Response of the Cerebral Circulation in Baboons to Changing Perfusion Pressure after Indomethacin

JOHN D. PICKARD, LINDSAY A. MACDONELL, ERIC T. MACKENZIE, AND A. MURRAY HARPER

SUMMARY An earlier study has demonstrated that indomethacin, a prostaglandin synthesis inhibitor, blocks the cerebrovascular response to hypercapnia. This response is believed to be mediated by a lowering of pH in the cerebral interstitial fluid. Should autoregulation of cerebral blood flow (CBF) to changing perfusion pressure also be mediated by a changing interstitial pH (the "metabolic" theory), then indomethacin should impair autoregulation. This hypothesis was tested in anesthetized baboons. CBF was measured by the intracarotid 133Xe clearance technique; the preparation and the indomethacin protocol were identical to those of our previous investigation. Arterial pressure was increased by the intravenous infusion of angiotensin and decreased by controlled hemorrhage. Indomethacin was given by continuous infusion into the internal carotid artery. Although it reduced resting CBF, the cerebrovascular response to changing perfusion pressure was unchanged. Because indomethacin affects the response to changing CO2 but not that to changing perfusion pressure, the mechanisms for these two reactions presumably are different and it is improbable that changing interstitial pH is responsible for autoregulation in the cerebral circulation.

CEREBRAL BLOOD FLOW is maintained relatively constant despite moderate changes in perfusion pressure, a phenomenon that commonly is termed autoregulation. Apart from possible neurogenic influences, there are two main hypotheses to account for autoregulation within the cerebral circulation: the myogenic and the metabolic. The myogenic theory asserts that cerebrovascular smooth muscle will constrict in response to an increase in transmural pressure, and relax following a reduction in pressure. This (the Bayliss effect) adjusts cerebrovascular resistance to changing perfusion pressure and tends to maintain constant blood flow within the brain. The metabolic theory asserts that autoregulation is a function of changes in metabolite concentration around the cerebral resistance vessels. The metabolite most commonly implicated is the cerebral interstitial fluid hydrogen ion.

It is widely believed that the increase in cerebral blood flow (CBF) induced by hypercapnia is due to a concomitant increase in the hydrogen ion concentration of the cerebral interstitial fluid. Indeed, it has been demonstrated that arterial hydrogen ion concentrations; acidity dilates and alkalinity constricts. Dissociated vasoparalysis is a well described clinical condition in which the cerebrovascular response to hypercapnia is impaired and that to changing perfusion pressure is intact (or vice versa). This phenomenon has been noted in a variety of different pathological conditions, suggesting that these two responses are not mediated by a common mechanism. An agent that selectively abolishes either the hypercapnic response or autoregulation might lead to some further understanding of the fundamental physiological responses of the cerebral circulation, as well as to further understanding of the mechanisms involved in dissociated vasoparalysis. Indomethacin, a potent inhibitor of prostaglandin synthesis, has been shown greatly to impair the increase in CBF associated with hypercapnia.
the effects of the intracarotid infusion of indomethacin on autoregulation within the cerebral circulation were studied in anesthetized baboons. It was hoped that this might help to distinguish between the fundamental mechanisms involved in autoregulation and those involved in the CO₂ responsiveness of the cerebral vasculature. The debate over whether or not indomethacin affects autoregulation of renal blood flow was a further stimulus to this work.13,14

Methods

Eight baboons (Papio anubis and Papio cynocephalus), weighing approximately 10 kg, were sedated with phencyclidine (12 mg, im) and anesthesia was induced with thiopental sodium (7.5 mg/kg, iv). After endotracheal intubation, anesthesia was maintained with 75% N₂O and 25% O₂ through an intermittent positive-pressure ventilator in open circuit. This was supplemented by half-hourly injections of phencyclidine (2–4 mg, im), and muscular relaxation was ensured by administration of suxamethonium. Normocapnia (arterial Pco₂ = 40 mm Hg) was maintained throughout the experiments either by adjusting the respiratory pump or by adding CO₂ to the inspired gas when required.

Both femoral arteries were catheterized. One artery was used for the measurement of mean arterial pressure (MAP) and for the withdrawal of arterial blood samples for the frequent measurement of arterial PCo₂, pH, PO₂, oxygen saturation, and hemoglobin concentration. The second femoral artery was used for bleeding the baboon into a warmed, heparinized reservoir when the lower limit of autoregulation was being tested. A catheter was introduced via the femoral vein into the inferior vena cava for administration of a slow saline infusion. Body temperature was maintained around 37°C by heating lamps.

To measure CBF, the right linguofacial trunk was cannulated for the injection of ¹³³Xe, dissolved in saline at 37°C. The main trunk of the right external carotid artery was ligated, as was the ascending pharyngeal artery (if this could be done without undue manipulation of the internal carotid artery). The ipsilateral scalp and temporal muscle were removed to minimize extracranial contamination of the ¹³³Xe clearance curve. CBF was measured from the parietal area following an intracarotid bolus injection of ¹³³Xe, and calculated from the height/area equation.15 A fine catheter was inserted into the superior sagittal sinus in order to measure sagittal sinus pressure (SSP) and cerebral venous oxygen saturation. The cerebral metabolic rate of oxygen consumption was calculated from the product of the CBF and corresponding arteriovenous oxygen content difference. Following completion of the surgical preparation, an hour was allowed before any baseline measurements of CBF were made.

In three baboons carotid angiography was performed prior to and during the infusion of indomethacin. The contrast agent used was 1–2 ml of meglumine iothalamate (Conray 280) warmed to 37°C. Following angiography, no determinations of CBF were made for 20–30 minutes because iodinated contrast media can transiently disturb cerebral autoregulation14 as well as cause a temporary increase in blood flow.15

Indomethacin was dissolved in a sodium bicarbonate-saline solution (NaHCO₃, 0.13 g/100 ml; NaCl, 0.81 g/100 ml) after the pH of this solution was adjusted to between 8.0 and 8.5 units. When the indomethacin had dissolved, the pH of the solution was corrected to that of the baboon's arterial blood, sufficiently slowly to avoid precipitation of the indomethacin. The biological half-life of indomethacin in such aqueous solutions at room temperature is 200 hours at pH 8.0 and 90 minutes at pH 10.0 (Merck Sharp and Dohme data). The solvent used has been shown previously to have no effect on CBF, cerebral oxygen consumption, or MAP.16 The indomethacin solution (0.2 mg/kg per min) was infused at 1 ml/min through the lingual artery catheter, using a concentric bitubular heating coil arrangement to ensure that the solution was administered at 37°C. The total dose of indomethacin ranged from 10 to 36 mg/kg given over a corresponding time period of from 50 minutes to 3 hours. When CBF was measured the infusion was interrupted for 20 seconds to inject the ¹³³Xe. Then the infusion was restarted after the withdrawal of a small volume of blood so that no secondary washout of ¹³³Xe occurred.

EXPERIMENTAL DESIGN

The lower range of autoregulation was examined by subjecting the baboons to stepwise bleeding. Great care was taken to ensure that the MAP was not reduced by more than 40% from the baseline MAP because retransfusion, after greater falls in MAP, may be accompanied by a reactive hyperemia and abolition of autoregulation within the cerebral circulation.4,18

The upper range of autoregulation was tested by increasing MAP by using an intravenous infusion of angiotensin. Angiotensin, as opposed to norepinephrine, was used because the former has been shown to be without effect on either CBF or cerebral oxygen consumption.17 The initial concentration of angiotensin employed was 5 × 10⁻⁸ g/ml but, as tachyphylaxis commonly developed, somewhat higher doses were necessary to achieve a stable level of hypertension. Excessive infusions of fluid were avoided by increasing the concentration of angiotensin. Again, care was taken to ensure that the MAP did not exceed 40% above the baseline value during the angiotensin infusion, because this figure is close to the "breakthrough" of autoregulation in anesthetized baboons.18

Autoregulation to induced hypotension was examined in six baboons and autoregulation to induced hypertension in four, both before and during the infusion of indomethacin. In two baboons both the upper and lower ranges of autoregulation were examined. In all baboons, whether induced hypotension or hypertension was being studied, the MAP was held steady for at least 5 minutes before and then 10 minutes during the estimation of CBF.

STATISTICAL ANALYSIS

Statistical analysis was performed in two ways. First, the figures for CBF, cerebral oxygen consumption, and other measured variables were divided into equal MAP bins...
(61–70 mm Hg, 71–80 mm Hg, etc.) and Student’s t-test analysis was performed between bins for all parameters. Second, linear regression analysis of cerebrovascular resistance (CVR) against MAP was performed by the least squares method, thus allowing further comparison between the control and indomethacin values. An analysis of covariance was used to test for similarity of the two regression slopes. CVR was defined as: CVR = (MAP – SSP)/CBF.

Results

CBF was always measured at least 20 minutes after the indomethacin infusion was begun. Indomethacin, by itself, had no effect on arterial PCO₂ or end-tidal CO₂ tension. Approximately 1 minute after the intracarotid indomethacin infusion was started the sagittal sinus pressure (SSP) began to decrease, the maximum decrease being seen about 10–15 minutes later.

The autoregulatory response of the cerebral circulation obtained prior to indomethacin infusion can be seen in the upper section of Figure 1. Intracarotid indomethacin infusion significantly reduced the absolute value of CBF at all levels of MAP by an average of 32%. The mean and standard errors for all the observations of CBF and calculated CVR in each bin are listed in Table 1, which also includes the results of the Student’s analyses. The bin with 61–70 mm Hg was used for comparison with all the other groups to provide the most stringent condition for this test. Within the range 61–120 mm Hg there was no statistical evidence for a relative change in CBF with changing perfusion pressure, either before or after indomethacin, although the CBF in the bin with 121–130 mm Hg was significantly higher than that of the bin with 61–70 mm Hg in the indomethacin-treated baboons. However, linear regression analysis indicated that the overall slope of this CVR/MAP relationship was not significantly altered by indomethacin; only the intercept was changed.

The regression equations found for CVR against MAP were:

Control: CVR = 0.42 + 0.010 MAP (r = 0.52; P < 0.001).

Indomethacin: CVR = 0.73 + 0.013 MAP (r = 0.62; P < 0.001).

The gradients of these regression equations are not significantly different on a test of covariance (F = 0.75; P, not significant) (Fig. 2).

No significant changes in cerebral oxygen consumption

### Table 1 Values of Cerebral Blood Flow and Cerebrovascular Resistance at Different Levels of Mean Arterial Blood Pressure (MAP) before and during Intracarotid Infusions of Indomethacin

<table>
<thead>
<tr>
<th>MAP bins (mm Hg)</th>
<th>Cerebral blood flow (ml/100 g per min)</th>
<th>Cerebrovascular resistance (mm Hg/ml per 100 g per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>61-70</td>
<td>Control Mean 59.7 ± 5.8 NS</td>
<td>Indomethacin† Mean 41.2 ± 3.8 NS</td>
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<td>71-80</td>
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<td>111-120</td>
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<td>121-130</td>
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</tbody>
</table>

* P value, when compared to the bin with 61–70 mm Hg. NS = not significant.
† Indomethacin infusion = 0.2 mg/kg per min; total dose of 10–36 mg/kg.
occurred, either at differing levels of MAP or between control and indomethacin values at any given MAP (Table 2). It was noticeable, however, that during indomethacin infusion the cerebral oxygen consumption, at the lower levels of MAP, was reduced from baseline values. This might be ascribed to methodological errors in the estimation of low oxygen saturation (in this instance, venous saturation) with the instrument used (Kipp and Zonen). However, reduction in cerebral oxygen consumption, with decreasing perfusion pressure, has been noted in other animal models and may not be an artifact. This point requires further elucidation.

Carotid angiography in three baboons demonstrated that indomethacin had no major occlusive effect on the larger visible intracranial vessels, even during hypercapnia when its effect on CBF was greatest.

Discussion

The results of this study demonstrate that indomethacin, a potent inhibitor of prostaglandin synthesis, does not affect the cerebral circulatory response to changing perfusion pressure, although the absolute values of CBF are lowered. The upper limit of autoregulation possibly was lowered during the infusion of indomethacin, but this effect might be seen with all vasoconstrictor agents, since the capacity for further vasoconstriction could be limited. It is not clear what criteria can be used to identify a minor impairment of autoregulation in a vasoconstricted vascular bed. Perhaps comparison with the cerebral circulation constricted by hypocapnia would be a suitable test: however, the upper limit for autoregulation during hypocapnia is not accurately known. The methods used in the present study cannot be used to demonstrate whether transient cerebrovascular responses to changes in perfusion pressure were affected by indomethacin.

Other conditions, such as carotid ligation and serotonin-induced internal carotid artery spasm, which impair the cerebral circulatory CO$_2$ response, tend to abolish or radically alter the vascular responses to changes in perfusion pressure within the brain. Even so, there was no angiographic evidence to suggest that any occlusion of either the internal carotid artery or the major intracranial vessels resulted from the intracarotid administration indomethacin.

Fujishima and his colleagues have shown that a reduction in brain metabolism is associated with not only a reduced CBF but also a markedly reduced response to CO$_2$, a finding that recently has been confirmed. However, indomethacin would appear to have no effect on the overall metabolic activity of the brain and thus reduced cerebral metabolism cannot account for the dissociation between the effect of indomethacin on the CO$_2$ response and autoregulation.

Despite the poor penetration of indomethacin into the brain (blood-brain partition coefficient of 0.02) and its high binding to plasma proteins (90%), the dose of indomethacin used in these experiments should be sufficient to block prostaglandin synthesis within the brain (concentration required to inhibit 50% of the prostaglandin synthetase activity in rabbit brain homogenates is 3.6 µM). This has been demonstrated in the spinal cord and cerebrospinal fluid of the ca and cerebral cortex of the rat. It remains to be demonstrated that this is true in the baboon.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Values of Cerebral Oxygen Consumption at Different Levels of Mean Arterial Blood Pressure (MAP) before and during Intracarotid Infusions of Indomethacin</th>
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</thead>
<tbody>
<tr>
<td>MAP bins (mm Hg)</td>
<td>61-70</td>
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<tr>
<td>Control</td>
<td>Mean</td>
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<td>± SEM</td>
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<td>Indomethacin*</td>
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<td>± SEM</td>
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<td>P*</td>
<td>&lt;0.05</td>
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</table>

* Indomethacin infusion = 0.2 mg/kg per min; total dose of 10-36 mg/kg.
† P values for control vs. indomethacin. NS = not significant.
The time course of on and off effects on blood flow was similar in brain to that described for the kidney, where it is possible to demonstrate that the time course of the reduction in prostaglandin output mirrors that of reduction in blood flow. A preliminary report by Vlahov suggests that prostaglandin F2α will reverse the effect of indomethacin on CBF in the cat. Our own unpublished data indicate that niflumic acid (Squibb), an inhibitor of prostaglandin synthesis that is chemically unrelated to indomethacin, mimics the action of the latter agent on the cerebral circulation.

Of the other, reported actions of indomethacin, none appears to explain the effect of indomethacin on the cerebral circulation. Both Flower and Pace-Acsiak and Cole draw attention to the fact that indomethacin can influence a variety of prostaglandin-metabolizing enzymes. The large reduction in levels of prostaglandins produced by indomethacin in the central nervous system of cat and rat suggest that this is not, overall, the significant mechanism. Furthermore, the prostaglandin dehydrogenases, whose inhibition would directly affect levels of active prostaglandins, are not affected except at much higher doses of indomethacin than enzymes that act later in the degradative pathway. As recently discussed by Flower and by Ryan and Zimmerman, all other effects of indomethacin occur at much higher doses. Of those actions not mentioned by these authors, the effects on calcium and other ionic fluxes in various human and guinea pig tissues required indomethacin concentrations of the order of 0.5 mM. Non-narcotic analgesics, including indomethacin, increase the potassium permeability and decrease the chloride permeability in molluscan neurons, but again concentrations around 1 mM were required.

Such membrane phenomena — when applied to the in vivo situation — would predict that indomethacin would result in vasodilation, not vasoconstriction as observed in the present study.

Both Tuvemo and Wide and Terragno and co-workers have demonstrated vascular wall synthesis of prostaglandins; when this synthesis is blocked by indomethacin, the tone of the vessel is reduced. Very similar findings have been noted in the bovine middle cerebral artery. It has been shown previously that indomethacin can effectively block the cerebrovascular responses to hypercapnia. Accordingly, we think it is unlikely that carbon dioxide (or the interstitial fluid hydrogen ion concentration) can be involved in the cerebrovascular response to changing perfusion pressure. However, this finding does not preclude other metabolic mediators such as potassium, adenosine, or other nucleotides from being involved in autoregulation.

Acknowledgments

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