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COMMUNICATIONS

The role of adenosine in cerebral vascular regulation during reductions in perfusion pressure

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The adenosine antagonist caffeine was used to test the hypothesis that endogenous adenosine contributes to the regulation of cerebral blood flow during acute reductions in mean arterial blood pressure. Caffeine, administered intraperitoneally in 20 and 40 mg kg^{-1} doses, failed to alter the autoregulatory flow responses to marked reductions in arterial blood pressure, showing that adenosine is not essential for cerebrovascular regulation during severe hypotension.

Cerebral blood flow (CBF) is able to respond rapidly to alterations in mean arterial blood pressure (MABP) so that flow is maintained relatively constant over a wide range of perfusion pressures. Under steady state conditions, in anaesthetized as well as conscious adult animals, autoregulatory mechanisms prevent CBF from changing appreciably over the arterial pressure range of approximately 60–150 mmHg (Busija & Heistad 1984). Below 60 mmHg pressure, there may be a decrease in CBF, even though the cerebral resistance vessels have dilated. The mechanism whereby cerebral autoregulation of blood flow occurs is unknown, but myogenic, neurogenic and metabolic theories have been proposed (Purves 1972; Kontos 1981; Busija & Heistad 1984). According to the metabolic hypothesis, a reduction in blood flow results in the release of a chemical factor that elicits dilation of cerebral vessels. Adenosine has been proposed as a possible metabolic regulator of cerebrovascular resistance. Adenosine is produced in the rat brain during (5 min) periods of sustained reduction in arterial blood pressure (Winn et al 1980). When MABP was decreased from 135 to 72 mmHg, within the range of cerebral autoregulation, brain adenosine concentrations doubled. With moderate to severe hypotension (MABP = 45 mmHg), brain adenosine levels increased almost six-fold. Adenosine is a potent vasodilator when applied topically to pial vessels (Berne et al 1974; Wahl & Kuschinsky 1976); and it has been proposed that adenosine plays an important role in regulating CBF during hypoxic and hypercapnic episodes (Busija & Heistad 1984; Phillis et al 1984, 1985a, b).

In the present study, we have used the adenosine antagonist, caffeine, to investigate the relationship between CBF regulation during reduced perfusion pressure and adenosine production. The hypothesis that adenosine regulates cerebrovascular resistance predicts that blockade of the central vascular adenosine receptors should reduce autoregulation, especially during severe hypotension when the increases in brain adenosine are most pronounced. Caffeine did not alter arterial pressure flow relationships, suggesting that adenosine is not essential for CBF regulation during acute hypotensive episodes. A venous outflow technique (Phillis et al 1984, 1985a) has been used to monitor changes in cerebral blood flow.

Methods

Studies were performed on eight male Sprague-Dawley rats (Charles River; 350–400 g). Anaesthesia was induced with 2–3% halothane to allow tracheotomy, and the animals were then maintained during surgery on a mixture of nitrogen (70%), oxygen (30%) and methoxyflurane. Body temperature was maintained at 37 °C by a heating pad controlled by a rectal probe. Both femoral arteries were cannulated and used for the continuous recording of arterial blood pressure, and to obtain arterial blood for the measurement of arterial O_2 tension (P_aO_2), arterial CO_2 tension (P_aCO_2) and pH. A femoral vein cannula was inserted to allow the return of cerebral venous blood to the animal. The animal was heparinized (1 u g^{-1} weight).

The retroglenoid veins were exposed bilaterally. The right vein was eventually tied off on the postglenoid foramen side of the large facial retroauricular veins that drain into it. The left vein was exposed and cannulated with an angiocatheter (Deseret 22 gauge, 1 in). The cannula tip was adjusted to be adjacent to the postglenoid foramen through which the transverse sinus drains into the vein. The angiocatheter was then tied into place with a ligature that prevented the contamination of cerebral venous blood with extracranial blood. Venous blood from the retroglenoid vein flowed through a drop counter and was then returned to the animal via a roller pump and the femoral cannula. A second angiocatheter was inserted into the cisterna magna for the recording of cerebrospinal fluid (CSF) pressure. (For further details of the surgical preparation see Phillis et al 1984, 1985a.)

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Pancuronium bromide (Pavulon, 1 mg kg^{-1}) was administered intravenously, and the animal connected to a ventilator and respired at a frequency of 70 strokes min^{-1} with a variable gas mixture of methoxyflurane (0·3%) in 30–40% oxygen in nitrogen. Arterial blood gases and pH were measured and ventilation was adjusted to obtain adequate blood–gas tensions. A period of 20 min was allowed for blood pressure and blood gas stabilization. Mean control arterial blood pressures ranged between 105 and 140 mmHg. Additional doses of heparin and pancuronium bromide were administered as required.

Following stabilization, the animals were administered a series of hypotensive challenges. Arterial blood (6 ml) was withdrawn slowly through an arterial cannula over 1 min. This was sufficient to reduce the mean arterial blood pressure of the eight animals used in this series from a control level of 120·2 ± 5·7 mmHg to 39·6 ± 2·75 mmHg (mean ± s.e.m.). Blood pressure was held constant at the reduced level for 2 min. Blood was then slowly reinjected over 1 min. In subsequent hypotensive episodes, the volume of blood withdrawn was adjusted, if necessary, to obtain the same fall in MABP as in the initial, control challenge. An interval of at least 15 min was allowed between successive hypotensive challenges.

Drop rate, arterial blood pressure and CSF pressure were recorded on a Grass polygraph. Drop rates were calculated from the polygraph records and converted into flow rates per 100 g brain min^{-1} using previously published conversion data (Phillis et al 1984).

In evaluating the effects of caffeine on CBF autoregulation, the response to a hypotensive challenge immediately preceding drug administration was used as a control and compared with the responses following intraperitoneal caffeine injection. Caffeine was administered intraperitoneally to all animals in two doses. After an initial dose of 20 mg kg^{-1}, the animal was exposed to two hypotensive episodes and then a further 40 mg kg^{-1} was administered and the responses to two further hypotensive challenges recorded. The data was analysed using a Student's *t*-test for paired data. Results are presented as means ± s.e.m.

Results

Blood gases in the normotensive animals were P_aO_2 139·2 ± 3·6 mmHg, P_aCO_2 30·6 ± 0·9 mmHg with a pH of 7·48 ± 0·01. When measured during the second minute of the stable phase of the hypotensive challenge, blood P_aCO_2 and pH were unaltered (P_aCO_2 30·0 ± 2·0 mmHg; pH 7·43 ± 0·02), whereas P_aO_2 tensions were significantly reduced (112·3 ± 8·8 mmHg; $P < 0·05$). CSF pressures, as measured by an angiocatheter inserted into the cisterna magna, were invariably low (<5 mmHg) and were further reduced during hypotensive episodes. A typical sequence of events during a hypotensive episode is illustrated in Fig. 1. During the 1 min period of blood withdrawal, the cerebral venous flow rate declined to 43% and then slowly increased during the stable phase of the challenge to 60% of the control flow rate. During the reinfusion period, flow rates increased rapidly, ultimately exceeding control flow rates by 22%. Flow rates then returned to control levels during the subsequent 3 min.

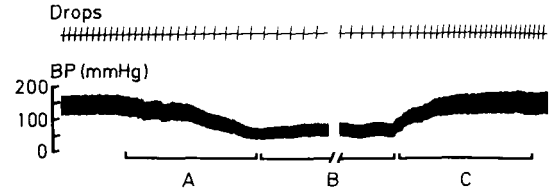

FIG. 1. Responses of cerebral blood flow (venous ouflow in drops) and arterial blood pressure to the withdrawal and reinfusion of 6 ml of blood. Period A, 6 ml of blood withdrawn in 1 min; Period B, 2 min hypotensive phase; period C, blood replaced in 1 min.

The animals studied showed a mean baseline cerebral blood flow of 28·5 ± 3·1 ml/100 g brain min^{-1} (Table 1). During the second minute of the stable phase of the hypotensive episode flow was reduced to 20·3 ± 2·1 ml/100 g min^{-1}. Upon re-infusion of the blood, CBF increased rapidly and was elevated above baseline flow rates during the initial two 1 min periods (Table 1). Flow rates returned to control levels during the subsequent 2–3 min.

Table 1. Effect of caffeine on cerebral blood flow (ml/100 g brain min^{-1}) during and after reductions in perfusion pressure (mean ± s.e.m.).

	Basal flow rate n = 8	During 2nd min of hypotension	During 1st min after re-infusion	During 2nd min after re-infusion
Pre-caffeine	28·5 ± 3·1 (4·5 ± 0·5)*	20·3 ± 2·1 (2·0) ± 0·2)†	35·5 ± 3·1 (3·7 ± 0·4)	31·0 ± 3·0 (4·1 ± 0·5)
Post-caffeine 20 mg kg^{-1}	29·4 ± 2·2**	20·7 ± 1·6**	38·7 ± 2·8**	30·4 ± 2·7**
Post-caffeine 60 mg kg^{-1}	25·6 ± 3·1**	18·5 ± 1·9**	38·6 ± 3·0**	33·0 ± 2·4**

* Figures in parentheses represent cerebral vascular resistances (CVR) calculated according to formula CVR = MABP/CBF.
** Flow rates after caffeine are not significantly different from pre-caffeine rates.
† CVR significantly reduced from control value ($P < 0·01$).

Table 1 also presents the mean changes in cerebrovascular resistance (CVR) calculated for each phase of the response to a hypotensive challenge. During the second minute of the stable phase of the pressure reduction, CVR was significantly decreased from control ($P < 0·01$), demonstrating relaxation of cerebral resistance vessels. Some relaxation of cerebral resistance vessels, as measured by CVR values, continued into the immediate post-hypotensive period, although these CVR values were not significantly ($P > 0·1$) different from control (Table 1).

Caffeine (20 mg kg^{-1}) did not alter cerebral venous flow rates in the normotensive animals (Table 1). After a total dose of 60 mg kg^{-1}, there was a small (but not

significant) decline in CBF. The effects of caffeine on cerebral venous flow rates during and immediately following a hypotensive episode are presented in Table 1. Caffeine did not alter the flow response to pressure change during the hypotensive episode, nor did it affect the intensity of the reactive hyperaemia upon reinfusion of blood.

Discussion

The major finding of this study is that the administration of an adenosine antagonist, caffeine, did not affect the cerebral vascular pressure–flow relationship when arterial pressure was reduced to 40 mmHg, even though a hypotensive challenge of this magnitude has been shown to increase brain adenosine levels six-fold (Winn et al 1980). Methylxanthine antagonism of adenosine in a variety of experimental situations, including cerebral vessels, has been extensively documented (Hardebo & Edvinsson 1979; Hirsh 1984). Caffeine was selected for use in our experiments on CBF both because it penetrates the blood brain barrier more readily than other methylxanthines such as theophylline (Sattin 1971), and also because it is the methylxanthine found in the highest quantities in popular beverages such as tea, coffee, colas and a number of medications.

The use of caffeine to probe the participation of adenosine in cerebral vascular autoregulation raises the question of whether caffeine is an effective adenosine antagonist at the dose levels used. Hirsh (1984), has identified a 20 mg kg^{-1} dose as being the maximal amount of caffeine that can be administered without incurring the risks of actions unrelated to adenosine antagonism. This, therefore, was the initial dose administered to our animals. In the light of its failure to alter the autoregulatory response, a second large dose (40 mg kg^{-1}) was administered to each animal.

We have already reported that, when used at these doses (20 and 40 mg kg^{-1}), caffeine, although not affecting basal flow rates, severely attenuates the reactive hyperaemia produced by an anoxic challenge to rats in comparable experiments (Phillis et al 1984), and we have also observed that caffeine (10 and 20 mg kg^{-1}) markedly attenuates the increase in CBF in rats exposed to 10% carbon dioxide in the inspired air (Phillis et al 1985b). There is further evidence to suggest that the increases in CBF during anoxia and hypercapnia involve adenosine release. Agents which enhance adenosine levels (uptake inhibitors, adenosine deaminase inhibitors) potentiate the reactive hyperaemia elicited by an anoxic challenge to rats using the same model (Phillis et al 1984, 1985a) and the increase in CBF elicited during hypercapnia (Phillis et al 1985b). On the basis of these positive results, it would appear that the failure of caffeine to affect the response evoked by hypotension is a valid indication that adenosine does not play a major role in the regulation of CBF during severe hypotension. This conclusion is somewhat surprising in the light of published findings of a large increase in brain adenosine during sustained (5 min), severe, alterations in systemic blood pressure (Winn et al 1980). The longer duration of the hypotensive challenge (5 min versus 2 min in the present experiments) used by Winn et al (1980) may furnish one explanation for this discrepancy. An alternative suggestion, which can reconcile the findings of the present experiments with those of Winn et al (1980), is that adenosine triphosphate rather than adenosine may be the primary purine involved in the autoregulation of CBF during hypotension (Burnstock 1982). The increased levels of brain adenosine measured after decreases in blood pressure may reflect an increased release of ATP and consequently higher levels of its breakdown product, adenosine. The present findings are, however, consistent with recent reports that adenosine is not essential for coronary vessel autoregulation during reductions in perfusion pressure (Gewirtz et al 1983; Hanley et al 1984; Dole et al 1985) even though it appears to be involved in myocardial reactive hyperaemia following coronary occlusion (Saito et al 1981; Dole et al 1985).

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