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Heterogeneous vasomotor responses of anatomically distinct feline cerebral arteries

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1 The vasomotor reactivity to a number of neurotransmitters and blood-borne substances was evaluated in several anatomically distinct arteries of the cat cerebral circulation. Few regional differences were observed in their vasoconstrictor responses to noradrenaline, dopamine, 5-hydroxytryptamine and prostaglandin $F_{2\alpha}$. Only the anterior cerebral artery reacted strongly to all vasoconstrictor agents.

2 Adenosine, acetylcholine and histamine induced pronounced relaxation in the vast majority of the major cerebral arteries. The relaxation elicited by adenosine showed a slight degree of heterogeneity between the arteries and the overall response accounted for $81 \pm 6\%$ of the pharmacologically-induced tone. On the other hand, the dilatation induced by acetylcholine and histamine varied as a function of the anatomical localization of the cerebral arteries. The acetylcholine-induced vasodilatation was significantly more pronounced in the middle cerebral, anterior communicating and anterior cerebellar arteries, with respective responses of 72, 66 and 83% of the induced tone as compared to 43% in the other vessels. However, all arteries were equally sensitive to acetylcholine with an overall mean pD_2 value of 7.47 ± 0.06. The most heterogeneous results were obtained with histamine and applied both to the magnitude of the maximal response and the sensitivity of the various arteries to this amine. The intensity of the relaxation varied from 20% (anterior communicating artery) to 118% (posterior cerebellar artery).

3 Among the neuropeptides studied, substance P and bradykinin were considerably less potent than vasoactive intestinal peptide on all the cerebral arteries. The least responsive vessel to bradykinin was the anterior cerebral artery with a maximal response of $22 \pm 5\%$ of the induced-tone and a pD_2 value of 7.56 ± 0.24. All vessels responded weakly to substance P and those from the vertebrobasilar circulation were significantly less sensitive to this neuropeptide with pD_2 values around 8.07 as compared to 9.82 in the more rostral arteries. Although all vessels were equally sensitive to vasoactive intestinal peptide, the dilator responses were significantly less pronounced in the middle cerebral and basilar arteries (maximal response of $86 \pm 5\%$ and $69 \pm 6\%$ of the induced-tone, respectively, as compared to $110 \pm 9\%$ in the other vessels).

4 The vertebrobasilar arteries were as reactive, if not more reactive, to vasoconstrictors than the vessels originating from the carotid circulation. In contrast, the dilator responses were less marked in most caudal arteries. Such dichotomies may be important in the regulation of local cerebral blood flow.

5 The results emphasize the considerable heterogeneity in the vasomotor responses to a given substance among the various cerebral arteries. Further, they suggest the presence of multiple receptor populations which mediate opposite effects and which are distributed in different proportions among the cephalic arteries.

Introduction

The cerebral circulation is innervated by a multiplicity of neurotransmitter systems (for review, see MacKenzie & Scatton, 1987), the distribution of which is heterogeneous throughout the various components of the cerebrovascular bed. For example, noradrenergic nerves are very dense in rostral, as opposed to caudal arteries of the circle of Willis and comparatively few fibres are associated with small

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pial arterioles (Edvinsson & MacKenzie, 1976; Kobayashi et al., 1981). Similarly, the cholinergic innervation is rather sparse in the vertebrobasilar vessels when compared to both those intracranial arteries that originate from the internal carotid artery (Edvinsson et al., 1972; Saito et al., 1985) and the small pial vessels (Hamel et al., 1986). Other than differences based purely on the anatomical location of the artery, both neurotransmitter concentrations and the density of innervation may be a function of vessel calibre within the cerebrovascular bed; such is the case for 5-hydroxytryptamine (Edvinsson et al., 1983a; 1984; Scatton et al., 1985). Another characteristic of the cerebral arteries resides in the heterogeneity of the vascular responses to agonists and antagonists not only between species (Chiba & Tsuji, 1985; Hamel et al., 1985; Sasaki et al., 1985; Usui et al., 1985) but also between anatomically distinct vessels (Toda, 1976) and, further, between different segments of the same vessel in a given species (Toda et al., 1984).

These various discrepancies have been attributed to differences either in the size of the vessels, the anatomical and/or physiological integrity of the arteries or to the methodological approaches employed. However, the heterogeneity in the innervation of the cerebral circulation could suggest a selective control or modulation of the irrigation of the brain at strategic points within the cerebrovascular tree.

In an attempt to evaluate and understand the variations in the cerebrovascular control, we studied the reactivity of several anatomically distinct cerebral arteries to various neurotransmitters and other vasoactive agents: noradrenaline (NA), dopamine, 5-hydroxytryptamine (5-HT), acetylcholine (ACh), vasoactive intestinal peptide (VIP), substance P prostaglandin $F_{2\alpha}$, histamine, bradykinin and adenosine. The great wealth of information available for the feline cerebrovascular system prompted us to investigate the regional heterogeneity in this species. Indeed, the cat is one of the laboratory animals most studied with regard to its perivascular neurotransmitter levels (Duckles, 1981; Duckles & Buck, 1982; Duckles & Said, 1982; Marco et al., 1985), as well as the histochemical and immunoreactive distribution of perivascular fibres (Edvinsson et al., 1972; Liu-Chen et al., 1983; Gibbins et al., 1984; Saito et al., 1985). Furthermore, the vasomotor responses of cerebral arteries both *in situ* and *in vitro* have been much investigated in the cat (Wahl & Kuschinsky, 1976, 1979; McCulloch & Edvinsson, 1980; Edvinsson et al., 1981; 1985b; Edvinsson & Fredholm, 1983; Wahl et al., 1983; Medgett & Langer, 1983; Uski & Andersson, 1984).

In the present study we describe the *in vitro* cerebrovascular reactivity of several arteries originating from either the carotid or the vertebrobasilar circulation in order to elucidate some of the mechanisms governing regional blood supply to the brain. A preliminary account of this work has been published in abstract form (Hamel et al., 1987).

Methods

Experiments were performed on cerebral arteries obtained from cats of either sex (2–2.5 kg) that were killed by decapitation under 4% halothane anaesthesia. The brains were rapidly taken out and the following cerebral arteries, as defined by Davis & Story (1944) were removed under a dissecting microscope: anterior cerebral, anterior and posterior communicating; middle cerebral; anterior and posterior cerebellar; basilar; and vertebral arteries. All vessels were kept on ice in a Krebs Ringer solution of the following composition (in mM): NaCl 118, KCl 4.5, $MgSO_4$ 1.0, KH_2PO_4 1.0, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 6.0.

Circular segments ($\simeq$3 mm long) of these arteries were mounted between two L-shaped metal prongs (the diameter of which varied according to the calibre of the vessel) in tissue baths containing the Krebs solution at 37°C and continuously gassed with 95% O_2 and 5% CO_2, in order to maintain a pH of 7.4. One of the metal prongs was connected to a force-displacement transducer (Grass FT03C) and the isometric circular tension developed by the smooth muscle was displayed on a potentiometric recorder (Lectromed MX412). The vessels were given a mechanical load of 4 mN and allowed to stabilize for 90 min, during which time the bathing fluid was replaced every 15 min (for further details, see Högestätt et al., 1983). The vessels were then exposed to a 124 mM potassium (K^+) solution in order to obtain the maximal constrictor response for each vessel segment. Thereafter, the vessels were allowed to recover for a 30 min period before testing the various vasoactive substances.

Log concentration-response curves were generated after cumulative addition of the vasoactive agent to the tissue bath. Vasoconstrictor substances were added directly to vessels equilibrated at the level of the passive load. For dilator agents, prostaglandin $F_{2\alpha}$ (2.5 μM) or 5-HT (3 μM) was used to evoke a stable constriction from which dilator responses could be evaluated. The reactivity of the various arteries was studied at random by changing the order in which the vasoactive substances were administered from one experiment to the other. Results are expressed as % of the maximal constriction obtained with 124 mM K^+ for constrictors and as % of the induced tone for dilator agents. The concentration of agonist which produced half maximal response (EC_{50}) was calculated.

Table 1 The regional cerebral arterial responses to various vasoconstrictor agents

Artery	K^+ (mN)	NA	Agent: Dopamine (% of the K^+-induced response)	$PGF_{2\alpha}$	5-HT
Anterior cerebral	8.0 ± 1.4 (10)	81 ± 14 (3)[f,g]	69 ± 10 (10)	66 ± 19 (3)	73 ± 11 (10)
Middle cerebral	17.8 ± 1.0 (12)[a,b]	47 ± 3 (7)	65 ± 7 (10)	66 ± 5 (8)	48 ± 6 (8)
Anterior communicating	23.3 ± 3.3 (7)[c,d]	46 ± 6 (2)	46 ± 3 (6)	NT	57 ± 6 (7)
Posterior communicating	12.8 ± 1.2 (3)	54 ± 2 (3)	44 ± 2 (3)	99 ± 13 (3)	56 ± 5 (3)
Anterior cerebellar	8.4 ± 1.6 (6)	44 ± 17 (4)	57 ± 2 (6)	NT	64 ± 7 (6)
Posterior cerebellar	9.6 ± 1.0 (6)	54 ± 8 (3)	52 ± 11 (5)	56 ± 4 (3)	57 ± 12 (6)
Basilar	15.1 ± 1.9 (15)[e]	46 ± 4 (11)	53 ± 6 (10)	80 ± 7 (11)	73 ± 5 (12)
Vertebral	7.3 ± 1.3 (4)	45 ± 6 (4)	34 ± 3 (4)	83 ± 8 (4)	71 ± 7 (4)

The data are presented as maximal response ± s.e. mean (expressed as a % of the K^+ response), based on the number of vessels denoted within parentheses (or as maximal response ± s.d. when $n = 2$). The responses to 124 mM K^+ alone are given in mN. NT = not tested. [a] $P < 0.05$ with respect to the posterior cerebellar artery and [b] $P < 0.01$ with respect to anterior cerebellar and vertebral arteries; [c] $P < 0.05$ with respect to the basilar artery and [d] $P < 0.01$ with respect to all other vessels except the middle cerebral artery; [e] $P < 0.05$ with respect to the anterior cerebral and vertebral arteries; [f] $P < 0.05$ with respect to both posterior communicating and posterior cerebellar arteries; [g] $P < 0.01$ with respect to all other vessels; by ANOVA and Duncan comparison tests.

Solutions and drugs

Acetylcholine chloride, noradrenaline hydrochloride, dopamine hydrochloride, 5-hydroxytryptamine hydrochloride, histamine dihydrochloride, adenosine hemisulphate, bradykinin triacetate, substance P triacetate and vasoactive intestinal peptide were purchased from Sigma (St Louis, MO, U.S.A.). Prostaglandin $F_{2\alpha}$ (Amoglandin) was obtained from Astra (Sweden).

All solutions were made up in 0.9% w/v NaCl solutions containing 0.2% ascorbic acid in order to avoid oxidation of the amine compounds. The drugs were added in volume of 50 µl to the tissue baths (total volume of 5 ml).

Statistical analyses

The vasomotor effects of a given substance were compared between the various cerebral arteries according to the intensity of the maximal response and sensitivity of the arteries towards this substance (EC_{50}). The data were analysed according to one way analysis of variance (ANOVA) for unequal sample size and the Duncan multiple comparison test. For comparison of the EC_{50} values, the concentrations (in M) were expressed as the corresponding pD_2 ($-\log EC_{50}$) values before analysis for statistical differences. Results were taken to be significant when $P < 0.05$ and are expressed as means ± s.e. mean.

Results

The 124 mM K^+ solution induced large vasoconstrictor responses in all vessels which varied from 7.3 to 23.3 mN (Table 1). The most marked vasoconstrictions elicited by K^+ were obtained in the anterior communicating, middle cerebral and basilar arteries with responses of 23.3, 17.8 and 15.1 mN, respectively (Table 1).

Effects of the vasoconstrictors: noradrenaline, dopamine, 5-hydroxytryptamine and prostaglandin $F_{2\alpha}$

In all arteries studied, these substances elicited reproducible vasoconstrictor responses. NA (10^{-9} to 10^{-4} M) induced a dose-dependent vasoconstriction of comparable magnitude in all vessels except for the anterior cerebral artery (Table 1). The extent of the constriction corresponded approximately to 50% of the maximal response obtained with 124 mM K^+. In the anterior cerebral artery, however, the NA-induced vasoconstriction was significantly greater than elsewhere and reached 81% of the maximal

Table 2 The sensitivity of regional cerebral arterial responses to various vasoconstrictor agents

Artery	Agent			
	NA	*Dopamine*	*$PGF_{2\alpha}$*	*5-HT*
Anterior cerebral	6.41 ± 0.15 (3)[a,b]	5.13 ± 0.07 (10)	6.54 ± 0.08 (3)	6.47 ± 0.11 (10)[l]
Middle cerebral	6.20 ± 0.10 (7)	5.27 ± 0.16 (10)	7.16 ± 0.04 (8)[i,j]	7.41 ± 0.09 (9)
Anterior communicating	5.92 ± 0.10 (2)	5.31 ± 0.14 (6)	NT	7.38 ± 0.13 (7)
Posterior communicating	5.74 ± 0.49 (3)	4.36 ± 0.14 (3)[e,f]	6.11 ± 0.04 (3)	7.43 ± 0.07 (3)
Anterior cerebellar	6.11 ± 0.26 (4)	4.44 ± 0.13 (6)[g,h]	NT	7.49 ± 0.12 (6)
Posterior cerebellar	5.82 ± 0.32 (3)	5.39 ± 0.18 (5)	6.50 ± 0.04 (3)	7.42 ± 0.12 (12)
Basilar	5.72 ± 0.02 (11)	5.19 ± 0.14 (10)	6.26 ± 0.13 (11)	7.37 ± 0.06 (12)
Vertebral	5.16 ± 0.22 (4)[c,d]	4.98 ± 0.11 (4)	6.66 ± 0.11 (4)[k]	7.62 ± 0.15 (4)

The data are presented as pD_2 ($-\log EC_{50}$) ± s.e. mean; based on the number of vessels denoted within parentheses (or as mean ± s.d. when $n = 2$). NT = not tested. [a] $P < 0.05$ with respect to the posterior communicating and basilar arteries and [b] $P < 0.01$ with respect to the vertebral artery; [c] $P < 0.05$ with respect to the posterior cerebellar artery and [d] $P < 0.01$ with respect to the anterior cerebral, middle cerebral and anterior cerebellar arteries; [e] $P < 0.05$ with respect to the vertebral artery and [f] $P < 0.01$ with respect to all other vessels except the anterior cerebellar artery; [g] and [h], see [c] and [f], respectively; [i] $P < 0.05$ with respect to the vertebral artery and [j] $P < 0.01$ when compared to all other arteries; [k] $P < 0.05$ with respect to the posterior communicating artery; [l] $P < 0.01$ with respect to all other cerebral arteries; by ANOVA and Duncan comparison tests.

K^+-induced constriction. The sensitivity of the arteries to NA was quite similar with an overall mean pD_2 of 5.85 ± 0.14 (Table 2), although the anterior cerebral artery was significantly more sensitive to this catecholamine (Table 2). In contrast, the vertebral artery was less sensitive to NA than the anterior, middle cerebral, anterior and posterior cerebellar arteries (Table 2).

The vasoconstriction induced by dopamine (10^{-9} to 10^{-4} M) also corresponded to about 50% of the K^+-induced maximal response in all the arteries (Table 1). Although not significant, the anterior and middle cerebral arteries exhibited slightly greater responses with contractions of more than 65% of the maximum response; the vertebral artery displayed a constriction of only 34% (Table 1). The sensitivity of the various cerebral arteries to dopamine was quite homogeneous with an overall mean pD_2 value of 5.01 ± 0.15. Only the posterior communicating and the anterior cerebellar arteries were significantly less sensitive ($P < 0.01$) to dopamine (Table 2).

5-HT (10^{-10} to 10^{-5} M) induced concentration-dependent vasoconstriction which accounted for approximately 60% of the maximal response to K^+ (Table 1). The intensity of the 5-HT-induced vasoconstriction tended to increase from the anterior circulation to the vertebrobasilar region. When the middle cerebral artery was compared to the vertebral and basilar arteries, the 5-HT induced vasoconstriction was significantly ($P < 0.01$) less in the middle cerebral artery than those in the basilar and vertebral arteries (Table 1). Except for the anterior cerebral artery, the sensitivity of the cerebral blood vessels to 5-HT was very homogeneous with an overall mean pD_2 value of 7.45 ± 0.04 (Table 2). The anterior cerebral artery was, however, significantly less ($P < 0.01$) sensitive to 5-HT than all the other cerebral vessels (Table 2).

Prostaglandin $F_{2\alpha}$ was also a potent vasoconstrictor agent in the cerebral circulation with an average response of 75 ± 7% of the K^+-induced maximal constriction and an overall mean pD_2 value of 6.54 ± 0.16. The posterior communicating artery was particularly responsive to prostaglandin $F_{2\alpha}$; on the other hand, this vessel was slightly, though significantly, less sensitive to prostaglandin $F_{2\alpha}$ with a pD_2 of 6.11 ± 0.04 (Table 2, $P < 0.05$ and $P < 0.01$, when compared to the vertebral and middle cerebral arteries, respectively). The middle cerebral artery was by far the most sensitive vessel to prostaglandin $F_{2\alpha}$ ($P < 0.01$ when compared to all the other vessels; Table 2).

When the effects of the various vasoconstrictors were compared on the same vessel, a similar pattern of reactivity and sensitivity was consistently observed in most of the vascular regions. The maximal vasoconstrictions obtained with NA and dopamine were, in general, less than those induced

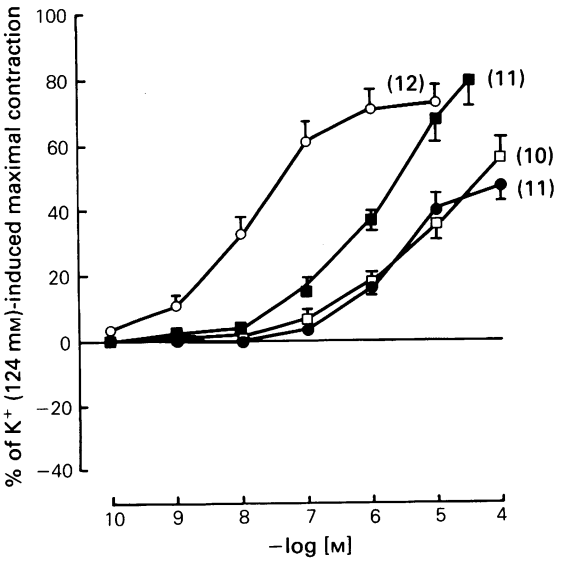

Figure 1 Concentration-response curves for the contractions induced by 5-hydroxytryptamine (○), noradrenaline (●), dopamine (□) and prostaglandin $F_{2\alpha}$ (■) in the feline basilar artery. Responses are expressed as % of the maximal contraction induced by 124 mM K^+. Each point represents the mean of the number of arterial segments denoted within parentheses; vertical lines indicate s.e. mean.

by 5-HT and prostaglandin $F_{2\alpha}$. A lower sensitivity to NA and dopamine of the vascular receptors, when compared to that of 5-HT, was also generally observed. This order of sensitivity is typified by the basilar artery (Figure 1).

Effects of vasodilators: adenosine, acetylcholine and histamine

Adenosine, ACh and histamine induced pronounced vasodilatation in most of the arteries investigated. Adenosine elicited homogeneous dilator responses, whereas the relaxation induced by ACh and histamine varied considerably as a function of the anatomical localization of the cerebral arteries. Adenosine-induced vasodilatations accounted for approximately 80% (overall mean maximal response: 81 ± 6%) of the induced tone when the average reactivity of all the arteries was considered (Table 3). The posterior communicating artery exhibited a slightly greater dilatation ($P < 0.05$, when compared to anterior and middle cerebral arteries), as did the anterior cerebellar and vertebral arteries ($P < 0.05$, when compared to the middle cerebral and to the anterior, middle cerebral and basilar arteries, respectively). No differences in the sensitivity to adenosine could be detected between the arteries, the pD_2 values ranged from 5.33 to 6.46 with an overall mean of 5.94 ± 0.16 (Table 4). Whatever the anatomical localization of the vascular segment, adenosine behaved as a pure vasodilator agent and was never found to elicit vasoconstriction even at high concentrations (Figure 2).

The ACh-induced vasodilatation was significantly more pronounced in the middle cerebral, anterior communicating and anterior cerebral arteries with dilatations of 72, 66 and 83%, respectively, of the induced tone, as compared to approximately 43% in the other vessels (Table 3). The sensitivities of the various cerebral arteries to ACh were very similar (overall mean pD_2 of 7.47 ± 0.06, Table 4), in spite of the marked differences in the vascular reactivity. As illustrated in Figure 2, ACh elicited a bimodal vascular response in all the feline cerebral arteries. Indeed, vasodilatation was systematically observed at concentrations lower than 10^{-6} M, whereas ACh induced notable vasoconstriction at higher concentrations, the magnitude of the constrictor response depending on the blood vessel studied.

Highly heterogeneous results were observed in the magnitude of the dilator responses induced by histamine (Table 3). The maximal relaxation induced by histamine varied from 20% to 118% of the pharmacologically induced tone. The middle cerebral, posterior communicating, anterior cerebellar and posterior cerebellar arteries were the most responsive vessels (see legend to Table 3). Similarly, marked differences were found in the sensitivity to histamine between the various cerebral arteries (Table 4). The most sensitive vessels were the anterior communicating and the vertebral arteries (Table 4). Despite showing marked dilatation to histamine, the anterior cerebellar artery was the least sensitive artery to this amine with a pD_2 value of 5.56 ± 0.18 ($P < 0.05$ or $P < 0.01$, when compared either to the posterior communicating or to the anterior communicating and vertebral arteries, respectively). In contrast to adenosine and ACh (see above), histamine sometimes behaved as a pure vasodilator agent (Figure 2), but at other times could also induce a vasoconstriction (Figure 2) in some vessels (e.g. the basilar artery) when the concentration exceeded 10^{-5} M. Whenever this constrictor effect was evident, the dilator response to histamine was considerably less in size than that observed in vessels in which histamine always induced vasodilatation.

Effects of neuropeptides: bradykinin, vasoactive intestinal peptide and substance P

All the neuropeptides studied induced relaxation at very low concentrations and these responses were observed irrespective of the anatomical localization

Table 3 The regional cerebral arterial responses to various vasodilator agents

Artery	Agent					
	Adenosine	*ACh*	*Histamine*	*Bradykinin*	*Substance P*	*VIP*
Anterior cerebral	63 ± 14 (5)	38 ± 6 (4)	27 ± 6 (4)	22 ± 5 (4)[o]	NT	NT
Middle cerebral	59 ± 6 (10)	72 ± 6 (6)[e]	96 ± 4 (6)[h,i]	43 ± 8 (7)	40 ± 5 (9)	86 ± 5 (12)[p]
Anterior communicating	76 ± 6 (4)	66 ± 7 (3)[f]	20 ± 6 (3)	53 ± 5 (4)	NT	NT
Posterior communicating	100 ± 1 (3)[a]	34 ± 3 (3)	62 ± 15 (3)[j,k]	49 ± 4 (3)	38 ± 10 (3)	100 ± 1 (3)
Anterior cerebellar	92 ± 21 (4)[b]	83 ± 19 (4)[g]	103 ± 4 (3)[l,m]	45 ± 9 (5)	NT	101 ± 23 (3)
Posterior cerebellar	79 ± 5 (6)	62 ± 7 (4)	118 ± 5 (5)[n]	31 ± 9 (6)	36 ± 11 (3)	128 ± 13 (4)
Basilar	71 ± 4 (7)	39 ± 6 (10)	37 ± 11 (9)	31 ± 1 (4)	33 ± 2 (8)	69 ± 6 (8)[q,r]
Vertebral	104 ± 14 (4)[c,d]	39 ± 10 (4)	33 ± 7 (4)	NT	19 ± 3 (4)	NT

The data are presented as maximal response ± s.e. mean, based on the number of vessels denoted in parentheses. The tone was induced with prostaglandin $F_{2\alpha}$ or 5-hydroxytryptamine as described in the text. [a] $P < 0.05$ when compared to the anterior and middle cerebral arteries; [b] $P < 0.05$ with respect to the middle cerebral artery; [c] $P < 0.05$ with respect to the basilar artery; [d] $P < 0.01$ with respect to the same vessels as in [a]; [e] $P < 0.05$ with respect to anterior cerebral, posterior communicating, basilar and vertebral arteries; [f] $P < 0.05$ with respect to posterior communicating artery and [g] $P < 0.01$ with respect to the same arteries as those listed in [e]; [h] $P < 0.05$ with respect to the posterior communicating artery and [i] $P < 0.01$ when compared to the anterior cerebral, anterior communicating, basilar and vertebral arteries; [j] $P < 0.05$ with respect to the anterior cerebral artery and [k] $P < 0.01$ with respect to the anterior communicating artery; [l] and [m], see [h] and [i], respectively; [n] $P < 0.01$ with respect to all vessels except the middle cerebral and anterior cerebellar arteries; [o] $P < 0.05$ with respect to the anterior communicating, posterior communicating and anterior cerebellar arteries; [p] $P < 0.05$ when compared to the posterior cerebellar artery; [q] $P < 0.05$ with respect to the posterior communicating and anterior cerebellar arteries and [r] $P < 0.01$ with respect to the posterior cerebellar artery; by ANOVA and Duncan comparison tests.

of the vascular preparation. The relative potencies of the neuropeptides were comparable in the arterial segments and are well represented by the posterior communicating artery (Figure 3). The most potent of the neuropeptides was VIP with relaxations being observed that ranged from 69 to 128% of the induced tone (Table 3). The intensities of the VIP-induced vasodilatations were less pronounced in the middle cerebral and, even more so, in the basilar arteries. The cerebral arteries exhibited a rather homogeneous sensitivity to VIP with an overall mean pD_2 value of 8.13 ± 0.11 (Table 4). The basilar artery was slightly, but not significantly, less sensitive to VIP (Table 4).

Bradykinin and substance P-induced comparable relaxations (overall mean maximal responses were 39 ± 5 and 33 ± 4%, respectively) which varied slightly between the arteries tested. The vessels of the vertebrobasilar region, however, exhibited a tendency to be less responsive to both these neuropeptides. This pattern of reactivity is well demonstrated with bradykinin (Table 3) despite the fact that, for this neuropeptide, the least responsive vessel was the anterior cerebral artery (Table 3). The anterior cerebral artery was also the least sensitive vessel to bradykinin with a pD_2 of 7.56 ± 0.24 (Table 4), as compared to an overall mean value of 8.68 ± 0.40 for the other arteries. Despite the tendency of the more caudal vessels of the cerebral circulation to respond weakly to substance P, no significant differences could be detected between the various arteries. The basilar and vertebral arteries were, however, significantly less sensitive to substance P than the other cerebral vessels (Table 4).

Discussion

The aim of the present study was to compare the vascular reactivity and sensitivity of anatomically distinct feline cerebral arteries to a number of vasoactive substances; no attempt was made to differentiate the receptor subtype(s) by which these agents may produce their overall effects. We recognize that the net vascular response might represent direct effects on multiple receptors and/or interactions with other neurotransmitter systems. Moreover, the technique employed does not allow the differentiation between endothelial- or medial-located receptors. Many of the substances investigated are known to

Table 4 The sensitivity of regional cerebral arterial responses to various vasodilator agents

Artery	*Agent*					
	Adenosine	*ACh*	*Histamine*	*Bradykinin*	*Substance P*	*VIP*
Anterior cerebral	6.23 ± 0.21 (5)	7.34 ± 0.27 (5)	6.35 ± 0.30 (4)	7.56 ± 0.24 (4)[f,g]	NT	NT
Middle cerebral	6.13 ± 0.30 (10)	7.48 ± 0.08 (9)	5.77 ± 0.13 (6)	8.24 ± 0.37 (7)	9.53 ± 0.39 (9)	8.36 ± 0.15 (12)
Anterior communicating	5.77 ± 0.47 (4)	7.26 ± 0.12 (8)	8.21 ± 0.40 (3)[a,b]	9.04 ± 0.37 (4)	NT	NT
Posterior communicating	6.46 ± 0.23 (3)	7.69 ± 0.15 (3)	6.97 ± 0.27 (3)[c]	9.34 ± 0.19 (3)	10.42 ± 0.30 (3)	8.05 ± 0.13 (3)
Anterior cerebellar	5.33 ± 0.09 (4)	7.58 ± 0.08 (4)	5.56 ± 0.18 (3)	8.98 ± 0.68 (5)	NT	8.22 ± 0.06 (4)
Posterior cerebellar	6.24 ± 0.15 (6)	7.64 ± 0.12 (4)	6.26 ± 0.31 (5)	8.63 ± 0.32 (6)	9.53 ± 0.92 (3)	8.32 ± 0.15 (4)
Basilar	5.97 ± 0.13 (7)	7.38 ± 0.10 (10)	6.61 ± 0.39 (9)	7.83 ± 0.63 (4)	8.51 ± 0.24 (8)[h]	7.73 ± 0.04 (8)
Vertebral	5.37 ± 0.31 (4)	7.40 ± 0.16 (3)	7.65 ± 0.63 (4)[d,e]	NT	7.63 ± 0.59 (4)[i,j]	NT

The data are presented as pD_2 ($-\log EC_{50}$) ± s.e. mean based on the number of vessels denoted within parentheses. NT = not tested. [a] $P < 0.05$ with respect to the posterior communicating and basilar arteries and [b] $P < 0.01$ when compared to all other arteries except the vertebral artery; [c] $P < 0.05$ with respect to the anterior cerebellar artery; [d] $P < 0.05$ with respect to the anterior cerebral and posterior cerebellar arteries and [e] $P < 0.01$ when compared to the middle cerebral and anterior cerebellar arteries; [f] $P < 0.05$ with respect to the anterior communicating and anterior cerebellar arteries and [g] $P < 0.01$ with respect to the posterior communicating artery; [h] $P < 0.05$ with respect to the posterior communicating artery; [i] $P < 0.05$ with respect to the middle cerebral and posterior cerebellar arteries and [j] $P < 0.01$ when compared with the posterior communicating artery; by ANOVA and Duncan comparison tests.

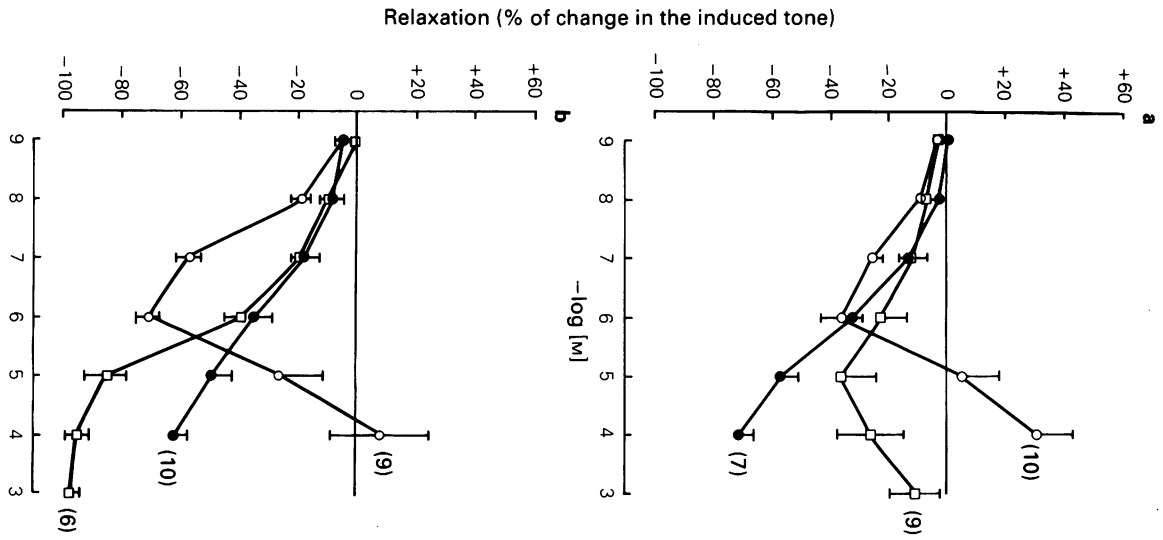

Figure 2 Concentration-response curves for the relaxations induced by acetylcholine (○), adenosine, (●) and histamine (□) on feline basilar (a) and middle cerebral (b) arteries. Adenosine consistently elicited relaxation. Acetylcholine, at low concentrations, induced a dilatation which was followed by a vasoconstriction at doses larger than 10^{-6} M. Histamine sometimes behaved like a pure vasodilator (in basilar arteries) but could also induce a vasoconstriction (in middle cerebral arteries). Responses are expressed as a % of the change in the pharmacological tone induced by increasing concentrations of the vasodilator agents. Each point is the mean of the number of segments denoted within parentheses; vertical lines indicate s.e. mean.

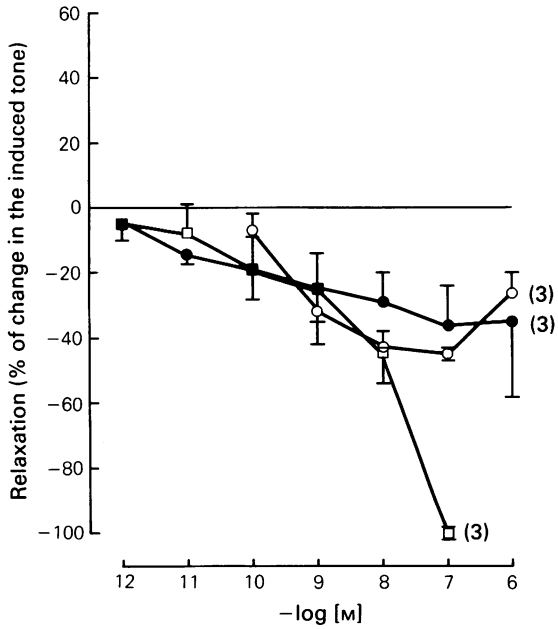

Figure 3 Concentration-response curves for the relaxations induced by bradykinin (○), substance P (●) and vasoactive intestinal peptide (□) on feline posterior communicating artery from the circle of Willis. Responses are expressed as a % of the change in the pharmacological tone induced by increasing concentrations of the peptides. Each point is the mean of the number of segments denoted within parentheses; vertical lines indicate s.e. mean.

rely on the integrity of the intimal layer to elicit their vascular responses (Lee, 1982; Lee *et al.*, 1984; Edvinsson *et al.*, 1985a; Hardebo *et al.*, 1985; Verrecchia *et al.*, 1986). Accordingly, the size of the metal prongs used for the isometric recording of the circular muscle tension was adapted to the internal size of the vessels and great care was taken to minimize any endothelial disruption.

Despite the limitations mentioned above, the present study clearly demonstrates that most vasoactive neurotransmitters or blood-borne substances exert their vascular effects according to the specific anatomical location of an artery in the cerebrovascular tree. The regional heterogeneity of the vasomotor responses was more pronounced with those substances that induce vasodilatation than with those that mediate vasoconstrictor effects. The present study also suggests that the vertebrobasilar vessels are more susceptible to vasoconstrictor agents than the vessels that originate from the carotid circulation. In contrast, dilator responses were more readily detected in vessels rostral to the basilar artery.

Overall, the regional differences in the cerebrovascular reactivity were such that the pattern of response for each agonist merits separate discussion.

Noradrenaline

The magnitude of the response to noradrenaline was homogeneous in the different vascular beds (despite a pronounced vasoconstriction being noted in the anterior cerebral artery). In contrast, the rostral vessels were by far the most sensitive to NA with a ten fold increase in potency compared to the vertebral artery. Such a regional pattern of NA-induced vasoconstriction would be difficult to demonstrate *in vivo* due to the inability of this monoamine to traverse the blood-brain barrier (i.e. the endothelium) and, subsequently, to influence directly the tone of cerebrovascular smooth muscle (MacKenzie & Scatton, 1987).

The present *in vitro* results suggest a limited cerebrovascular effect of NA—especially in the hindbrain, where high concentrations of the amine would be necessary to induce vasoconstriction. Such observations are in good agreement with the comparatively rich perivascular noradrenergic innervation of the anterior circulation as has been reviewed previously (Edvinsson & MacKenzie, 1976; MacKenzie & Scatton, 1987). The regional differences also accord with those *in vivo* studies in which it was shown that sympathetic nerve stimulation induced a decrease in cerebral blood flow in the areas perfused by the carotid circulation, such as the caudate-putamen, and not in structures (e.g. geniculate body) irrigated by the vertebrobasilar system (Sercombe *et al.*, 1975). Nonetheless, the anterior and middle cerebral arteries differed greatly in their responses to exogeneously applied NA, despite the fact that their density of innervation has been found to be comparable (Edvinsson & MacKenzie, 1976). Further, these two arteries exhibited a very high sensitivity to NA which contrasts with the very low sensitivity observed in the caudal circulation. Such differences could reflect regional variations in the sensitivity and/or density of constrictor as well as dilator adrenoceptors in the various cerebral arteries. In support of this hypothesis, a preferential association of dilator β-adrenoceptors with the basilar artery has been demonstrated in another species, the rat (Winquist & Bohr, 1982).

Dopamine and prostaglandin $F_{2\alpha}$

In contrast to NA and 5-HT, neither dopamine nor prostaglandin $F_{2\alpha}$ nerves have been found in cerebral blood vessels. Nonetheless, both dopamine and prostaglandin $F_{2\alpha}$ have potent vasoconstrictor effects on cerebrovascular smooth muscle *in vitro*; the

overall pattern of the intensity of response and sensitivity of the cerebral arteries to dopamine mimics that already described for NA.

In situ, the direct pial response to dopamine is vasoconstriction, a response that is thought to be mediated through 5-HT-receptors and α-adrenoceptors (Edvinsson *et al.*, 1985b). Despite this, the existence of dilator D_1-dopamine receptors in the cerebrovascular bed has been shown (Edvinsson *et al.*, 1978a; 1985b). The regional balance between the poorly characterized vasoconstrictor dopamine-sensitive receptors and the dilator D_1-dopamine receptors could be variable. The low sensitivity to dopamine observed in our present investigation could well reflect the simultaneous activation of multiple receptors that elicit opposing effects.

The vasoconstrictions induced by prostaglandin $F_{2\alpha}$ were relatively large in rostral as well as caudal regions of the arteries. The intensity of the vasoconstriction varied from 56 to 99% of the maximal vasoconstriction induced by K^+. However, the maximal response to prostaglandin $F_{2\alpha}$ was less than those found earlier for the middle cerebral and basilar arteries of the cat (Uski *et al.*, 1981; Uski & Andersson, 1984). This discrepancy is somewhat surprising since the same species and the same source of prostaglandin $F_{2\alpha}$ were used. One possible explanation could reside in the use of halothane (this study) as compared to sodium pentobarbitone as the anaesthetic before decapitation. It is well known that the *in vitro* effects of halothane are to relax cerebrovascular smooth muscle and to blunt the contractile effects of various vasoactive agents (Harder *et al.*, 1985).

5-Hydroxytryptamine

5-HT was the most potent constrictor neurotransmitter in all the feline cerebral arteries studied. This observation agrees with the presence of a dense plexus of 5-HT immunoreactive fibres found in all major cerebral arteries (Edvinsson *et al.*, 1983a; 1984). No quantification, however, has been made of the differential density of innervation of the various cerebral arteries, nor have 5-HT levels been measured in specific vascular beds (Edvinsson *et al.*, 1983a; 1984; Marco *et al.*, 1985). Our results suggest a gradient of increased reactivity towards the vessels in the vertebrobasilar region with, however, no differences whatsoever in the sensitivity to 5-HT between the arteries. Only the anterior cerebral artery could be excluded from the general pattern, in that this vessel displayed an intense response coupled with a low sensitivity to 5-HT. Interestingly, an innervation of intraparenchymal blood vessels by 5-HT has been demonstrated in the brainstem (Chan-Palay, 1976; Di Carlo, 1977; Kapadia & de Lanerolle, 1984), an observation which could be consistent with a role for 5-HT in the control of cerebral perfusion and, particularly in caudal brain areas. This hypothesis might be further supported by the fact that the perivascular 5-hydroxytryptaminergic innervation originates from the mesencephalic raphé nuclei of the brain stem (Reinhard *et al.*, 1979; Edvinsson *et al.*, 1983a; Scatton *et al.*, 1985). However, it has been shown that part of the characterized 5-hydroxytryptaminergic innervation might be due to the capacity of sympathetic perivascular nerves to accumulate and release 5-hydroxytryptamine (Verbeuren *et al.*, 1983; Levitt & Duckles, 1986; Saito & Lee, 1987).

The slight variation in the magnitude of the 5-HT vasoconstriction could be explained by different densities of 5-HT vasoconstrictor receptors or, alternatively, as a variable ratio of constrictor, as opposed to dilator, 5-HT receptors in these vessels. A 5-HT receptor mediating cerebral vasodilatation has been described both *in vitro* (Edvinsson *et al.*, 1978b) and *in situ* (Harper & MacKenzie, 1977). However, the similar pD_2 values noted between the arteries would favour the first hypothesis (that of differing receptor densities) unless 5-HT has a similar affinity at both constrictor and dilator sites. Alternatively, there could be significant variations in receptor reserve; the more spare receptors, then the higher would be the pD_2 for a given efficiency of coupling. Indeed, most *in vitro* studies have failed to demonstrate an interaction of 5-HT with a single population of receptors in cerebral arteries (Edvinsson *et al.*, 1978b, Bradley *et al.*, 1986, Peroutka *et al.*, 1986) and it has been argued that 5-HT interacts with multiple receptor subtypes in the cerebral arteries (Young *et al.*, 1987).

Adenosine

The importance of adenosine in the control and regulation of the cerebral circulation has been inferred from a number of studies. *In situ* and *in vivo* experiments have shown marked increases in arteriolar calibre and significant increases in cerebral blood flow after adenosine administration (Wahl & Kuschinsky, 1976; Forrester *et al.*, 1979). Our *in vitro* study shows a significant dilator effect of adenosine upon the cerebral circulation with the minimal relaxation corresponding to 59% of the induced tone. Further, the sensitivity to adenosine was similar in the various arteries studied and corresponded to that previously found in the feline middle cerebral artery (Edvinsson & Fredholm, 1983).

Histamine

The individual cerebral arterial responses to hista-

mine were highly variable; differences in sensitivity of three orders of magnitude were observed and the histamine-induced relaxation varied between 20 and 118% of the pharmacologically-induced tone. The considerable regional variability of the cerebral arterial responses is difficult to explain on an ontogenic basis, as arteries derived from both carotid and vertebrobasilar systems display marked differences in their reactivity and sensitivity to histamine (cf. Bevan *et al.*, 1982). This extreme heterogeneity in the cerebrovascular reactivity to histamine might well suggest a histaminergic modulation at selective and strategic points within the cerebral circulation. For example, given the reactivity of the cerebellar arteries to histamine, one could speculate that the cerebellum might be the target of histaminergic influences on the brain vasculature.

The vasodilatation induced by histamine in feline cerebral arteries is mediated almost exclusively by H_2-receptors, as evidenced by *in vivo*, *in situ* and *in vitro* studies (Edvinsson & Owman, 1975; Wahl & Kuschinsky, 1979; De Ley *et al.*, 1982; Edvinsson *et al.*, 1983b), primarily localized on the muscular layer. Indeed, the involvement of endothelial receptors appears unlikely since, *in vivo*, the systemic administration of histamine produces increases in regional cerebral blood flow only after osmotic disruption of the blood-brain barrier (Gross *et al.*, 1981).

Acetylcholine

As was the case with histamine, the ACh-induced vasodilatations differed considerably within groups of vessels originating in the same vascular bed. The vertebrobasilar region, for example, included both vessels with weak and strong vasodilator responses. The low reactivity of the basilar artery agrees with the low density of acetylcholinesterase-positive fibres (Edvinsson *et al.*, 1972; Edvinsson & MacKenzie, 1976) and the minimal release of authentic ACh noted after depolarization (Duckles, 1981; Hamel *et al.*, 1986) in this vessel. However, the ACh-induced relaxation in the basilar, middle cerebral and cerebellar arteries cannot be correlated with either, firstly, the endogeneous ACh levels (Duckles, 1981), secondly, choline acetyltransferase (ChAT) activity (Florence & Bevan, 1979; Bevan *et al.*, 1982) or, thirdly, the density of ChAT-immunoreactive nerve fibres (Saito *et al.*, 1985) found in these arteries. Indeed, high concentrations of ACh and considerable ChAT activity had been found in the basilar, as compared to the middle cerebral, artery; in the present study, the latter was significantly more responsive to ACh than the basilar artery.

In contrast to the marked heterogeneity in the intensity of the vascular response, all cerebral arteries exhibited a comparable sensitivity to ACh. ACh is thought to act on endothelial receptors to induce vasodilatation (Furchgott & Zawadzki, 1980; Lee, 1982) and, at higher concentrations, on muscular receptors to mediate vasoconstriction; both types of responses are muscarinic (Edvinsson *et al.*, 1977; Furchgott & Zawadzki, 1980). The anterior cerebral and posterior communicating arteries, which have been shown to be densely innervated by cholinergic nerves, possibly possess a predominant population of vasoconstrictor cholinoceptors which could explain their weak vasodilator responses. The concensus from the literature and our present study would be that there exists a mismatch between density of innervation, levels of cerebrovascular ACh and intensity of vascular response for this neurotransmitter. The exact localization of vasodilator, as opposed to vasoconstrictor, ACh receptors could be of considerable value to help explain these dichotomies.

Bradykinin

Except for one single study (Hanko *et al.*, 1981), bradykinin administered either *in situ* or *in vitro* always resulted in a relaxation of feline cerebral vessels (Wahl *et al.*, 1983; Whalley & Wahl, 1983). Our study is in accordance with the majority of the previous results, but further demonstrates that the regional vasodilator effects of bradykinin are essentially homogeneous in the cerebrovascular bed. The bradykinin-induced relaxations were found to be of feeble intensity when compared to those elicited by adenosine, histamine and ACh. These findings contrast with the *in situ* studies in which it was demonstrated that bradykinin induced higher maximal responses than adenosine or histamine (Wahl *et al.*, 1983), despite a similar vascular sensitivity for these compounds. The maximal responses elicited by bradykinin in the present study corresponded to approximately 40–50% of the induced tone which, however, agrees with the bradykinin activity previously found for the cat middle cerebral artery and pial arterioles (Wahl *et al.*, 1983). Only the anterior cerebral artery was significantly less responsive (exhibiting both a low sensitivity and low maximal response). However, a weak vasodilator response was also observed in the basilar and vertebral arteries. This latter observation is in support of previous *in vitro* studies on the cat basilar artery (Toda, 1977; Whalley & Wahl, 1983).

Although the intracarotid injection of bradykinin was without effect on cerebral perfusion and metabolism, the ventriculo-cisternal perfusion of bradykinin markedly increased cerebral blood flow and glucose use (Unterberg *et al.*, 1985); these experiments suggest that, *in vivo*, intravascular bradykinin is inactive.

Substance P

As with bradykinin, substance P-induced vasodilatations did not display marked regional differences among the feline cerebral arteries investigated. Immunocytochemical studies have shown a lower frequency of substance P-immunoreactive fibres in vessels located in the more caudal portion of the circle of Willis (Edvinsson *et al.*, 1981; Liu-Chen *et al.*, 1983). Our regional study failed to detect such heterogeneity in the vasomotor responses and, instead, agree with the homogeneous levels of substance P observed in cat cerebral arteries (Duckles & Buck, 1982). The vertebral and basilar arteries, however, were significantly less sensitive to substance P than the vessels located rostrally in the circle of Willis. The dilator action of substance P requires the integrity of the endothelial cells (Edvinsson *et al.*, 1985a). The low response induced by substance P might suggest that this neuropeptide would induce minor changes in vessel calibre and, would also be unimportant in the regional control of brain perfusion in the cat. Such an observation would agree with the suggested role of substance P-immunoreactive fibres in the transmission of intracranial pain (Duckles & Buck, 1982), rather than in the regional control of cerebrovascular reactivity.

Vasoactive intestinal peptide

VIP was by far the most potent vasodilator substance investigated in the present study. All vessels were equally sensitive to VIP despite the significant regional variation observed in the intensity of the vasodilator response. The basilar and middle cerebral arteries were the least responsive vessels to this neuropeptide. Such findings correlate well with the regional levels of VIP-immunoreactive material and density of perivascular VIP nerve fibres which were found to be lower in these two specific segments of the cerebrovascular bed in the cat (Edvinsson *et al.*, 1980; Duckles & Said, 1982; Gibbins *et al.*, 1984), and in the rat (Kobayashi *et al.*, 1983; Matsuyama *et al.*, 1983). The strong vasodilator effects of VIP were also observed *in vivo* (McCulloch & Edvinsson, 1980; Wilson *et al.*, 1981), but the regional changes in cerebral blood flow elicited by VIP could only be detected after either intraventricular administration (Wilson *et al.*, 1981) or after opening the blood-brain barrier (McCulloch & Edvinsson, 1980). The VIP induced flow increases were accompanied by parallel changes in brain metabolic activity (McCulloch & Edvinsson, 1980). In view of this, it seems likely that the vasodilatation observed *in vivo* is secondary to an increase in cerebral metabolism.

However, a direct effect of VIP on cerebral blood vessels is supported by the activation of the vascular adenylate cyclase by VIP (Huang & Rorstad, 1983; 1984; Edvinsson *et al.*, 1985a) and the presence of specific binding sites for this neuropeptide (Suzuki *et al.*, 1985; Poulin *et al.*, 1986) in cerebral arteries. All these observations are consistent with a direct effect on cerebrovascular smooth muscle, which could imply that the vasodilator actions of VIP are not necessarily secondary to augmented metabolic activity. Further support for this opinion can be adduced from recent investigations, which showed that VIP was the vasodilator neurotransmitter released by perivascular nerve fibres from feline cerebral arteries by electrical field stimulation (Brayden & Bevan, 1986). The direct action of VIP on blood vessels is likely to be exerted on vascular smooth muscle, in view of previous findings which have shown that the VIP-induced dilatation is not endothelium-dependent (Lee *et al.*, 1984; Edvinsson *et al.*, 1985a; Hardebo *et al.*, 1985) and that VIP receptors are exclusively localized on the tunica media (Poulin *et al.*, 1986).

General conclusions

The principal aim of this study was to map, in detail, the reactivity of major, named cerebral arteries to a range of vasoconstrictor and vasodilator agents of putative physiological importance. We considered that such an investigation was essential prior to the precise characterization of receptor types, or subtypes, in any given cerebral artery. The heterogeneous reactivity of the brain arteries to vasoactive agents has been discussed in depth above but, from the overall pattern of responsiveness, some salient features emerge.

Apart from the anterior cerebral artery which reacted strongly to all the vasoconstrictor substances, the more readily contractile vessels were found amongst the arteries originating from the vertebrobasilar circulation. The ability to constrict did not appear related to the contractile capacity of the arterial segments, as agonists induced either contractions of weak or high intensity in arteries exhibiting a strong response to K^+. Similarly, a potent vasomotor response to a given substance was not predictive of a high vascular sensitivity towards this agent. An inverse relationship was actually seen for histamine where the least reactive vessels tended to be the most sensitive to this amine. In these vessels, high concentrations of histamine induced a contraction, a response which was absent in arteries which relaxed strongly to histamine. This observation could well be compatible with the presence of both dilator and constrictor receptors in the former vessels, as opposed to a single population of receptors medi-

ating relaxation in the latter cerebral arteries. Altogether, these findings imply the presence of multiple receptor populations in the cerebral arterial regions, receptors which, once activated, can lead to opposite vasomotor responses. Such results clearly emphasize the need for proper identification of the vasomotor responses in a given arterial segment before detailed pharmacological characterization of the vascular receptors.

The differences between the reactivity of the major cerebral arteries is remarkable and one might pose the question as to whether or not a comparable heterogeneity of responsiveness exists in the smaller, downstream vessels. It should be remembered that, although large cerebral arteries can participate in the control of cerebral blood flow (Faraci *et al.*, 1987), blood supply to the brain is primarily regulated in a highly focal manner and that it is the arteriolar elements rather than major cerebral arteries that are responsible for the focal changes in brain perfusion. Certainly, any extrapolation from one vascular bed to another in the brain is fraught with difficulties and any global expression of the effects of a vasoactive agent on the cerebrovascular bed should be treated with the utmost caution.

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