BRIEF COMMUNICATIONS

The Effect of O₂ and CO₂ on Prostaglandin Levels in the Cat Cerebral Cortex

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SUMMARY. We measured the effect of arterial hypercapnia, arterial hypocapnia, and arterial hypoxia on the concentrations of PGE₂, PGF₂α, and 6-keto-PGF₁α in quickly frozen cortical tissue from anesthetized cats. In order to correlate PG levels with the effects of these alterations in blood gases on the cerebral circulation, pial arteriolar diameters were measured with a microscope during normoxic and normocapnic conditions and then after blood gas intervention, immediately before tissue freezing. PGs were analyzed by high pressure liquid chromatography, deuterated standards, and gas chromatography/mass spectrometry. During control conditions with normal arterial blood gases (Pao₂ = 87 mm Hg and Paco₂ = 30 mm Hg) the levels of PGE₂, PGF₂α, and 6-keto-PGF₁α were 193 ± 57, 169 ± 34, and 31 ± 4 ng/g wet weight. Lowering Paco₂ to 17 mm Hg had no significant effect on PG levels and decreased arteriolar diameter by 12%. Increasing Paco₂ to 56 mm Hg was associated with a significant 60% reduction on PGE₂ (P < 0.05), a statistically marginal 38% reduction in PGF₂α (P < 0.1), and no change in 6-keto-PGF₁α, while pial arterioles dilated 32%. Arterial hypoxia (Pao₂ = 21 mm Hg) was associated with a significant 60% reduction on PGE₂ (P < 0.05), a statistically marginal 38% reduction in PGF₂α (P < 0.1), and no change in 6-keto-PGF₁α, while vessel diameter increased by 32%. The results show that PG levels in brain tissue do not increase during severe arterial hypoxia or during arterial hypocapnia, while during moderate hypercapnia the level of PGE₂ decreases. The results do not support previous reports that prostaglandins are mediators of the vascular responses associated with these conditions. (Circ Res 51: 652–656, 1982)

WE have measured prostaglandin (PG) levels in brain tissue during alterations in arterial blood gases for two basic reasons. First, ischemia caused by bilateral common carotid occlusion has been shown to increase the free arachidonic acid levels in gerbil brain (Yoshida et al., 1980), and reperfusion following carotid occlusion is associated with a long-lasting increase in brain PG levels (Gaudet et al., 1980). These effects of ischemia on PGs are important because the conversion of arachidonic acid to PGs produces oxygen free radicals which cause cerebral arteriolar lesions (Kontos et al., 1980) and loss of arteriolar reactivity (Wei et al., 1981). Whereas tissue O₂ levels decrease and tissue CO₂ levels increase during the ischemia produced by carotid occlusion, the effect of altering arterial O₂ or CO₂ levels on brain PG levels is poorly understood.

Second, Pickard and MacKenzie (1975) and others have reported that indomethacin (10–20 mg/kg) inhibition of rat, baboon and rabbit brain PG synthesis decreases resting cerebral blood flow and virtually abolishes an increase in brain blood flow to arterial hypercapnia (Pickard and MacKenzie, 1973; Bill, 1979; Sakabe and Siesjo, 1979; Dahlgren et al., 1981, Dahlgren and Siesjo, 1981). They conclude that PGs are important for resting tone of the cerebral arterioles and that hypercapnia induces an increase in dilator PGs or a decrease in constrictor PGs. However, Wei et al. (1980) and Busija and Heistad (1982) found that inhibition of PG synthesis with a lower dose of indomethacin (3 mg/kg) had no effect on resting pial arteriolar diameter or on the response to arterial hypercapnia in cats and rabbits. Three features of the above experiments are noteworthy. First, all the evidence in favor of the participation of PGs in the cerebral vasodilatation response to hypercapnia is based on the use of indomethacin. Two studies in which inhibitors of cyclooxygenase other than indomethacin were used gave negative results. Wei et al. (1980) found that AHR 5850 did not alter the response of cat pial arterioles to hypercapnia, and Pickard et al. (1977) found that, in humans, orally administered aspirin did not affect the increase in cerebral blood flow during hypercapnia. However, one might anticipate negative results with aspirin, since Abdel-Halim et al. (1978) have shown that aspirin does not inhibit PG synthesis in the brain. Second, except for the reports of Wei et al. (1980) and Busija and Heistad (in press), there was no evidence of the effectiveness of PG synthesis inhibition. Third, the studies that reported an effect of indomethacin on the blood flow response to arterial hypercapnia utilized rather high doses of
indomethacin (10–20 mg/kg). This is important, since Abdel-Halim et al. (1978) have shown that 3 mg/kg indomethacin produces a maximal inhibition of brain PG synthesis and that higher levels of indomethacin than are required to inhibit PG synthesis also inhibit cyclic AMP-dependent protein kinase (Kantor et al., 1978), phospholipase A_2 (Kaplan et al., 1978), phosphodiesterase (Flores and Sharp, 1972), and prostaglandin dehydrogenase (Face-Asciak and Cole, 1975). In view of these considerations, we believe a more direct approach to study the role of PGs in the brain blood flow response to changes in arterial blood gases is necessary. We, therefore, have examined the effect of arterial hypercapnia, arterial hypocapnia, and arterial hypoxia on the concentration of PGs in quick-frozen cat cerebral cortical tissue. In addition to these biochemical measurements, we have recorded changes in pial arteriolar diameter and blood pressure in order to facilitate interpretation of the biochemical data.

Methods

Animal Set-up and Experimental Protocol

The animal set-up and alteration of gas levels have been published previously in more detail (Levasseur et al., 1975; Wei, 1980). Experiments were carried out in 40 cats anesthetized with pentobarbital (30 mg/kg, iv). After completion of tracheostomy, each cat was ventilated with a positive-pressure respirator and received 0.4 mg/kg of decamethonium bromide intravenously for skeletal muscle paralysis. The end-expiratory CO_2 of the animal was monitored continuously with a Beckman infrared CO_2 analyzer and, during control periods, was maintained at a constant level of about 30 mm Hg during room air breathing by adjustment of the respirator rate and volume. Arterial blood pressure was measured via the femoral artery. Arterial blood samples were collected periodically for the determination of P02, PCO2, pH, and hematocrit. Blood gases and pH were measured with Radiometer electrodes; hematocrit was measured by a micromethod.

Pial precapillary vessels were visualized through an acutely implanted cranial window, as described in detail previously (Levasseur et al., 1975). The window was implanted over the parietal cortex to allow visualization of the vessels in the suprasylvian and ectosylvian gyri. Intracranial pressure in the space under the cranial window was measured continuously with a Statham strain-gauge connected to the window and maintained at a normal level of 5 mm Hg by adjusting the height of an open tube filled with artificial CSF.

Arterial hypercapnia was induced by inhalation of gas containing 7.5% CO_2 in air. Arterial hypocapnia was induced by hyperventilation via an increase in the rate and volume of the respirator. Arterial hypoxia was induced by inhalation of 7% oxygen in nitrogen. Each gas mixture or level of ventilation was maintained for 8 minutes before measurements were begun. Pial arterioles were visualized via a Leitz microscope equipped with a Vickers image-splitting device, described in detail previously (Levasseur et al., 1975). Five to eight vessels, covering a wide range of vessel caliber, were studied in each animal.

Only cats that displayed pial arteriolar dilation in response to hypercapnia or hypoxia or vasoconstriction in response to hypocapnia were utilized. The experimental design was as follows: after implantation procedures had been completed, the cat was placed under the microscope and control observations of blood pressure, intracranial pressure, and arterial vessel diameter were made. The blood gases then were altered, a steady state response attained, and physiological parameters were measured again. The control animals were set up exactly the same, except the blood gases were not altered during tissue removal. After the physiological measurements, the cranial window was gently removed, approximately 1 g of brain tissue scraped out with a spatula, immediately placed on a flat piece of dry ice, and smash frozen by hitting the tissue with another flat piece of dry ice. Using this procedure, the brain tissue was removed and frozen to a 1-mm thick wafer within 8 seconds after starting removal.

Analysis of Prostaglandins

The PG analysis was conducted basically as in our previous studies (Birkle et al., 1981; Ellis et al., 1981), however some modifications, suggested by the work of Abdel-Halim et al. (1980), have been made to increase the purity of the isolated PGs. The frozen brain wafer was weighed, cracked into small pieces, dropped into cold 0.9% NaCl (pH 7.4, 10 ml/g wet wt tissue) containing 3 μg/ml indomethacin, and homogenized. Twenty-five microliters of a combined standard were added to each sample for each prostaglandin measured. Each combined standard contained 1 μg of tetradeterated PGE_2 or PGA_2, or 6-keto-PGF_1a (Upjohn Co.) and 0.08 μCi of high specific activity tritiated PGE_2 or PGF_2α or 6-keto-PGF_1α (New England Nuclear). The deuterated standard allows mass spectrometric quantitation of the PGs, whereas the tritiated compounds allow one to follow the PGs through various stages of isolation and purification. The homogenized samples were then centrifuged at 4°C for 45 minutes at 10,000 g, the supernatant removed and adjusted to pH 3 with 88% formic acid and extracted 2 times with 2 volumes of ethyl acetate:methanol (95:5, vol/vol). All organic solvents were glass distilled and purchased from Burdick and Jackson. The pooled extracts were roto-evaporated, and traces of acid and water were removed by the addition of acetone and roto-evaporation of the azeotrope. Following evaporation, the samples were dissolved in 0.5 ml of chloroform and applied to a 5-g column of silicic acid (CC-4) (Mallinkrodt). First, the neutral lipids were eluted with 40 ml of chloroform and then the PGs were eluted with 60 ml of ethyl acetate and roto-evaporated to dryness. The sample was next dissolved in 1 ml of chloroform and filtered with 0.45-μm Millipore filter. The chloroform then was evaporated with N_2 and the PG sample dissolved in the high pressure liquid chromatography (HPLC) solvent. The various prostaglandins were separated by high pressure liquid chromatography (Waters Assoc.) utilizing a reverse phase fatty acid analysis column (Waters Assoc.). The sample was dissolved in 0.5 ml of the column solvent and eluted isocratically using a mixture composed of 76.7% water, 23% acetonitrile, 0.2% benzene, and 0.1% acetic acid. The flow rate was 2 ml/min and 1-minute fractions were collected and 0.2 ml assayed for radioactivity.

After HPLC isolation, PGE_2, 6-keto-PGF_1α, and PGF_2α were derivatized for gas chromatography-mass spectrometry (GC/MS) quantitation by converting the carboxyl group to the methylester derivative with diazomethane, the keto group to the methoxime derivative, and the hydroxyl functions to the trimethylsilyl ether derivative. Quantification was performed with a Hewlett-Packard 5985A GC/MS computer system, using electron impact and the selected ion-monitoring mode of operation. The glass gas chromato-
graph column was 0.9 m long with an internal diameter of 2 mm, and packed with 3% OV 101. The helium flow rate was 36 ml/min. The initial column temperatures were maintained for 1 minute after injection and then increased at a rate of 10°C/min for 2 minutes. The mass spectrometer was tuned with pentafluorotributyl-amine using mass 502 in order to optimize the high mass. Electron energy was 70 eV. As outlined by Green at al. (1978), the ions monitored were m/e 508 and 512 for PGE2, m/e 423 and 427 for PGF2\alpha, and m/e 598, 602, 508, and 512 for 6-keto-PGF1\alpha. Analysis of various known amounts of protonated prostaglandins showed that the response of the GC/MS system was linear over the desired range of prostaglandin concentrations. For statistical analysis of PGs, we performed analysis of variance followed by a multiple range test. P values of 0.05 or less indicate statistical significance.

Results

Table 1 shows the physiologic parameters immediately before tissue removal and Table 2 shows the cortical PG levels under the different conditions of blood gas alterations. The control PG levels and the physiological changes produced by the alterations in blood gases are similar to those we observed previously in other studies (Wei et al., 1980, Ellis et al., 1981). The average hematocrits in the four groups of cats ranged from 34 to 37, with no significant differences between the groups.

Arterial hypoxia was associated with no statistically significant changes in the concentrations of PGE2, PGF2\alpha, or 6-keto-PGF1\alpha. It should be noted, however, that the decrease in the concentration of PGE2 during hypoxia (P < 0.1) was near statistical significance. Arterial hypercapnia was associated with a statistically significant decrease in the concentration of PGE2 (P < 0.05), a trend toward decreased PGF2\alpha (P < 0.1), and no change in the concentration of 6-keto-PGF1\alpha.

Whereas the arterial pH decreased by approximately 0.2 pH unit during hypercapnia, this small decrease in pH has been shown to have little or no effect on PG synthesis (White and Glassman, 1976; Rose and Collins, 1974). Arterial hypocapnia was associated with no significant changes in prostaglandin concentration. Since PGF2\alpha generally tended to decrease when PGE2 significantly decreased, we analyzed all PGs in each sample in all groups for covariance. We found that PGE2 and PGF2\alpha covaried at the P < 0.0001 level of significance. This implies that factors which alter the synthesis or removal of PGE2 in a given animal similarly alter PGF2\alpha in that particular animal.

Discussion

This study shows that a severe decrease in tissue oxygen does not significantly increase cat cerebral cortical PG levels. The possibility that an increase in PG concentrations did indeed occur but could not be detected because of insensitivity of methods may be safely discarded, since we have previously shown a
significant increase in cat cortical PGs following concussive brain injury, which, like hypoxia and hypercapnia, produces pial arteriolar dilation, increased blood pressure (Ellis et al., 1981), and increases in cerebral blood flow (DeWitt et al., 1981). We caution that our present results do not preclude the possibility that more severe hypoxia or hypercapnia, or a combination of these as might occur in ischemia, would lead to an increase in PG levels. In fact, Gaudet et al. (1980) showed that PG levels are increased for 1–2 hours in gerbil brain upon reperfusion following a 5-minute bilateral common carotid artery occlusion. Also, Yoshida et al. (1980) have shown an increase in brain free arachidonic acid during gerbil carotid artery occlusion and an increase in lipid hydroperoxides following reperfusion. Taken together, our results and those of others cited above suggest that even more extreme hypoxia, perhaps in combination with hypercapnia, is necessary to initiate in vivo arachidonate release and, subsequently, an increase in PG levels.

Our approach has been to examine cortical PG levels in the belief that local PG concentration influences local arteriolar diameter. Alternatively, one might measure cerebral blood flow and arteriovenous differences in PG levels and calculate brain PG synthesis and release. Neither of these approaches can, by itself, elucidate the specific cell type synthesizing the PGs or identify the local concentration of the various PGs in a specific compartment of the brain. However, several reports concerning PG synthesis in whole brain and microvessels isolated from brain tissue show that, in whole rat, cat, and human brain, PGD₂ or PGF₂α and PGE₂ are the predominant PGs synthesized, while 6-keto-PGF₁α represents only 6–10% of the total PGs formed by whole brain tissue. These studies also show that 6-keto-PGF₁α is 68–82% of the total PG produced by cerebral microvessels isolated from human, cat, and rat brain (Abdel-Halim et al., 1980a; Abdel-Halim et al., 1980b; Birkle et al. 1981). This has led to the general conclusion that brain 6-keto-PGF₁α is likely to be mainly of vascular origin while PGE₂, PGF₂α, and PGD₂ are predominantly of extravascular origin. Since the vasculature represents a relatively small percentage of the whole brain weight, we speculate that the PGE₂ and PGF₂α concentrations, as reported here, are reasonable estimates of PGE₂ and PGF₂α in the extravascular space. It might also be noted that, since PGs are not stored in tissue or extensively metabolized by the brain (Wolfe and Coceani, 1979), the PGs present in the extravascular space will pass through the vasculature and, therefore, likely influence vascular smooth muscle function even though they may not be synthesized by the vasculature. Also important is the fact that—because evidence indicates that 6-keto-PGF₁α is mainly of vascular origin—our 6-keto-PGF₁α level, expressed as ng/gram whole brain tissue, probably is a substantial underestimate of the 6-keto-PGF₁α concentration in the vasculature. Our inability to demonstrate a decrease in 6-keto-PGF₁α during hypercapnia may be related to this fact, or may even suggest an increase in PGI₂ formation during hypercapnia, since 6-keto-PGF₁α did not decrease whereas PGE₂ fell significantly, and the decrease in PGF₂α approached statistical significance. However, we believe that PGI₂ is unlikely to be the mediator of hypercapnia-induced vasodilation since our previous studies have shown that local PGI₂ concentration of 3 μg/ml is needed to induce dilation comparable to that induced by hypercapnia (Ellis et al., 1979).

Our finding that the cortical concentration of vasodilator PGE₂ decreases during hypercapnia contradicts the view that increases in dilator PGs are mediators of the cerebrovascular changes associated with hypercapnia. In fact, the decrease in PGE₂ observed during hypercapnia and during hypoxia seems to reflect a washout of PGs from this tissue as a result of the associated increase in cerebral blood flow. Such an increase in blood flow is well known to occur under these conditions, and its occurrence in the present experiments is supported by findings of increased blood pressure in the face of substantial pial arteriolar dilation. Therefore, our finding of a decreased PGE₂ concentration during hypercapnia may not necessarily be due to decreased PGE₂ synthesis but, perhaps, to an overriding increased washout during normal or altered rates of brain PG synthesis. Again, measurement of arteriovenous differences in PG levels and cerebral blood flow would be necessary before conclusions might be drawn concerning alteration in the rate of brain PG synthesis and release.

Alternatively, it might be suggested that pial arteriolar dilation during hypercapnia is due to a decrease in vasoconstrictor PGs. This possibility may be eliminated in the present experiments, since PGD₂, CO₂, E₂, and I₂ are all vasodilators of cat pial arterioles (Ellis et al., 1979), and a careful examination of the literature and our own unpublished findings show that PGF₂α has no direct effect on the systemic or cerebral vasculature in the cat (Koss and Nakano, 1974; Gillis et al., 1981; Ellis, unpublished observations). It might therefore be argued that, in other species such as rodents, in which PGF₂α causes cerebral arteriolar vasoconstriction (Rosenblum, 1977), a decrease in vasoconstrictor PGF₂α may be contributing to the cerebral vasodilation produced by arterial hypercapnia. We feel that this is unlikely for two reasons. First, important physiological mechanisms for regulation of blood flow in higher vertebrates are generally universal, with little species variation. This generalization is likely to apply to the effect of CO₂ on the cerebral circulation. Second, the concentration of PGF₂α in quickly frozen rodent brain (Gaudet et al., 1980) is very much less than that needed to induce cerebral arterial vasoconstriction (Rosenblum, 1977). In summary, our findings do not support the view that an increase in vasodilator PGs mediates the vasodilation of hypercapnia. We suggest, therefore, that the intriguing decreases in resting blood flow and the inhibition of the vasodilator response to hypercapnia following administration of large doses of indomethacin observed by several investigators may not be due to...
an inhibition of PG synthesis but may be due to other unknown factors.

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