3404

Blood Vessels *15*: 128–147 (1978)

Pharmacological *in vitro* Analysis of Amine-Mediated Vasomotor Functions in the Intracranial and Extracranial Vascular Beds

C. Owman, L. Edvinsson and J. E. Hardebo

Departments of Histology and Neurology, University of Lund, Lund

Key Words. Cerebral circulation · Adrenergic, cholinergic, peptidergic nerves · Amine receptors · Vasoconstriction and dilatation · Neurogenic control of cerebral blood flow

Abstract. The cerebrovascular system (the extra- as well as intracerebral vessels) receives a well-developed innervation by the adrenergic (originating in the superior cervical sympathetic ganglia) and cholinergic nerves. In addition, recent immunohistochemical studies have shown the presence of vasodilatory peptidergic nerve fibres. There may be an association of cerebral blood vessels also with various aminergic nerve systems of intracerebral origin. Besides the nerves, amine-containing mast cells are often located in close relation to the brain vessels. The specific receptors mediating the contractile and dilatory response of the various vasoactive amines – noradrenaline, dopamine, 5-hydroxytryptamine, histamine, acetylcholine – have been characterized in detail in pharmacological experiments. There is a number of major differences in the reactivity of cerebral vessels as compared to vasomotor functions in the peripheral circulation. The observations and results provide a basis for a better understanding of physiological neurogenic control mechanisms in the cerebral circulation, and they may also have implications for the interpretation of pathophysiological phenomena related to, for example, migraine and the vasospasm following subarachnoid hemorrhage.

Introduction

Detailed knowledge about amine receptor functions in, for example, the coronary, renal, and mesenteric vascular beds has formed the basis for our understanding of various types of circulatory control functions and pathophysiological mechanisms, as well as for a rational pharmacological approach to the clinical treatment of some important cardiovascular disorders. Such basic information has, for a long time, been lacking for the

cerebral circulation, mainly because it has been a generally accepted idea – and it is unfortunately still believed by several – that brain vessels are unique in being devoid of nerves, and hence that the control of cerebral blood flow is exerted entirely via metabolic factors, such as changes in the arterial CO_2 tension and extracellular pH. This early misconception is partly due to a lack of histological methods for unequivocal demonstration of perivascular nerves in the brain, and it has been further prepetuated by some electron microscopic reports. In these studies the investigators either could not find any nerves at all in relation to small brain vessels [32], or they could only demonstrate such nerve fibres running at the surface of the blood vessels without penetrating deeper into the smooth muscular media [28]. This is particularly unfortunate, since we know that a negative electron microscopic observation is usually of little value because of the extremely small areas of a sectioned tissue preparation that become available for analysis at the high resolution required. We know today that the localization of nerves only at the surface of the blood vessel is, in fact, the characteristic organisation of a vascular autonomic innervation. There has, of course, been a number of physiological observations indicating that the brain circulation might be influenced by neurogenic mechanisms, but with the very meagre structural basis for such mechanisms it has often been thought that positive results were artifacts of the experimental situation caused by variables beyond the control of the investigator.

Cerebrovascular Innervation

With the introduction more than a decade ago of new neurohistochemical techniques with a unique degree of sensitivity and specificity [16], it became possible to show that the cerebrovascular bed receives an extensive sympathetic innervation, which in some regions is better developed than in most other vascular beds. It has since been shown that the cerebral vessels are directly reached by several different systems of nerve terminals not only with an extracranial (such as the sympathetic), but probably also of intracerebral, origin. Apart from these more easily definable pathways, the cerebral blood flow may also be changed in response to altered activity of the extensively distributed aminergic systems running within the brain, directly or via hitherto unknown mediators.

Most of the studies on the cerebrovascular innervation have so far been focused on autonomic nerves [26]. Thus, a well-developed system

of adrenergic fibres originates in the superior cervical sympathetic ganglia to supply the pial vascular bed as well as the intracerebral system of resistance vessels. The sympathetic supply of vessels belonging to the carotid system is more extensive than that of the vertebral system. A considerable heterogeneity has been found with regard to the number of arterioles receiving adrenergic terminals in the brain parenchyma, as illustrated in figure 1. There is direct experimental evidence suggesting that this regional heterogeneity in the amount of cerebrovascular innervation may be directly related to the degree of vasomotor reaction that can be evoked by sympathetic nerve stimulation or by the administration of sympathomimetic amines [34].

The cerebrovascular sympathetic nerves are accompanied by an equally well-developed system of fibres (not affected by sympathectomy) containing specific acetylcholinesterase (as evidenced by histochemical staining in conjunction with specific inhibitors) and therefore designated as cholinergic [11]. The origin of these fibres is not fully known, but many of them appear to run in the facial nerve, turn off in the region of the geniculate ganglion to continue in the greater superficial petrosal nerve to the plexus of the internal carotid artery, where they form synapses.[1] In the pial vascular system, the plexuses of adrenergic and cholinergic axon terminals are closely related to one another, with a membrane-to-membrane distance of only 25 nm, forming the structural basis for an axo-axonal interaction (see further below). It should be noted that, so far, cholinesterase-positive fibres have been found only in association with the pial vascular systems, whereas the intracerebral vessels seem to be devoid of such nerves (although it cannot be fully excluded that this simply reflects the relatively low sensitivity of the histochemical technique for demonstration of acetylcholinesterase).

Recently, quite another system of nerve fibres has been visualized by the immunohistochemical demonstration of 'vasoactive intestinal polypeptide' (VIP) [21]. The axons have principally the same relationship to brain vessels as the adrenergic nerves, but cervical sympathetic ganglionectomy has shown that the VIP-containing fibres have another origin;

[1] There is some evidence in the literature that the intracranial arteries are supplied also with afferent nerve fibres. The amount of acetylcholinesterase in this type of nerves appears to be so low that substantial staining is obtained only after prolonged incubation. This would mean that acetylcholinesterase staining under conditions such as those employed in our studies on brain vessels only reveals the efferent (parasympathetic) fibres.

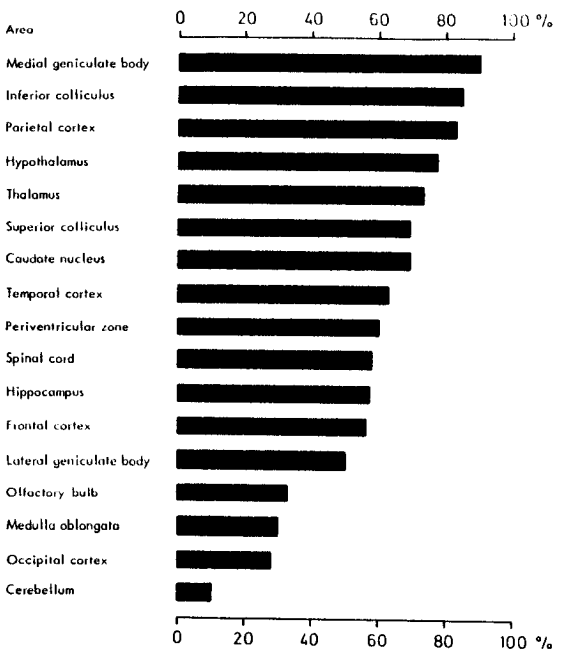

Fig. 1. Fluorescence microscopic evaluation of the percentage of arterioles in various brain regions of baboons supplied by adrenergic sympathetic nerves, visualized in sections from formaldehyde-treated tissue preparations.

they may even arise from intracerebral sources. Preliminary tests under *in vitro* conditions suggest that the VIP system exerts vasodilatory effects also in brain vessels [21]. It is thus not unlikely that the VIP nerves may be involved in the nonadrenergic, noncholinergic cerebral vasodilatory mechanism suggested by the studies of LEE *et al.* [22].

Intracerebral noradrenergic nerves, originating in the locus coeruleus and adjacent regions, have a close association with intracerebral arterioles [23], and it has therefore been implicated that this widespread system may have a direct influence on cerebral microcirculation [30]. There is experimental evidence to support this assumption; but since there are also indications from denervation studies in conjunction with electron microscopy that only the above-mentioned sympathetic terminals innervate intracerebral arterioles in a true sense, we then have to assume some kind of long distance diffusion of transmitter from the intracerebral noradrenaline neurons to the vascular smooth muscle. This may, in fact, be the

process by which other systems of intracerebral nerves affect cerebral blood flow – unless, as mentioned above, some kind of second mediator is involved.

Nonmyelinated nerve fibres of unknown origin, resembling autonomic axons and containing a majority of moderately electron-dense synaptic vesicles also innervate capillary pericytes and endothelial cells in the hypothalamus, as shown by electron microscopy [31]. This has interesting implications in view of the recent finding that immunohistochemically visible contractile proteins (actin and myosin) are present in the cytoplasm of both types of cells [27]: it may provide a mechanism for a neurogenic vasomotor control also at the capillary level and/or it may underlie the changes in permeability of the blood-brain barrier that can be induced by sympathetic nerve stimulation [20].

Contractile Receptor Functions in Cranial Vessels

Release of *noradrenaline* from the sympathetic nerves of brain vessels, either induced by administration of an indirectly acting sympathomimetic amine, such as tyramine [6, 25], or by electrical stimulation of the perivascular nerves [3], produces vasoconstriction; the sympathetic nerve stimulation is associated with a reduction in local cerebral blood flow, provided the brain region in which the measurements are made has a sufficiently well-developed adrenergic innervation of its resistance vessels [34]. For this reason, primary pharmacological interest has been directed to the cerebral vasoconstriction mediated by the sympathetic neurotransmitter, noradrenaline, and related sympathomimetic amines. The potency ratio for such amines in their contractile effects, together with the mode of inhibition caused by specific reversible or irreversible competitive inhibitors, has shown that the vasoconstriction is mediated by α-receptors [13].

The affinity of noradrenaline for the α-receptors, expressed in terms of the dissociation constant, K_A, which is obtained after irreversible inhibition of a fraction of the receptors, resembles that found for the rat portal vein, but is slightly lower than that reported for the rabbit aortic strip [13]. The relation between the actual noradrenaline-induced response and the α-receptor occupancy showed that the contraction is not directly proportional to the fraction of receptors occupied: half maximum contraction of the vessel (ED_{50}) is achieved with only about 11% of the

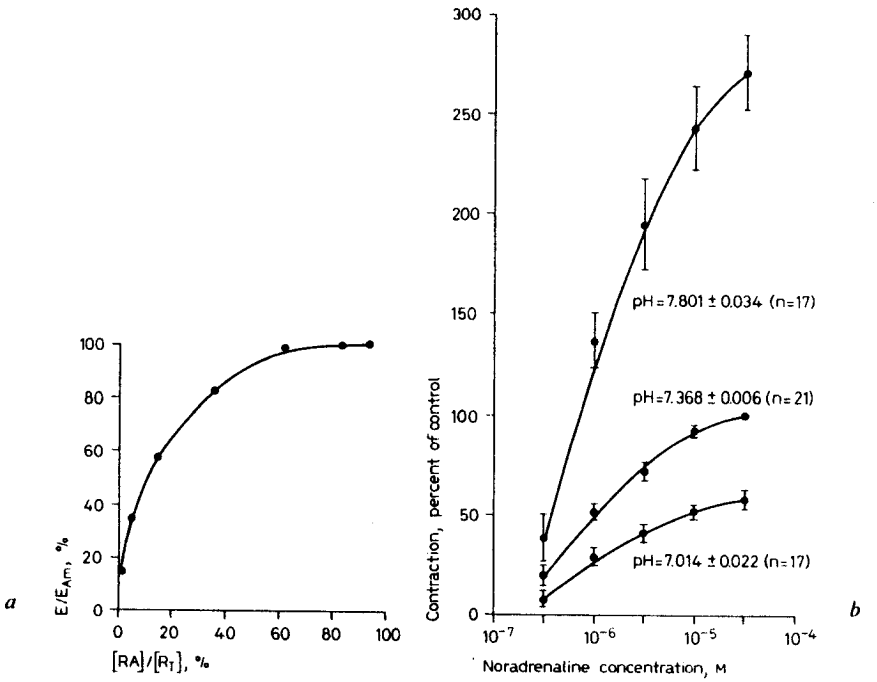

Fig. 2. α-Receptor response in isolated middle cerebral artery from cat. *a* Relation between contractile response and fractional receptor occupancy. E = Effect obtained after the noradrenaline administration; E_{Am} = maximum response; [RA] = concentration of the complex between α-receptor and noradrenaline; $[R_T]$ = total concentration of α-receptors. *b* Dose-response relation of noradrenaline at different pH of the Krebs-Ringer buffer solution. The experiments were performed on sympathectomized or cocaine (10^{-6} M)-treated vessels in the presence of normetanephrine and propranolol. pH was changed by adding small quantities of 0.1 N HCl or 0.1 N NaOH at constant pCO_2. Values are mean ± SEM (number of experiments).

receptors occupied, and maximum contraction (E_{Am}) is reached when about 75% are occupied by noradrenaline (fig. 2a).

When pH is increased (without changing pCO_2) in the medium in which the test artery is suspended, the response to a given dose of noradrenaline is markedly enhanced (fig. 2b), whereas a reduced degree of contraction is obtained at pH levels below normal [15]. Alterations of pCO_2 levels (at constant pH) had little or no effect on the cerebrovascular reactivity to noradrenaline. These observations support the idea that perivascular pH, rather than pCO_2, is of primary importance in the so-called

metabolic regulation of the vessel diameter. It is suggested that the mechanism of action of pH may involve, at least in part, the sympathetic neuroeffector apparatus by modulating the vasomotor action of noradrenaline at the α-receptors. The exact localization of the pH effect is difficult to determine, but it seems probable that it is partly involved in the excitation-contraction coupling process [15]. In view of the relatively large modifications of active tension produced by relatively small changes in pH, and since blood flow varies with the fourth power of vessel radius, it seems likely that such an interaction may play a significant role in the local regulation of cerebral blood flow.

There is reason to believe that the α-receptor present in the intracranial vasculature differs from α-receptors in peripheral smooth musculature: the dissociation constant for the receptor antagonist complex (K_B) does not conform with corresponding values for other smooth muscle tissues, the ED_{50} values for sympathomimetic amines are comparatively high, particularly for phenylephrine which, moreover, only acts as a partial agonist in the intracranial vascular bed [13].

Within 3 days after sympathetic denervation of the brain vessels, an approximately threefold increase in the sensitivity to noradrenaline develops [6]. A similar degree of supersensitivity is seen (in innervated vessels) with cocaine, supporting the prejunctional nature of this phenomenon. It has recently been found in rabbits that pial vessels also achieve an increased sensitivity to noradrenaline of the same magnitude within 3 days after a cisternal injection of autologous blood [35]. This change in reactivity is associated with a reduction in the content of noradrenaline of the perivascular nerves, and an impairment of the mechanism for transmitter uptake. The findings have led to the assumption that a prejunctional type of receptor supersensitivity to noradrenaline develops in conjunction with a subarachnoid hemorrhage, and may be involved in the development and maintenance of the associated cerebral vasospasm [12].

Numerous investigations have been concerned with the cerebrovascular effects of *histamine*, particularly because of the clinical relevance of the problem. A number of conflicting results have been obtained with histamine: from no alteration in cerebral blood flow, strong vasoconstriction, or a vasodilation; and it has even been suggested that the action of histamine on cerebral blood flow has reflected artifacts related to effects on the extracranial circulation, systemic effects, or to actions on vascular permeability. This confusing situation, together with the recent observa-

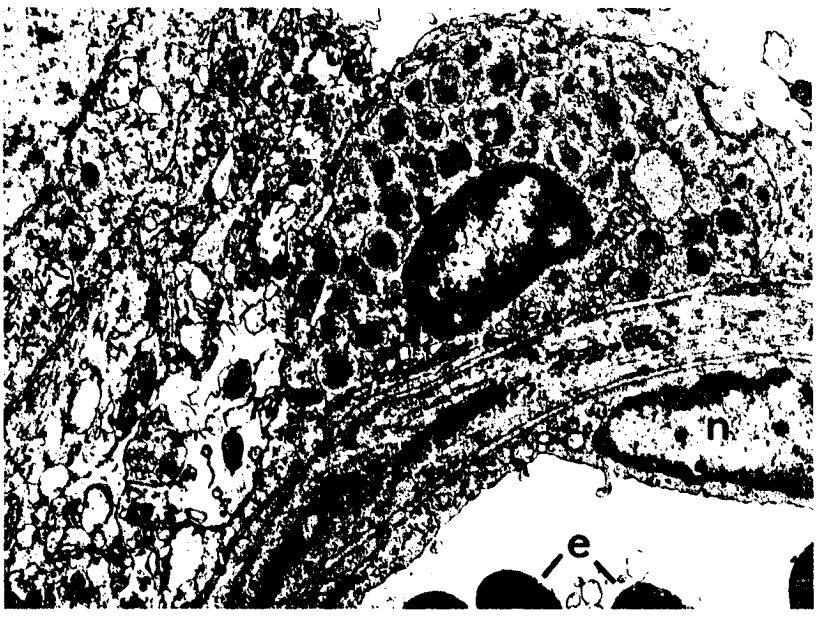

Fig. 3. Electron micrograph of mast cell abutting on the wall of a small intracerebral arteriole of a cat. There is a close relationship between the membranes of the mast cell and the underlying muscle cell, the gap being approximately 20 nm wide and occupied by the fused basement membrane. n = Nucleus of endothelial cell; e = erythrocytes. × 5,000.

tion that mast cells in brain often have a perivascular localization sometimes with a distance to the arteriolar smooth musculature of only 20 nm [7] (fig. 3), has stimulated to a more detailed pharmacological analysis of the histamine receptors under standardized *in vitro* situations [14]. Under these conditions, histamine produces both vasodilation (see below) and vasoconstriction (fig. 4). In extracranial arteries (branches from the external maxillary or lingual arteries), the vasoconstriction produced by histamine is quite remarkable with an ED_{50} value that is only one-tenth of that found for intracranial arteries (middle cerebral artery used as model); the maximum contraction obtained in the latter was only about one-third of that seen with extracranial arteries. The response of the extracranial vessels was inhibited in a competitive manner with increasing doses of mepyramine, showing that the contractile effect was mediated by the H_1 type of receptors; the mean K_B value was found to be in the

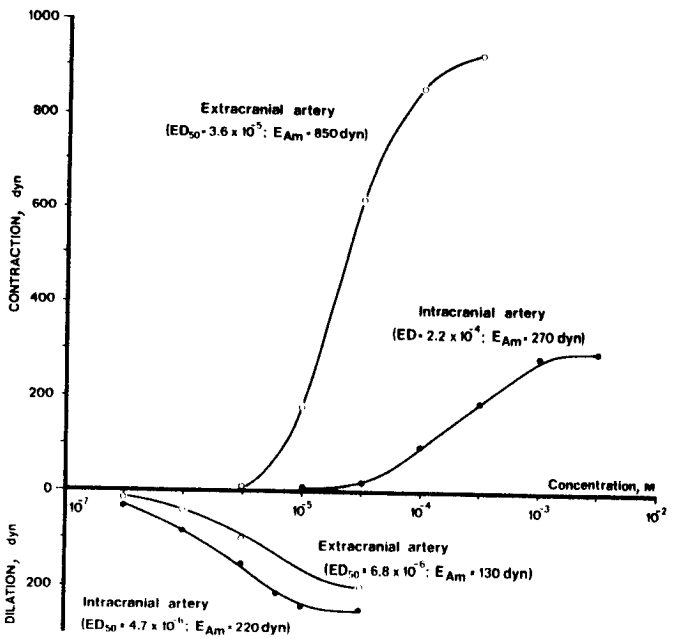

Fig. 4. Composite picture of representative curves comparing the effects of histamine on feline intra- and extracranial arteries *in vitro*. Contractile responses were obtained with H_2 receptors blocked. Dilation effects were recorded during blockade of H_1 receptors at an active tonic contraction of 250 dyn resulting from 5-HT 3×10^{-6} M.

same order of magnitude as those previously reported for other tissues. In the intracranial vessels, however, both of the specific H_1 receptor antagonists tested, mepyramine and chlorpheniramine, produced a reduction in the slope of the dose-response curve and in the maximum effect of histamine, suggesting that the contractile effect on the vascular smooth muscle in these vessels is of a nonspecific nature. This emphasizes the importance of testing the vasomotor actions of any biogenic amine over the full dose-response range, prior to and after the addition of various specific antagonists, before a conclusion can be drawn about the type of receptors mediating a particular response. The results also suggest that histamine-induced vasoconstriction in the brain may have more of a pharmacological than a physiological interest.

It has been suggested that *5-hydroxytryptamine* (5-HT), released from blood platelets after subarachnoid hemorrhage, might be a pathophysio-

logical factor in the development of the associated cerebral vasospasm [29]. We have known for a long time [24] that 5-HT is the most potent vasoconstrictor amine for brain vessels, and it has been shown that sufficient concentrations may occur in human cerebrospinal fluid following subarachnoid hemorrhage and in serum to produce a strong contraction of brain vessels [1]. From a clinical viewpoint there is much evidence suggesting that 5-HT is also involved in the etiology of migraine.

Reports are in conflict concerning the effect of 5-HT on the intra- and extracranial circulation. Thus, a reduction of cerebral blood flow with a large increase of the extracranial blood flow have been measured. On the other hand, some investigators have observed a reduction in both intra- and extracranial blood flow. As all these experiments have utilized complicated *in vivo* models, and it is not possible to decide whether they have involved only specific 5-HT receptors in the vascular wall, a quantitative pharmacological characterization of the receptors mediating the 5-HT-induced vasomotor response has been carried out on isolated vascular segments from cat and man *in vitro*. The responses of vascular segments from the intra- and extracranial vascular beds have been compared simultaneously under identical conditions [9]. The potency rank for the contractile response was the same in intra- and extracranial arteries, being in the order of 5-HT = LSD (*d*-lysergic acid diethylamide) > tryptamine. One difficulty in the pharmacological characterization of vascular responses as being mediated by specific 5-HT receptors is the lack of sufficiently well-defined 5-HT receptor antagonists: available substances influence not only the 5-HT receptors but, to a varying degree, also adrenergic, cholinergic and histaminergic receptors. Hence, they have only a narrow dose range in which the antagonism can be regarded as specific for one or the other of these receptors. Methysergide has been shown to be a specific 5-HT receptor antagonist on smooth muscle [19] and its effect on adrenergic receptors is, at least *in vitro*, negligible. Furthermore, methysergide does not interfere with the smooth muscle effects of acetylcholine and histamine and has, like other lysergic acid derivatives, a structural similarity with 5-HT. Structurally, LSD resembles methysergide and is commonly used as a 5-HT antagonist. Methysergide produced a parallel shift of the dose-response curves towards higher concentrations of 5-HT, and the calculated pA_2 value for the interaction between 5-HT and this antagonist agreed with those reported from experiments on rabbit aorta and rat fundus strips [9]. It has been suggested that part of the contractile 5-HT effect might involve stimulation of α-adrenergic recep-

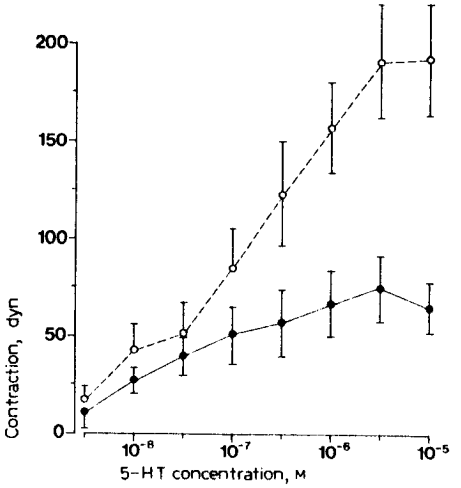

Fig. 5. Dose-response curves showing the increased *in vitro* sensitivity to 5-HT in arteries from rabbits treated with 1–2 ml of intracisternal blood 3 days before the test (○) as compared with normal basilar arteries (●). The curves represent values of mean ± SEM from tests on arteries from 10 animals.

tors. However, moderate concentrations of the α-antagonist, phentolamine, which was shown to produce a parallel shift in the dose-response curve of noradrenaline [13], did not affect the constriction induced by 5-HT. Phenoxybenzamine, an irreversible α-antagonist, tested in a dose that completely abolished the noradrenaline response, only slightly reduced the 5-HT effect. We therefore conclude that 5-HT mediates its contractile effect on extracranial arteries via specific 5-HT receptors. In both cat and man, the intracranial vessels were found to be more sensitive than the extracranial to 5-HT. The contractile effect of 5-HT on intracranial vessels was also inhibited by methysergide, but the inhibition involved a reduction in the maximum contraction and the slope of the dose-response curve; the antagonistic effect was reversible in the sense that it was easily eliminated by washing. As for the extracranial arteries, α-receptor antagonists had little or no effect on the response. The results showing that the potency rank for 5-HT and related agonists is the same in both types of vessels, together with the finding that intracranial arteries are more sensitive than the extracranial arteries to 5-HT, indicate that a 5-HT receptor is involved in the contraction in intracranial arteries, al-

though there seems to be a difference between the two types of vessels regarding the detailed nature of the receptor [9].

With regard to the possible role of 5-HT in the pathophysiology of cerebral vasospasm following subarachnoid hemorrhage [29], the sensitivity of rabbit pial arteries to 5-HT was studied 3 days after cisternal injection of autologous blood to simulate a subarachnoid bleeding [35]. When tested on the basilar artery *in vitro*, 5-HT produced about a three times stronger contraction of the vessel from rabbits receiving the cisternal blood injection compared with arteries from control animals (fig. 5). The mechanism does not seem to involve the sympathetic nervous system, since removal of the superior cervical ganglion has no influence on the reactivity of the cerebral arteries to 5-HT. The results agree with the idea that 5-HT is somehow involved in the pathogenesis of cerebral vascular spasm, which corroborates the observations that blood from dogs pretreated with reserpine, which is known to inhibit the accumulation of 5-HT in the blood platelets and thus reduce their 5-HT concentration, failed to produce spasm when injected into the subarachnoid space of normal dogs [36].

Dilatory Receptor Functions in Cranial Vessels

A sympathomimetic dilatory response is easily obtained in isolated brain vessels under conditions when the contractile α-receptor is inhibited (by e.g. phenoxybenzamine) and – if a high degree of dilatation already prevails under the experimental conditions used – after the vessel has been tonically contracted (by, e.g., 5-HT, KCl, prostaglandin $F_{2\alpha}$). The relative potency for drugs producing dilatory response has been found to be in the order *isoproterenol* > *noradrenaline* = *adrenaline* > *terbutaline* [13]. The effect is inhibited in a competitive way by propranolol [13] and practolol [33], showing that a β-receptor is involved, and the pA_2 values obtained agree with those reported for other isolated tissues [18]. From the finding that isoproterenol was 2,500 times more potent than terbutaline in its dilatory action, and that the potency ratio for noradrenaline to adrenaline was 1.2:1, it was concluded that the β-receptor is of the β_1 type. Further support for the view that the cerebrovascular β-receptor should be classified as a β_1 has recently been obtained under *in vivo* conditions, where changes in local blood flow of the caudate nucleus in nonanesthetized rabbits were followed by the thermoclearance tech-

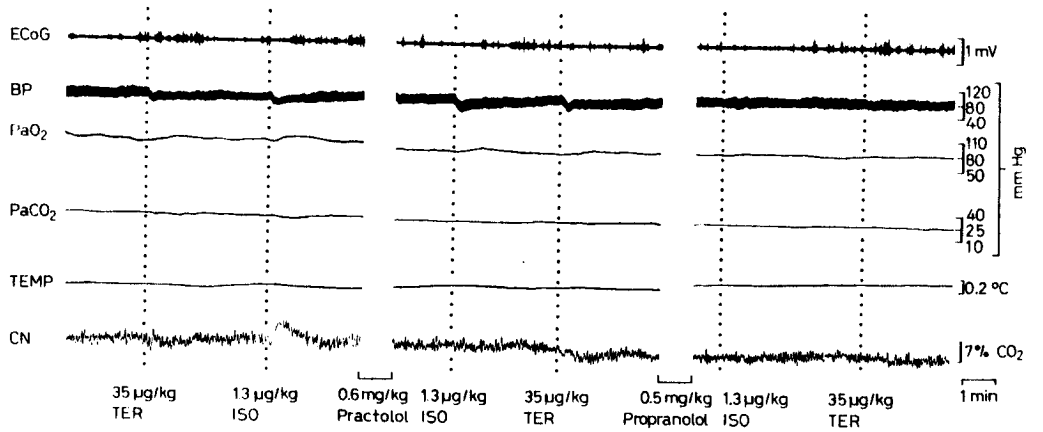

Fig. 6. Representative experiments performed by the thermoclearance technique in a nonanesthetized rabbit, illustrating that different effects of isoproterenol (ISO) and terbutaline (TER) on the blood flow in the caudate nucleus (CN), on the arterial blood pressure (BP) and associated variables. ECoG = Electrocorticogram; $PaCO_2$ and PaO_2 = partial pressure of CO_2 and O_2 in the aorta; TEMP = temperature in the caudate nucleus on opposite side of flow measurement. The calibration is indicated to the right. The BP (a) is reduced after addition of the two agonists in nontreated animals, whereas only isoproterenol increases the caudate nucleus blood flow. Practolol (b) inhibits only the cerebral vascular response to isoproterenol, whereas propranolol (c) abolishes all effects.

nique [33]. As illustrated in figure 6, isoproterenol increased the caudate blood flow and reduced systemic blood pressure. The β_2-receptor agonist terbutaline, on the other hand, had only systemic effects. The caudate nucleus flow response was selectively blocked by the β_1-receptor antagonist, practolol, whereas propranolol inhibited all effects (central as well as systemic) of both agonists. This would mean that the β-receptor in the brain vessels resembles that of the coronary circulation, whereas other parts of the peripheral vascular bed seem to possess the β_2 type of receptors. This is supported by the fact that the potency ratio for the dilatory response induced by isoproterenol and terbutaline in extracranial arteries is only approximately 100:1.

The *cholinergic vasodilation* in the brain provides a particularly complex problem since it involves both postjunctional actions on the vascular smooth musculature and prejunctional effects mediated by the above-mentioned axo-axonal relationship between the adrenergic and cholinergic perivascular nerves [11]. In isolated tonically contracted middle

cerebral arteries acetylcholine and carbamylcholine produce a dose-dependent dilatation in concentrations up to a level of 10^{-6} M. The response is inhibited in a competitive manner by atropine, showing that the effect is mediated by the muscarinic type of cholinergic receptor [8]. (At high doses, above 10^{-6} M, acetylcholine produces a dose-dependent contraction and this effect, too, is inhibited in a competitive manner with atropine. It may be assumed that the dilatory effect, occurring in the lower dose range, is the physiologically more relevant response, which agrees with observations that increased cerebral blood flow is obtained with parasympathomimetic compounds under *in vivo* conditions.) The experiments, performed on sympathectomized vessels, would thus indicate the presence of a direct cholinergic vasodilator receptor mechanism in the pial system. The salivary glands represent another example of a vascular bed where the vasodilation does not occur only through inhibition of the sympathetic vasoconstrictor fibres [5].

The close relationship between the perivascular adrenergic and cholinergic axon varicosities in the pial arteries [11] suggested that the cholinergic system might influence not only the vascular smooth muscle cells, but also adjacent adrenergic axons in the neuroeffector area. Indeed, it was found [8] that the release of noradrenaline from the perivascular nerves of pial vessels during transmural electrical stimulation *in vitro* was inhibited by acetylcholine or nicotine (whereas the spontaneous release of noradrenaline from nonstimulated vessels was not altered in the presence of nicotine). The effect of either compound could be counteracted by hexamethonium (but not by atropine); hexamethonium alone enhanced the transmitter release during the sympathetic nerve stimulation. Acetylcholine, released during stimulation of the perivascular cholinergic nerves, (of sympathetically denervated vessels) would thus tend to inhibit the release of noradrenaline from the adjacent adrenergic nerves via a nicotinic type of cholinergic receptor [8]. That a ganglionic mechanism might have been involved can be ruled out on an anatomical basis: ganglion cells have never been observed in that part of the pial vasculature used in the experiments [11] and, accordingly, Borodulya and Pletchkova [4] only observed cholinergic ganglion formations in the intracranial portion of the internal carotid artery. Moreover, since nicotine alone did not affect the spontaneous efflux of tritium, and atropine did not counteract the inhibitory effect of nicotine on the electrically induced release of noradrenaline, the possibility that the nicotinic receptors are located on the cholinergic axons running close to the adrenergic neurons can be ruled

out. Since the adrenergic sympathetic nerves appear to be under a vasoconstrictor tone [34], it follows that the cholinergic system, besides its direct (postjunctional) vasodilatory action mediated via the muscarinic receptors in the vascular smooth muscle, can promote pial vasodilation also indirectly, through a (prejunctional) inhibition of the noradrenaline release via the nicotinic receptors present on the perivascular sympathetic nerves. The interaction between the adrenergic and cholinergic systems has been confirmed in measurements of blood flow in the caudate nucleus of nonanesthetized rabbits utilizing the above-mentioned thermoclearance technique [2]: A direct, dose-dependent increase in flow was achieved with carbamylcholine (infused at a rate of 2–4 μg/kg/min). Furthermore, this agent also produced a 50% inhibition of the flow reduction evoked by stimulation of the superior cervical ganglion. Such a mechanism should be distinguished from that which involves the well-known cholinergic (muscarinic) receptors on adrenergic nerve fibres in tissues lacking a direct innervation by cholinergic nerves, as recently discussed by FOZARD and MUSCHOLL [17]; the physiological role of the latter arrangement seems less evident.

As mentioned above, *histamine* in high doses produces a cerebral vasoconstriction which does not involve specific histamine receptors [14]. When this response is blocked by mepyramine, histamine is able to dilate isolated brain vessels (fig. 4). This dilatory response, however, is inhibited in a competitive way by burimamide, showing that it is mediated by specific histamine receptors of the H_2 type. A similar response, though with a lower maximum effect, was obtained in extracranial arteries (fig. 4), and the values for pA_2 (and K_B) obtained in experiments involving competitive reversible inhibition by burimamide were in the same order of magnitude as those reported for other tissues. The interesting action of histamine on the cerebral circulation thus appears to be the vasodilatation, which can be evoked at low doses and is blocked in a competitive manner by antihistaminic drugs.

Most investigations on the vascular action of *5-hydroxytryptamine* have focused on its vasoconstrictor effects, probably because of its suggested involvement in the development of cerebral vasospasm following subarachnoid hemorrhage, and because of the fact that it is the most potent vasoconstrictor known for brain vessels [1, 24]. In the course of our studies on the characterization of receptors mediating the vasoconstrictor effects of 5-HT [9], it was found that this amine could also dilate both intracranial and extracranial arteries, provided they had been ton-

ically contracted by prostaglandin $F_{2\alpha}$ and the 5-HT-induced contractile effects were blocked by phenoxybenzamine. Under these conditions, both 5-HT and tryptamine dilated the intracranial and extracranial vessels in a dose-dependent way: the maximum effect of 5-HT was approximately 200 dyn, whereas the action of tryptamine on the intracranial vessels was much less pronounced. Judging from the ED_{50} values, 5-HT was more potent than tryptamine in its dilating effect on both types of vessels. Neither methysergide nor LSD were able to produce a parallel shift of the dose-response curves with 5-HT, but instead reduced the maximum effect successively without markedly affecting the ED_{50} value. The dilatory response of 5-HT could, on the other hand, be blocked in a competitive manner by increasing concentrations of two different β-receptor antagonists, propranolol and INPEA. The K_B values, and the corresponding value for pA_2, were, in the extracranial arteries, similar to the corresponding values obtained with isoproterenol as agonist. In the intracranial circulation the pA_2 values for the dilatory action of 5-HT against the two β-antagonists were found to be intermediary between those obtained for the dilatation by isoproterenol in middle cerebral arteries [13] and for isoproterenol-induced effects in peripheral tissues [18]. This supports previous evidence suggesting that intracranial vascular β-receptors are different from those in the extracranial vascular bed.

Concluding Remarks

The well-developed adrenergic and cholinergic innervation of the cerebrovascular system is described in relation to the characteristics of several amine receptors mediating contractile and dilatory functions in the vessels. Comparison is made with the corresponding receptors in the extracranial circulation. Because of the limited space, the discussion in this short treatise is mainly based on results obtained in our own laboratory; a more comprehensive review on amine mechanisms in the cerebrovascular bed has recently appeared [10], and full reference to work from other laboratories is given in this and in the original articles mentioned in the present paper.

A rather detailed understanding of the various receptor mechanisms mediating the effects of perivascular nerves and several vasoactive amines on cerebral blood flow is now beginning to develop. The complex situation is summarized in figure 7. It is a general impression that there is a

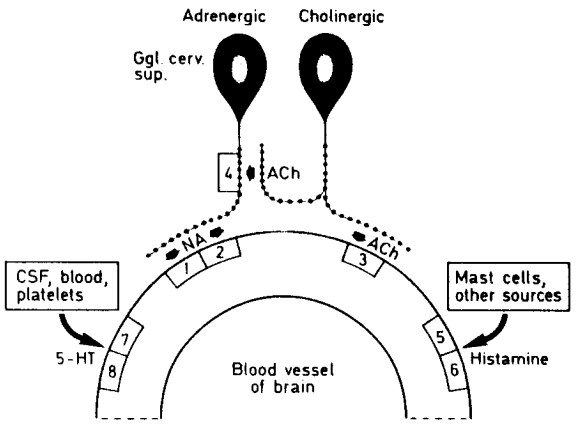

Fig. 7. Schematic representation of the postganglionic autonomic innervation and other sources of amines acting on brain vessels, together with the corresponding receptors and some of their pharmacological characteristics, including pA_2, which measures competitive antagonist affinity.

Receptor No.	Receptor type	Response	Inhibitors tested	Mode of inhibition	pA_2
1	alpha	+	piperoxan	competitive (reversible)	7.06
			phentolamine	competitive (reversible)	
			phenoxybenzamine	competitive (irreversible)	
			dibenamine	competitive (irreversible)	
2	$beta_1$	−	propranolol	competitive	9.17
			INPEA	competitive	
			practolol	competitive	
3	muscarinic	− and +	atropine	competitive	10.43 and 10.07
4	nicotinic	−	hexamethonium	competitive	
5	histamine (nonspecific)	+	mepyramine	noncompetitive	
			chlorpheniramine	noncompetitive	
6	histamine H_2	−	burimamide	competitive	5.51
7	serotonin	+	methysergide	noncompetitive (reversible)	
			LSD	noncompetitive (reversible)	
8	beta (serotonin)	−	methysergide	noncompetitive	
			LSD	noncompetitive	
			propranolol	competitive	8.29
			INPEA	competitive	8.47

+ = Contraction; − = dilation (inhibition in case of nicotinic receptors).

number of major differences in the reactivity of cerebral as compared with peripheral vessels. These are reflected in quantitative as well as qualitative differences in the characteristics of the pharmacological receptors. Since this type of knowledge forms the basis for a more rational approach in the investigations of neurogenic and aminergic control mechanisms in the cerebrovascular bed, it is important to realize that an understanding will not be obtained by simple extrapolation from the peripheral vascular beds. Moreover, it is now also evident that nerve systems other than the aminergic ones, for example peptide nerves, may mediate vascular actions in the brain, and that the neurogenic and so-called metabolic mechanisms may interact to control cerebral blood flow.

References

1 Allen, G. S.; Henderson, L. M.; Chou, S. N., and French, L. A.: Cerebral arterial spasm. II. *In vitro* contractile activity of serotonin in human serum and CSF on the canine basilar artery, and its blockage by methylsergide and phenoxybenzamine. J. Neurosurg. *40:* 442–450 (1974).

2 Aubineau, P.; Sercombe, R.; Edvinsson, L., and Owman, C.: Cholinergic influence on the cerebrovascular bed mediated through actions on the vascular smooth musculature and on the perivascular sympathetic nerves, in Owman and Edvinsson, Neurogenic control of the brain circulation, pp. 331–342 (Pergamon Press, Oxford 1977).

3 Bevan, J. A. and Bevan, R. D.: Localized neurogenic vasoconstriction of the basilar artery. Stroke *4:* 760–763 (1973).

4 Borodulya, A. V. and Pletchkova, E. K.: Distribution of cholinergic and adrenergic nerves in the internal carotid artery. Acta anat. *86:* 410–425 (1973).

5 Burgen, A. S. V. and Emmelin, N. G.: Physiology of the salivary glands; in Barcroft, Davson, and Paton Monographs of the physiological society, pp. 1–279 (Edward Arnold, London 1961).

6 Edvinsson, L.; Aubineau, P.; Owman, C.; Sercombe, R., and Seylaz, J.: Sympathetic innervation of cerebral arteries. Prejunctional supersensitivity to norepinephrine after sympathectomy or cocaine treatment. Stroke *6:* 525–530 (1975).

7 Edvinsson, L.; Cervos-Navarro, J.; Larsson, L.-I.; Owman, C., and Rönnberg, A.-L.: Regional distribution of mast cells containing histamine, dopamine or 5-hydroxytryptamine in the mammalian brain. Neurology, Minneap. *27:* 878–883 (1977).

8 Edvinsson, L.; Falck, B., and Owman, C.: Possibilities for a cholinergic action on smooth musculature and on sympathetic axons in brain vessels mediated by muscarinic and nicotinic receptors. J. Pharmac. exp. Ther. *200:* 117–126 (1977).

9 Edvinsson, L.; Hardebo, J. E., and Owman, C.: Pharmacological analysis of 5-hydroxytryptamine receptors in isolated intracranial and extracranial vessels of cat and man. Circulation Res. (in press, 1978).

10 Edvinsson, L. and MacKenzie, E.: Amine mechanisms in the cerebral circulation. Pharmac. Rev. *28:* 275–353 (1976).

11 EDVINSSON, L.; NIELSEN, K. C.; OWMAN, C., and SPORRONG, B.: Cholinergic mechanisms in pial vessels. Histochemistry, electron microscopy and pharmacology. Z. Zellforsch. *134:* 311–325 (1972).

12 EDVINSSON, L.; OWMAN, C.; SAHLIN, C., and SVENDGAARD, N.-AA.: Changes in uptake and accumulation of norepinephrine in sympathetic nerves of pial vessels following experimental subarachnoid hemorrhage. J. Neurosurg. (in press, 1978).

13 EDVINSSON, L. and OWMAN, C.: Pharmacological characterization of adrenergic alpha and beta receptors mediating vasomotor response of cerebral arteries *in vitro*. Circulation Res. *35:* 835–849 (1974).

14 EDVINSSON, L. and OWMAN, C.: A pharmacological comparison of histamine receptors in isolated extracranial and intracranial arteries *in vitro*. Neurology, Minneap. *25:* 271–276 (1975).

15 EDVINSSON, L. and SERCOMBE, R.: Influence of pH and pCO_2 on alpha-receptor mediated contraction in brain vessels. Acta physiol. scand. *97:* 325–331 (1976).

16 FALCK, B.: Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. Acta physiol. scand. *56:* suppl. 197, pp. 1–25 (1962).

17 FOZARD, J. R. and MUSCHOLL, E.: Do adrenergic fibres have muscarinic inhibitory receptors? A reply. J. Pharm. Pharmac. *26:* 662–664 (1974).

18 FURCHGOTT, R. F.: The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory; in BLASCHKO and MUSCHOLL Handbook of experimental pharmacology, pp. 283–335 (Springer, Berlin 1972).

19 GYERMEK, L.: Drugs which antagonize 5-hydroxytryptamine and related indolealkylamines; in EICHLER and FARAH Handbook of experimental pharmacology, vol. 29, pp. 471–528 (Springer, New York 1966).

20 HARDEBO, J. E.; EDVINSSON, L., and OWMAN, C.: Influence of the cerebrovascular sympathetic innervation on the blood-brain barrier function. Acta physiol. scand. *Suppl. 452:* 65–67 (1977).

21 LARSSON, L.-I.; EDVINSSON, L.; FAHRENKRUG, J.; HÅKANSON, R.; OWMAN, C.; SCHAFFALITZKY DE MUCKADELL, O., and SUNDLER, F.: Immunohistochemical localization of a vasodilatory polypeptide (VIP) in cerebrovascular nerves. Brain Res. *113:* 400–404 (1976).

22 LEE, T. J.-F.; SU, C., and BEVAN, J. A.: Nonsympathetic dilator innervation of cat cerebral arteries. Experientia *31:* 1424–1425 (1975).

23 LINDVALL, M.; CERVOS-NAVARRO, J.; EDVINSSON, L.; OWMAN, C., and STENEVI, U.: Non-sympathetic perivascular nerves in the brain. Origin and mode of innervation studied by fluorescence and electron microscopy combined with stereotaxic lesions and sympathectomy; in HARPER, JENNETT, MILLER and ROWAN, Blood flow and metabolism in the brain, pp. 1.7–1.9 (Churchill Livingstone, Edinburgh 1975).

24 NIELSEN, K. C. and OWMAN, C.: Contractile response and amine receptor mechanisms in isolated middle cerebral artery of the cat. Brain Res. *27:* 33–42 (1971).

25 NIELSEN, K. C.; OWMAN, C., and SPORRONG, B.: Sympathetic nervous control of pial arteries. Tyramine-induced contraction of the isolated middle cerebral artery of the cat; in RUSSEL Brain and blood flow. Proceedings of the Fourth International Symposium on the Regulation of Cerebral Blood Flow, pp. 244–247 (Pitman Medical, London 1971).

26 OWMAN, C.; EDVINSSON, L.; FALCK, B., and NIELSEN, K. C.: Amine mechanisms in

brain vessels, with particular regard to autonomic innervation and blood-brain barrier; in CERVOS-NAVARRO Pathology of cerebral microcirculation, pp. 184–200 (Walter de Gruyter, Berlin 1974).

27 OWMAN, C.; EDVINSSON, L.; HARDEBO, J. E.; GROSCHEL-STEWART, U.; UNSICKER, K., and WALLES, B.: Immunohistochemical demonstration of actin and myosin in brain capillaries. Proc. 8th Int. Symp. Cerebral Function, Metabolism and Circulation, Copenhagen 1977, pp. 384–385.

28 PEASE, D. C. and MOLINARI, S.: Electron microscopy of muscular arteries. Pial vessels of the cat and monkey. J. Ultrastruct. Res. *3:* 447–468 (1960).

29 POOL, J. D.: Cerebral vasospasm. New Engl. J. Med. *259:* 1259–1264 (1958).

30 RAICHLE, M. E.; HARTMAN, B. K.; EICHLING, J. O., and SHARPE, L. G.: Central noradrenergic regulation of cerebral blood flow and vascular permeability. Proc. natn. Acad. Sci. USA *72:* 3726–3730 (1975).

31 RENNELS, M. L. and NIELSON, E.: Capillary innveration in the mammalian central nervous system. An electron microscopic demonstration. Am. J. Anat. *144:* 233–241 (1975).

32 SAMARASINGHE, D. D.: The innervation of the cerebral arteries in the rat: an electron microscope study. J. Anat. *99:* 815–828 (1965).

33 SERCOMBE, R.; AUBINEAU, P.; EDVINSSON, L.; MAMO, H.; OWMAN, C., and SEYLAZ, J.: Pharmacological evidence *in vitro* and *in vivo* for functional beta$_1$ receptors in the cerebral circulation. Eur. J. Physiol. *368:* 241–244 (1977).

34 SERCOMBE, R.; SUBINEAU, P.; EDVINSSON, L.; MAMO, H.; OWMAN, C.; PINARD, E., and SEYLAZ, J.: Neurogenic influence on local cerebral blood flow. Effect of catecholamines or sympathetic nerve stimulation as correlated with the sympathetic innervation. Neurology, Minneap. *25:* 954–963 (1975).

35 SVENDGAARD, N.-AA.; EDVINSSON, L.; OWMAN, C., and SAHLIN, C.: Increased sensitivity at the basilar artery to norepinephrine and 5-hydroxytryptamine following experimental subarachnoid hemorrhage. Surg. Neurol. *8:* 191–195 (1977).

36 ZERVAS, N. T.; KUWAYAMA, A.; ROSOFF, C. B., and SALZMAN, E. W.: Cerebral arterial spasm. Modification in inhibition of platelet function. Archs Neurol., Chicago *28:* 400–404 (1973).

Prof. CHRISTER OWMAN, Department of Histology, Biskopsgatan 5, *S-223 62 Lund* (Sweden)