Cerebral Pressure Autoregulation and Vasoreactivity in the Newborn Rat

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ABSTRACT

Perinatal brain injury has been associated with impaired cerebral blood flow (CBF) pressure autoregulation. The brain of 3- to 5-d-old rat pups is immature and similar to that of a preterm infant, and therefore we tested cerebral vasoreactivity in that animal. CBF pressure autoregulation was tested in 20 Wistar pups during normocapnia and hypercapnia, respectively. Hypotension was induced by hemorrhage and cerebral perfusion was monitored with laser Doppler flowmetry and near-infrared spectroscopy. Systolic blood pressure was measured noninvasively from the tail. During normocapnia, the autoregulatory plateau was narrow. Resting systolic blood pressure (SBP) was 39.2 mm Hg and CBF remained constant until SBP decreased below 36.0 mm Hg (SE 0.8). Below the lower limit, CBF declined by a mean of 2.7% per mm Hg [95% confidence interval (CI), 2.4–3.0%], and hemoglobin difference (HbD) and total hemoglobin (HbT) changed proportionally to CBF. After inhalation of carbon dioxide, CBF increased significantly by a mean of 17.7% (95% CI,

13.7–22.8%). The CBF-CO₂ reactivity was estimated to 13.4% per kPa (95% CI, 2–24.8%), $p = 0.026$. Over the range of SBP (6–54 mm Hg), a linear relationship between CBF and SBP was found during hypercapnia, indicating abolished pressure autoregulation. A linear correlation between CBF and HbD was found ($r = 0.80$). CBF pressure autoregulation and reactivity to CO₂ operate in the newborn rat. This model may be useful for future investigations concerning perinatal pathophysiology in the immature brain. (*Pediatr Res* 57: 294–298, 2005)

Abbreviations

CBF, cerebral blood flow
HbD, hemoglobin difference
HbT, total hemoglobin
LDF, laser Doppler flowmetry
NIRS, near-infrared spectroscopy
SBP, systolic blood pressure

In very preterm infants, brain injury has been associated with impaired pressure autoregulation of CBF. Accordingly, periventricular hemorrhage may result from abrupt hyperperfusion whereas periventricular leukomalacia has been related to ischemia (1–9).

Cerebral pressure autoregulation refers to vascular mechanisms whereby CBF is held within constant levels during variations in perfusion pressure. Perfusion is thus maintained over a range of pressures by appropriate alterations in the diameter of precapillary arterioles. However, the lower limit of autoregulation is reached when the vessels become fully dilated and, beyond that point, any further decrease in pressure results in proportional drops in CBF. Several conditions may affect the autoregulatory plateau and increased CBF as well as complete pressure passiveness secondary to vasodilatation is

observed during hypercapnia, hypoglycaemia, hypoxia, and seizure (10–13).

Cerebral pressure autoregulation has been documented in several newborn animals such as piglets, lambs, and puppies (14–19). Most of these animals have rather mature brains at birth, and to compare vascular physiology with that of a preterm infant, the newborn rat pup served as a model (20,21). Because we could not control ventilation in this immature animal, we evaluated the cerebrovascular response to hemorrhagic hypotension during normocapnia and carbon dioxide inhalation. The hypothesis was that pressure autoregulation operates normally in the healthy immature brain.

METHODS

Litters of 1-d-old Wistar rats together with their dams were purchased from a commercial breeder (Møllegaarden, Lille Skensved, Denmark). They were housed in individual cages, and all pups were reared by their dams until the experiment. The adult rats were fed a standard diet. Twenty-one pups were studied between the d 3 and 5 of life (mean, 4.1 d). Their weights ranged between 8.5 and 14.1 g (mean, 10.8 g). The Danish Animal Experiments Inspectorate approved the experimental protocol.

Preparation of animals. Anesthesia was induced by inhalation of 3.5% isoflurane with 30% oxygen. The surgical operation was performed by removal

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of the scalp for the laser Doppler probe and skin incision in the back for a temperature probe, while ECG electrodes were attached to the chest. Thereafter, isoflurane was reduced to 1% while 30% oxygen was continued, and this concentration was maintained throughout the experiment. All pups were breathing spontaneously and respiration frequency and heart rate were monitored continuously (Diascope, Simonsen & Well Medico Technique, Albertslund, Denmark).

Doxapram was administered intraperitoneally (2 mg/kg/h) to maintain normal respiration rates without apneas and hypoxic episodes.

Transcutaneous P_{CO_2} (tcP_{CO_2}) and P_{O_2} (tcP_{O_2}) were monitored from the abdominal skin using an electrode temperature of 44.5°C (TINA, Radiometer A/S, Copenhagen, Denmark). A previous study in this model has shown that tcP_{CO_2} is reliable, whereas tcP_{O_2} is not (22,23). Normal core temperature of the rat was secured by heating pads (36.7–37.3°C).

CBF was measured by LDF (Perimed 5010, Stockholm, Sweden). The equipment works with a laser light source of 780 nm and an interoptode distance of 250 μ m. Using glue, the optical probe was fixed to the skull over the left parietal cortex. This procedure secured stable measurements from the same area of cerebral cortex over time.

NIRS monitored changes in oxy- (HbO₂) and deoxyhemoglobin (HHb), which are expressed in micromolar (Niroscope, Radiometer A/S). Subsequently, changes in HbT and HbD were derived from the following formulas: $[HbO_2 + HHb]$ and $[HbO_2 - HHb]$. NIRS operates with four different wavelengths (774, 806, 845, and 910 nm), and two optodes (fiber diameter being 3 mm). A source and a receiver were placed on either side of the head corresponding to the parietal lobes.

SBP was measured noninvasively from the tail. The artery was occluded by cuff inflation, and SBP was defined as the cuff pressure when pulsatile flow, detected by another laser Doppler probe, reappeared. All calculations of SBP were performed by commercial software (Perisoft for Windows 2.10, Perimed, Stockholm, Sweden). Intra-arterial access is impossible in such animals, which is why the present technique was not evaluated with direct measurements.

Experimental protocol. Following surgical preparation, all rats were stabilized for 15 min.

Two protocols were designed to investigate the cerebrovascular response to hemorrhagic hypotension during normocapnia and hypercapnia, respectively. At rest, baseline values for CBF, HbT, HbD, and SBP were recorded five times over a 15-min period. Thereafter, 2000 IU of heparin was injected subcutaneously. After another 15 min, isoflurane was increased briefly to 3.5% and hemorrhagic hypotension was induced by amputation of the forepaw. During the experiment, SBP pressure was measured repeatedly with 2- to 3-min intervals until death from hemorrhage (Fig. 1).

Identical procedures were used in the second study. After baseline readings, the rat was held hypercapnic by increasing the inspired fraction of CO₂ to 8%. After another 5 min, heparin was given (Fig. 1). The animal was decapitated at the end of each experiment.

Definitions and data analysis. SBP, CBF, HbT, HbD, and tcP_{CO_2} data were converted from analog to digital and they were stored on a personal computer for later analysis. Brain laser Doppler measurements were recorded in arbitrary

perfusion units (PU) and thus for each rat they were recoded into percentage change from the mean of the five baseline values.

Statistics. Parametric analyses were used and differences between rats and within the baseline series of five measurements were tested by means of one- and two-way ANOVA.

Pressure autoregulation and its lower limit were calculated as described by Pedersen *et al.* (24). In brief, the lower autoregulatory limit was obtained from the intersection of two lines found by the least square technique, and a constraint was put on the horizontal line.

The CBF-SBP and the CBF-CO₂ reactivities were calculated by linear regression. Because of repeated measures design, all regression analyses included one dummy variable for each rat.

The program SPSSPC 10 (SPSS Inc., Chicago, IL) was used and the level of significance was set to $p = 0.05$.

RESULTS

Blood pressure. A well-defined signal from the tail laser Doppler ensured that blood pressure measurements were obtained reliably from resting conditions and until SBP dropped below approximately 15 mm Hg (Fig. 2). Below this level, laser Doppler signals often became too noisy to allow exact calculations.

In the pups, resting SBP averaged 39.2 mm Hg (range, 27–58 mm Hg). SBP values did not differ systematically from the first through the fifth measurement and the test-retest variability was calculated to 12%. Similarly, resting blood pressure did not vary significantly among age groups (3, 4, and 5 d).

Pressure autoregulation in normocapnia. A series of 10 animals was studied successfully, and they were all alive at the end of the experiment. Blood pressures were measured approximately 25 times in each rat with simultaneous monitoring of CBF, HbT, and HbD. In a few rats, blood pressure declined slightly after heparin injection, most likely due to intraperitoneal bleeding, whereas the others were stable until amputation.

For the whole group, CBF remained constant until SBP decreased below 36.0 mm Hg (SE 0.8). Thus, below the lower autoregulatory limit CBF declined in parallel with blood pressure, in mean 2.7% per mm Hg [95% confidence interval (CI), 2.4–3.0%] (Fig. 3).

Pressure autoregulation was also observed with regard to HbT and HbD, respectively, as HbT and HbD were maintained until SBP decreased below 35 mm Hg (SE 1.2). It is noteworthy that HbT did not increase as a result of vasodilatation as SBP declined toward the lower limit (Fig. 4).

Hypercapnia and pressure autoregulation. Eleven rats were exposed to hypercapnia during the second study. Results from one animal were discarded because of unexplained low resting blood pressure (mean, 15 mm Hg) leaving 10 animals for the analysis. In that group, resting SBP was slightly lower but not significantly different from values in the above study. All rats were alive during the period of study.

When CO₂ was introduced, CBF increased by a mean of 17.7% (95% CI, 13.7–22.8%). From tcP_{CO_2} readings, the CBF-CO₂ reactivity was estimated to 13.4% per kPa (95% CI, 2–24.8%), $p = 0.026$ (Fig. 5). CO₂ did not affect SBP ($p = 0.90$) and minor variations in SBP had no influence on CBF when SBP was incorporated as an independent variable in the multiple regression analysis (CBF-SBP reactivity: -0.15% per mm Hg, (95% CI, -7.7 – 7.4%), $p = 0.97$; CBF-CO₂ reactivity: 13.5% per kPa (95% CI, 0.2 – 26.7%), $p = 0.047$).

Figure 1. Protocol for the first and the second experiment.

Figure 2. Four systolic blood pressure measurements calculated with Perisoft for Windows 2.10 (Perimed, Stockholm, Sweden). Cuff pressure and LDF in the tail (PU) are displayed with time.

Figure 3. Changes in CBF during hemorrhagic hypotension in 10 normocapnic rats. Below 36.0 mm Hg (SE = 0.8), CBF decreases linearly with SBP, indicating the lower limit of pressure autoregulation (2.7% per mm Hg).

Similarly, mean HbT increased by $0.87 \mu\text{M}$ per kPa (95% CI, 0.09 – $1.64 \mu\text{M}$), $p = 0.032$, whereas mean HbD increased by $2.94 \mu\text{M}$ per kPa, (95% CI, 0.87 – $5.0 \mu\text{M}$), $p = 0.011$, during hypercapnia.

Pressure autoregulation under hypercapnia was not well defined, as the software detected a lower limit of 34 mm Hg but with a very high SE of 6.9 mm Hg. Instead, a linear relation was found between CBF and SBP, and over the SBP spectrum (SBP ranging from 6 to 44 mm Hg), CBF declined by a mean of 2.7% per mm Hg decrease in SBP (95% CI, 2.0–3.4%), $p < 0.001$ (Fig. 6).

HbT and HbD versus CBF. Changes in HbT were closely related to changes in CBF ($r = 0.86$, $p < 0.001$) as HbT

Figure 4. HbT during hemorrhagic hypotension in 10 normocapnic rats. Below 35.0 (SE 1.2) mm Hg, HbT decreases linearly with SBP, indicating the lower limit of pressure autoregulation.

increased by a mean of $0.140 \mu\text{M}$ per percentage change in CBF (95% CI, 0.126 – $0.153 \mu\text{M}$).

Likewise, changes in HbD averaged $0.227 \mu\text{M}$ per percentage change in CBF (95% CI, 0.200 – $0.254 \mu\text{M}$; $r = 0.80$, $p < 0.001$) (Fig. 7).

DISCUSSION

The present study demonstrates that pressure autoregulation operates in the very immature brain and that the vessels are

Figure 5. Response of CBF and SBP in 10 rats during hypercapnia. After five baseline recordings, CO₂ was added to the inspiratory air. The data were normalized according to the average of the first five measurements. Mean values and SEM are presented.

Figure 6. Changes in CBF during hemorrhagic hypotension in 10 hypercapnic rats. Over the SBP spectrum, CBF increased 2.7% per mm Hg increase in SBP, indicating abolished pressure autoregulation.

responsive to CO₂. During resting conditions, however, the vasodilatory reserve appears to be limited, as 1) the lower limit of pressure autoregulation is reached after a minor decrease in SBP, 2) the CO₂ reactivity is lower than that of 21% per kPa observed in older animals (25), and 3) HbT does not increase when SBP decreases within the autoregulatory plateau. It is rational that the cerebrovascular resistance decreases by vasodilatation to ensure constant CBF levels when SBP drops toward the lower limit of pressure autoregulation. Thus, HbT should increase concomitantly with this vasodilatation. How-

Figure 7. The relation between HbD and CBF during normocapnia and hemorrhagic hypotension ($r = 0.80$).

ever, resistance is attributed to the cerebral arterioles, which comprise <5% of the total cerebral volume, and these minor changes in diameter may not be observed by NIRS. It should be noted that isoflurane was used for anesthesia, and this gas induces muscular relaxation and thereby cerebral vasodilatation in the mature brain (26). It is therefore possible that the cerebrovascular resistance is slightly higher in nonexperimental settings. However, a counteracting effect of doxapram is possible as mild cerebral vasoconstriction has been reported after its use (27–29).

A pressure-passive CBF occurs when blood pressure drops below the lower limit and the cerebral vascular resistance is minimal. From baseline SBP of 39.2 mm Hg and assuming of a perfusion pressure of 35 mm Hg, pressure-passiveness implies a linear decrease in CBF of 2.9% per mm Hg. This number is almost similar to those observed in the present study during normo- and hypercapnia.

LDF was used for monitoring changes in CBF. At a wavelength of 780 nm and interoptode distance of 250 μ m, light penetrates approximately 1 mm into tissue. The reported pressure autoregulation and CO₂ reactivity therefore relate to the cortical area (30). On the other hand, changes in HbT and HbD account for all cerebral vessels, because near-infrared light penetrates the whole brain. It has been proposed that HbD and CBF are highly interrelated (4), and the present data support that hypothesis. The relationship between mixed vascular hemoglobin oxygen saturation (HbD) and CBF relies on constant cerebral artery oxygen saturation and a constant cerebral metabolic rate of oxygen (CMRO₂). The pups did not experience hypoxia during the experiment but we did not measure CMRO₂, and any variations in CMRO₂ may explain why changes in CBF only accounted for 64% of the variation in HbD. Redistribution of CBF from cortical areas to midbrain structures during hypotension may be another factor. Finally, it is also possible that reductions in microvascular hematocrit resulting from shifts of fluid from the tissue into the vascular compartment may affect LDF readings during hemorrhage.

The linear relation between changes in HbT and CBF should be discussed. CBF is determined by perfusion pressure, blood viscosity, and the diameter of arteries and arterioles, whereas

HbT is mainly determined by the diameter of arterioles and venules. Thus, the arteriolar diameter is the only common denominator for both CBF and HbT. As a consequence, other relationships may be observed when testing pressure autoregulation using other methods than hemorrhagic hypotension. For example, small and inconsistent decreases in HbT as well as a poor relationship between HbT and CBF were found when systemic arterial hypotension was produced by partial occlusion of the ascending aorta (31).

It is impossible to insert arterial lines in the newborn rat, and blood pressure was measured noninvasively from the tail. A similar technique is used in commercial equipment for bigger animals. It cannot be excluded that SBP was overestimated, although an acceptable reproducibility was achieved by careful placement of the cuff and fast and reliable detection of capillary flow by LDF.

In many instances, the brain of the newborn rat is comparable to that of a preterm infant. Vannucci et al. (32) have previously reported that a 7-d postnatal pup is analogous to a premature child at 32–34 wk of gestation; *i.e.* cerebral cortical layering is complete, the germinal matrix is involuting, and white matter has yet to myelinate. The fact that we used slightly younger pups makes the result equivalent with conditions in even more preterm babies. Apparently, the cerebral vasoreactivity is fully developed in the immature rat pup, although we did not investigate the response to hypoxia or hypocapnia because of technical constraints with regard to mechanical ventilation. We hope that future investigations in this model may provide insight in mechanisms underlying brain pathology in sick preterm infants, especially with regard to periventricular hemorrhage and leukomalacia.

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