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 ganglia 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 cholinceptor agonists and adrenergic fibers does occur. Cholinceptor 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 cholinceptor 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 ear central 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 ear central artery and measured on a blood gas analyzer (Instrumentation Laboratory 313).

During each experiment injection cannulae were

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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") was obtained from the formula HBF = XB where B is the decay parameter of the monoeXponential 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 acted via 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 jg 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. Barbiturate (pentobarbital sodium, Sigma) was chosen as it has a well-established depressant effect on neurons17 but no effect on axons16 or blood vessels1 at the concentrations used, at least in vitro. In our experiments 60 jg of barbiturate in a final volume of 5 nl xenon-saline was injected into the hypothalamus 30 min before injecting 1 ng 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 dilation ought to be blocked not only by barbiturate, but also by a local injection of HCO3-. This hypothesis was tested by the bilateral injection of 40 mM NaHCO3 (Merck) into 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,15 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 anesthetized13 and conscious14 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 j9-adrenoeceptor antagonists and by chemical sympathectomy of the hypothalamus using 6-hydroxydopamine.15
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.1 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.
ANIMALS | TRIALS | ΔHBF | SEM | P |
--- | --- | --- | --- | --- |
3 | 15 | 9.0 | 1.3 | <0.001 |
3 | 15 | -2.1 | 0.9 | N.S |
3 | 15 | 0.6 | 1.7 | N.S |

**FIGURE 3.** The effect of barbiturate and bicarbonate on the vasodilation induced by stimulation of the INP. Note that both barbiturate and bicarbonate abolish the vasodilation caused by stimulation of the INP.

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. Inter-individual 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 cholinceptor agonists do not act directly on blood vessels or neurons but act, instead, by increasing the sensitivity of adrenoceptors to released norepinephrine or by causing the release of norepinephrine from adrenergic nerves. There are, however, no data in the literature supporting an increase in adrenergic receptor sensitivity mediated by acetylcholine or cholinceptor 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 vasoconstrictor tone produced by the sympathetic (non-INP) fibers: muscarinic inhibition of norepinephrine release might be 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,
mecamylamine. However, 5 μg of homatropine, a substance with less muscarinic antagonist action than atropine, but greater nicotinic antagonist activity, could only slightly reduce the dilatation due to INP stimulation. It would thus appear that the muscarinic antagonism may be effected at a dose where nicotinic antagonism is ineffective. Although multiple agonists and antagonists must be tested to characterize fully this cholinergic response, our data would suggest that the muscarinic excitation is the more likely physiological response. The often reported abolition of cerebrovascular dilatation by the specific muscarinic antagonist atropine may then be due to inhibition of norepinephrine release. Our experiments reported here would support that view. Stimulation of the INP by electrical or pharmacological means, causes a vasodilatation which can be blocked by either adrenoceptor or cholinocceptor antagonists. Hypercapnia-induced vasodilatation, which may be due to effects of the INP, can be blocked by atropine and by lesions in the INP.

While the effect of exogenous MC on INP stimulation is small, the observation that atropine and mecamylamine will block INP stimulation mediated vasodilation is consistent with the idea that endogenous acetylcholine is released during stimulation. However, it is not clear whether acetylcholine is released from the adrenergic fibers or from adjacent cholinergic nerves. It is unlikely that adrenergic nerves themselves contain acetylcholine. No direct histochemical test for acetylcholine is yet available and it has not been possible, therefore, to show acetylcholine in adrenergic nerves. Further, decreases in the acetylcholine content of tissues which occur after section of adrenergic nerves do not prove that adrenergic nerves contained acetylcholine because cholinergic nerves also run in the same nerve bundle as adrenergic nerves. The enzyme responsible for acetylcholine synthesis — choline acetyl-transferase — has also not been found in adrenergic nerves.

For all these reasons it is unlikely that the source of acetylcholine is adrenergic nerve fibers; more likely, the source is parasympathetic fibers. Further, the origin of the facial nerve, which carries parasympathetic fibers to other cerebral vessels, is closely associated with the INP in the midbrain. Thus, while stimulating the INP it is highly likely that we are also stimulating adjacent cholinergic nerves.

The experiments reported here are consistent with the idea that endogenous acetylcholine can increase hypothalamic blood flow. This vasodilatory effect depends on an intact INP and is likely to be due to a release of norepinephrine from INP nerve terminals. It is unlikely that the vasodilatation is due to inhibition of vasoconstrictor tone. Further, our findings illustrate that the relationship between nerves, neurotransmitters and cerebral vessels is complex. Figure 4 shows that there may be at least 5 different ways in which catecholamine release may change intraparenchymal vessel diameter. Three of these mechanisms act directly on blood vessel receptors; 2 act indirectly. The direct actions are vasocostriction of the vessels mediated by α-adrenoceptors, vasodilatation mediated by circulating catecholamines acting on vessel β-adrenoceptors and possibly vasodilatation mediated by fibers of the INP innervating vessel β-adrenoceptors. The most important vasodilator mechanism, however, appears to be indirect and caused by increased neuronal activity. The increase in neuronal activity is caused by norepinephrine released from the INP acting on neuronal β-adrenoceptors which in turn leads to a fall in local pH and consequent vasodilatation. The release of INP norepinephrine depends on either the effects of stimulation of the INP or on acetylcholine released from adjacent cholinergic nerves.

References

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