Intracranial pressure and brain redox balance in rabbits

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The effects of elevated intracranial pressure (ICP) on intracellular oxygenation and cerebrocortical blood volume (CBV) were studied in rabbits. Intracellular oxygen (O_2) concentration was assessed as the level of pyridine nucleotide concentration ([NADH]) oxidation/reduction balance and relative cerebrocortical blood volume (CBV) were measured with a fiberoptic fluororelectrometer probe placed on the cerebrocortical surface. Experiments were conducted in six urethane anaesthetized, normocarbic animals at different fractions of inspired O_2 (FIO_2). During gradual increases in ICP, [NADH] began to increase (representing decreased intracellular mitochondrial PO_2) for all values of FIO_2 as ICP exceeded a threshold of 18 ± 2.2 cmH $_2$ O ($P < 0.05$). The decline in intracellular oxygenation with elevated ICP was inversely related to FIO_2 ($P < 0.05$). With ICP greater than 18 ± 2.2 cmH $_2$ O, intracellular mitochondrial oxygenation showed an improvement between an FIO_2 of 0.21 and 0.5 ($P < 0.05$) but increasing FIO_2 from 0.5 to 1.0 resulted in no statistically significant improvement in tissue redox balance. The CBV, largely representing tissue capillary blood, increased when ICP reached greater 18 ± 1.2 cmH $_2$ O probably reflecting local autoregulation or venous distension ($P < 0.05$). However, above 30 ± 1.1 cmH $_2$ O, CBV decreased ($P < 0.05$). The results demonstrate the interdependence of inspired oxygen concentration, elevated ICP, and brain intracellular oxygenation, and suggest that brain oxygen utilization deteriorates above an ICP of about 18 cmH $_2$ O.

Key words

BRAIN: cerebral blood volume, cortical metabolism, intracranial pressure;
MEASUREMENT TECHNIQUE: fluororelectrometry, pyridine nucleotide fluorescence;
METABOLISM: oxygen consumption, redox state.

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Les effets d'une pression intracrânienne élevée (ICP) sur l'oxygénation intracellulaire et le volume sanguin cérébrocortical (CBV) ont été étudiés chez les lapins. La concentration en oxygène intracellulaire (O_2) a été évaluée alors que le niveau de la balance oxidation/réduction de la concentration de nucléotide pyridine ([NADH]) et le volume sanguin cérébrocortical relatif (CBV) étaient mesurés à l'aide de la sonde d'un fluororélectromètre à fibre optique placée à la surface cérébrocorticale. Les expériences ont été menées chez six animaux normocarbiqes anesthésiés à l'uréthane et à différentes fractions d'oxygène inspiré (FIO_2). Lors des augmentations graduelles de l'ICP, la [NADH] a augmenté pour toutes les valeurs de FIO_2 lorsque l'ICP dépassait un seuil de 18 ± 2.2 cm H $_2$ O (représentant une diminution de la PO_2 mitochondriale intracellulaire) ($P < 0.05$). La diminution de l'oxygénation intracellulaire en relation avec une ICP élevée était inversement proportionnelle à la FIO_2 ($P < 0.05$). Avec une ICP plus grande que 18 ± 2.2 cm H $_2$ O, l'oxygénation intracellulaire mitochondriale montrait une amélioration entre une FIO_2 de 0,21 et de 0,5 ($P < 0.05$) mais l'augmentation de la FIO_2 0,5 à 1,0 ne provoquait pas une amélioration statistiquement significative de la balance tissulaire réduction-oxydation. La CBV, représentant principalement le sang capillaire tissulaire, augmentait lorsque l'ICP atteignait des valeurs de plus de $18 \pm 1,2$ cm H $_2$ O, probablement secondaire à l'autorégulation locale ou à une distension veineuse ($P < 0,05$). Cependant, à des valeurs au-dessus de $30 \pm 1,1$ cm H $_2$ O, la CBV diminuait ($P < 0,05$). Les résultats démontrent l'étroite dépendance entre la concentration d'oxygène inspirée, l'augmentation de l'ICP et l'oxygénation cérébrale intracellulaire, et suggère que l'utilisation cérébrale en oxygène se détériore à des valeurs de l'ICP d'environ 18 cm H $_2$ O.

The unique and indispensable role of oxygen (O_2) in energy metabolism makes the problem of O_2 supply to living tissues of material interest to all fields of medical biology. Under normal conditions, the brain is dependent upon a continuous supply of blood in order to obtain a continuous flow of O_2 as well as glucose to the cells since they have a high rate of O_2 utilization and low O_2 and energy reserve.¹ It is accepted that this flow occurs solely via the process of diffusion and that the driving force for this diffusion is the partial pressure difference between the

capillary and the cell.² Any alterations in the matching of cerebral blood flow (CBF) to O₂ requirements may result in blockade of electron transport through the mitochondrial respiratory chain and a net decrease in ATP production.^{1,3} A basic variable of cellular metabolism is the oxidation/reduction state. The redox state of cytoplasmic oxidized/reduced pyridine nucleotide concentration ([NADH]/[NAD⁺]) is the fundamental description of the status of energy transduction in the cell since both glycolysis and the Krebs cycle involve redox coupling with NADH/NAD⁺. Chance *et al.*⁴ have shown that NADH is the most sensitive component to O₂ utilization in the respiratory chain and therefore is most competent to serve as an indicator of intracellular O₂ concentration. Various components of the respiratory chain exhibit different optical properties in the reduced and oxidized forms.⁵ Using the autofluorescence of the mitochondrial NADH, the excitation and emission spectra of NADH can be recorded using a fluorometer-reflectometer-spectrophotometer on the surface of the living tissues.⁵⁻⁶

Elevated intracranial pressure (ICP) is an important pathophysiologic feature of head injury, post-ischaemic reperfusion, and a variety of disease states including cerebral oedema, infection, and haemorrhage.⁷ A reduction in blood flow to the brain and to the brain stem and consequent imbalance between oxygen supply and oxygen demand are viewed as the key mechanisms of injury due to elevated ICP.⁷⁻¹⁰ Despite the importance of these mechanisms, there is little information concerning the relationship of ICP to brain tissue oxygenation.

This study was designed to examine the effect of progressive changes in ICP on cerebrocortical oxygenation and CBV by measuring intracellular redox balance as an index of brain tissue oxygenation, using the technique of time-sharing multichannel fibreoptic fluoro-reflectro-spectrophotometry.

Abbreviations

CBF:	cerebral blood flow
CBV:	cerebrocortical blood volume
CPP:	cerebral perfusion pressure
CSF:	cerebral spinal fluid
CF:	corrected fluorescence
CVP:	central venous pressure
FiO ₂ :	fraction of inspired oxygen
ICP:	intracranial pressure
MAP:	mean arterial pressure
[NADH]:	pyridine nucleotide, reduced concentration
[NAD ⁺]:	pyridine nucleotide, oxidized concentration
PaCO ₂ :	arterial carbon dioxide partial pressure
PaO ₂ :	arterial oxygen partial pressure

Methods

Animal preparation

With the approval of the Institutional Committee on Animal Research, mean arterial pressure (MAP), central venous pressure (CVP), and ICP were continuously recorded in six adult male New Zealand White rabbits, weighing 2.8–3.5 kg. Anaesthesia was induced with urethane 20 mg·kg⁻¹ and muscle paralysis was induced with pancuronium 0.15 mg·kg⁻¹. The trachea was intubated and mechanical ventilation with 100% O₂ was established. The PaCO₂ was maintained between 32–38 mmHg. Inspired and end-tidal CO₂, O₂, N₂ gas concentrations were analyzed with a Perkin-Elmer 1100 Mass Spectrometer (Perkin-Elmer, Norwalk, CT 06856). A thermistor rectal temperature probe was inserted and connected to a servocontrol heat lamp and a heating pad to maintain body core temperature at 39° C.

The rabbit was placed in the prone position and the head was clamped in a stereotaxic apparatus. The scalp was retracted and a burr hole was drilled in the parietal bone for the insertion of an epidural pressure bolt. The bolt was cemented in place with eight per cent cyanoacrylate cement. A vertical 50 cm fluid-filled glass tube connected the bolt to a calibrated differential pressure transducer. A 22-gauge spinal needle was then inserted into the cisterna magna and connected to a mock cerebrospinal fluid (CSF) filled manometer. The mock CSF contained the following constituents in mEq·L⁻¹: Na⁺ 151; K⁺ 3; Ca²⁺ 2.5; Mg²⁺ 1.2; Cl⁻ 134; HCO₃⁻ 25; urea 6; and dextrose 3.7.¹¹ Injections or withdrawals of mock CSF through this needle were made to control ICP. A second burr hole with a diameter of approximately 0.6 cm was made in the contralateral parietal bone to permit regional NADH and blood flow measurements. The dura-mater remained intact for this burr hole.

Measurements of tissue blood volume and oxygenation

Cerebrocortical NADH fluorescence and ultraviolet reflectance were measured with a CF-1 fibreoptic time-sharing fluorometer/reflectometer (Johnson Research Foundation, University of Pennsylvania) originally designed by Chance *et al.*¹² and Jobsis *et al.*¹³ Mitochondrial NADH concentration was detected as an indirect measure of intracellular oxygenation.¹² Excitation light with a wavelength of 366 nm was conveyed to the cerebrocortical surface by way of the central fibres of a trifurcated fibreoptic cable. The tip of the cable was positioned with a pressure-tight fit precisely at the level of surrounding cranium. The circumferential fibres of the cable returned the emitted NADH fluorescent light (450 nm) and the reflected light (366 nm) from the tissue to the filtered photomultiplier tubes. When illuminated by the

excitation light the reduced nucleotide NADH fluoresces at a wavelength of 450 nm, whereas the oxidized NAD^+ does not.¹² Because the tissue haemoglobin concentration in the region illuminated by the fibreoptic probe absorbs some of the incident light, the amount of light reflected back to the fibreoptic light guide decreased when there was a larger volume of blood in the adjacent region 0–3 mm beyond the probe. Changes in the intensity of the reflected light can be used to estimate changes in CBV, which are proportional to changes in cerebrocortical blood flow.¹⁴

Artifactual changes in NADH fluorescence caused by changes in incident light intensity and from changes in blood volume in the detection region were corrected by the methods of Jobsis *et al.*¹³ and Harbig *et al.*¹⁵ The method is based on the observation that artifacts are proportional to the magnitude of reflected light. A proportionality constant is obtained as follows: a transient dilution of cerebrocortical blood is caused by the rapid injection of 0.3–0.7 ml of normal saline into the ipsilateral carotid artery. The haemodilution transiently increases light intensity in both the fluorescence and reflectance channels. A corrected fluorescence (CF) is defined by assuming that the true concentration of cerebrocortical NADH did not change, although signal intensity was altered in both reflectance and fluorescence channels. The correction factor, k , is the ratio of the haemodilution-induced reflectance and fluorescence signals. True changes in tissue NADH are then calculated from the equation: $\Delta\text{CF} = \Delta F - k(\Delta R)$, where ΔF and ΔR are the recorded changes in the signals measured at 450 and 366 nm, respectively.¹⁶ Output signals of the two photomultiplier tubes were calibrated by equating the reflectance and fluorescence signals during ventilation with 100% O_2 and a PaCO_2 maintained at 35–37 mmHg.

While we cannot measure the absolute level of NADH in the mitochondria, we can specify the change of NADH concentration at different O_2 concentrations (e.g., hyperoxia, hypoxia, and anoxia)¹⁷ and compare the relative NADH variations at different FiO_2 levels. The oxidation-reduction state of pyridine nucleotide was calculated by expressing the compensated fluorescence intensity relative to the fluorescence value when the nucleotide pool was fully oxidized and fully reduced. The fully oxidized value was obtained by ventilating each animal with an atmosphere of 5% CO_2 in O_2 . This should result in luxury cerebral perfusion and maximal mitochondrial oxidation. The fully reduced signal was obtained after subjecting the brain to three to four minutes of anoxia at the end of each experiment. The redox state of the cytosolic NADH/ NAD^+ couple is a continuous function of FiO_2 between 1.0 and 0.07.¹⁷ The fluorescence signal was expressed as a percentage of this range (relative [NADH]).

Experimental protocol

The experimental preparation was performed during intermittent positive pressure ventilation with 100% O_2 . Normocarbica was maintained. The effect of changes in ICP on CBV and redox state was studied under conditions of different, constant fractions of inspired O_2 (1.0, 0.75, 0.5, 0.21). At each FiO_2 , ICP was increased in a stepwise fashion, and steady-state values (less than five per cent change over five minutes) for redox balance, relative blood volume, and ICP were recorded. Blood pressure was maintained at a constant level by adjusting the level of anaesthesia and by intravenous fluid administration.

Statistics

The mean $\pm$ standard deviation (SD) for MAP, PaCO_2 , PaO_2 , pH, and CVP were determined. Repeated-measures analysis of variance and the Tukey HPC test for multiple comparisons were used to determine statistical significance for within-group at each FiO_2 for changes in CBV and [NADH]. One-way analysis of variance and the Tukey HPC test for multiple comparisons were used to determine statistical significance for intermediate FiO_2 groups at each comparable level of ICP for CBV and [NADH]. A $P < 0.05$ was considered statistically significant.

Results

A total of 42 steady-state measurement periods were made in six rabbits. Mean ($\pm$ SD) values for MAP, 69 ± 3.0 ; PaCO_2 , 37 ± 0.02 ; pH, 7.37 ± 0.02 ; and CVP, 4.7 ± 0.6 were not significantly different among the different rabbits, or among different treatment periods in the same rabbit. The PaO_2 obtained at FiO_2 of 0.21, 0.50, 0.75, and 1.00 were respectively 134.0 ± 10.3 , 247.9 ± 9.0 , 390.2 ± 19.7 , and 505.3 ± 14.9 . Vasoactive drugs were not used to control MAP during changes in ICP between 0 and 35 cmH_2O .

Figure 1 shows the relationship between ICP and CBV, probably reflecting tissue capillary blood, at different levels of FiO_2 . While ICP was increased from normal to $18 \pm 1.2 \text{ cmH}_2\text{O}$, CBV was inversely related for all values of FiO_2 . The CBV reached statistical significance only for FiO_2 of 0.21 when compared with the other values of FiO_2 at each ICP level below $18 \pm 1.2 \text{ cmH}_2\text{O}$ ($P < 0.05$). The CBV increased as ICP increased above $18 \pm 1.2 \text{ cmH}_2\text{O}$ for all values of FiO_2 ($P < 0.05$), then declined dramatically when ICP passed $30 \pm 1.1 \text{ cmH}_2\text{O}$ ($P < 0.05$). The rate of change of CBV above $18 \pm 1.2 \text{ cmH}_2\text{O}$ in ICP was different from the baseline for each value of FiO_2 ($P < 0.05$) but there were no statistical differences among the values of FiO_2 at each ICP above 18 ± 1.2

FIGURE 1 Effects of intracranial pressure on reflectometrically measured cortical blood volume. *Statistically significant differences within groups at each intracranial pressure level and the baseline of that group ($P < 0.05$). + Statistically significant differences among groups at each ICP level ($P < 0.05$). Bars represent $\pm$ SD.

cmH₂O. The CBV observed at 35 ± 0.9 cmH₂O was not statistically different from the baseline values of each value of FiO₂.

Figure 2 shows the relationship of ICP to brain tissue oxygenation, expressed as relative cerebrocortical [NADH] levels. While ICP was increased from normal to 18 ± 2.2 cmH₂O [NADH] was inversely related for all values of FiO₂. The [NADH] was statistically different only for FiO₂ of 0.21 when compared with the other values of FiO₂ at each ICP level below 18 ± 2.2 cmH₂O ($P < 0.05$). The [NADH] (representing decreased intramitochondrial O₂ tension) increased as the ICP was increased above 18 ± 2.2 cmH₂O for all levels of FiO₂ ($P < 0.05$). This represented a cerebral perfusion pressure (CPP) of approximately 50 mmHg. The diminution in intramitochondrial O₂ tension observed above 18 ± 2.2 cmH₂O in ICP was statistically different from the baseline for each value of FiO₂ ($P < 0.05$). While increasing FiO₂ from 0.21 to 0.5 caused a significant improvement in brain redox balance ($P < 0.05$), changing FiO₂ from 0.5 to 1.0 had no statistically significant effect.

Discussion

This study demonstrated that inspired O₂ concentration and ICP are both important variables affecting brain redox balance. A key finding was that cerebrocortical redox balance began to deteriorate as ICP was increased beyond 18 ± 2.2 cmH₂O, and this was independent of the level of inspired O₂. At a given ICP, brain redox balance could be improved by increasing FiO₂ from 0.21 to 0.50 but with

FIGURE 2 Effects of intracranial pressure on brain redox balance at different levels of inspired O₂. *Statistically significant differences within groups at each intracranial pressure level and the baseline of that group ($P < 0.05$). + Statistically significant differences among groups at each ICP level ($P < 0.05$). Bars represent $\pm$ SD.

diminishing effect above an FiO₂ of 0.5. A gradual increase in NADH fluorescence was detected as ICP was increased above 18 cmH₂O. This suggests that a change in intracellular O₂ availability began to occur as CPP decreased below about 50 mmHg. The MAP was constant in these experiments until ICP exceeded 35–40 cmH₂O. The NADH fluorescence was observed to be a function of inspired oxygen level, even within the normoxic range. This is consistent with previous observations in rabbits,¹⁶ but it is at odds with *in vitro* data from isolated mitochondria that show that maintenance of normal redox balance are independent of arterial oxygen concentration until a critical concentration is reached.¹⁸ It is noteworthy that a relationship seems to exist between the predicted defect in brain-tissue oxygen supply and the cerebral venous oxygen-tension values.¹⁸ Few studies have examined the effect of changing FiO₂ (PaO₂) on redox balance *in vivo*. A continuous relationship between NADH fluorescence and O₂ availability *in vivo* probably reflects a steep O₂ diffusion gradient between arterial blood and mitochondrion.

Increasing NADH fluorescence occurred with increasing ICP even though local CBV appeared to increase. Several factors may have contributed to this occurrence. Firstly; it is important to recognize the limitations of reflectometric measurements of blood flow. Usually, brain blood volume correlates well with global cerebral blood flow¹³ and reflectometric measurements of CBV correlate well with changes in cerebral blood flow caused, for example, by increasing PaCO₂.¹⁵ Under conditions of

increased ICP this may not be so. It is generally accepted that intravascular pressure must be slightly greater than brain-tissue or ICP pressure or the more compliant vessels would collapse.¹⁹ Mild venous outflow obstruction may occur at 18 ± 1.2 cmH₂O, and increases up to about 30 ± 1.1 cmH₂O. Above 30 ± 1.1 cmH₂O, tissue blood volume decreases precipitously as venous outflow is collapsed or blood is squeezed from the tissue by the pressure applied against the reflectometric probe. Thus, reflectometric measurements of tissue blood volume may not reflect blood flow during high ICP elevation. Secondly, since increases in brain tissue blood volume were seen as ICP increased above the level causing increased NADH, the increase in blood volume may reflect autoregulatory vasodilation in brain surface vessels. Clearly, independent methods of blood flow measurement are needed to distinguish between these possibilities.

Previous work in our laboratory has demonstrated the physiological relevance of our techniques of *in vivo* fibreoptic fluorometry.¹⁶ Measurements of brain and redox state are sensitive to perturbations of blood pressure, PaCO₂, PaO₂, haemorrhage, haemodilution, and treatments with cyanide or acetazolamide. Because the fibreoptic probe used in these experiments was positioned flush with the internal surface of the cranium with an intact dura-mater, brain redox measurement did not interfere with ICP.

Urethane is a commonly used veterinary anaesthetic and its use is routine for studies of the central nervous system due to its lack of effect on systemic haemodynamics and its minimal impact on cerebral physiology.^{20,21} It is unlikely that urethane obscured the true effects of changes in oxygen delivery and ICP on brain redox balance.

The results of this study suggest that even mild increases in ICP cause measurable decreases in brain oxygenation. These changes can be ameliorated by increasing the FiO₂, but at higher levels of ICP, increased FiO₂ has diminished effect on brain redox balance. This is consistent with the fact that above a PaO₂ of approximately 100 mmHg, little additional gain in O₂ transport is achieved by increasing PaO₂.

This conclusion may have relevance in human intensive care settings where ventilator management of patients with intracranial injury takes place. However, further investigation is required to confirm this relevance.

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