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ADRENOCEPTORS IN INTRACEREBRAL RESISTANCE VESSELS

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1 The effects of tyramine and isoprenaline on hypothalamic blood flow (HBF) were measured in conscious rabbits.

2 Injections of small doses of tyramine caused an increase in HBF while larger doses caused a decrease in HBF.

3 Isoprenaline injections also produced an increase in HBF.

4 The vasodilatation induced by isoprenaline and the small dose of tyramine was blocked by propranolol.

5 The vasoconstriction induced by the larger doses of tyramine was abolished by phenoxybenzamine.

6 Chemical sympathectomy of the hypothalamus with 6-hydroxydopamine and depletion of biogenic amines by reserpine also abolished tyramine-induced vasoconstriction.

7 These results suggest the presence of α- and β-adrenoceptors in cerebral resistance vessels, and that these receptors may be activated by released (endogenous) noradrenaline.

Introduction

The functional importance of the cerebrovascular nerves is a subject of considerable controversy. Early observations around the turn of the century (Bayliss, Hill & Gulland, 1895; Hill & MacLeod, 1901) suggested that these vasomotor nerves were of minor importance in the regulation of cerebral blood flow. This view is still widely held.

Removal of the superior cervical ganglion appears to have little effect on total cerebral blood flow (Shackleford & Hegedus, 1966; Waltz, Yamaguchi & Regli, 1971a), or on the ability of the cerebral vasculature to autoregulate (Eklöf, Ingvar, Kagström & Olin, 1971) or respond to CO_2 (Waltz, Yamaguchi & Regli, 1971b). On the other hand, James, Millar & Purves (1969) have demonstrated a decrease in cerebral blood flow following stimulation of the cervical sympathetic nerves, and an enhancement of the cerebrovascular response to CO_2 following cervical sympathectomy. Also, D'Alecy & Feigl (1972) reported significant cerebral vasoconstriction following stimulation of the stellate ganglion in the anaesthetized dog; this response was blocked by α-adrenoceptor antagonists (D'Alecy, 1973).

The results of experiments with vasoactive drugs are also conflicting. Carpi (1972) reviewed 42 papers in which the effect of adrenaline or noradrenaline (NA) on cerebral blood flow was examined. No consistent pattern emerges. If anything, more authors have reported a vasodilator effect of these two drugs than vasoconstriction. Most of these experiments have involved the intravenous or intracarotid infusion of catecholamines, often in anaesthetized animals. Some of the variability in the results may be due to the anaesthetics used, to extra-cranial effects particularly where there are rich anastomotic channels between the intra- and extra-cranial vessels, to autoregulatory responses to the systemic blood pressure effects of these drugs particularly when given intravenously, or possibly, to difficulty of access of these drugs to adrenoceptors which probably lie on the adventitial side of the media of the resistance vessels. Also, there is rapid and avid binding of NA by the nerves which supply cerebral blood vessels (Rosenblum, 1973), so that less may be available for stimulation of NA receptor sites on the vessels. Another possibility applicable to both sympathetic stimulation and catecholamine infusion, is that the effects may be non-uniform,

i.e. involve a redistribution of blood flow from some areas to others. In this situation measurement of total cerebral blood flow is useless.

In experiments designed to assess the effect of local injections of adrenoceptor agonists on hypothalamic blood flow (HBF) in conscious rabbits, it has been shown (Rosendorff, 1972) that small doses of NA cause vasodilatation and larger doses local vasoconstriction. These effects were abolished by the addition of β- or α-adrenoceptor antagonists respectively, suggesting that there are adrenoceptors of both types in cerebral resistance vessels. However, the doses used were likely to produce local concentrations of NA considerably higher than those following the release of endogenous transmitter. We have, therefore, attempted to answer the question: Do adrenoceptors in or around resistance vessels within the brain respond to released endogenous transmitter?

Methods

The area of brain studied was the hypothalamus, chosen because it is a homogeneously perfused region rich in grey matter with a relatively high concentration of endogenous NA. The technique for measuring local blood flow in the hypothalamus of the conscious rabbit has been described in detail elsewhere (Cranston & Rosendorff, 1971; Rosendorff & Cranston, 1971; Rosendorff, 1972). The method is a modification of that described by Monnier & Gangloff (1961); headplates with holes drilled at 3 mm intervals were screwed to the skulls of New Zealand White rabbits anaesthetized with pentobarbitone and weighing 2.5-3.0 kg. Holes were also drilled through the skull at co-ordinates aA and aB (Monnier & Gangloff, 1961) on both sides.

At the time of the experiments, not less than a week later, injection cannulae were placed so that their tips lay in identical positions in the hypothalamus on either side of the midline. Local blood flow was determined by injection of 15 μCi of ^{133} xenon dissolved in 5 μl of 0.9% w/v NaCl solution (saline) into the hypothalamus. Following each injection, the clearance of the radioactive isotope was measured with an external, collimated scintillation detector, a ratemeter and pulse-height analyser, a logic and routing assembly, and recorded on an ultraviolet recorder, teletype printer and punched tape. The hypothalamic blood flow (HBF, as ml 100 g^{-1} min^{-1}) was then computed from the formula, $HBF = \lambda \cdot \log_e 2/T\frac{1}{2}$, where $\lambda = 0.74$, the brain tissue to blood partition coefficient for the rabbit hypothalamus (Rosendorff & Luff, 1970), and $T\frac{1}{2}$ = the half-decay time of the clearance curve. HBF values were obtained from an IBM 360/50 computer programmed to produce a best-fit regression of log counts above background vs time (0 to 100 seconds). Analysis of HBF values was in all cases done from monoexponential clearance curves. Curves with straight line correlation coefficients of less than 0.85 were rejected. This is considerably more rigorous than the 0.77 required for significance at the 1% level of confidence. This ensured that only steady-state flows, at least during the period of xenon clearance, were analysed. The number of flow rates measured was 548 of which only 31 (5.7%) were rejected because of a correlation coefficient less than 0.85.

One side of the hypothalamus was the control side (^{133}Xe in saline only) and the other side was the test side (^{133}Xe in saline plus the tests substance). Injections were made into each side alternately, at intervals of at least 10 minutes. Changes in blood flow on the test side were calculated by subtraction of the mean of the control flows preceding and succeeding the test flow, from the test flow. The change in flow was then expressed as a positive or negative value relative to the control flows. Three to eight injections were made into each side during any one experiment. Previously (Rosendorff, 1972) it has been shown that there was no systematic difference in HBF between the two sides of the midline if no vasoactive substances were injected. Also it has been demonstrated that, at least within the volume of distribution of the injected ^{133}Xe, hypothalamic resistance vessels autoregulate within the mean arterial blood pressure range of 40-140 mmHg and retain CO_2-sensitivity (Cranston & Rosendorff, 1971).

Drugs

The drugs tested were the β-adrenoceptor agonist isoprenaline (Isuprel, Winthrop), the β-adrenoceptor blocker propranolol (Inderal, I.C.I.), the α-adrenoceptor blocker phenoxybenzamine (Dibenyline, Smith, Kline & French) and tyramine (4-hydroxyphenethylamine, Sigma). Tyramine causes the release of endogenous NA from noradrenergic nerve terminals (Burn & Rand, 1958). In some experiments, detailed below, local chemical sympathectomy was performed by the use of 6-hydroxydopamine (Sigma), and in others, reserpine (Serpasil, Ciba) was used to deplete nerve terminals of biogenic amines (Rosenblum, 1973).

Results

Isoprenaline

The presence of a β-receptor mediated vasodilatation was established by injection of isoprenaline

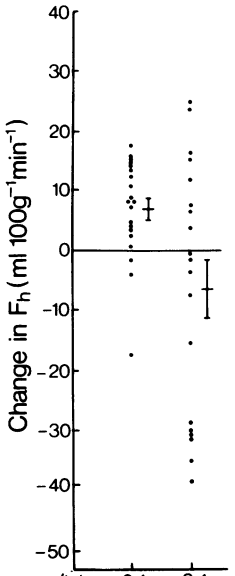

Figure 1 Effect of isoprenaline on hypothalamic blood flow (F_h) in the conscious rabbit. Abscissae: dose of isoprenaline, and isoprenaline plus 20 μg propranolol (Prop). Ordinates: F_h compared to mean control values (see text). Note that propranolol abolishes vasodilatation.
n = number of ^{133}Xe curves on the test side; $\bar{x}$ = mean F_h; P = significance level for the difference of each test mean from control mean (= 0) by Student's t test.

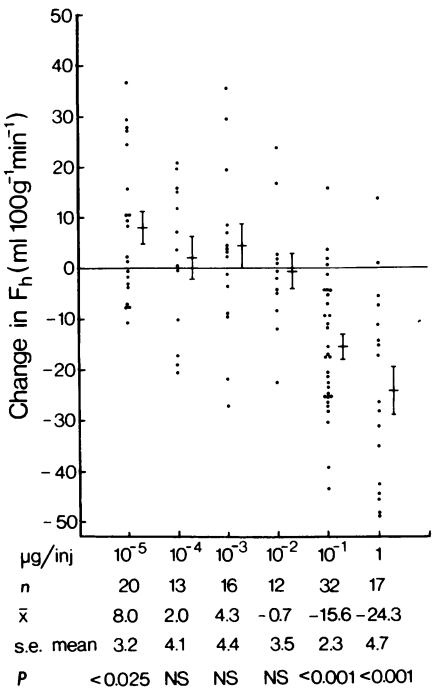

Figure 2 Effect of tyramine on hypothalamic blood flow (F_h) in the conscious rabbit. Abscissae: dose of tyramine in μg per injection. Ordinates: F_h compared to control values (see text). Vertical bars represent the s.e. mean. Note that the 10^{-5} μg (10 pg) per injection dose of tyramine causes an increase in mean F_h, while the 10^{-1} (100 ng) and 1.0 μg per injection doses cause a decrease in mean F_h.
n = number of ^{133}Xe curves on test side; $\bar{x}$ = mean F_h; P = significance level for the difference of each mean from control flows (= 0) by Student's t test.

(0.4 μg/injection). Isoprenaline produced a significant ($P < 0.005$) mean increase of 6.9 ml 100 g tissue^{-1} min^{-1} in HBF (Figure 1). This effect was abolished by the addition of 20 μg of propranolol to the injectate (Figure 1).

Tyramine

Figure 2 shows the dose-response relationship for tyramine. The smallest dose tested (10 pg per injection) produced an increase in mean HBF of 8.0 ml 100 g^{-1} min^{-1}, while doses of 100 ng and 1.0 μg per injection reduced HBF by mean values of 15.6 and 24.3 ml 100 g^{-1} min^{-1}, respectively. Intermediate doses had no significant effect.

The increase in HBF produced by the 10 pg dose of tyramine could be abolished by the addition of 20 μg propranolol to the injectate. The flow was reduced to 8.5 ml 100 g^{-1} min^{-1} less than the control side, which was significantly different ($P < 0.02$) from the response produced by the 10 pg dose of tyramine alone (Figure 3). This result suggests that a β-adrenoceptor responds to endogenous noradrenaline released from nerve terminals by small doses of tyramine.

The vasoconstrictor effect of 100 ng tyramine per injection was abolished, and possibly reversed, by the addition of 50 μg of phenoxybenzamine to the injectate (Figure 3). After the addition of phenoxybenzamine to the injectate the HBF increased to a mean value of 13.2 ml 100 g^{-1} min^{-1} ($P < 0.025$) greater than control flows. Depletion of biogenic amine stores by daily intramuscular injection of reserpine 1 mg/kg for three days also abolished the vasoconstrictor effect of tyramine as did 6-hydroxydopamine (300 μg)

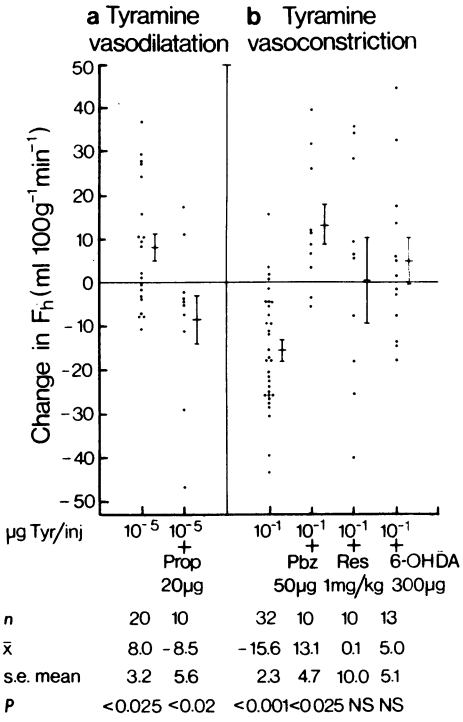

Figure 3 (a) Effect of propranolol (Prop 20 μg per injection) added to the 10^{-5} μg (10 pg) tyramine (Tyr) injection. The mean F_h is changed from +8.0 ± 3.2 ml 100 g^{-1} min^{-1} to −8.5 ± 5.6 ml 100 g^{-1} min^{-1}. These two results differ significantly ($P < 0.02$).
(b) Effect of phenoxybenzamine (Pbz 50 μg per injection) added to the 10^{-1} μg (100 ng) tyramine injection. The mean F_h is changed from −15.6 ± 2.3 ml 100 g^{-1} min^{-1} (which is significantly less than control = 0, $P < 0.001$, $n = 32$) to +13.1 ± 4.7 ml 100 g^{-1} min^{-1} (which is greater than control = 0, $P < 0.025$, $n = 10$). Pretreatment with reserpine or 6-hydroxydopamine (6-OHDA) (see text), abolishes the vasoconstrictor effect of 10^{-1} μg of tyramine.

injected into the hypothalamus 4 days before the experiment. These results suggest that vasoconstriction is dependent on α-adrenoceptor activation by NA released from nerve terminals by the larger doses of tyramine.

Discussion

Pial and other arteries have a dense adrenergic innervation and respond to sympathetic stimulation and denervation. The major controversy relates to the intracerebral (intraparenchymal) resistance vessels. Harper, Deshmukh, Rowan & Jennett (1972) have suggested that there is no autonomic control of the intracerebral vessels, and that these vessels are influenced only by metabolic factors. Sympathetic stimulation would result in constriction of extracerebral vessels only, and since extra- and intracerebral resistance vessels are in series, this will cause a fall in intracerebral arterial pressure. An autoregulatory adjustment would then cause the intracerebral vessels to dilate, and this tends to maintain an adequate flow. One piece of evidence in support of this idea is that the cerebral blood flow response to sympathetic stimulation would be more marked when the intracerebral vascular resistance was already reduced, e.g. by hypercapnia. Several studies (Meyer, Gotoh, Akiyama & Yoshitake, 1967; James *et al.*, 1969; Harper *et al.*, 1972) have confirmed this.

However, we have demonstrated both previously and in this study, the existence of adrenoceptors associated with intracerebral vessels at least in the hypothalamus. The present results also suggest that these receptors respond to the release of endogenous NA. The mechanism of this response is still unresolved. It is possible that there are both α- and β-receptors on or close to the vascular smooth muscle. There is, however, an alternative explanation for these findings. Recent studies have shown that NA, applied to neurones by microiontophoresis, may increase (Boakes, Bradley, Brooks & Wolstencroft, 1968; Johnson, Roberts & Straughan, 1969) or decrease (Biscoe & Straughan, 1966; Phillis & York, 1967) the firing rate. Neuronal excitation has also been reported with isoprenaline (Johnson, Roberts, Sobieszek & Straughan, 1969), and NA excitation may be blocked by α- and β-adrenoceptor antagonists, and more consistently with β-antagonists (Brawley & Johnson, 1973).

The results described in this paper are therefore also compatible with the idea that one or both of the α- and β-adrenoceptors are extravascular, possibly on neuronal membranes, and that the action of the α- and β-stimulating agents may be primarily on neurones. The vasomotor effects might be secondary to a change in local metabolic activity induced by changes in neurone firing rates. This hypothesis is more likely to apply to the β-mediated vasodilatation; NA-induced inhibition of neurones sufficient to cause the large reduction in HBF of 15.6 ml 100 g^{-1} min^{-1} is probably less likely. This approach has, on the other hand, been criticized by Stone (1971) who suggests that the neuronal effects are secondary to the changes in vascular calibre. However, irrespective of the

mechanism our results suggest that intracerebral blood flow, at least in the hypothalamus, is likely to be affected by released transmitter from noradrenergic nerve terminals.

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