ORIGINAL ARTICLE

Mechanisms of CBF Augmentation during Hypoxia in Cats: Probable Participation of Prostacyclin, Nitric Oxide and Adenosine

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Abstract. To elucidate the mechanisms of CBF augmentation during hypoxemic hypoxia, we applied continuous monitoring of CBF and metabolism to examine the participation of prostacyclin, nitric oxide (NO), and/or adenosine in these mechanisms. Cats ($n = 32$) were anesthetized and ventilated artificially. Cerebrovascular reactivity to hypoxia was assessed by changes in CBF, brain tissue oxygen and carbon dioxide tensions, and mean arterial blood pressure (Δ CBF, Δ BrPO₂, Δ BrPCO₂, and Δ MABP) during a 3-min inhalation of 10% O₂ + 90% N₂ before and after the intracarotid administration of (1) indomethacin (1 mg/kg, a cyclooxygenase inhibitor, $n = 11$), (2) L-N^G-monomethyl-arginine (LNMMA, 1 μ mol/kg/min, an inhibitor of nitric oxide synthesis, $n = 11$), and (3) caffeine (20 mg/kg, an adenosine antagonist, $n = 10$). BrPO₂ decreased significantly in all groups during the produced hypoxemic hypoxia. CBF significantly increased in this state before the administration of indomethacin, LNMMA, or caffeine, whereas it contrastively did not significantly increase after the administration of indomethacin or caffeine. In addition, CBF significantly decreased under hypoxia during the administration of LNMMA. Taken together, these results suggest that prostacyclin (PGI₂), nitric oxide (NO), and adenosine may jointly participate in CBF augmentation during hypoxia. (Keio J Med 43 (1): 31–36, March 1994)

Key words: cerebral blood flow, vasodilatation, indomethacin, L-N^G-monomethyl-arginine (LNMMA), caffeine

Introduction

General agreement exists concerning the relation between hypoxemic hypoxia and cerebral blood flow (CBF), *i.e.* CBF does not substantially change during alterations of arterial oxygen tension (PaO₂) within the physiological range, whereas cerebral arterioles dilate and CBF increases when PaO₂ drops below 50–60 mmHg (hypoxemia).^{1,2} Although the long-standing lactic acidosis theory² has been applied in many cases to explain the brain's hypoxic hyperemia mechanism, several other mechanisms have also been postulated and discussed.

Of several vasodilators to be considered, participation of eicosanoids in the regulation of CBF received initial attention.^{3–5} Among them, prostaglandin I₂ (PGI₂, prostacyclin), which is a product of cyclooxygenase, is one of the vascular system's main metabolites of

arachidonic acid and is dominantly provided by the endothelial cells.⁶ Since prostacyclin relaxes or dilates blood vessels, there is a possibility it affects hypoxic hyperemia of the brain.

Vascular dilation by various kinds of vasoactive substances is also known to be mediated by endothelium-derived relaxing factors (EDRFs),⁷ with one EDRF having been specifically identified as nitric oxide (NO), *i.e.* it is formed from the terminal guanidino nitrogen of L-arginine via the action of NO synthase (NOS).^{8,9} Continuous NO production appears to occur under resting conditions in cerebral vascular beds, thereby imparting a vasodilatory "tone".¹⁰ However, until recently limited information was available on the relationship between hypoxic hyperemia and NO.^{9,11}

A third vasodilator gaining research attention is the nucleoside adenosine. Among the high energy phosphate

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compounds found in the brain, adenosine triphosphate (ATP) exists in a concentration of 3 $\mu\text{mol/g}$, being most abundant among the purine nucleotides. When the brain reaches a hypoxic condition, ATP decreases immediately, adenosine begins to increase within a few seconds followed by an increase in inosine, and finally hypoxanthine increases.¹² Since adenosine affects cerebral vasodilatation, it has been proposed as one modulator of CBF regulation.^{12–15}

To elucidate the mechanisms of CBF augmentation during hypoxemic hypoxia, the present study uses continuous monitoring of CBF and metabolism^{16–18} to examine the participation of prostacyclin, NO, and/or adenosine in these mechanisms.

Materials and Methods

Thirty-two cats weighing 2.3–3.4 kg (mean 2.8 kg) were anesthetized with an intraperitoneal injection of α -chloralose (50 mg/kg) and urethane (500 mg/kg) and locally with 0.5% procaine hydrochloride. After tracheostomy, they were immobilized with alcuronium chloride (0.6–0.8 mg/kg) and a tracheal cannula was connected to a variable speed respirator pump (Harvard Model 662, Harvard, MA, USA). As the initial condition the respiration rate was set at 20 strokes/min and the stroke volume at 10 ml/mg. Abdominal aortic pressure was measured with a pressure transducer (Statham P23IDb, Gould, Oxnard, CA, USA) connected to a polyethylene tube inserted into the abdominal aorta through the left femoral artery. A polyethylene tube was inserted into the right femoral artery for blood gas sampling and another into the right femoral vein for intravenous injection of drugs. A fourth tube inserted retrogradely into the left lingual artery enabled drug injection into the carotid artery. Throughout the experiment the rectal temperature of cats was kept at 37°C by means of heating blankets.

A sampling tube connected to a gas analyzer (Engström Eliza CO₂ and O₂ Analyzer) was inserted into the tracheal cannula to continuously record alveolar CO₂ and inspiratory O₂ (F_IO₂) concentration. The skull was fixed in a stereotactic head holder (Type SN-1, Narishige, Tokyo, Japan) and the scalp and dura over the left parietal cortex region were removed. PO₂ and PCO₂ electrodes and a laser Doppler probe (Type ALF21, Advance, Tokyo, Japan) were gently attached to the exposed cerebral cortex by adjustable rods with springs.^{16,17} Cortical blood flow was also monitored using a thermistor placed gently on the cortex,^{16,18} *i.e.* variations in brain temperature qualitatively reflect continuous changes in CBF, with increases in temperature indicating increased CBF and vice versa.^{16,18} The output signal from the PO₂ electrode was amplified by a gas analyzer (Model 160, Beckman Toshiba, Tokyo, Japan), while that from the

PCO₂ electrode by a pH meter (Model 22, Radiometer, Copenhagen, Denmark). Both parameters were continuously recorded on a polygraph (Type R-56M3, Rikadenki, Tokyo, Japan). Electrode calibration was performed with 100% N₂ and 5% O₂ + 95% N₂ for the PO₂ electrode; and 5% CO₂ + 95% N₂ and 10% CO₂ + 90% N₂ for the PCO₂ one. Arterial blood PO₂, PCO₂, and pH were measured with a blood gas analyzer (PHM71, Radiometer, Copenhagen, Denmark).

Cerebrovascular reactivity to hypoxia was assessed by changes in CBF, brain tissue oxygen and carbon dioxide tensions, and mean arterial blood pressure (ΔCBF , ΔBrPO_2 , ΔBrPCO_2 , and ΔMABP) during and after a 3-min inhalation of 10% O₂ + 90% N₂. Arterial blood was sampled before and immediately after this 3-min inhalation.

The animals were divided into 3 groups for the following described experiments.

Experiment 1: Eleven cats were used to examine cerebrovascular reactivity to hypoxia before and after an intracarotid injection of indomethacin (a competitive inhibitor of cyclooxygenase, 1 mg/kg). CBF was evaluated by the laser Doppler method.

Experiment 2: Eleven cats were used to examine cerebrovascular reactivity to hypoxia before, during, and 60 min after cessation of an intracarotid continuous injection of L-N^G-monomethyl-arginine (a competitive inhibitor of NOS, LNMMA, 1 $\mu\text{mol/kg/min}$). ΔCBF was evaluated by the thermistor method.

Experiment 3: Ten cats were used to examine cerebrovascular reactivity to hypoxia before and after an intracarotid injection of caffeine (a competitive antagonist of adenosine, 20 mg/kg). ΔCBF was evaluated by the thermistor method.

The data of ΔBrPO_2 , ΔBrPCO_2 , and ΔMABP are shown as mean $\pm$ SD. The ΔCBF data obtained by the laser Doppler method are shown as % changes from the pre-hypoxic control state, whereas that by the thermistor method by the number of the animals whose CBF (brain temperature) increased or decreased from the pre-hypoxic control state. Statistical analysis was performed using the Student's paired t-test for all data except ΔCBF by the thermistor method in which the sign test was adopted. A p value less than 0.05 was considered significant.

Results

Table 1 summarizes changes in the arterial blood gas of 10 cats before and just after the 3-min inhalation of 10% O₂ + 90% N₂, where PaO₂ at the end of hypoxia

Table 1 Blood Gas Analysis

	PaO ₂ (mmHg)	PaCO ₂ (mmHg)	pH
Control	95.4 ± 11.7	32.5 ± 3.4	7.290 ± 0.061
Hypoxia	38.5 ± 6.0	30.4 ± 5.7	7.346 ± 0.067
	p<0.01	n.s.	n.s.

Changes in the arterial blood gas of 10 cats before (Control) and immediately after a 3-min inhalation of 10% O₂ + 90% N₂ (Hypoxia). Mean ± SD, n.s.: non-significant change.

was 38.5 ± 6.0 mmHg; being significantly lower (p<0.01) than the control value (95.4 ± 11.7 mmHg). Note arterial carbon dioxide tension (PaCO₂) and pH did not significantly change.

Cerebrovascular response to hypoxia following indomethacin administration (Experiment 1)

Figure 1 indicates the effect of hypoxia (10% O₂ + 90% N₂) on CBF and metabolism before and after the administration of indomethacin (n = 11). Before indomethacin administration, CBF significantly (p<0.05) increased to a maximum of 17.1 ± 18.5% at 3 min, with a significant (p<0.01) decrease correspondingly occurring in BrPO₂ (−11.6 ± 8.0 mmHg). In contrast, after the administration, CBF did not increase significantly at 3 min (6.4 ± 22.3%) in spite of a significant

(p<0.01) decrease in BrPO₂ (−13.7 ± 8.0 mmHg). BrPCO₂ showed non-specific changes before and after the administration of indomethacin, a behavior indicating indomethacin did not substantially affect cerebral metabolism in the present experimental condition. Since the amount of decrease in BrPO₂, as well as the amount of increase in MABP are not different before and after the administration of indomethacin, CBF augmentation during hypoxia was obviously suppressed by its administration.

Cerebrovascular response to hypoxia following LNMMA administration (Experiment 2)

Figure 2 shows the effect of hypoxia (10% O₂ + 90% N₂) on CBF and metabolism before, during, and after the administration of LNMMA (n = 11). Before LNMMA administration, CBF at 3 min significantly increased (p<0.01) in all 11 animals, with a corresponding significant decrease (p<0.01) in BrPO₂ (−20.5 ± 16.4 mmHg). However, CBF at 10 min significantly decreased (p<0.01) during the administration in all 11 animals in spite of a significant decrease (p<0.01) in BrPO₂ occurring at 3 min (−10.9 ± 8.2 mmHg). CBF at 2 min once again significantly increased (p<0.01) (increased in 7 animals/unchanged in 4) 60 min after cessation of

Fig 1 Changes in cerebrovascular reactivity to hypoxia (laser Doppler method) before and after the administration of indomethacin (n = 11). *, **: Significant change from 0 min.

Fig 2 Changes in cerebrovascular reactivity to hypoxia (thermistor method) before, during and after the administration of LNMMA (n = 11). *, **: Significant change from 0 min. Bar height for CBF represents the number of affected animals, i.e. the positive length indicates the number of animals with an increased CBF and negative length those with a decreased value. The circles, triangles, or squares correspond to a difference between the numbers of increased and decreased animals; the number of unchanged animals was not included.

LNMA. BrPCO₂ showed no remarkable changes before, during and after the administration of indomethacin, which indicates LNMA did not substantially affect cerebral metabolism in the present experimental condition. Thus, like indomethacin, the amount of decrease in BrPO₂ and increase in MABP were not comparatively different before, during, or after the administration of LNMA, hence CBF augmentation during hypoxia was suppressed by its administration.

Cerebrovascular response to hypoxia following caffeine administration (Experiment 3)

Figure 3 indicates the effect of hypoxia (10% O₂ + 90% N₂) on CBF and metabolism before and after the administration of caffeine. Before the administration, CBF at 3 min significantly increased ($p < 0.01$) (increased in 9 animals/unchanged in 1), with a corresponding significant decrease ($p < 0.01$) occurring in BrPO₂ (-19.0 ± 6.7 mmHg). After administration, however, no significant increase in CBF took place (increased in 5 animals/unchanged in 2/decreased in 3) in spite of a significant decrease ($p < 0.01$) in BrPO₂ (-21.3 ± 7.4 mmHg) at the same time. BrPCO₂ showed no remarkable changes before and after the administration of caffeine, which indicates caffeine did not substantially affect

cerebral metabolism in the present experimental condition. Because no difference was observed between the amount of decrease in BrPO₂ or increase in MABP before and after the administration, this leads to a similar conclusion for caffeine as was obtained in Experiments 1 and 2 for indomethacin and LNMA, respectively.

Discussion

Since laser Doppler and thermistor methods have less or no quantitative basis in comparison with, for example, the hydrogen clearance method,^{16–18} some limitations exist in interpreting our results. However, these two techniques are presently the most reliable ones for obtaining continuous CBF data recording. Although hypoxia induced by a 3-min inhalation of a mixture of 10% O₂ + 90% N₂ is relatively mild,^{1,2} it nonetheless reduces the PaO₂ level below 50 mmHg (Table 1). Therefore, we consider this method to be an effective way to simulate hypoxic hyperemia.

Sakabe and Siesjö¹⁹ observed no relationship between prostacyclin and hypoxic hyperemia, whereas McCalden *et al*²⁰ obtained opposite results. Here, CBF augmentation during hypoxia was suppressed by the administration of indomethacin, a competitive cyclooxygenase inhibitor; thereby suggesting that prostacyclin participates in such augmentation. A hypothesized reactivity mechanism is described as follows. Depolarization of the endothelial cell membrane under brain hypoxia causes an influx of Ca²⁺ ions with intracellular free Ca²⁺ accumulating. Consequently, phospholipase A₂ is activated, cell membrane phospholipids are hydrolysed, and free fatty acids are released.²¹ The resynthesis (reacylation) from free fatty acids to phospholipids is known to be disturbed by the depletion of ATP, and so free fatty acids, particularly arachidonic acid, accumulate.⁴ This increase in arachidonic acid would then be metabolized to prostacyclin by cyclooxygenase, and since endothelium-derived prostacyclin dilates vascular smooth muscle and indomethacin inhibits the cyclooxygenase pathway, conversion from arachidonic acid to cyclic endoperoxide is suppressed; hence prostacyclin is no longer formed²² and hypoxic hyperemia is ultimately repressed.

Until recently, only a limited number of studies have addressed the relationship between NO and hypoxic hyperemia in the brain *in vivo*. Kozniowska *et al*¹¹ concluded that NO modulates hypoxic changes of CBF, but that this is probably not the main factor responsible for increases in CBF. Pelligrino *et al*⁹ also reported that NO synthesis is critically involved in the cerebral hyperemic response to hypercapnia but not to hypoxia. Nevertheless, here we clearly demonstrate that CBF augmentation during hypoxia is suppressed by the administration of LNMA, a competitive NOS inhibitor; suggesting NO participates in this augmentation. The reactivity

Fig 3 Changes in cerebrovascular reactivity to hypoxia (thermistor method) before and after the administration of caffeine ($n = 10$). *, **: Significant change from 0 min. See Fig 2 for explanation of CBF results.

mechanism in this case is hypothesized as follows. NO release from the endothelium has been found to increase under hypoxia.²³ In such a condition, the intracellular free Ca^{2+} ions in the endothelium increase and activate NOS, and this in turn increases NO production from L-arginine.²⁴ The main action of NO is to stimulate soluble guanylate cyclase which causes an increase in cGMP in vascular smooth muscle.²⁵ If LNMMA antagonizes L-arginine and suppresses NO production by NOS, then hypoxic hyperemia would subsequently be repressed.

Phillis *et al*,¹² Winn *et al*,¹³ Morii *et al*,¹⁴ and Puiroud *et al*¹⁵ have all investigated the relationship between adenosine and hypoxic hyperemia and provided supportive data, yet it has still not been determined whether the increase in adenosine during hypoxic hyperemia is the cause or result.¹³ As shown in Fig 3, CBF augmentation during hypoxia was suppressed by the administration of caffeine, a competitive adenosine antagonist, suggesting that adenosine participates in CBF augmentation during hypoxia. The reactivity mechanism is hypothesized as follows. Under hypoxia, cytosolic adenosine of brain parenchyma increases by metabolic processes such as the degradation of ATP to adenosine. Cytosolic adenosine is then released outside the cell by a nucleoside transporter. Adenosine's action is mediated by receptors on the cell surface which are classified as A_1 , A_2 , and A_3 receptors. In fact, vascular dilatation by adenosine is known to be mediated by the A_2 receptor.²⁶ Since adenosine can activate adenylate cyclase through the A_2 receptor of vascular smooth muscle and stimulate production of cyclic AMP, the influx of Ca^{2+} ions from outside the smooth muscle cell and Ca^{2+} release from sarcoplasmic reticulum would consequently be inhibited and dilation of the vascular smooth muscles occur. As caffeine blocks adenosine receptors, this prevents muscle dilation even though the amount of adenosine increases due to hypoxia.

Apart from the cerebral circulation, a multifactorial hypoxic coronary vasodilatation hypothesis has been proposed for the coronary circulation, *i.e.* Park *et al*²⁷ reported that NO is an important mediator of the early phase of hypoxic coronary vasodilation, whereas additional mechanisms and/or factors, including adenosine and vasodilatory prostaglandins, contribute to the late phase. This hypothesis is supported in the present study because we confirmed that CBF augmentation during hypoxia is suppressed by inhibition of either prostacyclin production, NO production, or adenosine action. These results subsequently suggest that prostacyclin (PGI_2), nitric oxide (NO), and adenosine may jointly participate in CBF augmentation during hypoxia. In addition, a possibility may exist for the multifactorial regulation of hypoxic hyperemia by endothelium-derived and brain parenchyma-derived substances.

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