Br. J. Pharmac. (1980), **68**, 449–459

PARASYMPATHOMIMETIC INFLUENCE OF CARBACHOL ON LOCAL CEREBRAL BLOOD FLOW IN THE RABBIT BY A DIRECT VASODILATOR ACTION AND AN INHIBITION OF THE SYMPATHETIC-MEDIATED VASOCONSTRICTION

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1 Two sorts of effect of carbachol on local cerebral blood flow (caudate nucleus) have been studied: (a) a direct action on the arterial smooth muscle; (b) an interaction with the adrenergic (sympathetic) constrictor system which innervates the vascular system of this nucleus.

2 Continuous measurements of the following variables were performed in lightly anaesthetized rabbits: local blood flow (caudate nucleus), arterial blood pressure, Pa_{O_2}, Pa_{CO_2}.

3 After blockade of the nicotinic synapses in the superior cervical sympathetic ganglion by local hexamethonium injection, carbachol was infused into the common carotid artery, thus minimizing systemic effects of this drug. Infusions of 0.6, 1.3 and 2.5 $\mu g\ kg^{-1}\ min^{-1}$ induced mean increases in caudate blood flow of about 8, 17 and 37% respectively, without notable modifications of other variables measured.

4 The dilator effect of 2.5 $\mu g\ kg^{-1}\ min^{-1}$ carbachol was reduced to a mean of 12% after intravenous injection of 0.5 mg/kg atropine, and could be totally abolished by higher doses (1 to 1.5 mg/kg).

5 Administration of 2.5 $\mu g\ kg^{-1}\ min^{-1}$ of carbachol diminished by more than 50% the reduction in caudate blood flow induced by postganglionic stimulation of the cervical sympathetic chain, but did not affect the reduction of flow obtained by intravenous infusion of noradrenaline (2.5 to 5.0 $\mu g\ kg^{-1}\ min^{-1}$). This inhibition of the adrenergic (sympathetic) system by carbachol was not modified by high doses of atropine (1 mg/kg i.v.).

6 We conclude that: (a) the local cerebral blood flow of a deep structure can be significantly modified by activation of vascular muscarinic receptors; (b) activation of non-muscarinic prejunctional cholinoceptors can cause inhibition of the sympathetic fibres innervating the vascular bed of the same structure.

Introduction

Studies on nervous regulation of cerebral blood flow (CBF) have been mostly confined to the possible role of the sympathetic adrenergic system. However, the cholinergic component of the autonomic innervation of the pial arteries is quantitatively at least as important as the adrenergic component (Laurentieva, Mchedlishvili & Plechkiva, 1968; Edvinsson, Nielsen, Owman & Sporrong 1972; Motavkin, Vlasov & Palashchenko, 1975; Denn & Stone, 1976). It has been demonstrated by electron microscopy that the terminals of both the adrenergic and the cholinergic system form synaptic-type contacts with the arterial smooth muscle fibres (Nelson & Rennels, 1969; Iwayama, Furness & Burnstock, 1970; Nielsen, Owman & Sporrong, 1971). Moreover, the fibres of the two systems run parallel in the adventitial layer, often enclosed in the same Schwann cell sheath, and the proximity of their terminals (25 nm) suggest the possibility of interaction by axo-axonal synapses (Edvinsson *et al.*, 1972).

By contrast with these precise data on the anatomy, there exists little experimental evidence of a cholinergic vasodilator action on cerebral arteries. However, it has been shown *in vitro* that acetylcholine and some cholinomimetic drugs produce a relaxation of pial arteries in a state of tonic contraction (Lee, Su & Bevan, 1975; Edvinsson, Falck & Owman, 1977). A recent paper (D'Alecy & Rose, 1977) has also demonstrated the possibility of a cholinergic dilator mechanism in the dog *in vivo*.

It seems likely that the paucity of physiological investigations results mainly from the absence of precise data on the origin of the intracranial cholinergic nervous system. This gap in our knowledge apparently excludes very clear demonstrations of the effects of stimulation and section of the parasympathetic

0007-1188/80/030449-11 $01.00

nerves, so that we must depend principally on a pharmacological approach. We have therefore employed the cholinomimetic drug, carbachol, in order to examine: (1) the possibility of a direct parasympathomimetic action on the smooth muscle of the cerebral arteries; (2) the possibility of an indirect parasympathomimetic action on CBF based on the hypothesis that the cholinergic fibres can modify the activity of the adrenergic fibres via receptors situated at the axo-axonal contacts mentioned above.

Methods

Continuous measurement of local cerebral blood flow

Details of this method have been given elsewere (Seylaz, 1968; Seylaz, Aubineau, Correze & Mamo, 1973); the principle and the essential stages are as follows. A resin-coated glass probe, diameter 0.7 to 0.8 mm, carrying a thermistor and a constantan heating filament, is implanted in the brain tissue where it is heated by the filament to 0.4 to 0.5°C above the tissue temperature. The latter is monitored by a second probe implanted in the equivalent contralateral structure, and the temperature increment is maintained constant by a negative feedback system (Hémastor, Electronique Appliquée). The current variations necessary to maintain the temperature increment are linearly related to the variations of blood flow in the brain tissue surrounding the probe, as demonstrated by *in vitro* experiments for flows up to 100 ml 100 g^{-1} min^{-1} (Seylaz, 1968). *In vitro* tests have also shown that the time constant of this instrumentation is 0.5 to 1 s for an abrupt change in thermal conductibility of the surrounding medium. These probes are implanted at least 2 weeks before the experiments: this delay is sufficient for the complete resorption of tissue reactions after implantation of a cannula (Edvinsson, Nielsen, Owman & West, 1971; Seylaz *et al.*, 1973) and the formation of a fine (100 μm) layer of scar tissue around the probe (Seylaz, *et al.*, 1973). This tissue cannot play any significant role in the blood flow measurement for two reasons: first, it is poorly vascularised in comparison with the surrounding cerebral tissue, and second, it can be shown from the theoretical analysis of the technique (Seylaz, 1968) that its volume (a few tenths of a mm^3) is relatively very small with respect to that of the sphere of activity of the probe (>10 mm^3). Moreover, we have previously shown that the vascular responses to certain drugs can be very different in two zones of the brain of the same animal (Aubineau, Seylaz, Sercombe & Mamo, 1973; Sercombe, Aubineau, Edvinsson, Mamo, Owman, Pinard & Seylaz 1975), although the tissue reactions around the implanted probes and the vasodilatation induced by CO_2 inhalation are independent of the site of implantation.

After this recovery period, preliminary atraumatic tests are carried out in the days immediately preceding the actual experiment to check the pattern and amplitude of the blood flow responses to defined stimuli (e.g. inhalation of air/CO_2 mixtures, injections of papaverine).

Probes were implanted stereotaxically bilaterally in the caudate nucleus in each of 13 rabbits, under diazepam anaesthesia (Valium, 2 mg/kg i.v.) and pentobarbitone (Nembutal, 30 to 40 mg/kg i.v.). The choice of the structure to be implanted was guided by previous investigations (Aubineau *et al.*, 1973; Sercombe *et al.*, 1975; Lacombe, Reynier-Rebuffel, Mamo & Seylaz 1977) and also by the results of a recent study of the effects of electrical stimulation of the cervical sympathetic chain on caudate blood flow which were studied with 2 complementary methods (Sercombe, Lacombe, Aubineau, Mamo, Pinard, Reynier-Rebuffel & Seylaz, 1979). One of these was the thermal clearance technique employed here, the other was a quantitative, instantaneous measurement obtained by the diffusion of [^{14}C]-ethanol (Lacombe *et al.*, 1977). Identical stimulations were used, the mean maximum decrease in flow obtained being 23.9 ± 1.6% (±s.e. mean) with the first method, and 24.4 ± 1.4% with the second. In view of the high reactivity of this structure to sympathetic stimulation and catecholamine injection (Sercombe *et al.*, 1975; Lacombe *et al.*, 1977), and the close correlation of the results of two different methods, it seemed particularly suitable for studying the influence of cholinomimetic agents on the adrenergic vascular innervation. After experimentation, the position of the probes in this nucleus was verified on histological sections of the formalin-fixed brain cut on a freezing microtome (Leitz 1310). The absence of local inflammation or haemorrhage was also checked.

Measurement of blood gases

Blood partial pressures of O_2 and CO_2 were measured in the aorta by a continuous mass spectrometric method (Seylaz, Pinard, Correze, Luft, Aubineau & Mamo, 1974). The sampling cannula consisted of a flexible stainless steel tube, of outside diameter 0.4 mm, the last few cm of which possessed several lateral orifices and were sheathed with a fine Silastic membrane. The high vacuum of the mass spectrometer draws out from the blood, across this membrane, minute quantities of gas which are continuously analysed by the mass spectrometer (Riber Q.M.M. 16); the signal obtained for each gas is directly proportional to the partial pressure in the tissue. The time constant of the system used in the present experiments was about 8 s. At the beginning of

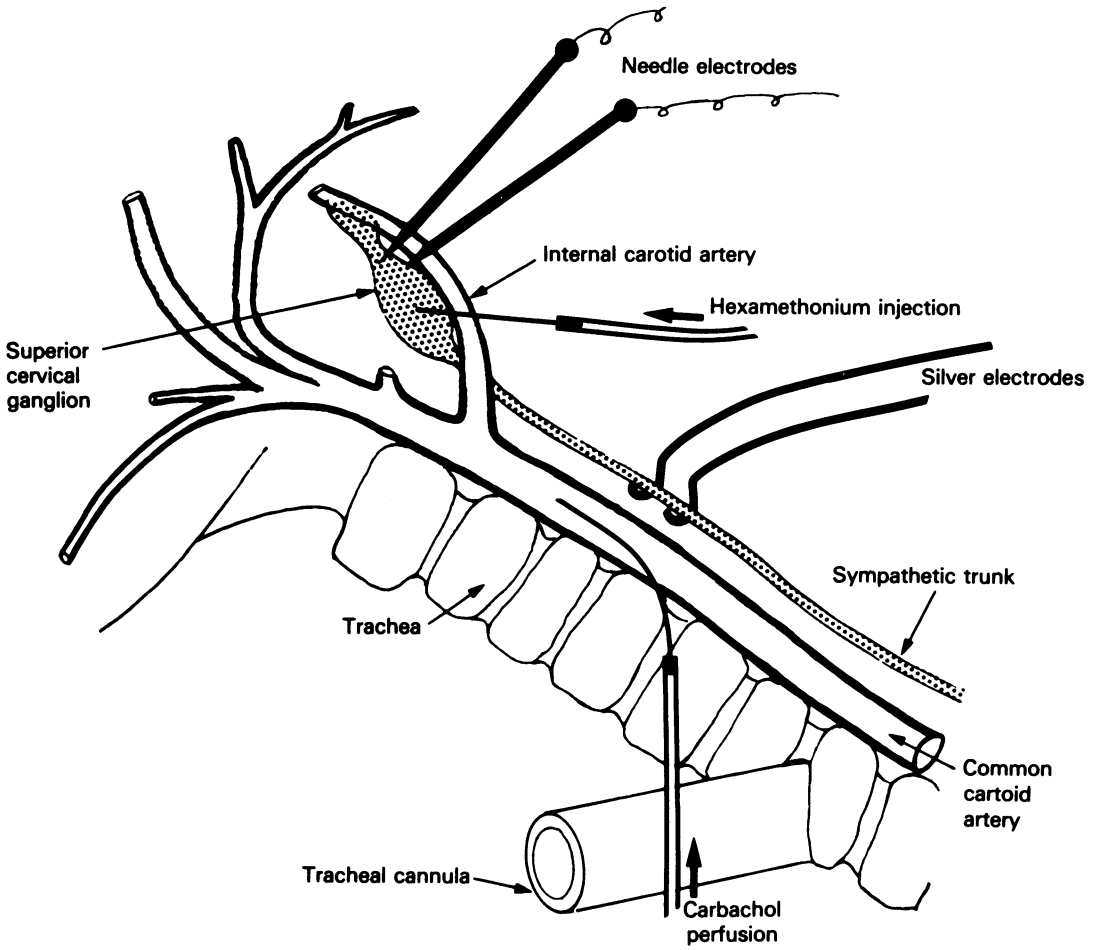

Figure 1 Diagram of the experimental arrangement in the neck region. Carbachol was infused into the common carotid artery via a 0.3 mm needle (o.d.) connected to a variable-speed perfusion pump. Hexamethonium could be injected by a 5 μl Hamilton syringe, via another 0.3 mm needle, into the superior cervical ganglion to block the nicotinic synapses. Notice the needle electrodes in the distal part of the ganglion (for postganglionic stimulation) and the preganglionic electrodes on the sympathetic trunk (for checking the ganglionic blockade).

each experiment the signals were calibrated by polarographic determination of the Pa_{O_2} and Pa_{CO_2} of blood samples (Radiometer BMS 3), and several other such determinations were made (including pH measurement) during the experiment.

General preparation of the animal

Two or three weeks after the implantation of the flow probes, the animals were anaesthetized with diazepam (2 mg/kg i.v.) and pentobarbitone (30 to 40 mg/kg i.v.). After tracheotomy, a femoral artery was exposed and catheterized, the catheter being pushed up into the aorta. This catheter of inside diameter 1.2 mm, not only guided the mass spectrometer cannula into the aorta, but served for continuous measurement of the arterial pressure (Statham transducer P 23 DB) and withdrawal of blood samples for polarographic determinations.

Preparation of the neck region

Figure 1 shows diagrammatically the final stage of this preparation. The sympathetic nerve and the superior cervical ganglion (SCG) were dissected under a binocular microscope; particular care was taken to preserve their vasculature. A pair of silver electrodes was placed on the preganglionic trunk. A hollow, 0.3 mm needle connected to a 5 μl Hamilton syringe, was implanted into the centre of the SCG. Two tungsten

needle electrodes, 0.1 mm diameter, were firmly placed on the distal part of the ganglion; they were used for postganglionic stimulation. Another hollow 0.3 mm needle, connected to a perfusion pump (Harvard, Model 901), was made to pierce the wall of the common carotid artery and held in place by collodion. The whole region was then covered with paraffin oil.

Experimental protocol

The animal was kept lightly anaesthetized during the whole experiment by small regular injections of pentobarbitone (2 to 4 mg/kg i.v.). Under these conditions the spontaneously breathing animal had a $Paco_2$ of 30 to 35 mmHg, a Pao_2 of 80 to 100 mmHg, and a mean arterial blood pressure of 90 to 110 mmHg. Rectal temperature was maintained at 38°C by a heating pad.

Pre- and postganglionic stimulation parameters were determined by trial stimulations such that a maximum effect was obtained on the caudate blood flow and on pupil diameter. Final parameters varied according to the animal, and were within the range 6 to 10 V, 12 to 25 Hz and 1 to 4 ms. The trains of pulses delivered at regular intervals by the stimulator (Digitimer, Devices) also varied in total duration according to the animal. The criteria for fixing this duration were that the control stimulation was sufficiently long to obtain a maximum effect on the blood flow while avoiding too long a stimulation, which, in most cases, was accompanied by an escape phenomenon and sometimes followed by a significant rebound. (Escape: after the initial fall, blood flow tends to return towards its original level despite the continuing stimulation; rebound: blood flow rises above its baseline level whether or not stimulation continues). This escape from sympathetic stimulation is present in many organs and has recently been described in the cerebral vascular bed (Sercombe *et al.*, 1979). The duration of the purely vasoconstrictor stimulations we used varied from 40 to 90 s according to the animal, but the same stimulation parameters (voltage, shock duration, frequency and total stimulation duration) were used in all the tests for a given animal. Furthermore, the interval between 2 stimulations was at least 4 min so that the effect of one stimulation could not be modified by possible late effects of the preceding stimulation. As can be seen in Table 1 the control blood flow response to stimulation varied very little.

After having performed several trial stimulations the nicotinic synapses of the SCG were blocked by a slow intra-ganglionic injection of a hexamethonium solution. Under the experimental conditions used, this blockade did not by itself cause any significant change in the caudate nucleus blood flow. We therefore checked its efficiency by verifying that the effects of preganglionic stimulation had disappeared, while the effects of postganglionic stimulation were unchanged or diminished by less than 10%. The purpose of this blockade was to eliminate a possible interfering action of carbachol through pharmacological stimulation of the ganglionic cell bodies in the SCG. Since some degree of recovery of the ganglionic cells could be detected about 45 min after a hexamethonium injection, we routinely checked this with preganglionic stimulations given about every 30 min; supplementary injections of hexamethonium were made if necessary.

Only after completion of this preparatory phase was a series of tests of the action of carbachol performed in each animal.

At the end of the experiment the rabbit was killed by an overdose of pentobarbitone, and the brain fixed by formalsaline perfusion before histological verification as already mentioned. The results presented were obtained from rabbits in which the probe was found to have been implanted in the heart of the caudate nucleus, with the major part of its sphere of sensitivity (a 2 mm radius sphere around the thermistor) in this structure. Notably insensitive recordings were found in particular in the rare cases in which the probe had penetrated too medially and slid into the ventricle; they were not included.

Drugs injected

Carbachol (carbamyl choline, Sigma) was infused into the common carotid artery at doses of 0.6, 1.3 and 2.5 $\mu g\ kg^{-1}\ min^{-1}$ in the form of isotonic saline solution at a concentration of 10 μg/ml. With this concentration the infusion rate in a 3 kg rabbit was always appreciably less than 1 ml/min and well below rates likely to disturb the cerebral circulation. Tests made by infusing up to 2 ml/min of physiological solution into the common carotid artery did not show significant change in caudate nucleus blood flow. Atropine sulphate (Meram, 0.3 to 1.5 mg/kg of sulphate) and noradrenaline bitartrate (Levophed, Sigma, 2.5 to 5.0 $\mu g\ kg^{-1}\ min^{-1}$ of base) were injected intravenously. Intraganglionic injections of hexamethonium bromide (Sigma) were administered as isotonic saline solutions containing 1 μg/μl of hexamethonium (salt). Quantities injected varied from 0.5 to 3 μl.

Results

Effects of carbachol infusion on caudate nucleus blood flow

Intracarotid infusion of an isotonic solution of carbachol after blockade of the nicotinic receptors in the

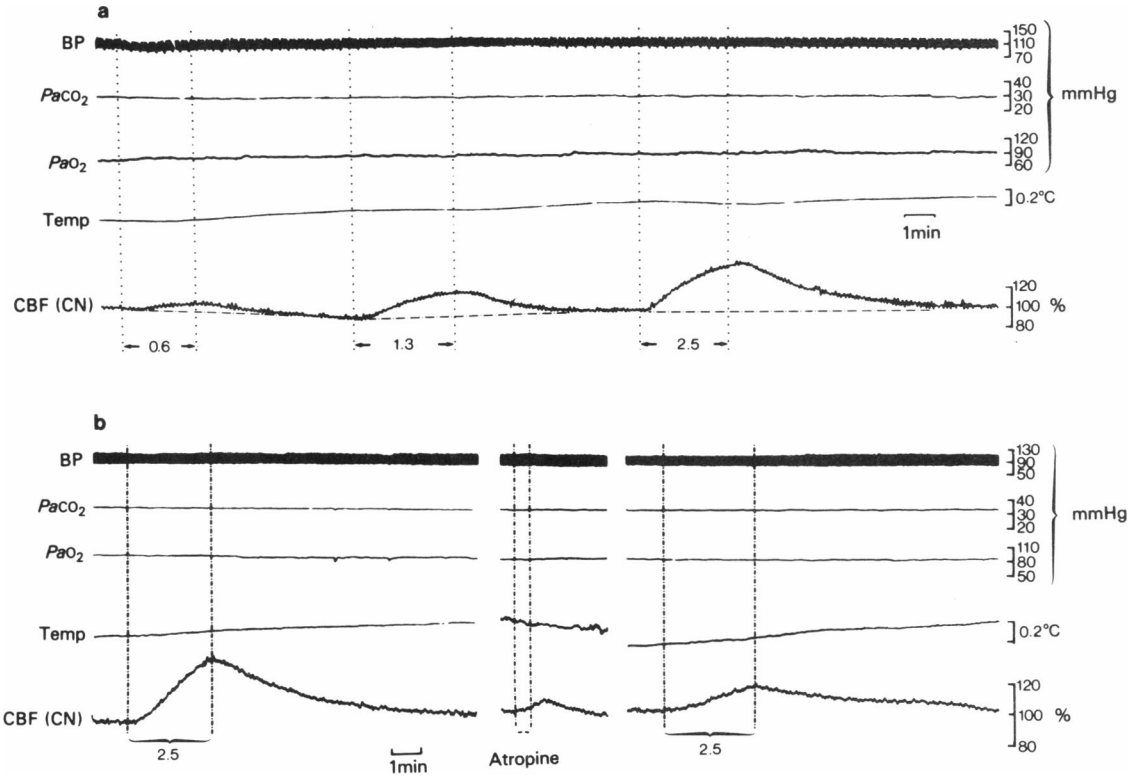

Figure 2 Continuous recordings of the effects of carbachol on caudate blood flow (CBF (CN)). (a) Effects of 0.6, 1.3 and 2.5 μg kg^{-1} min^{-1}; (b) effects of 2.5 μg kg^{-1} min^{-1} in another animal before and after an intravenous injection of 0.5 mg/kg of atropine. BP: arterial blood pressure; $Paco_2$: arterial blood partial pressure of CO_2; Pao_2: arterial blood partial pressure of O_2. Temp: temperature in the caudate nucleus. Neither carbachol nor atropine affected Pao_2 and $Paco_2$. The slight variations of BP and the temperature variations (max. 0.2°C) were unrelated to the injections except that in (a) the slight decrease in temperature corresponded to the increase of flow induced by the highest dose. Notice that the atropine did not totally block the increase in flow caused by carbachol, but reduced it by about 2/3 at the dose employed. Periods of about 5 min separated the different parts of the recording (b).

SCG induced dose-dependent increases in the caudate blood flow. For the doses used (0.6, 1.3, 2.5 μg kg^{-1} min^{-1}), no notable variation in blood gas partial pressures was ever observed. Mean blood pressure diminished by a maximum of 5 mmHg at the highest doses tested (2.5 μg kg^{-1} min^{-1}). The effects of 3 doses (0.6, 1.3 and 2.5 μg kg^{-1} min^{-1}) injected in the same animal within a period of 20 min are shown in Figure 2a. Control injections in the same conditions of isotonic saline alone did not induce any modification of the variables measured. Figure 2b shows the effects of 2.5 μg kg^{-1} min^{-1} of carbachol in another rabbit and partial blockade by intravenous atropine (0.5 mg/kg) of these effects on blood flow. In 4 animals previous intravenous injection of 0.5 mg/kg of atropine reduced by about 2/3 the increase in flow induced by 2.5 μg kg^{-1} min^{-1} of carbachol (Figure 3), and this difference was significant ($P < 0.01$). Higher doses of atropine (1 to 1.5 mg/kg i.v.) used in 3 animals totally abolished the dilator response to carbachol. Atropine itself did not induce any modification of the variables measured, except for caudate blood flow which was sometimes subject to transient changes, usually increases, after rapid and large injections (Figures 2 and 5).

Carbachol-induced inhibition of the vascular response to sympathetic stimulation

The effects of carbachol on the changes in blood flow in response to postganglionic stimulation (after blockade of the nicotinic receptors in the SCG) was tested

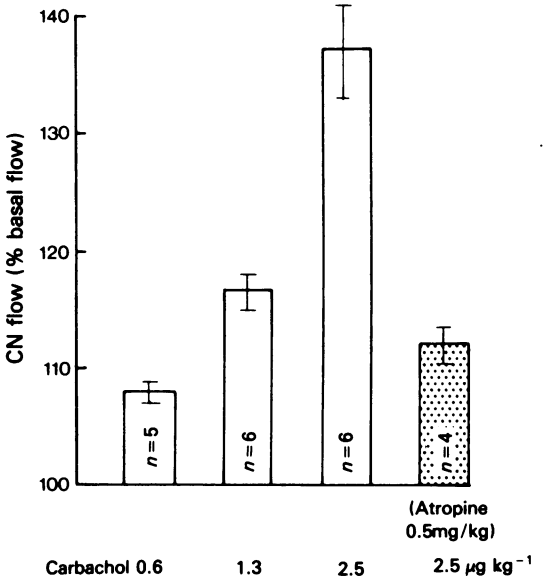

Figure 3 Histogram showing the mean effects of carbachol on caudate blood flow. Open bars: the increase in flow induced by 0.6, 1.3 and 2.5 μg kg^{-1} min^{-1} of carbachol infused into the common carotid artery. Stippled bar: the effects of 2.5 μg kg^{-1} min^{-1} of carbachol after an intravenous injection of 0.5 mg/kg of atropine in 4 of the 6 animals tested at this dose. Vertical lines show s.e. means. The paired t test on these 4 pairs of results indicated a statistically significant difference ($P < 0.01$).

in 10 rabbits. Figure 4 shows 2 examples of these tests. Mean effects of identical control and test stimulations are given in Table 1, showing that carbachol infusion reduces the effect of stimulation by about one half. This difference was highly significant ($P < 0.001$). It can be seen in Figures 4 and 5 that carbachol seemed to exaggerate the rebound of the blood flow often visible after the stimulation, and that the maximum of the constrictor effect of the stimulation occurred at shorter latency under the influence of carbachol.

The inhibition of the vasoconstriction induced by the sympathetic stimulation was reversible 5 to 10 min after the end of the carbachol infusion, this latency varying with the duration of the infusion (Figure 4). However, it was found, that intravenous injection of atropine in doses which blocked the direct, dilator effect of carbachol did not influence the inhibitory action of the latter on the vascular effects of sympathetic stimulation (3 tests with 1 to 1.5 mg/kg atropine and 2.5 μg kg^{-1} min^{-1} carbachol, Figure 5).

In contrast to the effects of stimulation, the decrease in flow induced by intravenous injection of noradrenaline was not modified by the infusion of carbachol (Table 1), whether or not preceded by an injection of atropine. The recording in Figure 5 shows an example of all these tests. It illustrates in particular the absence or insignificance of effects on the blood pressure, Pa_{O_2} and Pa_{CO_2}, except for the rise in blood pressure due to the injections of noradrenaline and the slight transient falls in blood pressure sometimes observed at the beginning of the sympathetic stimulations (pre- and postganglionic).

These results are summarized by Figure 6 and Table 1.

Discussion

The density and the structure of the plexus of cholinergic fibres on the pial arteries is such (Laurentieva *et al.*, 1968; Nelson & Rennels, 1969; Iwayama *et al.*, 1970; Edvinsson et al., 1972; Motavkin *et al.*, 1975; Denn & Stone, 1976) as to suggest that this system could play a significant role in CBF regulation. By means of direct observation of the pial arteries, the existence of a dilator system, probably cholinergic, has been demonstrated by several authors: the tests consisted of either stimulation of the vagus nerves in the neck (Forbes & Wolff, 1928; Chorobski & Penfield, 1932; Cobb & Finesinger, 1932) or selective blockade with atropine of autoregulatory responses to a fall in systemic blood pressure (Mchedlishvili & Nikolaïshvili, 1970). More recent work with a method for measuring global CBF showed that acetylcholine substantially diminished the cerebral vascular resistance, and that this action could be blocked with atropine (D'Alecy & Rose, 1977). To date, histochemical techniques have not yet revealed the presence of cholinergic fibres on intracerebral vessels (arterioles and small arteries), although the existence of adrenergic fibres is generally admitted (Edvinsson, 1975). The present results with carbachol show, however, that the parasympathomimetic influence in a deep structure, such as the caudate nucleus, can be considerable. Our evidence suggests that carbachol acts on blood flow in this structure by (i) action on vascular cholinoceptors, and (ii) by decreasing the vasoconstrictor action of the adrenergic (sympathetic) system through presynaptic inhibition of sympathetic fibres.

Presence of vascular cholinoceptors

The dose-dependent increase in caudate blood flow induced by carbachol was not accompanied by any significant alterations of the other parameters measured likely to contribute to the flow change (BP, Pa_{CO_2}, Pa_{O_2}). This action of carbachol cannot be attributed to a ganglionic effect, either, since the nicotinic synapses of the SCG were blocked by hexameth-

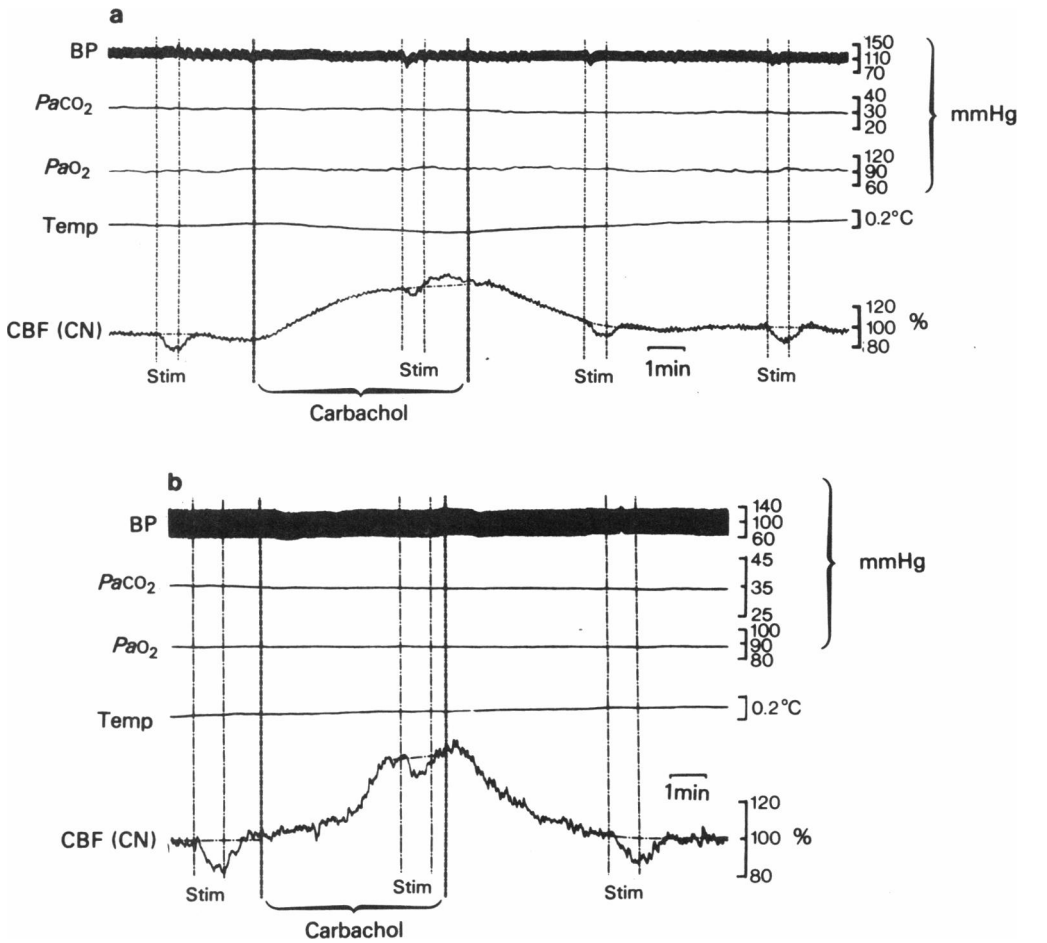

Figure 4 Influence of intracarotid infusion of carbachol 2.5 μg kg^{-1} min^{-1} on the effects of sympathetic stimulation on caudate blood flow. Recordings from 2 different animals. (a) Stimulations at 10 V, 4 ms and 12 Hz (Stim) in animal No. 8 of Table 1. (b) Stimulations at 6.6 V, 1.5 ms and 25 Hz (Stim) in animal No. 2 of Table 1. Notice in both examples the reduction by about 1/2 of the effects of stimulation during the carbachol infusion. At the end of the upper recording, about 8 min after the end of the infusion, the effect of stimulation was again practically the same as before the infusion. The lower recording shows only the beginning of the reversibility. Notice in (a) that no relation can be observed between the small variations in arterial pressure and the flow level, this being corroborated in (b) with a more stable arterial pressure. In neither recording were Pao_2 and $Paco_2$ influenced by the carbachol infusion. Same abbreviations as Figure 2.

onium. The vasodilatation therefore appears to be a local phenomenon, and evidence that, to a large extent this reaction depends on a specific muscarinic action on vascular receptors, is provided by the antagonistic action of atropine. Total blockade of the dilator effect could be obtained at doses of 1 to 1.5 mg/kg of atropine. This result confirms that obtained recently by D'Alecy & Rose (1977) who blocked the dilator action of acetylcholine on cerebral blood vessels of the dog with atropine (1 mg/kg i.v.). However, it is generally considered that 0.1 to 0.5 mg/kg intravenously is a sufficient dose of atropine to block peripheral cholinergic transmission in various animals, whereas in the present work we obtained only a 2/3 reduction in the effect of carbachol on caudate blood flow with a dose of 0.5 mg/kg. Apart from the possibility that atropine reaches the cerebral vascular receptors less readily than carbachol, this absence of

total blockade may perhaps be explained by a small action of carbachol on the metabolism of the caudate nucleus, causing a rise in flow secondarily. Preliminary results obtained in our laboratory in the rabbit (2 animals) and the monkey (1 animal) suggest that the dose of carbachol which produces an increase in flow of about 40% does not induce distinct (>5%) modifications in tissue P_{O_2} and P_{CO_2} measured continuously by mass spectrometry (Seylaz & Pinard, 1978) in the caudate nucleus. Although preliminary, the results compare closely with those obtained by D'Alecy & Rose (1977). They found that injection of 27 μg/min of acetylcholine into the common carotid arteries of dogs gave rise to a mean increase of 55% of whole brain blood flow and that simultaneously the cerebral venous P_{CO_2} fell by 7.5%; this fall corresponds to a drop in tissue P_{CO_2} of only 4.5% according to the data of Pontén & Siesjö (1966). In comparison, intravenous injection in the rabbit of 2.5 mg/kg of papaverine causes caudate nucleus blood flow to augment by 70% and the tissue P_{CO_2} to diminish by 7.5% (Pinard & Seylaz, 1978; Seylaz, Pinard, Dittmar & Birer, 1979).

The slightness of the variation of the tissue P_{CO_2} in these 3 cases, and the fact that the action of papaverine has been shown to be purely vascular (Benzi, 1975), provides evidence for an essentially direct vascular action of carbachol in the present experiments. It seems probable therefore that, although the metabolic needs of the brain tissue may be raised with carbachol, the direct vasodilator action of this drug suffices to maintain normal tissue gas levels except when blocked by atropine (0.5 mg/kg i.v.).

A final point which can be brought up here is that there was apparently no cholinergic tone under our experimental conditions, as evidenced by the absence of direct effects of atropine on caudate blood flow. As regards the absence of an increase in local CBF after blockade of the nicotinic synapses of the SCG, it apparently demonstrates an absence of tonic nervous activity passing via these synapses in our preparations. This result may not be fundamentally in contradiction to previous observations but may be related to the depth of anaesthesia (moderately deep) and the type of sympathectomy (acute ganglionic blockade, as opposed to postganglionic section).

Prejunctional cholinergic inhibition of sympathetic fibres

A recent electron microscopy study has shown that in the pial arteries the terminals of the adrenergic and cholinergic axons can establish close contacts with a gap between the basal membranes of only 25 nm (Edvinsson *et al.*, 1972). The presence of prejunctional cholinergic inhibition of adrenergic fibres has been demonstrated in other vascular beds, in particular in the mesentery (Malik & Ling, 1969), the heart (Haeusler, Thoenen, Haefely & Huerlimann, 1968), and the saphenous vein (Vanhoutte & Shepherd, 1973). However, the essential data on this question have been established using a preparation of rabbit ear artery: it has been shown not only that many cholinomimetic compounds could reduce the efflux of noradrenaline during stimulation of the sympathetic fibres (Rand & Varma, 1970; Steinsland, Furchgott &

Table 1 Comparison of flow decreases induced by postganglionic stimulations of sympathetic chain and by noradrenaline (NA), before and during carbachol infusion

	Postganglionic sympathetic stimulation				*Infusion of 4 μg kg^{-1} min^{-1} of NA (i.v.)*			
R No.	n	*Control*	n	*With carbachol*	n	*Control*	n	*With carbachol*
1	12	25.4 ± 0.6↓	2	3.7↑	2	17.0↑	1	18.0
2	8	24.7 ± 0.7↓	5	10.0 ± 1.2↓	2	20.0↑	1	17.8
3	8	12.5 ± 0.6↓	5	3.7 ± 0.4↓				
4	7	34.8 ± 0.6↓	2	17.3↑				
5	6	27.1 ± 0.6↓	4	13.6 ± 0.6↓	1	22.5	1	24.2
6	2	22.2↑	1	13.3	1	17.8	1	18.0
7	12	27.0 ± 0.5↓	3	13.6 ± 2.2↓	2	24.6↑	1	25.8
8	6	18.7 ± 1.3↓	3	7.7 ± 1.7↓				
9	18	13.7 ± 0.2↓	3	7.3 ± 1.1↓				
10	9	14.9 ± 2.4↓	3	2.0 ± 1.0↓				
	88	22.1 ± 2.2↓	31	9.2 ± 1.6↓	8	20.4 ± 1.4↓	5	20.8 ± 1.7↓
		P (paired *t* test): $P < 0.001$					$P > 0.5$	

R No.: number of the rabbit; *n*: number of tests in each rabbit. Decreases of flow are expressed as a percentage of the normal flow level taken as 100%: ↓ Mean decrease ±s.e. mean.; ↑ mean decrease. All carbachol infusions: 2.5 μg kg^{-1} min^{-1}.

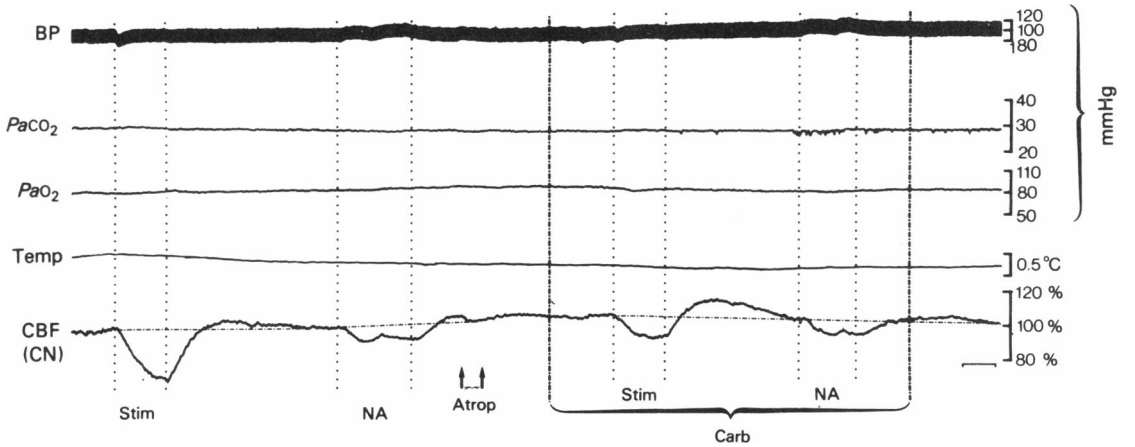

Figure 5 Effects on caudate blood flow of postganglionic sympathetic stimulation (Stim 6 V, 2 ms, 20 Hz) and intravenous injection of noradrenaline (NA 2.5 μg kg^{-1} min^{-1}). The dilator effect of carbachol (Carb) was blocked by intravenous injection of high dose of atropine (Atrop, 1 mg/kg). Atropine did not antagonize the inhibitory effect of cabachol on the decrease in blood flow induced by sympathetic stimulation. This inhibition was greater than 50%. In contrast the action of noradrenaline on blood flow was unmodified by the injections of atropine and carbachol. Pao_2 and $Paco_2$ were not affected by any of the tests, nor was BP, except for the expected rise on noradrenaline injection and the slight, transient fall at the beginning of stimulation. (Similar falls occurred spontaneously and had no effect on caudate blood flow). Same abbreviations as Figure 2.

Kirpekar, 1973; Allen, Glover, McCulloch, Rand & Story, 1975), but also that the vasoconstriction caused by this stimulation was strongly inhibited by the same compounds although they did not alter the vasoconstriction induced by exogenous noradrenaline (Malik & Ling, 1969; Steinsland *et al.*, 1973). Blockade of this inhibition by atropine suggests that the receptors responsible were muscarinic (Fozard & Muscholl, 1974).

Sympathetic stimulation and intravenous noradrenaline injections have been shown in previous investigations to induce decreases in cerebral blood flow, the size of which varied in relation to the density of adrenergic vascular innervation in the cerebral structures studied (Sercombe *et al.*, 1975; Lacombe *et al.*, 1977). This decrease in blood flow was particularly marked in the caudate nucleus, the arterioles of which are richly innervated. In the present study, we have shown that the vasoconstrictor effect of sympathetic stimulation could be strongly inhibited by simultaneous infusion of carbachol. Examination of the time course of the variations of blood flow indicate that the inhibition essentially works by acceleration of the escape from the vasoconstriction. This escape phenomenon has been observed in numerous vascular beds (Ross, 1971) and has recently been investigated in the cerebral vascular bed (Sercombe *et al.*, 1979). It is quite clear in Figures 4 and 5 that, whereas the maximum fall in flow occurred at the end of the control stimulations (the duration of the stimulations being adjusted accordingly), it occurred much earlier during the same stimulations delivered under the influence of carbachol infusion. The level of flow tended to rise despite the persistence of the stimulation, and, despite a maximum fall much smaller than in the control situation, the stimulation under carbachol was followed by a larger rebound than before (especially visible in Figure 5). We should emphasize that in no case could these phenomena be attributed to modifications of the other variables recorded.

The similarity of the reactions to exogenous noradrenaline under control conditions and during carbachol infusion shows that the inhibitory action of the latter does not occur through the vascular adrenoceptors. In addition, since the SCG was blocked by hexamethonium, the inhibitory effect of carbachol could not be due to nicotinic depolarization of ganglionic cells. It seems logical therefore to locate this inhibition on the axons of the adrenergic system, i.e., on the varicosities and nerve terminals.

Support for this hypothesis is provided by a recent study which showed that acetylcholine considerably reduced the efflux of [^{3}H]-noradrenaline induced by electrical stimulation of the sympathetic fibres on the cat middle cerebral artery *in vitro* (Edvinsson *et al.*, 1977). This result corroborates the findings obtained in other vascular beds (already cited), except for the fact that this effect was mimicked by nicotine, and

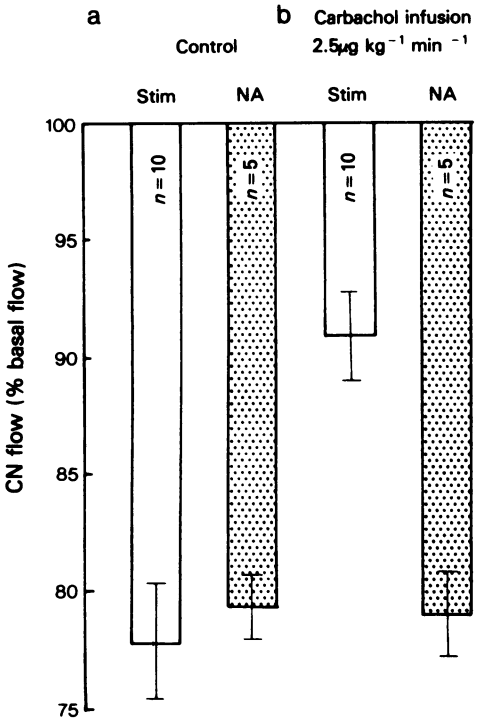

Figure 6 Histogram summarizing the effects of postganglionic sympathetic stimulation (Stim) and of intravenous injection of noradrenaline (NA) on caudate blood flow. (a) Control, indicates mean effects of control stimulations in 10 animals, and mean effects of control injections of 4 μg kg^{-1} min^{-1} of noradrenaline in 5 of these animals. (b) The mean effects of the same tests made during carbachol infusion (2.5 μg kg^{-1} min^{-1} intracarotid). The reduction of the effects of stimulation was significant ($P < 0.001$, paired t test), but the effects of noradrenaline were not significantly different ($P > 0.5$, paired t test).

blocked, not by atropine, but by hexamethonium. The present results apparently confirm that atropine does not antagonize the inhibitory action of carbachol on the sympathetic fibres. It has not yet been possible to confirm that hexamethonium specifically blocks this action *in vivo* because of the large disturbances of systemic blood pressure and other variables induced by this compound. Present evidence therefore suggests that a cholinergic mechanism exists at the adrenergic terminals which can inhibit the secretion of noradrenaline and thereby considerably reduce the vasoconstrictor effect induced by stimulation of these fibres.

Such a mechanism might explain the results of McKee, Denn & Stone (1976) who showed that electrical stimulation of the fastigial nucleus augmented CBF, probably through activation of the parasympathetic system. This dilator action was diminished, but not abolished, after postganglionic sympathectomy and degeneration of the sympathetic perivascular fibres. To explain these findings, the authors proposed a twofold neurogenic vasodilator mechanism, one part of which acts by inhibition of the sympathetic system. This appears to be compatible with the present findings which can be explained by the presence of a single cholinergic dilator system with two parallel modes of action, one by direct action on the vascular smooth muscle, the other by prejunctional inhibition on the sympathetic terminals.

In the light of the possibility of a two-fold cholinergic influence, as shown by the present results, it seems necessary to re-examine the question of the cholinergic innervation of intracerebral arteries. If there were really no intracerebral cholinergic fibres, as suggested by work so far published and already cited, the vascular cholinoceptors demonstrated here could only be activated by circulating acetylcholine. In view of the cholinolytic properties of blood, such a hypothesis seems untenable, and it appears more reasonable to suppose that suitably refined techniques for demonstrating cholinergic nerves have not yet been applied to intracerebral vessels.

Although no direct proof of the functioning of the cholinergic system innervating the cerebral arteries is given in this paper, the evidence forms a possible functional basis for its role in CBF regulation, and suggests that it may act via close interaction with the adrenergic (constrictor) system. The nature of the physiological role of these two systems remains to be determined.

We are indebted to Mademoiselle Françoise Emery for her secretarial help, and to Madame Solange Damato and Madame Claudine Grivas for their technical assistance. This work was supported by grants from the Centre National de la Recherche Scientifique (R.C.P. 319) and the Institut National de la Santé et de la Recherche Médicale (No. 7522136).

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(Received February 2, 1979.
Revised April 25, 1979.)