Cholinergic Regulation of Intracerebral Noradrenergic Pathway-Induced Hypothalamic Vasodilatation


SUMMARY Stimulation of the intracerebral noradrenergic pathway (INP) increases hypothalamic blood flow as measured in conscious rabbits using a 133Xenon washout technique. This increase is abolished by the intra-hypothalamic injection of 0.65 μg of the muscarinic antagonist atropine and by 5 μg of the nicotinic antagonist mecamylamine. Further, 1 μg of the cholinomimetic methacholine produces a similar vasodilatation. While methacholine enhances the vasodilatation on stimulation of the INP, destruction of the pathway abolishes the entire vasodilator response to methacholine. Removal of the superior cervical sympathetic ganglion does not abolish vasodilatation. A role for endogenous acetylcholine in the INP-induced vasodilatation is thus proposed. This vasodilatation appears to act via an increase in neuronal activity with a resultant lowering of local pH, as 60 μg barbiturate and intra-hypothalamic bicarbonate abolish the dilatation completely. The cholinergic vasodilatation reported here is probably an excitatory effect on the INP and is not likely to be due to an inhibition of sympathetic vasoconstrictor tone.

THERE IS LITTLE DOUBT that autonomic fibers can affect intraparenchymal cerebral blood flow, although the importance of these nerves in the regulation of cerebral blood flow is not clear. Adrenergic fibers arising in the superior cervical ganglia are vasoconstrictor, and those running in the intracerebral noradrenergic pathway are vasodilator. Cholinergic fibers have been demonstrated in pial vessels and in the anterior cerebral artery. There is also a diffuse intracerebral network of cholinergic fibers associated with intraparenchymal blood vessels. Cholinergic nerves are usually considered to be vasodilator.

Cholinergic and adrenergic fibers often run together within the same Schwann cell sheath, and it has been suggested that this close association offers the possibility of interaction in the cerebral as well as other vascular beds.

Recently we demonstrated that in the hypothalamus of conscious rabbits interaction between cholinocceptor agonists and adrenergic fibers does occur. Cholinocceptor agonists cause a vasodilatation, an effect blocked by atropine, chemical sympathectomy of intrahypothalamic adrenergic nerves using 6-hydroxydopamine, and by the β-adrenoceptor antagonist propranolol. However, our experiments were not able to identify whether endogenous acetylcholine could cause similar effects, nor were we able to identify a source of endogenous acetylcholine.

We have, therefore, investigated the possible sources of endogenous acetylcholine and whether endogenous acetylcholine could produce effects similar to those of exogenous cholinocceptor agonists.

Method

Twenty-two New Zealand white rabbits of both sexes and weighing between 2 and 3 kg were used. The hypothalamus was chosen as the test region because of its ease of access, its relatively large mass of homogeneously perfused grey matter, and because it is well supplied with adrenergic nerves. It also has a high rate of turnover of acetylcholine. Access to the hypothalamus was gained using a modification of the method of Monnier and Gangloff. Two weeks before experimentation, rabbits were anesthetized using 30 mg/kg Nembutal (Abbott) and perspex headplates were screwed to their skulls. Holes drilled through the headplates at coordinates aB-15 mm allowed stereotaxic access to each side of the hypothalamus. At the time of experimentation hypothalamic blood flow (HBF) was measured in conscious rabbits using the 133Xenon clearance technique. To measure the effects of various drugs and procedures on HBF, one side of the hypothalamus was designated the control side, the other the test side. Blood flow on each side of the hypothalamus is the same, so that any changes in HBF measured on the test side compared to the control side must depend on the experimental drug or procedure. Also, we measured arterial blood pressure, blood gas tensions and pH to exclude any effect of the experimental procedures on these variables. Blood pressure was measured using an indwelling catheter in the carotid artery, and recorded on a Beckman dynograph using a Statham P23AA strain gauge and a strain gauge coupler. Blood samples for blood gas analysis and pH were taken from the carotid artery and measured on a blood gas analyzer (Instrumentation Laboratory 313). During each experiment injection cannulae were...
placed so that their tips lay in identical positions in the hypothalamus on each side of the midline. Injections into the control side contained 15 jCi "xenon dissolved in 5 nl saline. The test side received the "xenon-in-saline plus the test substance in the same volume. Injections were given into each side alternatively at intervals of 10 min, to allow for xenon clearance and the return of radioactivity to pre-injection levels. After each injection the clearance of the radioactive isotope was measured for 500 sec using an external collimated scintillation counter and recorded on magnetic tape using a Hewlett Packard 2644A video terminal. HBF was then calculated from the "xenon clearance curve on an IBM 370/158 computer using a non-linear regression analysis. HBF (ml/100 g tissue min"1) was obtained from the formula HBF = X*B where B is the decay parameter of the monoeponential clearance curve and X the tissue-blood partition coefficient for xenon. For the rabbit hypothalamus X = 0.74.18

The first series of experiments was designed to establish whether cholinergic modification of norepinephrine release activated adrenergic nerves arising in the superior cervical ganglia or via those arising in the medulla and running in the intracerebral noradrenergic pathway (INP). In these experiments 1 ng methacholine chloride (Merck) was injected into the hypothalamus after bilateral superior cervical ganglionectomy or after destruction of the INP. This dose of methacholine has been shown previously1 to produce a consistent vasodilator effect. Superior cervical ganglia were removed under Nembutal anesthesia 2 weeks prior to experimentation without damage to the adjacent vagus nerves and blood vessels. In other animals destruction of the INP was achieved by injecting 300 fig 6-hydroxydopamine (6-OHDA) into the pathway at co-ordinates aF-16 mm11 using a concentric needle electrode delivering 3V with a pulse width of 1 msec at a frequency of 5 Hz. Atropine (M. L. Laboratories), Homatropine HBr (Boehringer Ingelheim) and Mecamylamine HCl (Merck, Sharp & Dohme) were used to assess whether stimulation released acetylcholine. Further, stimulation of the INP is considered to cause a vasodilatation via an increase in neuronal metabolism,1 an effect abolished by barbiturate.1 The effect of barbiturate on a methacholine-induced vasodilatation was thus investigated.

The rationale of these experiments was that if the vasodilatation is due to an increase in neuronal metabolism, then specific neuronal depressants would inhibit the response. Barbiturate (pentobarbital sodium, Sigma) was chosen as it has a well-established depressant effect on neurons17 but no effect on axons18 or blood vessels1 at the concentrations used, at least in vitro. In our experiments 60 fig of barbiturates in a final volume of 5 nl xenon-saline was injected into the hypothalamus 30 min before injecting 1 fig methacholine (MC).

There is evidence that changes in blood flow in the hypothalamus, consequent to cerebral metabolism, may be effected through changes in local pH.11 If the increased neuronal metabolism induced by stimulation of the INP causes a vasodilatation via a lowering of local pH, then this dilatation ought to be blocked not only by barbiturate, but also by a local injection of HCO3- in the hypothalamus, with and without stimulation of the INP in the reticular formation.

Absolute values of HBF measured by the 133xenon washout technique are affected by the number of erythrocytes in the perfusing vessels,13 which is a function of both the diameter of the vessels and the viscosity of the blood. However, absolute HBF values are of minor importance in our experiments as the technique is designed to measure changes in flow in each half of the hypothalamus virtually simultaneously. Basal flows for each half of the hypothalamus in an individual rabbit are similar. When saline alone is injected into both the control and test sides of the hypothalamus, blood flow is the same on both sides. This is true both for anesthetized14 and conscious15 rabbits. Further, blood flow remains constant in an individual rabbit, as measured by injections of saline alone over a 90 min period. Thus, while absolute control flows may vary in different rabbits, flows remain constant in individual rabbits.

The changes in flow produced by each experiment were calculated by subtracting each test flow from the preceding control flow, and the results analyzed by 2-way analysis of variance to account separately for inter-individual and inter-treatment differences. Tests of significance were based on the residual variance calculated using the several observations obtained on each animal for each procedure (F test). Each rabbit was used once only for each experiment and was not used more than 3 times in total. In each experiment equal numbers of trials were performed on each animal to avoid individual bias.

Results

In all experiments the analysis of variance revealed inter-individual variation in absolute values of HBF. The mean control flow for all the experiments was 33.4 ± 1.1 ml/100 g tissue/min (X ± SEM). The analysis of changes in flow revealed the following effects of the various drugs and procedures.

Effect of Methacholine

Figure 1 shows that a 1 ng dose of methacholine (MC) causes a vasodilatation.1 This effect can be blocked by atropine, by 9-adrenerceptor antagonists and by chemical sympathectomy of the hypothalamus using 6-hydroxydopamine.14
Figure 1. The effect of barbiturate, cervical sympathectomy and INP destruction on methacholine induced vasodilatation. Note that methacholine (MC) increases HBF, an effect reduced by barbiturate (BARB) and destruction of the INP (INP lesion). Vasodilatation is not affected by removal of the superior cervical ganglia (Cerv. Sym.)

Effect of Barbiturate

Figure 1 also shows that when 60 µg of barbiturate (BARB) is injected into the hypothalamus 30 min before the methacholine, the vasodilatation is abolished. This result suggests the MC vasodilatation is due to the stimulation of neurons and the elaboration of vasodilator metabolites.

Effect of Sympathectomy

Figure 1 shows that removal of the superior cervical ganglia does not abolish the MC-induced vasodilatation. However destruction of the INP does abolish the cholinergic vasodilatation. These results indicate that MC acts only in the presence of an intact INP.

Effect of Stimulation

Figures 2 and 3 show the effect on HBF of stimulation of the INP, an observation we have reported before. This response is enhanced by the addition of methacholine. While homatropine only slightly reduces the effect, the vasodilatation produced by INP stimulation is abolished by both atropine and mecamylamine. The vasodilatation produced by INP stimulation could, therefore, be explained on the basis of stimulation of cholinergic nerves adjacent to the INP or to the release of acetylcholine from adrenergic fibers. Irrespective of the origin of the acetylcholine, it seems clear that endogenous acetylcholine is released and can affect cerebral blood vessels.
While it was clear from earlier work that barbiturate could abolish the dilatation on stimulation of the INP, further evidence for a role of metabolites and pH in particular is presented here. Figure 3 shows that 5 μl of 40 mM bicarbonate abolishes the vasodilatory effect of stimulation of the INP.

Discussion

In all the experiments, analysis of variance revealed inter-individual variability in HBF. The factors contributing to this include the radial diffusion of xenon, trauma, and variation of HBF with time. Interindividual differences are not considered important as each animal served as its own control: flows in half the hypothalamus are compared to flows in the other half virtually simultaneously. Control flows vary between experiments as a result of one additional factor, and that is that control injections are not identical for each of the different experiments. However, control flows on each side of the hypothalamus are consistent in a particular rabbit. It has been shown that there is no disruption of hypothalamic tissue on light microscopic examination in animals used for up to 4 experiments with multiple injections on each occasion. Auto-regulation, a sensitive index of functional vasomotor integrity, could be demonstrated in the hypothalamus, within a range for mean arterial blood pressure of 41–140 mm Hg, and CO2 responsiveness of HBF was maintained.

Further, in all rabbits tested there were no significant changes in blood pressures, blood gases or pH during the experiments. The changes in HBF observed in the experiments reported here are, therefore, dependent on the local effects of the drugs and procedures.

Our experiments suggest that methacholine-induced vasodilatation of intraparenchymal blood vessels is indirectly caused by a release of norepinephrine. The vasodilatation seems to be due to stimulation of neuronal β-adrenoceptors, an increase in neuronal activity and the evolution of metabolites. The evidence for this is that barbiturate, which has no effect on blood vessels or axons but does depress neurons, blocks the effect of methacholine. These findings also support the idea that cholinergic mechanism of norepinephrine from sympathetic nerves. There are, however, no data in the literature supporting an increase in adrenergic receptor sensitivity mediated by acetylcholine or cholinergic agonists, whereas evidence for cholinergic modification of norepinephrine release is abundant.

The source of the norepinephrine appears to be the INP. Not only is the vasodilator response to INP stimulation similar to that of methacholine, but destruction of the INP abolishes the effect of methacholine. Further, removal of the superior cervical ganglia, the only other major source of norepinephrine for the hypothalamus, has no effect on the methacholine mediated vasodilatation. A completely different explanation of the vasodilatation might be that methacholine causes a muscarinic inhibition of vasoconstrictror tone produced by the sympathetic (non-INP) fibers: muscarinic inhibition of norepinephrine release is the likely physiological mechanism of cholinergic modulation of adrenergic effects. However, removal of the superior cervical ganglia has no effect on methacholine vasodilatation (fig. 1). Further, there is little evidence of vasoconstrictor tone in cerebral vessels. Also, removal of the superior cervical ganglia does not result in the disappearance of cholinergic fibers which implies that cholinergic fibers are not closely associated with cervical sympathetic nerves. For these reasons muscarinic inhibition of norepinephrine release from sympathetic nerves arising in the superior cervical ganglion is unlikely. The fact that muscarinic inhibition is not occurring implies that there is cholinergic excitation of norepinephrine release from INP terminals, mediated via receptors that appear to combine the properties of both muscarinic and nicotinic receptors, in that they are blocked by both 0.65 μg of the muscarinic antagonist atropine, and 5 μg of the nicotinic antagonist,
chemically tested to see whether this cholinergic response is due to either adrenoceptor or cholinergic neurotransmitters. However, this question concerning the origin of the acetylcholine released during INP stimulation cannot be answered directly from the available literature. The presence of acetylcholine in adrenergic nerves has been reported in some experiments, but not in others. Moreover, the release of acetylcholine from adrenergic nerves could also be due to the action of cholinergic neurotransmitters on INP nerve terminals. For example, norepinephrine released from INP nerve terminals could act on acetylcholine receptors to release acetylcholine from adrenergic nerve terminals.

The experiments reported here are consistent with the idea that endogenous acetylcholine can increase hypothalamic blood flow although its direct role in vasodilatation is not clear. The vasodilatory effect of INP stimulation depends on an intact INP and is likely to be due to a release of norepinephrine from INP nerve terminals. The vasodilatory effect of norepinephrine released from INP nerve terminals is unlikely to be due to inhibition of vasoconstrictor tone. Further, the release of acetylcholine from adrenergic nerve terminals is likely to be due to the release of acetylcholine from adrenergic nerve terminals. The vasodilatory effect of norepinephrine released from INP nerve terminals is likely to be due to the release of acetylcholine from adrenergic nerve terminals. The vasodilatory effect of norepinephrine released from INP nerve terminals is likely to be due to the release of acetylcholine from adrenergic nerve terminals.

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