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 (Yo-shida 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 vasodilation 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 A2 (Kaplan et al., 1978), phosphodiesterase (Flores and Sharp, 1972), and prostaglandin dehydrogenase (Pace-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 on 40 cats anesthetized with pentobarbital (30 mg/kg, iv). After completion of tracheostomy, each animal was ventilated with a positive-pressure respirator and received 0.4 mg/kg of decamethonium bromide intravenously for skeletal muscle paralysis. The end-expiratory CO2 of the animal was monitored continuously with a Beckman infrared CO2 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 PCO2, 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% CO2 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 imaging-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 scooped 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 PGE2 or PGF2α, or 6-keto-PGF2α (Upjohn Co.) and 0.08 µCi of high specific activity tritiated PGE2 or PGF2α or 6-keto-PGF2α (New England Nuclear). The deuterated standard allows mass spectrometric quantitation of the PGs, whereas the triitated 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 azetropes. Following evaporation, the samples were dissolved in 1 ml of chloroform and applied to a 5-g column of silicic acid (CC-4) (Mallinckrodt). 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 N2 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, PGE2, 6-keto-PGF1α, and PGF2α were derivatized for gas chromatography-mass spectrometry (GC/MS) quantitation by converting the carboxyl group to the methoxime derivative with diazomethane, the keto function to the methine 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 et al. (1978), the ions monitored were m/e 508 and 512 for PGE2, m/e 423 and 427 for PGF2a, and m/e 598, 602, 508, and 512 for 6-keto-PGF1α. 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 change in the concentrations of PGE2, PGF2α, or 6-keto-PGF1α. 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 PGF2α (P < 0.1), and no change in the concentration of 6-keto-PGF1α.

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 PGF2α 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 PGF2α 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 PGF2α 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

<table>
<thead>
<tr>
<th>PG</th>
<th>Group</th>
<th>Control (n = 15)</th>
<th>Hypoxia (n = 8)</th>
<th>Hypercapnia (n = 8)</th>
<th>Hypocapnia (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/g wet wt (mean ± se)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td></td>
<td>193 ± 57</td>
<td>106 ± 25†</td>
<td>77 ± 20*</td>
<td>115 ± 28</td>
</tr>
<tr>
<td>F2α</td>
<td></td>
<td>169 ± 34</td>
<td>129 ± 30</td>
<td>104 ± 29†</td>
<td>163 ± 34</td>
</tr>
<tr>
<td>6-keto-F1α</td>
<td></td>
<td>31 ± 4</td>
<td>21 ± 10</td>
<td>34 ± 10</td>
<td>26 ± 7</td>
</tr>
</tbody>
</table>

* P < 0.05, compared to control.
† P < 0.1, compared to control.

Although 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 PGF2α 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 PGF2α 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 PGF2α in that particular animal.
significant increase in cat cortical PGs following con-
cussive brain injury, which, like hypoxia and hyper-
capnia, 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 combi-
nation 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
tissue 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 hy-
percapnia, 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 influ-
ences local arteriolar diameter. Alternatively, one
might measure cerebral blood flow and arteriovenous
differences in PG levels and calculate brain PG syn-
thesis 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 predomi-
antly 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 esti-
mates 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 Cocceani, 1979), the PGs present in the
extravascular space will pass through the vasculature
and, therefore, likely influence vascular smooth mus-
cle 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 con-
centration in the vasculature. Our inability to dem-
onstrate a decrease in 6-keto-PGF1α during hypercap-
nia may be related to this fact, or may even suggest
an increase in PG12 formation during hypercapnia,
since 6-keto-PGF1α did not decrease whereas PGE2
fell significantly, and the decrease in PGF2α ap-
proached statistical significance. However, we believe
that PG12 is unlikely to be the mediator of hypercap-
nia-induced vasodilation since our previous studies
have shown that a local PG12 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 vas-
odilator PGE2 decreases during hypercapnia con-
tricts the view that increases in dilator PGs are media-
tors 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 pres-
ent experiments is supported by findings of increased
blood pressure in the face of substantial pial arterio-
dialation. 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, measure-
ment of arteriovenous differences in PG levels and
cerebral blood flow would be necessary before con-
clusions might be drawn concerning alteration in the
rate of brain PG synthesis and release.

Alternatively, it might be suggested that pial arteri-
olar dilation during hypercapnia is due to a decrease
in vasoconstrictor PGs. This possibility may be elimi-
nated in the present experiments, since PGD2, COX,
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 vas-
culature 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 rod-
ents, in which PGF2α causes cerebral arteriolar
vasoconstriction (Rosenblum, 1977), a decrease in
vasoconstrictor PGF2α may be contributing to the
cerebral vasodilation produced by arterial hypercap-
nia. We feel that this is unlikely for two reasons. First,
important physiological mechanisms for regulation of
blood flow in higher vertebrates are generally univer-
sal, 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 sum-
mary, our findings do not support the view that an
increase in vasoconstrictor PGs mediates the vasodilation
of hypercapnia. We suggest, therefore, that the intri-
guing decreases in resting blood flow and the inhibi-
tion of the vasoconstrictor response to hypercapnia fol-
lowing 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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