Evidence for a Dual Innervation Affecting Local Blood Flow in the Hypothalamus of the Conscious Rabbit

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SUMMARY We have attempted to evaluate the role of adrenergic nerves which arise from the superior cervical ganglia or which are intracerebral throughout their course, in the control of local cerebral blood flow (CBF). Hypothalamic blood flow (HBF) was measured in the conscious rabbit by the $^{133}$Xe-clearance technique. Stimulation of the upper brainstem, using 5-Hz, 3-V, 1-msec, square wave pulses, increased HBF by a mean of 7.6 ml/100 g per min ($P < 0.005$). This effect was abolished by the intrahypothalamic injection of the $\beta$-adrenoceptor blocker, propranolol, and by chemical sympathectomy of the hypothalamus or of the upper brainstem with 6-hydroxydopamine, but was not altered by bilateral cervical ganglionectomy.

Intrahypothalamic injection of 0.1 $\mu$g of tyramine caused a mean decrease in HBF of 15.6 ml/100 g per min ($P < 0.001$). This effect of intrahypothalamic injection of tyramine was abolished by bilateral cervical sympathectomy but not by chemical sympathectomy of the upper brainstem. These results support the idea that local CBF, at least in the hypothalamus, is mediated by two distinct pathways. The first consists of the sympathetic nerves which arise in the cervical ganglia, and which activate intrahypothalamic $\alpha$-receptors to cause constriction. The second is an entirely intracerebral noradrenergic pathway which stimulates $\beta$-receptors to cause vasodilatation.

We have suggested, as a very speculative hypothesis, that there may be two noradrenergic systems which regulate cerebral blood flow (CBF). The first, originating in the sympathetic ganglia in the neck, supplies mainly extracerebral vessels such as those around the circle of Willis and pial arteries and, to a lesser extent, the intracerebral vessels. The second, or intracerebral, adrenergic system arises in the medulla, and its fibers run with the intraparenchymal vessels of the brain. Although it has not yet been demonstrated that these fibers satisfy the ultrastructural criteria for true vascular innervation, we have proposed that activation of this pathway causes dilation of the intracerebral resistance vessels, and that this effect is mediated by $\beta$-adrenergic receptors.

This paper is a report of the results of experiments designed to assess the physiological effects of each of these pathways on local (hypothalamic) blood flow in conscious rabbits.

Methods

The hypothalamus was chosen as the test region because it is included in the intracerebral noradrenergic pathway. Also, it is a homogeneously perfused and relatively large region of subcortical gray matter that responds to physiological stimuli such as $CO_2$ and changes in blood pressure in much the same way as does flow to the total gray matter. The technique for measurement of local blood flow in the...
hypothalamus of conscious rabbits has been described in detail elsewhere. Our experiments were conducted on 41 New Zealand white rabbits weighing 2.5-3.5 kg. At the time of the experiments two injection cannulas were placed (through a previously implanted head-plate) so that their tips lay in identical positions in the hypothalamus on either side of the midline. Local blood flow was determined by injecting into the hypothalamus 15 μCi of 133Xe dissolved in 5 μl of saline. After each injection, the clearance of the radioisotope was measured by an external, collimated scintillation detector, a rate meter and pulse height analyzer, and a logic and routing assembly, and recorded on a teletype printer and punchtape. In the analysis of the clearance curves, the minimum acceptable peak to background ratio was 50.√N, where N is the background radioactivity. Hypothalamic blood flow (HBF) was then calculated on an IBM 360/50 computer using a nonlinear regression analysis. We rejected any curve that did not fulfill the criteria for data validity of Dell et al. or that was multieponential. This ensured that only steady state flows, at least during the period of xenon clearance, were measured. HBF values were calculated from the formula HBF = λβ, where β is the decay parameter of the monoexponential clearance curve and λ is the tissue-blood partition coefficient (0.74 for the rabbit hypothalamus).

For statistical analysis of the results we used Student's paired t-test. In these experiments two possible sources of variation may occur: the change in blood flow caused by treatment and an idiosyncratic change in blood flow. We controlled the idiosyncratic response by performing similar numbers of trials on each animal used, and by using each animal only once in each experiment. The sensitivity of change dependent on treatment was elucidated by a number of trials on each animal. To test whether these treatment-dependent changes in blood flow were significant, N was obtained from the number of trials performed. In all experiments in which there were significant changes in mean blood flow the change occurred in the same direction in each animal in the group.

One side of the hypothalamus was designated the control side (133Xe in saline only) and the other side was the test side (133Xe in saline plus the test procedure). Injections were made into each side alternately at intervals of at least 10 minutes. We determined changes in HBF on the test side by subtracting from the test flow the mean of the control flows preceding and succeeding the test flow. The change in flow was then expressed as a positive or negative value relative to the control flow, in ml/100 g of tissue per min.

The usefulness and limitations of this technique have been carefully assessed and fully discussed elsewhere. It has been shown that there is no systematic difference in HBF between the two sides of the midline and that there is no disruption of hypothalamic tissue on light microscopic examination in animals used for up to four experiments with multiple injections on each occasion. Autoregulation, 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. Changes in vascular reactivity with time are small, but were in any event allowed for by the experimental design which compares HBF on either side of the midline at closely consecutive times.

In the present experiments, we assessed the possible role of the sympathetic nerves and of the intracerebral noradrenergic pathway in control of HBF as follows. In one series of experiments on 19 rabbits we measured HBF during unilateral electrical stimulation of the intracerebral pathway in the brainstem, before and after ipsilateral β-adrenoreceptor blockade with propranolol in the hypothalamus, or chemical sympathectomy with 6-hydroxydopamine (6-OHDA) in the hypothalamus or brainstem, or cervical sympathectomy. 6-OHDA destroys noradrenergic nerve fibers, and was injected into the hypothalamus or brainstem on the test side at coordinates aB-15 and aF-16, respectively, at least 4 days before the experiment. Bilateral superior cervical sympathectomy was performed 2 weeks before experiments on HBF; in the rabbit the superior cervical ganglia are easily removed without damage to adjacent nerves and blood vessels. In all experiments the vagi were left intact.

We also studied the effect of vasoconstrictor doses of tyramine on HBF in 22 rabbits. Tyramine causes the release of endogeneous NE from intact adrenergic nerve terminals. Vasconstrictor doses of tyramine were injected into the hypothalamus before and after ipsilateral α-adrenoreceptor blockade in the hypothalamus by phenoxybenzamine, chemical sympathectomy by injection of 6-OHDA into the hypothalamus or brainstem, or cervical sympathectomy.

**Results**

**EFFECT OF BRAINSTEM STIMULATION ON HBF**

The first series of experiments was designed to show the effect of stimulation of the intracerebral noradrenergic pathway on HBF. A concentric needle electrode delivering 3 V with a pulse width of 1 msec was placed in the pathway in the upper brainstem region at the coordinates aF-16. At these coordinates the tip of the electrode lies in the reticular formation rostral to the locus ceruleus and caudal to the median forebrain bundle. The cannulas for injecting 133Xe into the hypothalamus were at aB-15 of the same coordinate system. A frequency of 5 Hz caused a significant (P < 0.005) ipsilateral increase in mean HBF of 7.6 ml/100 g per min, which is of the order of 11% of the mean resting HBF. There was no change in HBF on the contralateral side (Fig. 1 and Table 1).

**EFFECT OF β-BLOCKADE, CHEMICAL SYMPATHECTOMY, AND CERVICAL SYMPATHECTOMY ON THE RESPONSE OF HBF TO BRAINSTEM STIMULATION**

The next series of experiments (Fig. 1 and Table 1) was designed to determine whether this effect was mediated by adrenergic nerves. The addition of 20 μg of the β-adrenergic receptor antagonist propranolol to the 133Xe-saline injection had no effect on resting HBF. However, the injection of propranolol into the hypothalamus during stimulation abolished vasodilation; this finding suggests that vasodilation caused by stimulation is a response mediated by β-
FIGURE 1
Effect on hypothalamic blood flow (HBF) of unilateral stimulation (5 Hz, 3 V, 1 msec) at the junction of pons and midbrain (aF-16). HBF is expressed as the change in test side flow compared to the contralateral (control) flow. Each point represents one measurement of test side flow, and the mean and standard error of the mean are shown as the horizontal and vertical lines. Values are shown for stimulation alone and for stimulation after the intrahypothalamic pretreatment of 300 μg of 6-hydroxydopamine (6-OHDA), which destroys noradrenergic nerve fibers. 12 The vasodilator effect of stimulation of the intracerebral adrenergic pathway is abolished by α-receptor blockade with propranolol or by chemical sympathectomy of the hypothalamus or upper brainstem, but is unaffected by cervical sympathectomy.

<table>
<thead>
<tr>
<th></th>
<th>PROP. 6-OHDA</th>
<th>6-OHDA CERV SYMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>20μg i.h.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300μg i.h.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300μg i.h.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>1.5</td>
<td>2.6</td>
</tr>
<tr>
<td>N</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.005</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N.S.</td>
</tr>
</tbody>
</table>

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MECHANISM OF TYRAMINE VASOCONSTRICTION

We have shown previously that the intrahypothalamic injection of 0.1 μg of tyramine decreased mean HBF by 15.6 ml/100 g per min. In our present experiments (Fig. 2 and Table 2) the addition of 50 μg of the α-adrenergic receptor antagonist phenoxybenzamine to the 133Xe-saline injectate had no effect on HBF. However, addition of phenoxybenzamine to the 133Xe-saline-tyramine injectate abolished the vasoconstrictor effect of the tyramine but caused a significant vasodilation. Tyramine vasodilation also was abolished by the injection of 300 μg of 6-OHDA into the hypothalamus 4 days before the experiment, and by cervical sympathectomy 2 weeks previously. In another group of rabbits the vasoconstrictor effect of the tyramine was retained after destruction of the intracerebral NE pathway by the injection of 300 μg of 6-OHDA at coordinate aF-16 (brainstem) 4 days previously. These findings suggest that at the dose used, tyramine causes vasoconstriction by the release of NE from adrenergic nerve terminals to activate α-adrenoreceptors, that these nerves have their origins in the superior cervical ganglia, and that this effect is independent of the intracerebral NE pathway.

DISCUSSION

Previous work has shown that the local intrahypothalamic injection of exogenous NE produces dose-dependent changes in HBF; small doses increase flow whereas larger doses decrease it. The dilator effect of the small dose was abolished by the β-receptor blocker propranolol, and the vasoconstrictor effect of the larger dose was blocked by the α-receptor blocker phenoxybenzamine. Similarly, we have shown that local injections of tyramine, which releases endogenous NE from adrenergic nerve terminals, cause a β-receptor mediated vasodilation when the dose is sufficiently large. We have shown also that intrahypothalamic injections of isoproterenol cause a increase in HBF. 24 These findings suggest that there are α- and β-receptors which affect blood flow in the hypothalamus; activation of β-receptors causes vasodilation, and activation of α-receptors causes vasoconstriction. The β-receptors have a lower threshold for NE than do the α-receptors and thus are activated by smaller doses of NE. The net effect of larger doses is an α-receptor-mediated vasoconstriction.

The nerves mediating vasoconstriction appear to arise in the superior cervical ganglia. Several authors have reported that these nerves have a vasoconstrictor action, although removal of the ganglia has little effect on total CBF or on the ability of the cerebral vasculature to respond to CO₂ or to autoregulate. 25 Our results also suggest a vasoconstrictor action for these nerves in that tyramine-
TABLE 1 Effect of Brainstem Stimulation on Hypothalamic Blood Flow (HBF)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>HBF (ml/100 g per min)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>133Xe-saline i.h.</td>
<td>14</td>
<td>28.3 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>133Xe-saline + propranolol, 20 µg i.h.</td>
<td>12</td>
<td>30.1 ± 3.9</td>
<td></td>
</tr>
<tr>
<td>133Xe-saline i.h.</td>
<td>30</td>
<td>45.9 ± 2.0</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>133Xe-saline i.h. during stimulation</td>
<td>25</td>
<td>53.5 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>133Xe-saline i.h.</td>
<td>20</td>
<td>40.1 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>133Xe-saline + propranolol, 20 µg i.h., during stimulation</td>
<td>17</td>
<td>34.2 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>133Xe-saline i.h.</td>
<td>22</td>
<td>29.4 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>133Xe-saline i.h. during stimulation after hypothalamic 6-OHDA</td>
<td>17</td>
<td>31.1 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>133Xe-saline i.h.</td>
<td>27</td>
<td>32.9 ± 2.7</td>
<td>NS</td>
</tr>
<tr>
<td>133Xe-saline i.h. during stimulation after brain stem 6-OHDA</td>
<td>21</td>
<td>28.9 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>133Xe-saline i.h.</td>
<td>17</td>
<td>33.9 ± 1.8</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>133Xe-saline i.h. during stimulation after cervical sympathectomy</td>
<td>15</td>
<td>42.1 ± 1.8</td>
<td></td>
</tr>
</tbody>
</table>

HBF values are expressed as means ± SE. i.h. = hypothalamic injection; 6-OHDA = 6-hydroxydopamine; N = number of experiments; NS = not significant (i.e., P > 0.05, by paired t-test).

The pathways involved in vasodilation are less clear. The intracerebral noradrenergic pathway, so convincingly delineated in the rat, remains to be demonstrated in the rabbit. However, the results presented here provide at least indirect evidence for the presence in the rabbit of an adrenergic pathway running in the reticular formation from the midbrain to the hypothalamus.

Harper et al. suggested that vasodilation is part of an autoregulatory response to extracerebral vasoconstriction. Our results show, however, that vasodilation can be produced after removal of the cervical ganglia and, therefore, after degeneration of the vasoconstrictor nerves to the extracerebral circulation. It also has been shown that vasodilation occurs after either electrical or pharmacological stimulation of the brainstem, whereas direct stimulation of cerebral β-adrenergic receptors by iso-

TABLE 2 Effect of Tyramine on Hypothalamic Blood Flow (HBF)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>HBF (ml/100 g per min)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>133Xe-saline i.h.</td>
<td>32</td>
<td>31.6 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>133Xe-saline + phenoxybenzamine, 50 µg i.h.</td>
<td>27</td>
<td>33.8 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>133Xe-saline i.h.</td>
<td>35</td>
<td>48.9 ± 2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>133Xe-saline + tyramine, 0.1 µg i.h.</td>
<td>32</td>
<td>33.3 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>133Xe-saline i.h.</td>
<td>12</td>
<td>48.5 ± 2.6</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>133Xe-saline + tyramine, 0.1 µg, + phenoxybenzamine, 50 µg i.h.</td>
<td>10</td>
<td>61.6 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>133Xe-saline i.h.</td>
<td>15</td>
<td>50.0 ± 3.6</td>
<td>NS</td>
</tr>
<tr>
<td>133Xe-saline + tyramine, 0.1 µg i.h., after hypothalamic 6-OHDA</td>
<td>13</td>
<td>55.0 ± 5.1</td>
<td></td>
</tr>
<tr>
<td>133Xe-saline i.h.</td>
<td>14</td>
<td>19.5 ± 1.7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>133Xe-saline + tyramine, 0.1 µg i.h., after brain stem 6-OHDA</td>
<td>12</td>
<td>10.4 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>133Xe-saline i.h.</td>
<td>20</td>
<td>32.7 ± 2.0</td>
<td>NS</td>
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<tr>
<td>133Xe-saline + tyramine, 0.1 µg i.h., after cervical sympathectomy</td>
<td>16</td>
<td>30.3 ± 2.1</td>
<td></td>
</tr>
</tbody>
</table>

HBF values are expressed as mean ± SE. Abbreviations are given in footnote to Table 1.
excluding all known causes of hypertension, it is likely that this population of patients is heterogeneous and that the sympathetic system would play a role in only a fraction of that population.

The metabolism of circulating catecholamines is rapid, and catecholamines liberated from the adrenal medulla and from sympathetic nerve fibers diffuse into the bloodstream;\(^13\) therefore, the level of circulating catecholamines at a given time may reflect the sympathetic tone under specific conditions. To be able to compare several individuals, it is therefore necessary to standardize the conditions under which blood is sampled. The maintenance of the supine position for 20 minutes was found to be adequate for estimating basal levels. The marked increase in circulating levels, in response to standing erect for 20 minutes, supports the hypothesis that circulating catecholamine levels may be a good index of sympathetic activity, because it is known that a change from the supine to the erect position is associated with sympathetic activation. Although basal levels were higher in hypertensive patients, the response to changes in position was slightly greater than in normotensive subjects, suggesting an increased sympathetic reactivity in that condition. These results differ from those reported by De Quattro and Chan,\(^8\) who showed that the response to 5 minutes of standing erect was normal or lower in hypertensive patients. Differences in time of blood sampling could account for the discrepancy.

The finding of elevated circulating catecholamine levels in rats made hypertensive by administration of DOCA and sodium also supports the hypothesis that circulating catecholamines may be an accurate index of sympathetic activity. In this model of hypertension, hyperactivity of the sympathetic system has been demonstrated and confirmed by several investigators.\(^8\),\(^9\),\(^10\) Although the etiological factors were similar for all animals, it is interesting to note that circulating levels are spread over a wide range in a pattern similar to that in essential hypertension. Moreover, since it also has been reported that sodium may be an important factor in the etiology of essential hypertension,\(^9\),\(^10\) these findings suggest that DOCA and sodium hypertension may constitute an excellent model for the study of essential hypertension. The large range of values in human and in experimental hypertension still remains unexplained.

Preliminary studies in animals suggest that the highest levels may be linked to a longer duration and to a greater severity of the hypertensive disease. It therefore is possible that circulating catecholamine levels may play an important role not only in the etiology and maintenance of human and experimental hypertension but also in the evolution of the disease and, since elevated catecholamine levels have been associated with myocardial infarction,\(^18\) in the incidence of cardiovascular complications.

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