurement of physiological variables

Thirty-five 6-week-old pigs (mixed German breed; body weight 12.6±1.7 kg) were used. The experimental protocol was approved by the committee of animal care and use of the Thuringian State Government. Six pigs were assigned to the normothermic sham group (SHAM-NT), and 14 in the normothermic traumatic brain injury (TBI-NT) group. The hypothermic sham group (SHAM-HT) comprised 6 and the hypothermic TBI group (TBI-HT) 10 pigs. Anesthesia was induced by an intramuscular injection of ketamine hydrochloride (20 mg/kg body wt.) and midazolam (1 mg/kg body wt.) and inhalation of 70% nitrous oxide in 30% oxygen. Anesthesia was maintained throughout the experiment by a continuous infusion of fentanyl (0.015 mg/kg body wt. per h) and midazolam (1.08 mg/kg body wt. per h). Muscle relaxation was achieved with i.v. pancuronium bromide (0.4 mg/kg body wt. per h).

A polyethylene catheter (inner diameter 1.5 mm) was advanced from the femoral artery to the abdominal aorta to record arterial blood pressure and obtain samples for blood gas analysis (model ABL50, Radiometer, Copenhagen, Denmark; alpha-stat management). Body temperature was controlled by a thermoprobe advanced about 10 cm into the rectum and was maintained throughout the general instrumentation at 38±0.3°C using a water-heated pad connected to a heating-cooling thermostat and by a feedback controlled heating lamp. Physiological parameters were recorded on a multi-channel polygraph (MP25K2, Astro-Med, USA).

Two holes were drilled in the left parietal bone and a fiberoptic catheter was implanted in the subcortical white matter for ICP measurements (Camino Laboratories, San Diego, USA). A thermocouple catheter (Licox pO2 Monitor, GMS, Kiel-Mielkendorf, Germany) was implanted in the parietal cortex. In all animals a craniotomy was made, centered between lambda and bregma over the left parietal cortex to fix a Plexiglas FP adapter with a 9-mm bore tube. The dura was kept intact. The burr holes were sealed with bone wax and covered with dental acrylic to fix the probes and FP adapter in place. CPP was calculated as the difference between mean arterial blood pressure and ICP.

**Experimental protocol**

Altogether, 23 randomly chosen animals were subjected to lateral FP injury (FP-TBI) using a device designed according to [27]. Briefly, the FP adapter was connected to a transducer housing, and this in turn was connected to the FP device. The device itself consisted of a cylindrical reservoir of Plexiglas 40 cm long and 5 cm in diameter. One end of the device was connected to the transducer housing and the other had a metal piston with a 4-mm exposed end of the piston was covered with a rubber pad. The entire system was filled with physiological saline solution (air bubble-free). FP-TBI was produced by allowing a 6.2-kg pendulum to strike the Plexiglas cork and generate a transient hydraulic pressure that traveled through the device and impacted upon the dura overlying the brain. The severity of the impact, determined by a transducer and recorded on a storage oscilloscope, was allowed to reach 3.5±0.3 atmospheres (atm) and 21, 23, 24, 26, atm which was similar in all groups investigated. This model of FP-TBI has not been fully characterized in terms of its behavioral and histopathological responses, but level of injury is considered to be severe to high TBI in different species and ages [5, 28, 29, 30, 31, 32]. Hypovolemic hypotension was then induced by removing blood (25 ml/kg body wt., duration: 18 min) through the femoral catheter, so that blood withdrawal was completed 21 min after FP injury and then maintained for another 9 min. The same volume of plasma expander (gelatine-polysuccinate, Gelafusum, Serum-Werke Bernburg, Germany) was reinfused within 10 min. In ten animals systemic hypothermia was started 1 h after FP injury by placing the animal on a cooling blanket and by forced air cooling (TBI-HT). Sham-operated animals received the same instrumentation, and the same experimental protocol was performed except for administration of FP-TBI and temporary blood loss. Six sham-operated pigs were cooled using the same procedure (SHAM-HT). Hypothermia was guided by the temperature measured within the parietal cortex. The target brain temperature of 32°C was reached after about 3 h, and maintained for 6 h, followed by a rewarming period of about 3 h. The remaining animals were kept normothermic throughout the whole experiment. The neurometabolic and cerebrovascular findings are a subject of another manuscript (Fritz et al., in preparation).

**Tissue fixation, histology, and immunohistochemistry**

Animals were killed at 24 h after trauma by transcerebral perfusion-fixation of the brain with a neutrally buffered solution containing 4% formalin after a short rinse with heparinized physiological saline solution. One animal of the TBI-NT-ICP group could not be adequately perfused. This brain was excluded from immunocytochemical processing, but not from TUNEL staining. Heads were...
then immersion fixed for 48 h at 4°C before the brains were removed from the skulls. Three 7-mm-thick slices from the frontolobal, temporoparietal brain, including diencephalon and hippocampal, and brain stem (ponto mid-brain site) (Fig. 1), were embedded in paraffin. They were cut into 7-μm-thick sections, which were stained with hematoxylin and eosin (H&E) for routine morphological, or prepared for immunohistochemistry. The observations were made per unit area procedure. Reactions for MAP-2 and TUNEL were performed twice on consecutive sections. βAPP immunolabeling was performed only once. The avidin-biotin-peroxidase complex (ABC) method (Vectastain Elite Kit, Vector Laboratories, Burlingame, CA), as described by Hicks et al. [33], was used for MAP-2 and βAPP immunohistochemistry. Each deparaffinized tissue section was incubated with: 10% non-immunized horse serum and 0.1% Triton X-100 for 1 h; with the primary antibody in phosphate-buffered saline (PBS) containing 5% normal horse serum at 4°C overnight; with the secondary antibody (horse anti-mouse IgG 1:200) at 4°C overnight; and with the ABC solution (1:100) for 1 h. The reaction product was visualized using 3,3-diaminobenzidine tetrahydrochloride as a chromogen. Control sections were incubated with normal horse serum in the absence of primary antibody and showed no staining. As primary antibodies, we used monoclonal anti-MAP-2 mouse serum (Amersham International, Amersham, UK; 1:500), and monoclonal anti-βAPP mouse serum (mAb 22C11; Boehringer Mannheim, Germany). Immunostained sections were evaluated without prior knowledge of the experimental procedures performed. MAP-2 immunoreactivity in the whole neocortex and hippocampus was measured using an image analysis program (IMAGE, Version 1.42, NIH Public Domain, USA). Images of coronal sections of the brain at the level of the hippocampus were digitized onto a computer screen. The intensity of immunostaining was determined as gray scale values between ‘white’ (0) and ‘black’ (255) by outlining the brain regions of interest. Subcortical white matter of temporoparietal brain sections were used as typically non-reactive sites for MAP-2 immunostaining. These background measurements were subtracted from the values obtained from the areas of interest.

Anti-βAPP reaction was scored as ranging from accumulation of βAPP in slightly dilated axons, to accumulation of βAPP in swollen axons (axonal swelling) or bulb formation (axonal bulbs) [34]. These features were assessed in the following brain areas: left and right hemisphere at the site of FP-TBI (i.e., radiatio optica, fasciculus subcallosus, corpus callosum, splenium, commissura fornicens), left and right diencephalon (fasciculus tectemini, thalamus), frontolobe (subcortical white matter at the level of fissura rhinalis anterior, rostral cap of nucleus caudatus), and brain stem (commissura colliculi inferioris, pedunculus cerebellaris superior, lemniscus lateralis, fasciculus tectemini, fasciculus longitudinalis medialis, lemniscus medialis) [35]. All βAPP-positive regions were taken into account. A rating score was given according to [36, 37]. If none of the features was present, a rating of 0 was given. If there was any staining of axons, however slight, a rating of 1 was made. When there were scattered areas or patches of axonal damage, a score of 2 was given. When there was extensive damage throughout large areas of white matter, the rating score was 3. In addition, numbers of axonal swelling and axonal bulbs were calculated separately (counts/0.5 mm², 20× objective, extrapolated for the whole βAPP-positive area) and are given as total number.

Fragmented DNA was detected in situ by the TUNEL method using a commercially available kit according to the manufacturer’s protocol (in situ cell death detection kit “AP”, Boehringer Mannheim, Germany). Sections were deparaffinized, pretreated with 20 mg/ml proteinase K, and washed in PBS prior to TUNEL staining. TUNEL staining was performed by incubation with fluorescein-conjugated digoxigenin-UTP and terminal deoxynucleotidyl transferase at 37°C for 1 h. DNA fragmentation was visualized using converter-alkaline phosphatase, NBT/BCIP and counterstaining with Kernechtrot.

Neocortical structures of parietal and temporal lobes medial and lateral to the middle suprasylvian sulcus (Fig. 1), the underlying white matter (i.e., radiatio optica, fasciculus subcallosus, corpus callosum, splenium, commissura fornicens), hippocampus, and diencephalon (corpus geniculatum laterale, corpus geniculatum medialis, pulvinar, pretectum, fasciculus tectemini) were investigated. A semiquantitative score was used to estimate TUNEL-positive and -negative cells. When there were no positive cells in the whole section, a score of 0 was given. In cases with TUNEL positivity, positive cells were counted in 5 microscopic fields (20× objective). The scores were: 1 for up to 5 positive cells per field, 2 for 6–20 positive cells, and 3 for >20 positive cells. In addition, the distribution of TUNEL-positive cells was estimated and set as 1 for location of TUNEL-positive cells in a single dot, 2 for positive cells located in scattered areas, and 3 for ubiquitous TUNEL-positivity in the area investigated. The two scores were added together in a single value with a maximal score of 6.

Statistical analysis

Unless otherwise indicated, data are reported as means ± SD. One-way analysis of variance (ANOVA) was used to determine effects of FP-TBI and hypothermia on physiological parameters. Post hoc comparisons were made with the Student-Newman-Keuls method or with unpaired t-tests as indicated. Immunomorphological data were compared by the Mann-Whitney U-test with the alpha-adj uncorrected procedures of Bonferroni and Holm [38,39]. The Fisher Exact Test was used to compare the distributions of treatment effects (normothermia vs hypothermia) in regard to occurrence of secondary ICP increase. Differences were considered significant when P<0.05.

Results

Physiological variables

Physiological parameters including intracranial pressure are shown in Table 1. These parameters remained unchanged throughout the experiment in sham-operated normothermic pigs (SHAM-NT). Temporary withdrawal of blood (25 ml/kg body wt.) immediately after FP-TBI induced a marked reduction in cardiac output of 37–49% (P<0.05) with a concomitant reduction in MABP and CPP (Table 1). At 8 h after TBI, ICP had increased considerably (29±24 mmHg) in seven of the normothermic animals (designated TBI-NT-ICP), whereas six normothermic animals (group TBI-NT) and all hypothermic animals...
Table 1  Physiological parameters of animals. Values are means ± SD (TBI traumatic brain injury, ICP intracranial pressure, SHAM-NT normothermic sham-operated animals, SHAM-HT hypothermic sham animals, TBI-NT normothermic animals with fluid percussion-induced TBI, TBI-NT-ICP normothermic animals with TBI, which developed a secondary increase of ICP, TBI-HT hypothermic animals with TBI, MABP mean arterial blood pressure, CPP cerebral perfusion pressure)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>End of blood withdrawal</th>
<th>8 h after TBI</th>
<th>24 h after TBI</th>
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<td>ICP (mmHg)</td>
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<td></td>
</tr>
<tr>
<td>SHAM-NT</td>
<td>8±2</td>
<td>8±2</td>
<td>7±3</td>
<td>6±2</td>
</tr>
<tr>
<td>SHAM-HT</td>
<td>9±3</td>
<td>7±2$</td>
<td>5±2$</td>
<td>4±2$</td>
</tr>
<tr>
<td>TBI-NT</td>
<td>9±3</td>
<td>7±3$</td>
<td>5±2$</td>
<td>4±2$</td>
</tr>
<tr>
<td>TBI-NT-ICP</td>
<td>9±2</td>
<td>13±9$</td>
<td>29±24*</td>
<td>45±27$*</td>
</tr>
<tr>
<td>TBI-HT</td>
<td>6±3</td>
<td>6±4</td>
<td>6±2</td>
<td>6±2</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td></td>
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<tr>
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<td>104±13</td>
<td>103±10</td>
<td>111±13</td>
<td>100±25</td>
</tr>
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<td>SHAM-HT</td>
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<td>112±7</td>
<td>102±13</td>
<td>93±19</td>
</tr>
<tr>
<td>TBI-NT</td>
<td>104±19</td>
<td>79±17$</td>
<td>82±17$*</td>
<td>85±16$</td>
</tr>
<tr>
<td>TBI-NT-ICP</td>
<td>103±13</td>
<td>82±23</td>
<td>88±10*</td>
<td>71±19$</td>
</tr>
<tr>
<td>TBI-HT</td>
<td>125±14*</td>
<td>95±22$</td>
<td>101±15$</td>
<td>82±12$</td>
</tr>
<tr>
<td>CPP (mmHg)</td>
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<td>105±6</td>
<td>97±14</td>
<td>89±19</td>
</tr>
<tr>
<td>TBI-NT</td>
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<td>73±19</td>
<td>77±18*</td>
<td>80±19</td>
</tr>
<tr>
<td>TBI-NT-ICP</td>
<td>95±13</td>
<td>67±27$*</td>
<td>58±27$*</td>
<td>29±29$*</td>
</tr>
<tr>
<td>TBI-HT</td>
<td>119±13*</td>
<td>88±22$</td>
<td>95±15$</td>
<td>76±12$</td>
</tr>
<tr>
<td>Cardiac output (ml/min/kg)</td>
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<td></td>
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<td></td>
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<tr>
<td>SHAM-NT</td>
<td>229±21</td>
<td>243±26</td>
<td>248±35</td>
<td>232±54</td>
</tr>
<tr>
<td>SHAM-HT</td>
<td>205±43</td>
<td>202±45</td>
<td>128±48$*</td>
<td>204±33</td>
</tr>
<tr>
<td>TBI-NT</td>
<td>244±18</td>
<td>126±19$**</td>
<td>257±55</td>
<td>302±51</td>
</tr>
<tr>
<td>TBI-NT-ICP</td>
<td>240±25</td>
<td>128±40$*</td>
<td>245±46</td>
<td>294±60</td>
</tr>
<tr>
<td>TBI-HT</td>
<td>214±26</td>
<td>137±37$**</td>
<td>109±10$*</td>
<td>200±42</td>
</tr>
<tr>
<td>Arterial $PO_2$ (mmHg)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>SHAM-NT</td>
<td>159±15</td>
<td>148±22</td>
<td>137±34</td>
<td>149±15</td>
</tr>
<tr>
<td>SHAM-HT</td>
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<td>142±19</td>
<td>157±31$^*$</td>
<td>133±39</td>
</tr>
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<td>139±26</td>
<td>139±26</td>
<td>133±38</td>
</tr>
<tr>
<td>TBI-NT-ICP</td>
<td>144±21</td>
<td>141±17</td>
<td>157±21</td>
<td>149±12</td>
</tr>
<tr>
<td>TBI-HT</td>
<td>146±26</td>
<td>155±15</td>
<td>180±32$^*$</td>
<td>145±34</td>
</tr>
<tr>
<td>Arterial $PCO_2$ (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHAM-NT</td>
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<td>41±1</td>
<td>43±3</td>
<td>40±6</td>
</tr>
<tr>
<td>SHAM-HT</td>
<td>41±5</td>
<td>40±4</td>
<td>43±3</td>
<td>37±1$^*$</td>
</tr>
<tr>
<td>TBI-NT</td>
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<td>42±2</td>
<td>41±3</td>
<td>41±1</td>
</tr>
<tr>
<td>TBI-NT-ICP</td>
<td>40±4</td>
<td>40±4</td>
<td>37±4$^*$</td>
<td>37±3</td>
</tr>
<tr>
<td>TBI-HT</td>
<td>40±3</td>
<td>38±1</td>
<td>41±4</td>
<td>39±4</td>
</tr>
<tr>
<td>Arterial pH</td>
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<td></td>
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</tr>
<tr>
<td>SHAM-NT</td>
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<td>7.49±0.02</td>
<td>7.48±0.01</td>
<td>7.52±0.06</td>
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<tr>
<td>SHAM-HT</td>
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<td>7.50±0.04</td>
<td>7.46±0.03</td>
<td>7.53±0.02$^*$</td>
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<tr>
<td>TBI-NT</td>
<td>7.45±0.01</td>
<td>7.44±0.04</td>
<td>7.46±0.05</td>
<td>7.51±0.04$^*$</td>
</tr>
<tr>
<td>TBI-NT-ICP</td>
<td>7.49±0.05</td>
<td>7.49±0.04</td>
<td>7.54±0.03$^*$</td>
<td>7.53±0.06</td>
</tr>
<tr>
<td>TBI-HT</td>
<td>7.48±0.04</td>
<td>7.47±0.03</td>
<td>7.47±0.04</td>
<td>7.49±0.06</td>
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<td>Brain temperature (°C)</td>
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<tr>
<td>SHAM-NT</td>
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<td>38.0±0.6</td>
<td>37.7±0.3</td>
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<tr>
<td>SHAM-HT</td>
<td>38.4±0.7</td>
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<td>32.3±0.5$^*$</td>
<td>38.8±0.5</td>
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<tr>
<td>TBI-NT</td>
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<td>38.6±0.9</td>
<td>38.9±1.0</td>
<td>39.1±1.0</td>
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<tr>
<td>TBI-NT-ICP</td>
<td>38.5±0.7</td>
<td>38.3±0.6</td>
<td>37.5±2.2$^*$</td>
<td>36.2±3.5</td>
</tr>
<tr>
<td>TBI-HT</td>
<td>38.8±0.7</td>
<td>38.5±0.5</td>
<td>32.4±0.4$^*$</td>
<td>38.7±0.6</td>
</tr>
</tbody>
</table>

*$^p<0.05; $indicates significant differences between groups compared to SHAM-NT group at the respective experimental stage; $indicates significant differences between baseline values and values obtained at different stages of the experimental procedure

did not show this secondary increase in ICP ($P<0.05$). Lowering of brain temperature to 32.2±0.5°C by surface cooling required 185±30 min, and this level of mild hypothermia was maintained for 360 min. During hypothermia cardiac output was similarly reduced in both hypothermic groups (SHAM-HT, 61±13%; TBI-HT, 54±7%; $P<0.05$). Arterial blood pressure was diminished 24 h after TBI in the TBI-NT-ICP and TBI-HT groups ($P<0.05$).

General morphological findings

In all traumatized animals, slight subarachnoidal hemorrhages were found near the craniotomy for FP-TBI administration, and small intracranial bleedings occurred in the white matter around gliding contusions. In animals from the TBI-NT-ICP group, subarachnoidal hemorrhages were also observed in the contralateral right hemisphere.
Slight subarachnoidal bleedings were present in the brain stem of all traumatized normothermic pigs, but in only three of seven animals in the TBI-HT group. At the site of trauma, circumscribed triangular hemorrhagic necroses (approximately 0.8 cm²), particularly of the gray matter, were seen. In animals from the TBI-HT group the peri-injury zones appeared macroscopically normal, but histologically showed some condensed neurons and vacuolation of tissue. There were no gross differences in the traumatic necroses between the groups. Small necrotic zones were located near the intracerebral catheters used for physiological data acquisition; they were not included in the analysis. The TBI-NT-ICP animals had increased brain weights (72.6±2.2 g; vs TBI-NT, 60.0±1.5 g; TBI-HT, 61.8±1.8 g; SHAM-NT, 59.8±2.2 g; and SHAM-HT, 66.3±2.1 g; P<0.05). Macroscopically, three TBI-NT-ICP animals showed severe and three animals moderate features of raised ICP, such as narrowing of sulci and flattening of gyri, reduction in size of the ventricles, tentorial and tonsillar hernia. One animal also showed brain stem infarction. Microscopically, these brains displayed microscopic signs of global neuronal hypoxia, namely shrunken and triangular cell bodies and nuclei, eosinophilic cytoplasm, and perineuronal edema. In addition, they showed blood vessel congestion. Neuronal injury in the hippocampus after secondary increase in ICP was observed in all subfields of both hemispheres, whereas in the TBI-NT group necrotic neurons were observed only in two cases and were restricted to the left hippocampus. In the TBI-HT group, the hippocampus displayed only small circumscribed areas of necrotic neurons at the trauma site without predilection of hippocampal fields (like in the TBI-NT group).

MAP-2 immunostaining

Immunostaining was performed twice in each case and each region with reproducible results. In SHAM-NT (Fig. 2A, D) and SHAM-HT (not shown), immunostaining for MAP-2 gave a strong, homogeneous reaction in dendrites and somata of neurons. The dendrites appeared as fine slender structures radiating through the cerebral cortex and hippocampus. The site of FP-TBI administration in all animals was totally devoid of MAP-2 immunostaining (see arrow in Fig. 2B) or showed MAP-2-positive fragments of damaged neuronal structures. Outside the site of FP-TBI administration, the neurons of TBI-NT animals displayed nearly normal MAP-2 immunostaining (Fig. 2B, E). In TBI-NT-ICP animals, however, neurons outside the FP-TBI administration site showed markedly decreased or negative MAP-2 labeling (Fig. 2C, F). Dendrites were sometimes immunoreactive, but only in their initial portions, and appeared as short MAP-2-positive stumps.

Fig. 2 MAP-2 immunostaining at low magnification in SHAM-NT (A), TBI-NT (B), and TBI-NT-ICP (C) groups. The site of FP-TBI administration was totally devoid of MAP-2 immunostaining (arrow in B). D, E MAP-2 immunoreactivity in traumatic animals outside the site of FP-TBI administration, at higher magnification. D Staining in dendrites and somata of neurons in SHAM-NT animals was strong and homogeneous. E Outside the site of FP-TBI administration, the neurons of TBI-NT animals displayed MAP-2 immunostaining comparable to that shown in D. F In TBI-NT-ICP animals, neurons outside the site of FP-TBI administration had a markedly decreased or negative MAP-2 labeling (MAP-2 microtubule-associated protein 2, SHAM sham-operated, ICP intracranial pressure, NT normothermia, NT-ICP NT – trauma with secondary ICP increase, HT hypothermia). A–C ×2; D–F ×100
Three animals showed this pattern of decreased MAP-2 immunostaining in both hemispheres, three animals mostly in the left hemisphere. Histologically, these neurons had condensed nuclei but otherwise looked normal. MAP-2 immunostaining in the TBI-HT animals was similar to that in controls (TBI-NT).

Quantification of immunostaining for MAP-2 was performed separately in the two hemispheres, i.e., in left traumatized and right non-traumatized neocortex, and in left and right hippocampus (Fig. 3). There was no significant difference in MAP-2 immunoreaction between the two sides. With the exception of the FP-TBI administration site, MAP-2 values were fairly similar in the neocortex and hippocampus of sham animals and TBI-NT animals. MAP-2 values were, however, dramatically reduced in neocortex and hippocampus of TBI-NT-ICP animals (when compared to the other groups) ($P<0.05$, Fig. 3).

**βAPP immunostaining**

The following seven areas were investigated: frontal cerebral hemispheres, left and right; cerebral hemispheres at the level of FP-TBI administration, left and right; diencephalon, left and right; and brain stem. βAPP immunoreactivity was quantified using an average rating score of βAPP immunostaining (0–3) according to McKenzie et al. [36]. βAPP-immunostained axons were not observed in sham animals. Axonal swelling and axonal bulbs were found in various degrees in all traumatized animals. No immunostaining was found in the frontal lobes of either hemisphere. In the TBI-NT animals, βAPP-positivity was moderate and mainly found in the white matter underlying the traumatization (three of seven animals, score 1–3) in the diencephalon (four animals, score mainly 1) and, to a lesser extent, in the brain stem (two animals, score 1–2), but was absent in the contralateral hemisphere. In the TBI-NT-ICP pigs, many βAPP-immunoreactive axons and axonal bulbs were observed in all brains (score mostly 3) and in all areas studied except the frontal lobes, being most abundant in the traumatized hemisphere and brain stem. Hypothermic animals displayed comparable amounts of axonal swelling and axonal bulbs in the left hemisphere (five of eight animals, score mainly 1–2) and left diencephalon (four animals, score 1–2) as in the TBI-NT group, but lacked βAPP reactivity in the other areas. Dif-

![Image of Fig. 3](image)

**Table 2** Regional βAPP immunostaining score and regional TUNEL score (βAPP β-amyloid precursor protein, LH left hemisphere, RH right hemisphere)

<table>
<thead>
<tr>
<th>Groups</th>
<th>SHAM-NT</th>
<th>SHAM-HT</th>
<th>TBI-NT</th>
<th>TBI-NT+ICP</th>
<th>TBI-HT</th>
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</thead>
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<tr>
<td>βAPP immunostaining score (maximum 3.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal brain (LH)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Temporoparietal brain (LH)</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>2.5 *</td>
<td>1.1</td>
</tr>
<tr>
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<td>0</td>
<td>0.3</td>
<td>0</td>
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<td>0.8</td>
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<td>1.0</td>
<td>0</td>
</tr>
<tr>
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<td>0.4</td>
<td>1.8 *</td>
<td>0</td>
</tr>
<tr>
<td>TUNEL score (maximum 6.0)</td>
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<td></td>
<td></td>
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<tr>
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<td>0</td>
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</tr>
<tr>
<td>Temporoparietal brain (cortex-RH)</td>
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<td>0</td>
<td>0</td>
<td>3.0</td>
<td>0</td>
</tr>
<tr>
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<td>0</td>
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<td>5.2 *</td>
<td>0.7</td>
</tr>
<tr>
<td>Temporoparietal brain (white matter-RH)</td>
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<td>0</td>
<td>0.3</td>
<td>5.2 *</td>
<td>0.7</td>
</tr>
<tr>
<td>Diencephalon (LH)</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
<td>4.4 *</td>
<td>0.7</td>
</tr>
<tr>
<td>Diencephalon (RH)</td>
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<td>0</td>
<td>0.3</td>
<td>5.3 *</td>
<td>0.7</td>
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<tr>
<td>Hippocampus (LH)</td>
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<td>4.0</td>
<td>0.5</td>
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<tr>
<td>Hippocampus (RH)</td>
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<td>0</td>
<td>0</td>
<td>4.0</td>
<td>0.5</td>
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* $P<0.05$, indicating significant differences between SHAM-NT animals and other groups
 Differences in βAPP immunoreactivity were statistically significant in TBI-NT-ICP pigs compared with SHAM-NT animals \((P<0.05)\), but not compared with TBI-NT or TBI-HT animals (Table 2). In addition, the number of βAPP-positive damaged axons and axonal bulbs was counted in all regions (Fig. 4). The number was lower in hypothermic than in TBI-NT-ICP pigs; however, it was comparable in the TBI-NT (without secondary ICP) and TBI-HT groups.

**Fig. 4** Graphic presentation of βAPP-positive axons per group counted in all areas. Significant differences from SHAM-NT are indicated by an asterisk.

Staining reactions were performed twice in each case and each region and yielded reproducible results. TUNEL staining was investigated in four brain areas of both hemispheres: neocortex, cerebral white matter, hippocampus, and diencephalon. TUNEL-positive cells were morphologically different. Most cells were characterized by a homogeneous dark nucleus (Fig. 5A), others showed cell shrinkage, nuclear condensation and fragmentation (apoptotic bodies) (not shown). A few cells demonstrated slight staining and without morphological changes of nuclei and cytoplasm, indicating nonspecific staining; these cells were not taken into further account. Most TUNEL-positive cells were glial cells, very few were neurons. TUNEL-positive cells were also found in the hippocampus, especially in the TBI-NT-ICP animals. Interestingly, the germinative zone of the granule cells of the dentate gyrus was selectively stained in these animals (Fig. 5B). The other animals lacked this reaction pattern (not shown).

The TUNEL reaction was semiquantified (see Materials and method) with a maximal score was 6.0. Brains of sham animals did not contain TUNEL-positive cells (score 0). TBI-NT animals displayed TUNEL-positive cells mainly in white matter areas adjacent to the FP-TBI administration site. All other areas had very low scores. TUNEL-positive cells in TBI-NT-ICP animals were abundant. The white matter of both hemispheres, the left and right hippocampus and the diencephalon were particularly involved, whereas TUNEL scores were lower in neocortex. The hippocampus, especially the germinative zone of the granular layer, showed a positive TUNEL reaction (Table 2, \(P<0.05\)). Hypothermic animals yielded TUNEL

**Fig. 5** A TUNEL-positive glial cells in the white matter of a TBI-NT animal. B Selective TUNEL positivity in the cells of the germinative cell layer of hippocampus in a TBI-NT-ICP animal. \(\times350\); \(\times150\)

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**TUNEL staining**

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scores similar to those of TBI-NT pigs, although scores in the hemisphere of trauma were lower in TBI-HT (0.7) than in the normothermic pigs (1.4). Moreover, the TUNEL score in TBI-HT group came from only one of six animals, whereas in TBI-NT animals, TUNEL positivity was found in three of six animals (data not shown).

Discussion

Effect of lateral FP-TBI on ICP

The juvenile pigs responded differently to the modified lateral FP injury, although the severity of the trauma, verified by a transient pressure increase near the epidural application site, was similar in each experiment. A significant transfer of the fluid pulse to the brain was likely administered, since previous studies using identical experimental conditions, but with an additional transducer contralateral to the FP application site, documented that a synchronous ICP transient occurred [40]. Of the 14 normothermic traumatized animals, 7 developed a secondary ICP increase after an interval of several hours. This can not be ascribed to variation in the experimental procedure. Assignment of animals to the different experimental procedures was randomized (by means of drawing by lot). Moreover, FP-TBI administration was standardized; in particular, identical FP conditions were achieved in all TBI groups, including the kind of FP administration, the height of the falling pendulum and the hydraulic pressure transient generated. The administered FP-induced hydraulic pressure transient did not differ between TBI groups. Furthermore, we performed extensive cardiovascular, respiratory, and neurophysiological monitoring, which is summarized in Table 1 and will be presented in more detail elsewhere (Fritz et al., in preparation). We were able to show that, soon after FP-TBI, and also after temporary blood loss, no statistically significant differences occurred between TBI groups (Table 1). Thus, animal preparation, FP-TBI administration, and blood withdrawal were similarly performed in all animals with similar physiological responses. Furthermore, management of anesthesia, analgesia, fluid replacement and ventilation were similar in all experiments. Thus, the divergent response of normothermic animals with regard to ICP is not related to the care and use of the animals, and at present cannot be explained. It is, nevertheless, notable that a rise in ICP was achieved in the present experimental setting, suggesting that the model is useful for investigating secondary injuries. Although discussed in detail elsewhere (Fritz et al., in preparation), two further findings are of note; (i) animals with a secondary ICP increase could not be predicted from the physiological data, and (ii) none of the hypothermic traumatized animals had a secondary ICP increase. Our model is in this response similar to neurotrauma in man, where development of a secondary ICP increase is also hard to predict [41].

The lack of a secondary ICP increase in an animal of the hypothermic group is noteworthy. Although the numbers were rather small and a secondary ICP increase was found only in about half of normothermic pigs after TBI, hypothermia-induced neuroprotection from ICP elevation was verified significantly (P=0.018, Fisher Exact Test). This is in line with findings in clinical studies, where hypothermia is related to a lowered incidence of ICP [16].

ICP monitoring was performed using a fiberoptic system which has been shown to record pressure accurately from within the lateral ventricle, from the subdural space over the surface of the cerebral hemispheres, and from within the brain tissue itself [42, 43]. We used the intraparenchymal approach because its offered an easy and standardized placement of the fiberoptic catheter.

Altered MAP-2 immunostaining correlates with diffuse brain oxygen deprivation

Under normal conditions, the cytoskeletal protein MAP-2 is abundantly expressed in neurons, almost exclusively in dendrites and perikarya [44]. It is degraded by calcium-activated neutral proteases, such as calpain [33]. MAP-2 immunostaining can serve as a sensitive marker for neuronal impairment after brain damage of various types, including ischemia [45, 46, 47, 48, 49]. It has also been used in models of neurotrauma. The intensity and location of decreased MAP-2 immunostaining depends on the mode and severity of traumatization. For instance, rats subjected to mild lateral FP injury (1.1–1.3 atm) showed less MAP-2 loss than rats with a moderate injury (2.3–2.5 atm) [50]. Diminished or lost MAP-2 immunolabeling was found either mainly at the contusion site [51] or both at the traumatized hemisphere and outside the contused regions [52]. In a study by Folkerts et al. [52], the effect was time dependent, being observed shortly after the traumatization, with most severe loss at 48 h and a partial recovery at 72 h. Similarly, Hicks et al. [33] demonstrated decreased staining from 10 min to at least 7 days after injury (at slightly different levels). Decreased or lost MAP-2 immunostaining was correlated with signs of neuronal death in histological sections [33], but sometimes preceded morphological changes [53]. Whether MAP-2 loss implies a state of transition leading to an irreversible level or a state of functional disturbance that can be remedied by restored protein synthesis, is not known.

In our study MAP-2 immunostaining was not significantly diminished after severe lateral FP injury, except after secondary ICP increase, and then at the FP-TBI administration site. The affected area was so small (0.8 cm²) in comparison with the remaining cortex that it did not influence the total MAP-2 cortical staining index. This result is in contrast to some findings in the literature, which show a substantial decrease of MAP-2 expression outside the FP-TBI administration site [33, 52]. The dramatic loss of MAP-2 reactivity in the normothermic animals with a secondary ICP increase is, however, in line with the literature. The diffuse reduction of MAP-2 immunoreactivity most likely reflects changes due to decreased cerebral blood flow and global ischemia. In the porcine model...
Although the TUNEL method was used mainly to assess apoptotic cells, labeling of necrotic cells could not be excluded. On the contrary, DNA fragmentation due to necrosis was in many cases more likely than apoptotic cell death. According to Rink et al. [63], cells with homogeneous dark nuclei are considered to be necrotic, while those with nuclear condensation and fragmentation are apoptotic. Therefore, the general term ‘TUNEL positivity’ has been used.

The TUNEL experiments yielded high scores in normothermic animals with a secondary ICP increase. It is important to note that the time period of 24 h between TBI and killing of the animals may not be long enough for the induction of significant apoptosis. Conti et al. [64] found apoptosis in adult rat brain 24 h after lateral FP injury only in the injured cortex, whereas 48 h or even weeks were needed for it to occur in other brain regions, such as hippocampus and thalamus. However, a study by Xu et al. [24] on cold-induced brain injury in rats demonstrated occurrence of apoptotic neuronal cell death already at 12 h with a peak at 24 h in the cortex and at 48 h in the white matter and hippocampus. Thus, apoptosis in brain injury is influenced by various factors including the experimental setting. Moreover, the mechanism of delayed cell death after brain injury remains to be determined. Clark et al. [65] investigated expression of the apoptosis-suppressor gene bcl-2, and Colicos and Dash [66] the role of apoptosis in spatial memory deficits. Alteration in the expression of immediate early genes and glutamate might trigger a rise in the intracellular free Ca$^{2+}$ concentration by activating NMDA receptors and voltage-dependent Ca$^{2+}$ channels. Hypothermia is able to reduce apoptosis via Ca$^{2+}$-mediated endonuclease activity, resulting in DNA fragmentation [67]. In a study of cold-induced brain damage [24], hypothermia for 12 h seemed to be more effective than hypothermia for 3 h, and hypothermia reduced apoptosis and essentially eliminated detectable DNA labeling not only at 24 and 48 h after injury but 1, 3 and 6 months later, arguing against the hypothesis of a simple delay of cerebral damage under hypothermia [68]. In our study, TUNEL-positive scores at the FP-TBI administration site were lowered after hypothermic treatment, indicating a protective effect.

In conclusion, severe FP-TBI combined with temporary blood loss consistently produced traumatic axonal injury with focal brain damage. Mild hypothermia was able to prevent a secondary increase in ICP and its sequelae of diffuse hypoxic-ischemic brain injury. However, mild hypothermia did not offer significant protection against traumatic axonal injury.

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References


The Effect of Mild Hypothermia on Plasma Fentanyl Concentration and Biotransformation in Juvenile Pigs

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Therapeutic hypothermia may alter the required dosage of analgesics and sedatives, but no data are available on the effects of mild hypothermia on plasma fentanyl concentration during continuous, long-term administration. We therefore assessed in a porcine model the effect of prolonged hypothermia on plasma fentanyl concentration during 33 h of continuous fentanyl administration. Seven female piglets (weight: 11.8 ± 1.1 kg) were anesthetized by IV fentanyl (15 μg·kg⁻¹·h⁻¹) and midazolam (1.0 mg·kg⁻¹·h⁻¹). After preparation and stabilization (12 h), the animals were cooled to a core temperature of 31.6 ± 0.2°C for 6 h and were then rewarmed and kept normothermic at 37.7 ± 0.3°C for 6 more hours. Plasma fentanyl concentrations were measured by radioimmunoassay, cardiac index by thermodilution, and blood flows of the kidney, spleen, pancreas, stomach, gut, and hepatic artery by a colored microspheres technique. Furthermore, in an additional 4 pigs, temperature dependency of hepatic microsomal cytochrome P450 3A4 (CYP3A4) was determined in vitro by ethylmorphine N-demethylation. Plasma fentanyl concentration increased by 25% ± 11% (P < 0.05) during hypothermia and remained increased for at least 6 h after rewarming. Hypothermia reduced the cardiac index (41% ± 15%, P < 0.05), as well as all organ blood flows except the hepatic artery. A strong temperature dependency of CYP3A4 was found (P < 0.01). Mild hypothermia induced a distribution and/or elimination-dependent increase in plasma fentanyl concentration which remained increased for several hours after rewarming. Consequently, a prolonged increase of the plasma fentanyl concentration should be anticipated for appropriate control of the analgesia/sedatives during and early after therapeutic hypothermia.

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Mild hypothermia has been effective after cardiac arrest, ischemic and traumatic brain injury (TBI), neonatal asphyxia, and neurosurgical procedures (1–3). However, there is controversy on the effects of hypothermia on patient outcome in these applications, especially after TBI (4). The results may be influenced by the complex effects of hypothermia, the modalities of hypothermia application, and side effects of hypothermia.

Background anesthetics seem to be an independent factor that may influence both the putative protective and detrimental effects of hypothermia. Indeed, hypothermia itself may alter the pharmacokinetics of anesthetics. The plasma concentration of propofol increases about 28% during mild hypothermia in humans (4). During reduced core temperature, a doubling of vecuronium-induced neuromuscular block duration has been demonstrated in humans (5). Additionally, the anesthetic/analgese regimen after TBI may dramatically influence the response to hypothermia (6).

The synthetic opioid fentanyl, when administered IV, is cleared predominantly by hepatic biotransformation (7). Fentanyl is metabolized by the microsomal cytochrome P450 3A4 (CYP3A4) isorm in humans (8), which is also present in the pig liver with similar activity (9). However, the effects of mild hypothermia on the action of continuously administered opioids for long-term analgesia and sedation (e.g., fentanyl) are not well understood.

We therefore examined, in a porcine model, the effect of mild hypothermia (32°C) on the plasma concentration of fentanyl during continuous long-term

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administration (33 h). We also investigated the effect of hypothermia on perfusion of organs that are involved in fentanyl metabolism and excretion, using color-labeled microspheres (CMS) (10). Furthermore, we determined temperature dependency of porcine hepatic microsomal CYP3A4 activity in vitro by ethylmorphine N-demethylation. We studied whether a temporarily decreased and subsequently reestablished body temperature, by altering the enzyme activity responsible for hepatic biotransformation, would influence the plasma concentration of fentanyl during continuous infusion.

**Methods**

The study protocol was approved by the committee of the Thuringian State Government for animal research. The animals were managed in accordance with the guidelines of the American Physiological Society.

**In Vivo Study**

Seven female juvenile pigs of mixed German domestic breed (6-wk-old, weight: 11.8 ± 1.1 kg) were initially sedated with ketamine hydrochloride (20 mg/kg) and midazolam (1 mg/kg), and anesthesia was maintained during surgery by 70% nitrous oxide in 30% oxygen and 2% isoflurane. After tracheotomy (tube size: 5.5-mm inner diameter) and insertion of a central venous catheter into the left external jugular vein for drug administration and fluid therapy (lactated Ring-er’s solution: 4 mL·kg⁻¹·h⁻¹), muscular relaxation was achieved with IV pancuronium bromide (0.4 mg·kg⁻¹·h⁻¹). The lungs of the pigs were mechanically ventilated using pressure-controlled ventilation (Servo Ventilator 900C; Siemens-Elema, Solna, Sweden) with an oxygen/air mixture (Fio₂ 0.35). Ventilation was controlled by continuous end-expiratory CO₂ monitoring and arterial blood gas check with a stat regime hourly.

The left brachial artery was cannulated for continuous monitoring of mean arterial blood pressure (MABP). A thermolodiution catheter for cardiac index (CI) measurement was inserted into the abdominal aorta 2 cm below the diaphragm via the femoral artery. A left atrium catheter (inner diameter 1.0 mm) was placed via a left thoracotomy for CMS injection. In addition, a catheter (inner diameter 0.5 mm) was inserted into a branch of the pulmonary artery for mixed venous blood sampling. Another catheter (inner diameter 1.4 mm) was advanced from the left femoral artery into the abdominal aorta in order to withdraw reference samples during regional blood flow measurement (see below).

An electrocardiogram was recorded from standard limb leads using stainless steel needle electrodes.

Body temperature was controlled by a rectal thermoprobe, maintained throughout instrumentation at 37.5°C ± 0.5°C using a water-filled pad connected to a heating cooling thermostat and a feedback-controlled heating lamp. Physiological variables were recorded on a multichannel polygraph (MT95K2; Astro-Med).

**Experimental Protocol and Management of Hypothermia.** After instrumentation, anesthesia was maintained throughout the experiment with a continuous infusion of fentanyl (15 μg·kg⁻¹·h⁻¹) and midazolam (1.0 mg·kg⁻¹·h⁻¹). During the baseline stabilization period of 12 h, values were recorded at 6 h (measuring point one, MP1) and 12 h (MP2). Systemic hypothermia was started 1 h after MP2 by means of a cooling blanket and forced air cooling. Hypothermia was guided by a rectal thermocouple probe. The animals were surface cooled within 217 ± 101 min to a core temperature of 31.6°C ± 0.2°C for 6 h (MP3). Afterward, animals were rewarmed (MP4) within 258 ± 55 min and kept normothermic at 37.7°C ± 0.3°C for a further 6 h (MP5) (Fig. 1).

**Measurements.** Blood samples for analysis of plasma fentanyl concentrations were injected into glass tubes. The plasma was separated in a refrigerated centrifuge (4°C) and placed in polypropylene tubes. Plasma fentanyl concentrations were measured by radioimmunoassay (Fa. Janssen Biotech, Olen, Belgium) at the five time points (MP1–5) (11). The assay sensitivity was 0.05 ng/mL, with intra- and intercoefficients of variation 6.0% and 6.9%, respectively. The assay was specific for fentanyl and did not include the metabolites.

Organ blood flows were measured using the reference sample CMS technique (10). Briefly, a known amount (approximately 3 × 10⁹ per injection) of polystyrene CMS (diameter: 15.5 ± 0.33 μm) in 0.01% Tween 80, surface coated with 1 of 5 dyes (blue, yellow, white, red, violet) (Dye-Trak; Triton Technology, San Diego, CA) were thoroughly vortexed and sonicated and immediately injected within 20 s into the left atrium. CMS injected had a different color for each measuring point (MP1–5). A blood sample was withdrawn from the descending aorta as the reference sample, beginning 15 s before the CMS injection and continuing for 2 min at a rate of 3 mL/min (syringe pump SP210iw; World Precision Instruments, Inc., Sarasota, FL). The CMS injection did not alter MABP. At the end of each experiment, the pigs were killed with potassium chloride and the organ tissue samples from pancreas, spleen, liver, kidney, stomach (cardia, fundus, and pylorus, 4–6 g each), and gut (duodenum, jejunum, and ileum, 4–6 g each) were removed for processing. Blood and tissue samples were digested and the dye content of the combined CMS was estimated by photometric absorption with a diode-array ultraviolet/visible spectrophotometer (model 7500, wave length range 300–800 nm with a 2-nm
optical band width; Beckman Instruments, Fullerton, CA). Calculations were performed using the MISS software (Triton Technology). The number of CMS was calculated using the specific absorbency value of the different dyes (provided by the manufacturer). Absolute tissue blood flows measured by CMS were calculated by the formula: flow_{tissue} = number of microspheres_{tissue} \cdot (flow_{reference} / number of microspheres_{reference}). Flows are expressed in mL/min per 100 g of tissue.

Assuming the oxygen capacity of hemoglobin to be 1.39 mL O2/g hemoglobin in pigs, blood O2 content was calculated as equal to grams of hemoglobin/mL \cdot 1.39 mL O2/g hemoglobin \cdot O2 saturation and expressed in mL/100 mL. Dissolved oxygen was added by calculation, using the measured PO2 and the temperature-corrected solubility coefficient for oxygen. The systemic oxygen consumption (VO2) was calculated by multiplying CI by the difference in arteriovenous O2 content. Systemic oxygen extraction rate was calculated by arteriovenous O2 content divided by arterial oxygen content.

In Vitro Study

**Biological Material.** To determine temperature dependency of CYP3A4 activity, 9000 g supernatants from pig liver specimens were used. Four juvenile pigs of mixed German domestic breed (age: 6 wk, weight: 12.7 ± 1.5 kg) were initially sedated with ketamine hydrochloride (20 mg/kg) and midazolam (1 mg/kg) and afterward killed for organ removal by IV injection of 10 mL of saturated magnesium chloride. Subsequently, the liver specimens (approximately 1 g each) were quickly removed and shock-frozen in liquid nitrogen and kept therein until processing. For preparation of the 9000 g supernatants, the specimens were homogenized in ice-cold 0.1 M sodium phosphate buffer pH 7.4 (1/3 w/v) and centrifuged at 9000 g for 20 min at 4°C. The protein content of the supernatants was determined by modified biuret method.

**Cytochrome P450-Dependent Monoxygenase Model Reactions.** Ethanolamine N-demethylation activity was assessed in the supernatants by photometrical determination of the reaction product formaldehyde (12). For this reaction, the supernatants were diluted 1:4 with 0.1 M sodium phosphate buffer pH 7.4. Reactions were performed in a shaking water bath for 10 min at the temperatures indicated (26°C, 32°C, 38°C, 44°C). Before starting the reaction with the substrate ethylmorphine, samples were allowed to equilibrate to the temperature for 5 min. The activities of the model reactions were referenced to the protein content of the supernatants.

Values were presented as means ± sd. One-way analysis of variance with repeated measures was performed within the group. Post hoc comparisons were done using paired Student’s t-test with Bonferroni correction for multiple comparisons. Differences were considered significant at P < 0.05. All statistical tests were done using the statistical package SPSS for Windows release 10.0 (SPSS Inc., Chicago, IL).

**Results**

Blood gases, arterial glucose and lactate contents, as well as systemic hemodynamics are presented in Table 1. Hypothermia resulted in a decrease in CI of 41% ± 15% and heart rate of 21% ± 4% (P < 0.05), whereas arterial glucose and lactate concentrations were slightly increased (P < 0.05). After rewarming, both CI and heart rate returned to baseline levels. MABP decreased 22% ± 8% (P < 0.05) during hypothermia, but remained slightly reduced after rewarming. Furthermore, during hypothermia, the systemic VO2 was reduced by 44% ± 11% (P < 0.05) and returned to prehypothermic values after rewarming.

The baseline plasma fentanyl concentration did not change during the prehypothermic period (MP1, MP2) (Fig. 2). At the end of the cooling period, the plasma fentanyl concentration increased by 25% ± 11% (MP3, P < 0.001). After rewarming (MP4) and 6 h of normothermia (MP5), plasma fentanyl concentration remained increased (P < 0.05).

As shown in Figure 3, hypothermia was associated with markedly reduced blood flows (P < 0.05) to the kidney (38% ± 32%), spleen (45% ± 33%), stomach (53% ± 31%), and gut (49% ± 26%). Pancreatic blood flow was also reduced during hypothermia (49% ± 46%), and remained so even after rewarming (P < 0.05). In contrast, hepatic artery blood flow remained unchanged throughout the experiment.

Hypothermia induced a strong temperature-dependent reduction in hepatic CYP3A4 activity (at 26°C: 48% ± 2%, at 32°C: 69% ± 1%, compared with values obtained at 38°C (P < 0.001). Temperature increase was associated with a modest reduced CYP3A4 activity (at 44°C: 94% ± 3%) (P < 0.01) (Fig. 4).
Table 1. Hemodynamic and Blood Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>MP1</th>
<th>MP2</th>
<th>MP3</th>
<th>MP4</th>
<th>MP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.49 ± 0.04</td>
<td>7.49 ± 0.03</td>
<td>7.41 ± 0.07*</td>
<td>7.51 ± 0.03</td>
<td>7.51 ± 0.07</td>
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<tr>
<td>Pao2 (mm Hg)</td>
<td>146 ± 18</td>
<td>152 ± 10</td>
<td>183 ± 17*</td>
<td>157 ± 6*</td>
<td>144 ± 13</td>
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<tr>
<td>Paco2 (mm Hg)</td>
<td>40 ± 4</td>
<td>39 ± 3</td>
<td>43 ± 3*</td>
<td>38 ± 4</td>
<td>38 ± 3</td>
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<tr>
<td>Arterial glucose (mM/L)</td>
<td>6.4 ± 1.2</td>
<td>6.5 ± 1.0</td>
<td>9.9 ± 3.1*</td>
<td>8.4 ± 3.6*</td>
<td>6.3 ± 1.0</td>
</tr>
<tr>
<td>Arterial lactate (mM/L)</td>
<td>1.4 ± 0.6</td>
<td>1.5 ± 0.5</td>
<td>2.6 ± 1.4*</td>
<td>1.4 ± 0.5</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>206 ± 11</td>
<td>200 ± 20</td>
<td>163 ± 9*</td>
<td>192 ± 22</td>
<td>197 ± 23</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>119 ± 17</td>
<td>110 ± 15</td>
<td>92 ± 5*</td>
<td>104 ± 10</td>
<td>96 ± 17*</td>
</tr>
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<td>CI (mL · min⁻¹ · kg⁻¹)</td>
<td>199 ± 34</td>
<td>195 ± 26</td>
<td>118 ± 30*</td>
<td>193 ± 46</td>
<td>198 ± 40</td>
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<td>O2ER (%)</td>
<td>44 ± 8</td>
<td>43 ± 9</td>
<td>43 ± 5</td>
<td>51 ± 9</td>
<td>49 ± 6</td>
</tr>
<tr>
<td>VO2 (mL · min⁻¹ · kg⁻¹)</td>
<td>5.4 ± 9.0</td>
<td>5.6 ± 1.6</td>
<td>3.0 ± 6.0*</td>
<td>5.1 ± 9.1</td>
<td>4.9 ± 6.4</td>
</tr>
</tbody>
</table>

Values are presented as means ± SD.

MP1 = baseline; MP2 = control of baseline; MP3 = after 6 h of hypothermia at 32°C; MP4 = return of normothermia; MP5 = end of the experiment; MP = measuring point; Pao2 = arterial oxygen partial pressure; Paco2 = arterial carbon dioxide partial pressure; HR = heart rate; MAP = mean arterial blood pressure; CI = cardiac index; O2ER = systemic oxygen extraction rate; VO2 = systemic oxygen consumption.

*P < 0.05 versus baseline (MP1).

Figure 2. The baseline plasma fentanyl concentration did not change during the first 12 h (MP1, MP2) before cooling (MP = measurement points for organ blood flow and plasma fentanyl concentration). At the end of the cooling period (MP3), the plasma fentanyl concentration increased significantly to 7.0 ± 0.6 ng/mL. After rewarming (MP4) and a further 6 h at normothermia (MP5), the plasma fentanyl concentration decreased significantly to 6.3 ± 0.6 ng/mL (P < 0.05) compared with MP3, but remained increased compared with baseline (MP1, MP2). Horizontal lines in the boxes represent median, the boxes represent the interquartile ranges (25th and 75th percentiles); error bars represent 10th and 90th percentiles, *P < 0.001, **P < 0.05, MP versus control.

Discussion

The present study demonstrates an increase of approximately 25% in the plasma concentration of fentanyl, when infused at a constant rate during a 6-hour period of hypothermia at approximately 32°C. This observation is consistent with other reports describing the effect of hypothermia on the pharmacokinetics of other drugs with a high hepatic extraction ratio such as propofol (4), propranolol (13), and fentanyl (14) and also those having a low hepatic extraction ratio, such as phenytoin (15) or metabolized by nonspecific esterases like remifentanil (16). However, these studies differed with regard to drug administration, observation time, and level of hypothermia. Although continuous fentanyl infusion over a prolonged period is a common approach for analgesia and sedation in intensive care medicine, there are few data on plasma fentanyl concentrations during hypothermia.

We chose a porcine model because of the similarities between swine and humans in the basic cardiovascular variables, including CI and VO2, as well as regional distribution of blood flows (17). Furthermore, there is a remarkable similarity in biotransformation pathways, because the activity of the most important CYP isoenzymes in humans, CYP3A4, also responsible for hepatic fentanyl metabolism, is present in pigs with comparable levels and activities (9).

Under normal conditions, fentanyl is cleared predominantly by hepatic biotransformation (7), and the metabolism is extensive and rapid under physiological conditions. Furthermore, fentanyl is preferentially oxidized to norfentanyl by hepatic microsomal cytochrome P450 3A isoform (8) and excreted renally. Other pathways such as intestinal metabolism seem less likely with parenteral administration (18).

There are several processes that may be responsible for an increase in plasma fentanyl concentration during hypothermia. The pharmacokinetics of fentanyl, a drug with a large distribution volume and a high hepatic extraction ratio, could be altered. After a bolus injection, distribution volume and total body clearance were markedly reduced during hypothermia (14). A reduced total body clearance can be a result of reduced hepatic biotransformation and/or slower distribution caused by reduced cardiac output and organ perfusion rate estimated in this study. Simulation studies have demonstrated that the amount of reduced perfusion reported in this study can be responsible for increased blood fentanyl concentration (19).
However, the experimental design does not allow discrimination between alterations in distribution and elimination influences.

With regard to hypothermia-dependent alterations of fentanyl elimination, several aspects have to be considered. We have shown that hepatic CYP3A4 activity in juvenile pigs is strongly temperature dependent. At 32°C, the conversion rate was reduced by about one-third. Therefore, a relevant component of the reduction in total body clearance seems to be a reduced hepatic biotransformation of fentanyl. Furthermore, total hepatic blood flow is assumed to be reduced, despite the surprising finding that only arterial hepatic influx remained unaltered during mild hypothermia. Under normal conditions, arterial hepatic blood flow represents no more than one-fifth of total hepatic blood flow in the juvenile pig (20). The other influx arrives via the portal vein. Indeed, there is no doubt that portal blood flow was markedly reduced during hypothermia, because all perfusion rates of splanchnic organs drained by the portal vein were reduced. Because fentanyl has a high liver extraction ratio, hepatic elimination of fentanyl is expected to be more sensitive to blood flow alterations than to enzymatic activity (21). We suggest that both mechanisms, i.e., reduced total liver blood flow and reduced hepatic CYP3A4 activity, are involved in the reduced hepatic elimination, which may in turn be responsible for the increased efficient levels of fentanyl during hypothermia.

Hemodynamic data support our opinion that hypothermia-dependent alteration of fentanyl turnover is not primarily caused by compromised hepatic energy metabolism. The expected effects of mild hypothermia on systemic hemodynamics and oxygen uptake were similar to that reported earlier with a comparable anesthetic/analgesic regimen (22). Our data demonstrate a marked decrease in CI (CI decreased by an average 41%), and concomitant decrease in whole body O2 uptake which suggests an appropriate decreasing of metabolic demand. This assumption is supported by an unaltered arteriovenous oxygen
Figure 4. Temperature-dependency of pig hepatic microsomal cytochrome P450 3A4 (CYP3A4) activity in vitro by etynorphine N-demethylation. MP = measurement points for organ blood flow and plasma fentanyl concentration. Values are presented as means ± SD, *P < 0.05 versus 38°C.

 extraction rate during and after mild hypothermia (Table 1). Furthermore, during hypothermia, similar reductions in blood flow have been shown for the splanchnic organs studied, suggesting portal blood flow to the liver appropriate to a reduced oxidative metabolism. The maintained hepatic artery influx tends to exclude hepatic hypoperfusion and restricted hepatic O2 availability for the reduced fentanyl metabolism.

Thus, a risk for increased blood concentrations during long-term administration of fentanyl depends mainly on portal blood flow, which is proportional to cardiac output. Hence, maintenance of cardiac output to normal values may suggest that fentanyl concentration is likely stable whereas a decrease in cardiac output should suggest decreasing fentanyl doses.

The reason for a delayed normalization of plasma fentanyl level after rewarming remains unclear. A prolonged inhibition of hepatic CYP3A4 activity seems rather implausible, because after long-term deep hypothermic liver preservation, hepatic drug extraction was reestablished within 30 min of rewarming (23). An augmented tissue accumulation of fentanyl during the hypothermic period of reduced clearance, and subsequent rebound of plasma levels after rewarming increases tissue blood flow, may be involved.

Additionally, it should be noted that hepatic CYP3A4 is also responsible for midazolam biotransformation, hence this drug could also accumulate during mild hypothermia. In this study, however, we did not estimate plasma midazolam levels. There is no information about the effects of hypothermia on midazolam pharmacology. Nevertheless, it is quite possible that a decreased midazolam metabolism during hypothermia might have resulted in increased midazolam concentration with a possible competition on fentanyl metabolism. Provided that a similar response occurs as with fentanyl, a prolonged accumulation of two drugs could overcome the transforming capacity of CYP3A4 for xenobiotics, resulting in a delayed normalization of the expected plasma levels. Furthermore, midazolam could presumably enhance the hemodynamic effect of hypothermia on regional blood flow and fentanyl transport to the liver. A biphasic response of portal blood flow to bolus administration of midazolam has been reported with an early increase and a following decrease. This is probably related to redistribution of blood within the splanchnic system (blood mobilization from spleen and intestine), whereas hepatic arterial flow decreased immediately after administration (24).

Although the underlying mechanisms of the persistent increase of plasma fentanyl concentration remain speculative, the description of the phenomenon may be of clinical relevance with respect to the recovery from sedation of intensive care unit (ICU) patients after therapeutic hypothermia. However, the range of concentrations usually required in ICU can be smaller than in anesthesia. Therefore, the consequences of hypothermia-related fentanyl accumulation per se seem less relevant. However, hypothermia can aggravate a fentanyl overdosage during continuous long-term administration and can result in negative side effects such as decreased intestinal motility, hypotension, reduced tissue extraction capabilities, prolonged ICU stay, increased costs, and acute withdrawal syndrome (25). Consequently, an optimal analgesic/sedative regimen during the application of hypothermia should be carefully chosen.

Nevertheless, we can conclude that hypothermia of 32°C induces a reduction of CI, hypoperfusion in several organs, and an increase in plasma fentanyl concentrations. With rewarming, there is a prolonged recovery phase which may include increased side effects and prolonged recovery from analgesia/sedation.

The authors acknowledge the advice of U. Jaeger, I. Witte, and L. Wunder and their skillful technical assistance (Institute for Pathophysiology and Pathobiochemistry, University Jena, Germany), and Don Bredle, PhD (Department of Kinesiology, University of Wisconsin, Eau Claire, WI), for advice in preparing the manuscript.

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A Pig Model with Secondary Increase of Intracranial Pressure after Severe Traumatic Brain Injury and Temporary Blood Loss

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ABSTRACT

There is a lack of animal models of traumatic brain injury (TBI) that adequately simulate the long-term changes in intracranial pressure (ICP) increase following clinical TBI. We therefore reproduced the clinical scenario in an animal model of TBI and studied long-term postinjury changes in ICP and indices of brain injury. After induction of anesthesia, juvenile piglets were randomly traumatized using fluid-percussion injury (FPI) to induce either moderate (mTBI = 6 pigs: 3.2 ± 0.6 atm) or severe (sTBI = 7 pigs: 4.1 ± 1.0 atm) TBI. Injury was followed by a 30% withdrawal of blood volume. ICP and systemic hemodynamic were monitored continuously. Repeated measurements of global cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMRO2) were performed at baseline, at the end of blood withdrawal, after volume replacement, and at 8 and 24 h postinjury. Histological and immunocytochemical studies have also performed. ICP peaked immediately following FPI (mTBI: 33 ± 16 mm Hg; sTBI: 47 ± 14 mm Hg, p < 0.05) in both groups. In the sTBI group, we noted a second peak at 5 ± 1.5 h postinjury. This second ICP peak was accompanied by a 50% reduction in CBF (44 ± 31 mL · min · 100 g−1) and CMRO2 (2.5 ± 2.0 mL · min · 100 g−1). Moderate TBI typically resulted in focal pathological change whereas sTBI caused more diffuse change, particularly in terms of the ensuing axonal damage. We thus describe an animal model of severe TBI with a reproducible secondary ICP increase accompanied by patterns of diffuse brain damage. This model may be helpful in the study of pathogenetic relevance of concomitant affections and verify new therapeutic approaches in severe TBI.

Key words: fluid-percussion injury; hemorrhagic hypotension; intracranial pressure; piglets; secondary injury

INTRODUCTION

Severe traumatic brain injury (TBI) is often associated with elevation of intracranial pressure (ICP). According to one estimate (Ward, 1994), approximately 60% of severely head-injured patients develop increased ICP. Intracranial hypertension following TBI is of considerable prognostic value (Ciricillo et al., 1992; Juul et al., 2000) and correlates with adverse outcome (Miller et al., 1981; Signorini et al., 1999).
Although several groups of investigators have developed animal models of TBI in an attempt to reproduce diverse aspects of pathophysiological responses, long-term ICP changes have been assessed in only a limited number of studies (Shibata et al., 1993). One reason for this might be that the majority of investigations used rodents (Statler et al., 2001). The lissencephalic nature of the rodent cortex appears less susceptible to simulating intracranial changes leading to secondary ICP increase, as frequently observed in humans after severe TBI. The marked structural difference in the human brain morphology with its appearance of complex gyri and sulci may be involved in the different response of ICP to TBI (Povlishock et al., 1994). In contrast, animals showing similarities to human brain morphology (i.e., cat, dog, sheep, pig) have been successfully used as models for TBI with ICP alterations (Lindgren and Rinder, 1969; Millen et al., 1985; Pfenninger et al., 1989; Sullivan et al., 1976). One major difficulty in these animal models of TBI was that the reported ICP increases varied. Some reported no ICP change (Lewelt et al., 1982). Others reported moderate (Engelborghs et al., 1998), or only short-lived ICP elevations (Sullivan et al., 1976). Alternatively, some reported pronounced ICP elevations (Pfenninger et al., 1989). The reasons for “inconsistencies” in ICP response include differences in the injury methods used, observational durations, and/or species-specific characteristics.

Given that none of the known experimental TBI approaches are able to simulate the complex pathophysiology and the large variety of events occurring during clinical TBI, a main disadvantage of the study of the pathological ICP alterations as a main consequence of secondary brain injury, was their lack of reproducibility. Because of these limitations, other experimental models such as epidural balloon inflation (Bauer et al., 1999; Nilsson et al., 1995), intracranial fluid infusion (Leffler et al., 1989), or cryogenic lesion (Kroppenstedt et al., 1999) were used to investigate the compromising effects of increasing ICP or CPP reduction on brain metabolism and cerebral circulation.

However, these approaches simplified the complex interactions of secondary brain injury responsible for the secondary ICP increase after severe TBI. Therefore, we sought to simulate a scenario of two sequential insults known to be frequent at the scene and relevant for the subsequent consequences: a TBI of different intensity followed by temporary blood loss. We used the fluid percussion (FP) injury, which is known to induce TBI with different extents of brain damage, dependent on the intensity of impact administered (McIntosh et al., 1989; Prins and Hovda, 2003; Sullivan et al., 1976), leading to focal or diffuse brain injury. Subsequently, a temporary blood loss was used to induce short-lived hypotension. The aim of this study was to describe a model providing a consistent response of secondary ICP elevation with subsequent neuropathological consequences.

**MATERIALS AND METHODS**

Experiments were carried out according to the guidelines for animal care and use. Laboratory animal protocols were approved by the Animal Research Committee of the Thuringian State government.

**General Instrumentation**

Nineteen female juvenile pigs of mixed German domestic breed (6 weeks old, weighing 11.1–13.9 kg) were sedated with ketamine hydrochloride (20 mg/kg b.w.) and midazolam (1 mg/kg b.w.), and were anesthetized with 70% nitrous oxide in 30% oxygen and 2 Vol-% isoflurane (Vapour 19.3, Draegerwerk AG, Lübeck, Germany). Tracheotomy (tube size: 5.5 mm I.D.) was performed, and a central venous catheter was inserted in the left external jugular vein for drug administration and fluid therapy (lactated Ringer’s solution: 4 mL/kg b.w./h). Anesthesia was then maintained throughout the experiment with continuous infusion of fentanyl (0.015 mg/kg b.w./h) and midazolam (1.1 mg/kg b.w./h). Therefore, nitrous oxide and isoflurane were exchanged by air. An inspired fraction of oxygen of about 0.35 was used. Muscle relaxation was achieved with pancuronium bromide (0.4 mg/kg b.w./h, i.v.). All animals were ventilated in a pressure-controlled mode (Servo Ventilator 900C, Siemens-Elema, Sweden). Ventilation was controlled by continuous endexpiratory CO2 monitoring and hourly arterial blood gas samples. The ventilator was set with a positive inspiratory pressure of 15–20 mbar and a positive endexpiratory pressure of 2–4 mbar. Respiratory rate and inspired oxygen fraction were titrated to maintain a PaCO2 of 35 to 40 mm Hg and Pao2 of 100–130 mm Hg.

Body temperature was controlled by a rectal thermometer, maintained throughout instrumentation at 37.5 ± 0.5°C using a water-filled pad connected to a heating-cooling thermostat and a feedback-controlled heating lamp. The urinary bladder was punctured and permanently drained (Cystofix, Braun Melsungen AG, Germany).

The left brachial artery was catheterized for continuous arterial blood pressure (ABP) monitoring, and a further catheter (inner diameter 1.4 mm) was advanced from the left femoral artery into the abdominal aorta and positioned 1 cm above the aortic bifurcation in order to withdraw reference samples for the colored microsphere measurements. A thermodilution catheter for cardiac in-
A lateral fluid percussion injury (FP-TBI). A self-made de-
vice designed according to Sullivan et al. (1976) was
used. Briefly, the fluid percussion adapter was con-
ected to a transduced housing, and this in turn was con-
ected to the fluid percussion device. The device itself consisted of a Plexiglas cylindrical reservoir 25 cm long and 50
mm in diameter. One end of the device was connected to
the transducer housing, and the other end had a metal pis-
tion mounted on O-rings. The exposed end of the piston
was covered with a rubber pad. The entire system was
filled (air bubble-free) with physiologic solution. FP-TBI
was produced by allowing a 6.2-kg pendulum to strike
the Plexiglas cork and generate a hydraulic pressure tran-
sient traveling through the device and impacting upon the
dura overlying the brain (the surface area amounted to
63.6 mm²). The severity of the injury was randomly var-
ed by modification of pendulum angle of deflection
(moderate TBI [mTBI, n = 6]: 21.2 ± 1.2°; severe TBI
[sTBI, n = 7]: 26.0 ± 3.6°). The resulting pressure pulse
was recorded on a high time resolution transducer (Type
5011, Kistler, Germany) and saved on an oscilloscope
(54600B Oscilloscope, Hewlett Packard, Colorado
Springs, CO). Accordingly, the pressure pulse differed
between mTBI (3.2 ± 0.6 atm) and sTBI (4.1 ± 1.0 atm),
estimated as the mean pressure pulse amplitude between
2nd and 5th msec after FP onset. Immediately after TBI
administration, a three-way stopcock mounted on the
Plexiglas cylindrical reservoir of the TBI device was
opened to allow decompression. Starting 3 min after tra-
matic injury, 30% of the calculated blood volume (BV,
was assumed to be 8% of body weight [Pownall and Dal-
ton, 1973]) was withdrawn continuously within 18 min.
After a delay of 14 min, the withdrawn BV was replaced
by a gelatin-based plasma expander (Gelafusal; Serum-
Werk Bernburg, Germany) within 10 min.
Repeated measurements of blood gases, electrolytes,
glucose, lactate, CI, and CBF (colored microspheres)
were performed at baseline at the end of blood withdrawal
(TBI and BW), immediately after volume replacement (1
h after TBI/VR), as well as 8 and 24 h after FPI. Another
six animals received all experimental procedures except
FP-TBI administration, blood withdrawal, and volume re-
placement, and served as control animals.
Immediately after the last measurement, animals were
prepared for perfusion fixation of the brain. For this pur-
pose, the chest was opened by a midline sternotomy. The
ascending aorta was carefully prepared, and a lax double
suture was placed. A stiff secured cannula (inner diame-
ter 2.2 mm) was prepared and flushed with warmed saline. Then 30% potassium chloride solution was in-
jected intracardially, so that the heart beat stopped im-
mediately, followed by a fast insertion of the prepared
perfusion cannula, which was tightly locked into the as-
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mediately, followed by a fast insertion of the prepared
perfusion cannula, which was tightly locked into the as-
cending aorta. Descending aorta, both subclavian arter-
ies, and the lower caval vein were clamped by forceps. Right cardiac atrium was opened widely, and subsequently about 500 mL of warmed heparinized physiological saline was infused to flush the perfused vessels from blood. When the returned solution was clear, the brain and adjacent tissues were perfused with 1500 mL of neutrally buffered 4% formalin solution. Afterwards, brain was fixed in situ by immersion in 4% formalin for 48 h at 4°C, then removed from the skull and processed for macro- and microscopical evaluation.

Analytical procedures and calculations

The regional CBF was measured by means of the reference sample color-labeled microsphere (Dye-Trak®, Triton Technology, San Diego, CA) technique, which represents a valid alternative to the radioactively labeled microsphere method for organ blood flow measurement in newborn piglets and avoids all disadvantages arising from radioactive labeling with long-lived isotopes (Walter et al., 1997). Application in piglets and methodical considerations have been presented and discussed in detail elsewhere (Bauer et al., 1996; Walter et al., 1997). Briefly, in random sequence, about 3 million colored polystyrene microspheres were injected into the left atrium. The injection line was then flushed with 2 mL of saline. A blood sample was withdrawn from the thoracic aorta as the reference sample, beginning 15 sec before the microsphere injection and continuing for 2 min at a rate of 3.53 mL/min (syringe pump SP210iw, World Precision Instruments Inc., Sarasota, FL). At the end of each experiment, the brains were removed and sectioned in the desired brain regions. For digestion, reference blood samples and tissue samples between 0.5 and 2.5 g were covered with an appropriate volume (approximately 3 mL/g) of digestive solution (4 N KOH with 4% Tween 80 in deionized water). All tissue and blood samples were digested for a minimum of 4 h at 60°C. In order to isolate the microspheres, each digested tissue sample was then filtered under vacuum suction through an 8-μm-pore polyester-membrane filter. Colored microspheres were quantified by their dye content. The dye was recovered from the microspheres by adding dimethylformamide. The photometric absorption of each dye solution was measured by a diode-array UV/visible spectrophotometer (model 7500, Beckman Instruments, Fullerton, CA). Calculations were performed using the MISS® software (Triton Technology, San Diego, CA). The number of microspheres was calculated using the specific absorbance value of the different dyes. All reference and tissue samples contained >400 microspheres.

Heart rate, ABP, arterial and brain venous pH, Pco2, and Po2, oxygen saturation, glucose, lactate, and hemoglobin values were measured immediately before the microsphere injection, respectively. Blood pH, Pco2, and Po2 were measured with a blood gas analyzer (model ABL50, Radiometer, Copenhagen, Denmark), blood hemoglobin and oxygen saturation were measured using a hemoximeter (model OSM2, Radiometer, Copenhagen, Denmark), and blood glucose and lactate contents were measured with an electrolyte, metabolite laboratory EML105® (Radiometer, Copenhagen, Denmark) and corrected to the body temperature of the animal at the time of sampling.

Assuming the oxygen capacity of hemoglobin to be 1.39 mL/O2/g hemoglobin in pigs (Bauer et al., 2001), blood O2-content was calculated as equal to g hemoglobin/mL · 1.39 mL O2/g hemoglobin · O2-saturation and expressed in mL/100 mL blood. Dissolved oxygen was added by calculation, using the measured pO2 and the temperature-corrected solubility coefficient for oxygen.

The absolute flows to the tissues measured by the colored microspheres were calculated by the formula: flowtissue = number of microspheres tissuenumber of microspheres reference · (flowreference/number of microspheres reference). Flows are expressed in milliliters per min per 100 g tissue by normalizing for tissue weight. The CMRO2 was obtained by multiplying the blood flow to the forebrain by the cerebral arteriovenous O2 content difference, where the blood flow to the forebrain includes all regions drained by the sagittal sinus (cerebral cortex, cerebral white matter, some deep gray structures: basal ganglia, thalamus, and hippocampus) (Coyle et al., 1993). Cerebral perfusion pressure (CPP) was calculated continuously as the difference between ABP and ICP. Brain cortical blood flow was normalized by estimation of a mean value of arbitrary units during baseline (3-min period) and expressed as percentage of baseline. Furthermore, ICP amplitude was continuously estimated off-line using data acquisition and analysis software package “Watisa 1.1” (GJB Datentechnik Bolten & Jannek GbR, Ilmenau, Germany). Spectral analysis of ICP (sample rate 50 Hz) was performed for time intervals of 900 sec using the software package “MathLab®” Version 7.0.1 (The Math-Works GmbH, Aachen, Germany) and expressed as spectral power density.

Macropathologic Evaluation

Macroscopic inspection after skull removal confirmed an intact dura mater below the fluid percussion adapter in all animals with TBI. To assess subarachnoid bleeding, dura mater was carefully removed from the fixed brains. A 10-point scale of a brain-bleeding–surface index (BBSI) was used to score bleeding extent. Total volume of intracerebral bleeding could not
be quantified exactly, because of anatomically and dissection considerations. Bleeding scores were estimated independently by two investigators blinded to study groups.

**Histology**

The 7-mm-thick coronal slices of the frontal, temporo-parietal brain, and brainstem were embedded in paraffin and cut into 7-μm-thick sections, which were stained with hematoxylin and eosin (H&E) for routine morphology or prepared for immunohistochemistry. For immunohistochemical analysis, tissue sections were permeabilized in 0.1% Triton X-100/PBS (3× for 5 min), followed by incubation in blocking buffer containing goat serum. The primary monoclonal antibody mouse anti-beta amyloid precursor protein (βAPP, MAb 22C11; Boehringer Mannheim, Indianapolis, IN; 1:200) was applied overnight at 4°C in TBS, 0.1% Triton X-100 and 5% goat serum. Cy3 or Cy2-linked goat anti-mouse secondary antibodies (1:200 for 2 h at 4°C, Jackson Immuno Research Laboratories, Inc., USA) were used to recognize the primary antibodies. Fluorescent signals were imaged by confocal laser scanning microscopy (Zeiss LSM 510) using standard filters. For negative controls, sections were incubated with normal goat serum in the absence of primary antibody and showed no immunostaining.

The total number of damaged βAPP-positive axons and axonal bulbs were estimated in the subcortical temporo-parietal white matter and thalamus of both ipsi- and contralateral hemispheres as well as in the brainstem (counts/0.5 mm², 20× objective, extrapolated for the whole βAPP-positive area). In addition, a semiquantitative rating score was used to evaluate the extent of traumatic induced axonal injury for βAPP immunostaining (McKenzie et al., 1996). This score allows a differentiation between mild, moderate, and severe axonal damage. For morphological orientation, an atlas of the pig brain was used (Felix et al., 1999).

**Statistical Analysis**

Comparisons between groups were made with one-way ANOVA. One-way ANOVA with repeated measures was performed within each group. Post hoc comparisons were performed with Student’s t-tests. Differences in frequencies of macro- and microscopical characteristics as well as patterns of ICP behavior were compared by the Fisher exact tests; immunomorphological data were compared by the Mann-Whitney U-test. The alpha-adjustment procedure of Bonferroni and Holm was performed to evaluate significant differences. Differences were considered significant when p was <0.05.

**RESULTS**

**Functional Effects**

Physiological data are shown in Table 1. Arterial blood gases and rectal temperature were within physiological ranges and not significantly altered from baseline in any group during the whole experiment. Baseline data of systemic and cerebral hemodynamics (Table 1) as well as of the cerebral oxygen metabolism were similar between all groups studied (Fig. 2). Furthermore, in the control group, physiological data remained similar throughout the experiment.

While the systemic hemodynamic response (ABP, HR, CI) did not differ between the two trauma groups, the higher intensity of FPI in the sTBI animals produced a biphasic ICP increase (Fig. 1). The first ICP peak of sTBI animals occurred 90 sec after the impact and was 1.5-fold greater than the corresponding peak in mTBI group (p < 0.05). The ICP increase in sTBI animals persisted until the blood withdrawal was started, whereas in the mTBI animals the ICP decreased immediately after reaching the peak (p < 0.05). A secondary ICP increase in sTBI animals occurred at 5 ± 1.5 h postinjury and persisted up to the end of the experiment (Table 1, p < 0.05). Reduction of cerebral perfusion pressure (CPP) was more pronounced after severe TBI and blood withdrawal (p < 0.05) and temporarily reestablished after volume replacement. A further progressive decrease occurred in sTBI animals (p < 0.05), whereas CPP remained sustained in mTBI animals. Brain temperature was slightly reduced in sTBI animals (p < 0.05).

Spontaneously occurring ICP patterns of compromised intracranial compliance appeared more frequently in the sTBI group (Fig. 3, Table 2, p < 0.05), whereas provoked ICP alterations due to tracheal aspiration occurred also regularly after moderate TBI and occasionally in untreated animals.

Under baseline conditions, juvenile piglets represent a CBF of 48 ± 11 mL · min⁻¹ · 100 g⁻¹ and CMRO₂ of 3.2 ± 1.0 mL · min⁻¹ · 100 g⁻¹. Compared to baseline, CBF increased following volume replacement and remained elevated up to the end of the experiment in mTBI animals (Fig. 2, p < 0.05). In sTBI animals, however, global CBF decreased after the end of blood withdrawal and recovered insufficiently after volume substitution, compared to baseline. At the end of the experiment, CBF of sTBI animals was reduced to nearly half of the mTBI CBF values (44 ± 31 mL · min⁻¹ · 100 g⁻¹ vs. 91 ± 18 mL · min⁻¹ · 100 g⁻¹, p < 0.05).

CMRO₂ of mTBI animals increased after blood loss to 120 ± 15% of baseline levels (p < 0.05), returned to baseline levels after volume replacement and remained unaltered throughout the recovery period. In contrast,
<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Control group (n = 6)</th>
<th>mTBI group (n = 6)</th>
<th>sTBI group (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial pO2 (mm Hg)</td>
<td>Baseline 146.3 ± 5.1</td>
<td>139.3 ± 25.6</td>
<td>144.4 ± 20.6</td>
</tr>
<tr>
<td></td>
<td>TBI &amp; BW 148.4 ± 22.3</td>
<td>138.9 ± 25.9</td>
<td>146.4 ± 12.4*</td>
</tr>
<tr>
<td></td>
<td>1 h after TBI/VR 154.2 ± 14.2</td>
<td>145.5 ± 28.6</td>
<td>150.1 ± 10.5</td>
</tr>
<tr>
<td></td>
<td>8 h after TBI 136.7 ± 34.1</td>
<td>139.2 ± 26.5</td>
<td>145.8 ± 11.8</td>
</tr>
<tr>
<td></td>
<td>24 h after TBI 149.0 ± 14.9</td>
<td>146.0 ± 22.0</td>
<td>144.6 ± 12.1</td>
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<td>Arterial pCO2 (mm Hg)</td>
<td>Baseline 39.0 ± 4.4</td>
<td>42.4 ± 2.3</td>
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<tr>
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<td>TBI &amp; BW 41.2 ± 1.5</td>
<td>41.9 ± 2.4</td>
<td>37.6 ± 4.3*</td>
</tr>
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<td>1 h after TBI/VR 40.3 ± 1.0</td>
<td>41.5 ± 1.7</td>
<td>37.5 ± 41.1*</td>
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<td>8 h after TBI 42.8 ± 3.5</td>
<td>40.5 ± 3.1</td>
<td>36.6 ± 3.8* †</td>
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<tr>
<td></td>
<td>24 h after TBI 39.8 ± 6.3</td>
<td>40.8 ± 1.4</td>
<td>36.7 ± 3.2* †</td>
</tr>
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<td>Arterial pH</td>
<td>Baseline 7.49 ± 0.03</td>
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<tr>
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<td>TBI &amp; BW 7.49 ± 0.02</td>
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<td>7.45 ± 0.07</td>
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<td>1 h after TBI/VR 7.49 ± 0.02</td>
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<td>7.54 ± 0.03 †</td>
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<td>24 h after TBI 7.52 ± 0.06</td>
<td>7.51 ± 0.04 †</td>
<td>7.53 ± 0.06 †</td>
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<tr>
<td>Arterial hemoglobin (mmol · L⁻¹)</td>
<td>Baseline 6.4 ± 0.6</td>
<td>5.9 ± 0.5</td>
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</tr>
<tr>
<td></td>
<td>TBI &amp; BW 6.1 ± 0.5</td>
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<td>5.4 ± 0.3 †</td>
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<td>1 h after TBI/VR 5.8 ± 0.4</td>
<td>3.2 ± 1.2 †</td>
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<td>8 h after TBI 5.4 ± 0.5 †</td>
<td>4.0 ± 0.9 †</td>
<td>4.0 ± 0.7 †</td>
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<td>24 h after TBI 4.9 ± 0.8</td>
<td>3.8 ± 1.1 †</td>
<td>3.9 ± 0.6 †</td>
</tr>
<tr>
<td>Blood glucose (mmol · L⁻¹)</td>
<td>Baseline 6.3 ± 0.5</td>
<td>6.7 ± 1.1</td>
<td>6.2 ± 0.9 †</td>
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<tr>
<td></td>
<td>TBI &amp; BW 6.5 ± 0.5</td>
<td>10.7 ± 3.3 †</td>
<td>11.0 ± 4.7 †</td>
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<tr>
<td></td>
<td>1 h after TBI/VR 6.3 ± 0.3</td>
<td>7.0 ± 1.3</td>
<td>9.9 ± 4.3 * †</td>
</tr>
<tr>
<td></td>
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<td>7.4 ± 1.9</td>
<td>7.8 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>24 h after TBI 6.1 ± 0.5</td>
<td>6.5 ± 1.0</td>
<td>6.8 ± 1.4</td>
</tr>
<tr>
<td>Arterial blood pressure (mm Hg)</td>
<td>Baseline 104 ± 12</td>
<td>103 ± 17</td>
<td>102 ± 12</td>
</tr>
<tr>
<td></td>
<td>TBI &amp; BW 107 ± 11</td>
<td>69 ± 23 †</td>
<td>65 ± 24 †</td>
</tr>
<tr>
<td></td>
<td>1 h after TBI/VR 110 ± 12</td>
<td>92 ± 17</td>
<td>99 ± 15</td>
</tr>
<tr>
<td></td>
<td>8 h after TBI 94 ± 11</td>
<td>75 ± 15</td>
<td>78 ± 18</td>
</tr>
<tr>
<td></td>
<td>24 h after TBI 108 ± 20</td>
<td>81 ± 11</td>
<td>72 ± 21 †</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>Baseline 176 ± 32</td>
<td>177 ± 9</td>
<td>166 ± 15</td>
</tr>
<tr>
<td></td>
<td>TBI &amp; BW 170 ± 23</td>
<td>208 ± 32</td>
<td>218 ± 38 †</td>
</tr>
<tr>
<td></td>
<td>1 h after TBI/VR 163 ± 21</td>
<td>178 ± 15</td>
<td>170 ± 14</td>
</tr>
<tr>
<td></td>
<td>8 h after TBI 154 ± 22</td>
<td>173 ± 24</td>
<td>179 ± 32</td>
</tr>
<tr>
<td></td>
<td>24 h after TBI 161 ± 24</td>
<td>170 ± 13</td>
<td>171 ± 15</td>
</tr>
<tr>
<td>Animal groups</td>
<td>Control group (n = 6)</td>
<td>mTBI group (n = 6)</td>
<td>sTBI group (n = 7)</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------</td>
<td>-------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td><strong>Cardiac index</strong> (mL · kg⁻¹ · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>248 ± 15</td>
<td>256 ± 33</td>
<td>260 ± 59</td>
</tr>
<tr>
<td>TBI &amp; BW</td>
<td>248 ± 27</td>
<td>137 ± 61†</td>
<td>128 ± 40†</td>
</tr>
<tr>
<td>1 h after TBI/VR</td>
<td>248 ± 23</td>
<td>267 ± 40</td>
<td>295 ± 49</td>
</tr>
<tr>
<td>8 h after TBI</td>
<td>267 ± 56</td>
<td>249 ± 60</td>
<td>256 ± 39</td>
</tr>
<tr>
<td>24 h after TBI</td>
<td>258 ± 45</td>
<td>289 ± 49</td>
<td>265 ± 74</td>
</tr>
<tr>
<td><strong>Whole body O₂ uptake</strong> (mL · kg⁻¹ · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.6 ± 0.9</td>
<td>6.4 ± 1.1</td>
<td>6.4 ± 2.1</td>
</tr>
<tr>
<td>TBI &amp; BW</td>
<td>6.3 ± 1.6</td>
<td>5.4 ± 1.6</td>
<td>5.1 ± 1.4</td>
</tr>
<tr>
<td>1 h after TBI/VR</td>
<td>6.1 ± 1.7</td>
<td>5.5 ± 1.0</td>
<td>5.9 ± 1.9</td>
</tr>
<tr>
<td>8 h after TBI</td>
<td>7.1 ± 1.8</td>
<td>6.3 ± 1.3</td>
<td>5.8 ± 1.0</td>
</tr>
<tr>
<td>24 h after TBI</td>
<td>6.0 ± 1.2</td>
<td>5.9 ± 1.9</td>
<td>5.2 ± 1.8</td>
</tr>
<tr>
<td><strong>Rectal temperature</strong> (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>38.9 ± 0.4</td>
<td>38.8 ± 0.7</td>
<td>38.7 ± 0.6</td>
</tr>
<tr>
<td>TBI &amp; BW</td>
<td>38.3 ± 0.4</td>
<td>38.9 ± 0.9</td>
<td>38.5 ± 0.7</td>
</tr>
<tr>
<td>1 h after TBI/VR</td>
<td>38.3 ± 0.6</td>
<td>83.4 ± 0.7</td>
<td>37.8 ± 0.6†</td>
</tr>
<tr>
<td>8 h after TBI</td>
<td>38.1 ± 0.3</td>
<td>38.5 ± 0.8</td>
<td>38.1 ± 0.4</td>
</tr>
<tr>
<td>24 h after TBI</td>
<td>38.0 ± 0.6</td>
<td>38.6 ± 0.7</td>
<td>38.1 ± 0.9</td>
</tr>
<tr>
<td><strong>Intracranial pressure</strong> (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8 ± 2</td>
<td>9 ± 3</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>TBI &amp; BW</td>
<td>8 ± 2</td>
<td>4 ± 7</td>
<td>16 ± 12</td>
</tr>
<tr>
<td>1 h after TBI/VR</td>
<td>8 ± 2</td>
<td>9 ± 8</td>
<td>18 ± 8</td>
</tr>
<tr>
<td>8 h after TBI</td>
<td>6 ± 3</td>
<td>7 ± 2</td>
<td>26 ± 21</td>
</tr>
<tr>
<td>24 h after TBI</td>
<td>4 ± 4</td>
<td>7 ± 3</td>
<td>48 ± 24*†</td>
</tr>
<tr>
<td><strong>Cerebral perfusion pressure</strong> (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>97 ± 13</td>
<td>94 ± 19</td>
<td>93 ± 12</td>
</tr>
<tr>
<td>TBI &amp; BW</td>
<td>99 ± 12</td>
<td>65 ± 26</td>
<td>50 ± 24†</td>
</tr>
<tr>
<td>1 h after TBI/VR</td>
<td>102 ± 12</td>
<td>83 ± 25</td>
<td>82 ± 14</td>
</tr>
<tr>
<td>8 h after TBI</td>
<td>88 ± 13</td>
<td>68 ± 17</td>
<td>52 ± 29†</td>
</tr>
<tr>
<td>24 h after TBI</td>
<td>104 ± 22</td>
<td>75 ± 13</td>
<td>31 ± 29*†</td>
</tr>
<tr>
<td><strong>Brain temperature</strong> (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>38.3 ± 0.8</td>
<td>38.8 ± 1.0</td>
<td>38.5 ± 0.7</td>
</tr>
<tr>
<td>TBI &amp; BW</td>
<td>38.0 ± 1.1</td>
<td>38.6 ± 0.9</td>
<td>37.4 ± 1.2*†</td>
</tr>
<tr>
<td>1 h after TBI/VR</td>
<td>38.3 ± 1.2</td>
<td>38.3 ± 0.6†</td>
<td>37.7 ± 0.7*†</td>
</tr>
<tr>
<td>8 h after TBI</td>
<td>37.9 ± 0.3†</td>
<td>38.9 ± 1.0</td>
<td>37.5 ± 2.3*</td>
</tr>
<tr>
<td>24 h after TBI</td>
<td>38.6 ± 0.9</td>
<td>38.8 ± 0.8</td>
<td>37.1 ± 2.7*†</td>
</tr>
</tbody>
</table>

*p < 0.05 mTBI versus sTBI.
†p < 0.05 value versus baseline.

Data expressed as mean ± SD.
FP, fluid percussion; TBI, traumatic brain injury; BW, blood withdrawal; VR, volume replacement; mTBI, moderate traumatic brain injury; sTBI, severe traumatic brain injury.
sTBI animals represented a triphasic behavior in CMRO₂ with a marked decrease immediately after TBI application and blood withdrawal (70 ± 6% of baseline levels) and a distinct increase of cerebral O₂ uptake after volume replacement (130 ± 12% of baseline), followed by a re-decline at 24 h postinjury (75 ± 24% of baseline; Fig. 2, p < 0.05).

Morphological Effects

In the control animals small amounts of bleeding and tissue distortions were observed around the FP-adapter. In all traumatized animals subarachnoid hemorrhage was seen on the ipsilateral hemisphere. In both traumatized groups small amounts of subarachnoid hemorrhage were noted over the brainstem. Small amounts of supratentorial intraparenchymal bleeding (mainly in subcortical white matter) was seen in all sTBI animals but only in one of six mTBI animals. Subarachnoid hemorrhage over the contralateral hemisphere and the cerebellum were seen in all sTBI animals. Consequently, the semi quantitative evaluation of the extent of bleeding showed a significantly higher score in the sTBI compared to the mTBI group (Table 3, p < 0.05). Four sTBI animals showed severe and the other three sTBI animals showed moderate features of raised intracranial pressure (narrowing of sulci and flattening of gyri, reduction in size of ventricles, tentorial and tonsillar hernia). In addition, one animal showed brainstem infarction.

Histological examination showed in all traumatized animals, slight subarachnoid hemorrhage near the craniotomy for FP-adapter, and small intraparenchymal bleeding in the temporoparietal subcortical white matter around gliding contusions. In animals of the sTBI group, subarachnoid hemorrhage was also observed at the contralateral right hemisphere as described already. In all traumatized animals, foci of triangular hemorrhagic necroses were found accompanied by an almost complete loss of nerve cells in the center of the injury. In the adjacent areas, shrunken neurons with eosinophilic homogenization of the cytoplasm and triangular dark nuclei were abundant. The next zone was characterized by shrunken neurons without cell homogenization. Adjacent to this region (approximately 1.5 cm from the center of trauma), morphologically intact neurons were observed in mTBI, whereas shrunken neurons with triangular cell bodies and nuclei, eosinophilic cytoplasm, and perineuronal edema could be found scattered throughout the whole ipsi- and contralateral temporoparietal cortex of sTBI animals (Table 4, p < 0.05). In the sTBI group, neuronal injury in the hippocampus was observed in all subfields of both hemispheres, whereas in the mTBI group, necrotic neurons were observed in only two cases and were restricted to the CA2 subfield of the ipsilateral hippocampus (Table 4, p < 0.05). Additional neuronal damage in the brainstem could be observed in three animals of the sTBI group, whereas no damage was detected in the brains of mTBI group. Taken together, mTBI animals showed mainly focal brain damage, whereas sTBI animals showed signs of both focal and diffuse damage.

Using βAPP staining, no axonal damage was found in the control animals. In animals of mTBI, moderate axonal damage was observed in the subcortical temporoparietal white matter near the contusion but not in the subcortical white matter of the contralateral hemisphere. Axonal damage was moderate in the ipsilateral
thalamus and sparse in the contralateral thalamus. Only one animal showed additional axonal damage in the left and right superior cerebellar peduncle and in the medial longitudinal bundle of the brainstem.

In brains of sTBI animals, moderate to severe axonal damage was detected scattered in the subcortical temporoparietal white matter, in the corpus callosum, and in the thalamus of the ipsilateral hemisphere. Moderate axonal damage was found in the temporoparietal subcortical white matter and in the thalamus of the contralateral hemisphere. Nearly all animals showed moderate to severe axonal damage in the left and right superior cerebellar peduncle as well as in the medial longitudinal bundle of the brainstem. In comparison to mTBI and control, sTBI showed significant pronounced axonal damage in the ipsi- and contralateral hemisphere as well as in the brainstem (Fig. 4, \( p < 0.05 \)). Taken together, mTBI animals represented focal axonal damage, whereas in sTBI animals signs of diffuse axonal damage (DAI) were found frequently.

**DISCUSSION**

We described a large animal model of TBI that closely simulates frequently observed courses of events after TBI in humans. Here we were able to show that a high-intensity FP-TBI together with a temporary induced marked blood loss provoked consistently a secondary ICP increase combined with disturbances of brain function and signs of diffuse brain damage. In contrast to previous studies (Dixon et al., 1987), which reported early ICP elevation immediately following trauma, corresponding to the time course of the primary ICP peak in this study, a secondary ICP elevation occurred gradually after a free interval of several hours. This was reproducible after high
FIG. 3. Representative recordings of arterial blood pressure (ABP), intracranial pressure (ICP), cerebral perfusion pressure (CPP), ICP amplitude (ICP amp), and brain cortical blood flow (LDF), derived from two different animals of sTBI group with typical patterns of transient ICP elevation. (A) Plateau wave of ICP, which usually occurs when cerebrospinal compensatory reserve is low. At the height of the wave, CPP and brain cortical blood flow are distinctly reduced despite maintained ABP. After the plateau wave, ICP falls below baseline level and cerebrospinal compensatory reserve appears to be improved. (B) “B” waves of ICP were shown in almost all animals suffering sTBI. They are suggested to indicate decreased intracranial compliance and enhanced risk of intracranial hypertension. (C) High, spiky wave of ICP caused by sudden increase in ABP. The initial period is associated with marked brain cortical blood flow reduction, whereas an outlasting cortical hyperemia appeared after ICP lowering induced by sustained CPP elevation. Frequency components of ICP recordings were resolved and separately presented by frequency analysis (lowest panel) and represent main components of ICP fluctuations, for example, showing pulse wave and harmonics (frequency range, 2–10 Hz), respiratory wave and the first harmonic (small frequency band at ~0.4 and ~0.8 Hz owing to artificial ventilation) and “slow waves” (frequency range, 0.05–0.0055 Hz, which represents 20-sec to 3-min period).
intensity FP-TBI, subsequent blood withdrawal and volume replacement. In contrast, a reduced intensity of FP-TBI was accompanied by a short-lasting ICP increase early after TBI induction. Subsequently, mild functional alterations of the brain were observed together with rather focal brain damage.

We used transient hemorrhagic hypotension as a complicating pathophysiological factor, followed by a substitution of colloids 35 minutes following injury. This scenario is comparable to the primary resuscitation management of severely head injured patients. Data from the Traumatic Coma Data Bank have been shown that early hypotension occurs in about 35% of the patients and is associated with a doubling of mortality (Chesnut et al., 1993). Secondary insults after experimental TBI have been shown to aggravate cerebral hypoperfusion and lead to energy failure and cerebral edema development. It has further been shown that animals subjected to a secondary hypoxic insult after TBI have worse motor and histological outcomes than those subjected to TBI alone (Bramlett et al., 1999; Clark et al., 1997; Ishige et al., 1988; Nawashiro et al., 1995).

Furthermore, this experimental approach offers improved possibilities to investigate resuscitation procedures on outcome after TBI and early hypotension. Despite clinical importance, there is still controversy about treatment standards because comprehensive prospective studies are missing.

Recently it has been shown that early aggressive resuscitation to re-establish preinjury arterial blood pressure after combined fluid-percussion injury and uncontrolled hemorrhage resulted in increased hemorrhage and failure to optimize cerebrovascular function. In contrast, a period of moderate hypotension was well tolerated and did not compromise cerebrovascular hemodynamics, as evidenced by physiological parameters that remained within the limits of cerebral autoregulation. In addition, a tendency of improved short-term survival was reported (Stern et al., 2000). In a similar pig model of severe FP-TBI and controlled hemorrhagic hypotension, it was shown that CPP directed therapy improved brain oxygenation and maintained the cerebrovascular CO2 reactivity within the first 5 h post-TBI. Brain edema was lower, but lung edema was greater, suggesting a higher propensity for pulmonary complications (Mattioli et al., 2003).

Indeed, respiratory disturbance represents a frequently occurring complication owing to severe TBI and hemorrhagic hypotension, which may cause hypoxic hypoxia and hypercapnia. We did not focus on this issue in this study. However, it represents a main area of clinical relevance, especially after resuscitation and should be considered in future to mimic more closely human severe brain injury. Indeed, the endangering consequences of respiratory disturbances are not only characterized by systemic O2 deficit and disturbed acid-base balance but also by specific effects on intracranial volume regulation early after brain trauma. Given, that under those circumstances intracranial compliance is rather compromised, any additional volume increase can be responsible for critical ICP elevation with concomitant CPP reduction. It is well-known that the components of respiratory insufficiency, that is, hypoxemia, hypercapnia, and acidosis, induce cerebral hyperemia, mainly in the cerebrovascular bed outside the traumatically injured brain tissue in order to maintain adequate O2 availability. However, the underlying vasodilation of resistance brain ves-

<table>
<thead>
<tr>
<th>Patterns of ICP behavior</th>
<th>Control</th>
<th>mTBI</th>
<th>sTBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low stable ICP</td>
<td>6/6</td>
<td>6/6b</td>
<td>0/7</td>
</tr>
<tr>
<td>Stable and elevated ICP</td>
<td>0/6</td>
<td>0/6</td>
<td>7/7a,b</td>
</tr>
<tr>
<td>&quot;B&quot; waves of ICP</td>
<td>0/6</td>
<td>0/6</td>
<td>6/7a,b</td>
</tr>
<tr>
<td>Plateau waves of ICP</td>
<td>0/6</td>
<td>0/6</td>
<td>4/7</td>
</tr>
<tr>
<td>High, spiky waves of ICP during sudden increases in ABP</td>
<td>0/6</td>
<td>0/6</td>
<td>3/7</td>
</tr>
<tr>
<td>Spiky waves of ICP caused by sucking out the trachea</td>
<td>2/6</td>
<td>4/6</td>
<td>7/7a</td>
</tr>
</tbody>
</table>

* Differences in frequencies in the appearance of specific patterns of ICP behavior between control group and sTBI group, at \( p < 0.05 \).

† Differences in frequencies in the appearance of specific patterns of ICP behavior between mTBI group and sTBI group, at \( p < 0.05 \).

Data pairs represent number of animals with specific ICP patterns per total number of experimental animals of each group.

ICP, intracranial pressure; mTBI, moderate traumatic brain injury; sTBI, severe traumatic brain injury; ABP, arterial blood pressure.

**Table 2. Appearance of Specific ICP Patterns Throughout the Observational Period**

<table>
<thead>
<tr>
<th>Patterns of ICP behavior</th>
<th>Control</th>
<th>mTBI</th>
<th>sTBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low stable ICP</td>
<td>6/6</td>
<td>6/6b</td>
<td>0/7</td>
</tr>
<tr>
<td>Stable and elevated ICP</td>
<td>0/6</td>
<td>0/6</td>
<td>7/7a,b</td>
</tr>
<tr>
<td>&quot;B&quot; waves of ICP</td>
<td>0/6</td>
<td>0/6</td>
<td>6/7a,b</td>
</tr>
<tr>
<td>Plateau waves of ICP</td>
<td>0/6</td>
<td>0/6</td>
<td>4/7</td>
</tr>
<tr>
<td>High, spiky waves of ICP during sudden increases in ABP</td>
<td>0/6</td>
<td>0/6</td>
<td>3/7</td>
</tr>
<tr>
<td>Spiky waves of ICP caused by sucking out the trachea</td>
<td>2/6</td>
<td>4/6</td>
<td>7/7a</td>
</tr>
</tbody>
</table>

* Differences in frequencies in the appearance of specific patterns of ICP behavior between control group and sTBI group, at \( p < 0.05 \).

† Differences in frequencies in the appearance of specific patterns of ICP behavior between mTBI group and sTBI group, at \( p < 0.05 \).

Data pairs represent number of animals with specific ICP patterns per total number of experimental animals of each group.

ICP, intracranial pressure; mTBI, moderate traumatic brain injury; sTBI, severe traumatic brain injury; ABP, arterial blood pressure.

**Table 3. Brain-Bleeding-Surface Index (BBSI) from Animals Suffering from Moderate (mTBI) and High Intensity FP-TBI (sTBI) Evaluated by Two Blinded Investigators**

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Control (n = 6)</th>
<th>mTBI (n = 6)</th>
<th>sTBI (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Investigator 1</td>
<td>1.4 ± 0.5</td>
<td>4.4 ± 1.5</td>
<td>6.6 ± 2.7</td>
</tr>
<tr>
<td>Investigator 2</td>
<td>1.8 ± 0.6</td>
<td>2.8 ± 0.8</td>
<td>6.4 ± 2.5</td>
</tr>
<tr>
<td>Mean</td>
<td>1.6 ± 0.6</td>
<td>3.6 ± 1.1†</td>
<td>6.5 ± 2.6n†</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \) mTBI versus sTBI.
† \( p < 0.05 \) value versus control group.

Data expressed as mean ± SD.
sels represent a distinct enlargement of intracranial blood volume, and hence an additional intracranial volume increase. Because traumatic brain injury induces disturbance of cerebrovascular autoregulation within the affected brain tissue with shift of the lower limit of the autoregulatory threshold to higher pressure values (Chan et al., 1992), even a limited CPP reduction can be responsible for CBF restriction with secondary regional ischemia.

The experimental approach, used in this study, did not clarify the underlying mechanisms responsible for secondary ICP increase. Nevertheless, sophisticated elaboration of the continuously monitored cardiovascular, ventilatory and neurological parameters allows us to exclude relevant alterations in ventilatory gas exchange or systemic hemodynamics in animals after mTBI and sTBI. However, a critical compromise in CPP was always induced by a pathologically increased ICP. Therefore, we suggest that intracranial processes are predominantly responsible for secondary ICP elevation. This assumption is supported by regularly occurring patterns of ICP increase in animals after sTBI suggesting a compromised intracranial compliance and increased risk to induce subsequently states of stable and elevated ICP (Fig. 3, Table 2, p < 0.05). Traumatic brain swelling appears to be the main cause for mass expansion and subsequent ICP increase. It has been reported that early after FP-TBI a transient ICP occurred regularly (Dixon et al., 1987). We suggest that the ICP increase immediately after FP administration is caused by the amount of the rapidly injected fluid volumes into the cranial cavity, which is trapped extradurally. In all cases of FP-TBI administration a dural integrity has been confirmed by inspection immediately after impact and at autopsy. The maintenance of early ICP increase may be caused by vascular swelling (Mararou and Shima, 1990). A further component of space occupation occurring early after FP-TBI is that of intracranial bleeding. The semi quantitative evaluation of the extent of bleeding showed a significantly higher score after high intensity FP-TBI compared to moderate intensity FP-TBI (p < 0.05). We suggest that the FP impact induced predominantly bleeding by cerebral and cerebellar vein disruption. However, because the ICP exhibited normalization with failed progression during blood withdrawal and several hours after volume replacement, we assume that intracranial bleedings stopped spontaneously. Nevertheless, enhanced masses of clotting blood may compromise intracranial compliance with increased risk of secondary ICP elevation after high intensity FP-TBI.

Furthermore, high intensity FP-TBI was associated with a more pronounced reduction of CPP at the end of

![FIG. 4. Total number of damaged β-APP positive axons and axonal bulbs in the ipsi- and contralateral hemisphere. Values are presented as means ± SE. Control group, open columns; moderate traumatic brain injury group (mTBI), shaded columns; severe traumatic brain injury group (sTBI), black columns. *Significant differences between mTBI and sTBI group, p < 0.05.](image-url)
A PIG MODEL WITH SECONDARY INCREASE OF ICP AFTER TBI

Intraparenchymatous or intracerebral hemorrhages frequently accompany and aggravate diffuse traumatic brain injury. Such bleeding patterns have also been reported in adults and children after severe traumatic brain injury and have been associated with worse outcome (Graham et al., 1989; Mattioli et al., 2003). Previous experimental studies have also shown evidence of significant diffuse bleeding in various TBI models (Adelson et al., 2001). The FPI model, although frequently used to study focal pathological or physiological responses (Armstead, 2002; Armstead and Kurth, 1994; Prins and Hovda, 2003), has frequently shown pathological evidence of diffuse brain injury (Dixon et al., 1987). Interestingly, patterns of diffuse brain injury were observed more frequently when TBI was accompanied by hemorrhagic hypotension and hypoxemia (Ito et al., 1996). In the present study, we also found an increase in incidence and quantity of intracerebral bleeding in the severe brain injury group.

Neuropathological consequences were associated with the TBI intensity. We observed a considerable number of βAPP-immunolabeled axons in the ipsi- and contralateral temporoparietal subcortical white matter, thalamus and brainstem in the sTBI animals. The intra-axonal accumulation of βAPP serve as a sensitive and early marker for the diffuse traumatic axonal injury (Buki et al., 2000; Maxwell et al., 1997) and underlines in addition to the histological findings diffuse brain damage in the sTBI group.

However, altogether we have to realize that despite the efforts towards standardization of experimental approaches to induce severe TBI a considerable heterogeneity in brain reactivity has been found. Time course of ICP alteration and related effects on CBF and brain oxidative metabolism as well as morphopathological consequences showed distinct quantitative differences despite maintained systemic conditions. Therefore, the need for further clarification of key pathogenetic mechanisms in the development of secondary brain injury due to TBI and related complications, like hemorrhagic hypotension or respiratory disturbances are necessary. The current animal model appears to be an experimental tool to provide useful findings.

In conclusion, we present a model of traumatic brain injury which closely simulates the clinical scenario. We have reproduced the secondary ICP increase after severe brain injury and described the complex hemodynamic, metabolic, functional, and histopathologic changes during the first 24 h. Beside the induction of secondary ICP increase this model is suitable to induce experimental focal or diffuse neuronal damage and traumatic axonal damage depending on the trauma intensity. This experimental model appears to be helpful in the study of new therapeutic approaches. Furthermore, it enables in-depth
studies such as resuscitation thresholds, effects of hypovolemia, hypoxemia, hypercarbia, hyperthermia, and their influence on neuropathology to be studied.

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