Role of Catecholamine Degradative Enzymes and the Adrenergic Innervation in Determining the Cerebrovascular Response to Infused Norepinephrine


SUMMARY Cerebral blood flow responses to intra-arterial infusion of norepinephrine (NE) at 0.55 \( \mu \text{g/kg/min} \) and 1.1 \( \mu \text{g/kg/min} \) were studied in 3 groups of baboons. The flow was measured by the intracarotid \(^{133}\text{xenon} \) clearance technique using a computer program to calculate flow (height over area — H/A) flow (initial slope — is) and cerebral metabolic utilization of oxygen (CMRO\(_2\)). The normal response to NE was to increase flow without significant changes in CMRO\(_2\). Blockade of catechol-o-methyl transferase (COMT) produced vasoconstrictor responses to these same NE doses. Monoamine oxidase blockade abolished the normal vasodilation. Denervation of the cerebral circulation with intracisternal 6-hydroxydopamine produced vasoconstrictor responses with flow (H/A) but not with flow (is). It is concluded that the extra-neuronal COMT enzyme is important in limiting the access of blood-borne NE to cerebrovascular constrictor receptors.

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WE HAVE RECENTLY suggested that extra-neuronal uptake and degradation of norepinephrine (NE) in cerebrovascular smooth muscle prevents the accumulation of constrictor doses of this amine at cerebrovascular alpha adrenoreceptors.\(^1,2\) Others have supported this hypothesis and shown that cerebral microvessels have a higher concentration of catechol-o-methyl transferase (COMT) and monoamine oxidase (MAO) than similar mesenteric vessels.\(^3,4\) These enzymes may be located in either the endothelium\(^5\) (preventing NE access from the blood to the tunica media) or in the medial smooth muscle cells\(^6\) (removing NE from the extracellular biophase around the adrenoreceptors). Previous studies have shown both sites to be important in limiting the cerebrovascular response to intraarterially infused NE.\(^2,3\) Osmotic disruption of the blood-brain barrier (BBB) was shown to induce a cerebral dilator response to intracarotid \(^{133}\text{xenon} \) associated with an increase in cerebral metabolic rate of oxygen (CMRO\(_2\)).\(^1\) We have demonstrated that BBB disruption and extraneuronal uptake inhibition cause a cerebrovascular constrictor response to NE.\(^2\) Thus, it seems clear that both the BBB and extraneuronal uptake are important in limiting medial NE concentrations. However, the relative roles of the enzymes COMT and MAO have still to be determined. The present study investigated the effects of COMT and MAO inhibition on the cerebrovascular response to NE in vivo in baboons.

In addition, it has been suggested that NE uptake into the adrenergic nerve plexus surrounding cerebral resistance vessels may be important in limiting responses to exogenous NE.\(^4,9\) This does seem possible when NE is topically applied, but should not significantly affect the responses to NE delivered by the intraluminal route. The present study investigated the effect of cerebrovascular adrenergic denervation by 6-hydroxydopamine (6-OHDA) on the cerebrovascular response to infused NE.

Methods and Materials

These experiments were carried out on 15 baboons (Papio ursinus) weighing 12.6 ± 3.0 (sp) kg. Cerebral blood flow (CBF) was measured by the intracarotid \(^{133}\text{xenon} \) injection technique previously described.\(^2\) The animals were sedated with an intramuscular injection of ketamine hydrochloride (Ketalar), 0.2 mg/kg body weight, and full anesthesia was induced with an intravenous injection of sodium pentobarbital (Nembutal), 20 mg/kg body weight. Small amounts of additional barbiturates were given as required, but measurements of CBF were always made during periods of stable electroencephalographic activity with the dominant frequency in the alpha range.

One of the carotid bifurcations was exposed and a fine catheter inserted retrograde into the lingual artery. The external carotid and all its remaining branches were ligated. A bolus of 30–50 \( \mu \text{Ci} \) of \(^{133}\text{xenon} \) in 0.2 ml of saline was injected via this lingual catheter into the internal carotid artery. The cerebral uptake and clearance of the xenon were monitored using an externally placed, highly collimated, 50-mm diameter sodium iodide crystal detector mounted over the parietal region. The contribution of extracerebral blood flow was minimized by reflecting a flap of skin and muscle from the area beneath the detector. In addition, a lead screen was placed over the anterior region to screen radiation from the orbits. The clearance curves were recorded for 15 min using a Nuclear Enterprises data logging system.
Computer Analysis

The xenon clearance curves were recorded on a Hewlett Packard 2644A video terminal and then analyzed on line by an IBM 370/158 computer. The computer program fitted a bioexponential curve to the raw data using the nonlinear least-squares method as described by Marquardt. The program computes the 18xenon brain/blood partition coefficient λ according to the formula of Veall and Mallett from the xenon solubility data of James et al. Flow values were determined from the initial slope of the clearance curve during the first 2 minutes (\( f_{h/a} \)) according to the method of Hjedt-Rasmussen et al. and by the stochastic method over 10 minutes (\( f_{h/a} \)) according to the method of Hjedt-Rasmussen et al.

Arterial and cerebral venous blood samples were taken before each flow determination and analyzed for hemoglobin and hemoglobin oxygen saturation percentage on an Instrumentation Laboratories 182 co-oximeter. These determinations were used to calculate the A-V oxygen difference across the brain and subsequently the cerebral metabolic rate for oxygen (CMRO₂) by substituting the A-VO₂ difference and the \( f_{h/a} \) in the Fick equation. In addition, arterial PO₂, PCO₂, pH and pressure were monitored and maintained within normal physiological limits.

After surgery the animals were allowed to stabilize for 30 min and then 2 baseline blood flow measurements were made. If these 2 values were similar the alteration in \( f_{h/a} \), \( f_\delta \) and CMRO₂ was documented by cumulative infusion of NE at 0.55 and 1.10 \( \mu g/min/kg \). These responses were determined in 3 groups of 5 animals.

These groups were as follows:

1) COMT blockade. The alterations in \( f_{h/a} \), \( f_\delta \) and CMRO₂ were determined under control conditions. Then 3, 4, dihydroxy-2-methyl propiophenone (Upjohn-0521) was infused at 1.0 \( \mu g/min/kg \) into the internal carotid catheter. After 60 min new baseline measurements were made and if these were stable the response to the same doses of NE was determined.

2) MAO blockade. The response to NE was determined before and after a 60 min infusion of tranylcypromine sulphate (Parnate, SKF) at 4.0 \( \mu g/min/kg \).

3) Denervation. The responses to NE were determined in animals pretreated one week previously with an intracisternal injection of 12.5 mg of 6-hydroxydopamine (6-OHDA) in 1 ml saline into the subarachnoid space.

Statistics

The mean alteration from baseline flow and metabolism when NE was infused was compared in groups 1 and 2 between the control and experimental situations using Student’s paired t-test.

The mean alteration in flow and metabolism in group 3 with NE were compared to the control responses of groups 1 and 2 using Student’s t-test.

In addition, the alteration in baseline flow and metabolism produced by the COMT blocker, the MAO blocker or the denervation was assessed.

Additional Statistics

The variance of each group in each t-test was compared by an F test. When the variances were significantly different a Wilcoxon rank sum test was used in place of the t-test.

Results

The table shows the mean alteration from baseline of \( f_{h/a} \), \( f_\delta \) and CMRO₂ with infusion of NE in the controls and treated animals. The control animals showed a significant increase in \( f_{h/a} \) and \( f_\delta \) from the baseline values of 33.9 ± 2.8 and 26.3 ± 2.5 ml/min/100g respectively (paired t-test, \( p < 0.05 \) compared to baseline control). This increase in flow was not accompanied by any significant increase in CMRO₂. Arterial blood pressure increased non-significantly from 102 ± 4.8 to 112.9 ± 5.4 and 111.6 ± 5.6 mm Hg at the two NE doses and Paco₂, PaO₂ and pH were maintained at 34.8 ± 0.2 mm Hg, 80 ± 1.4 mm Hg and 7.49 ± 0.01 respectively throughout these experiments. Therefore, the increase in flow could be attributed to cerebral vasodilation in response to the infusion of the NE.

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Control</th>
<th>COMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_{h/a} ) (ml/min/100g)</td>
<td>+9.7 ± 3.6</td>
<td>+15.5 ± 4.8</td>
</tr>
<tr>
<td>( f_\delta ) (ml/min/100g)</td>
<td>+1.8 ± 4.0</td>
<td>+0.1 ± 2.2</td>
</tr>
<tr>
<td>CMRO₂ (ml/min/lOOg)</td>
<td>-9.0 ± 7.2</td>
<td>-6.8 ± 8.2</td>
</tr>
</tbody>
</table>

**TABLE 1**

Alteration from Baseline in Cerebral Blood Flow (\( f_{h/a} \) and \( f_\delta \)) and Oxygen Consumption (CMRO₂) with Infusion of Norepinephrine in Controls, COMT Inhibited, MAO Inhibited and Adrenergically Denervated Groups. Values are Means ± One Standard Error.

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Control</th>
<th>COMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_{h/a} ) (ml/min/100g)</td>
<td>+11.5 ± 2.2</td>
<td>+14.1 ± 3.2</td>
</tr>
<tr>
<td>( f_\delta ) (ml/min/100g)</td>
<td>+2.1 ± 2.7</td>
<td>+0.6 ± 2.3</td>
</tr>
<tr>
<td>CMRO₂ (ml/min/lOOg)</td>
<td>-0.6 ± 4.6</td>
<td>-0.5 ± 4.5</td>
</tr>
</tbody>
</table>

*\( p < 0.05 \) when compared to the control response.
alterations in flow were significantly different from control (table). CMRO<sub>2</sub>, arterial blood pressure, PCO<sub>2</sub>, PO<sub>2</sub>, and pH were not different from the controls. Therefore, COMT blockade caused the CBF response to NE to change to a constrictor one and this new response was significantly different from the normal increased flow.

**MAO Blockade**

Tranylcypromine alone caused an increase in f<sub>H/A</sub>, f<sub>IA</sub> and CMRO<sub>2</sub> to 40.6 ± 3.7, 34.9 ± 3.1 and 2.6 ± 0.1 ml/min/100g respectively (p < 0.05 for f<sub>IA</sub> and CMRO<sub>2</sub>, paired t-test). The subsequent alterations in flow and CMRO<sub>2</sub> with NE infusion were to slightly increase flow but decrease CMRO<sub>2</sub> (table). These responses were significantly different from control (p < 0.05, paired t-test). Therefore, MAO blockade abolished the dilator response to NE seen in the controls.

**Denervation**

The animals treated with 6-OHDA showed mean baseline f<sub>H/A</sub>, f<sub>IA</sub> and CMRO<sub>2</sub> values of 42.9 ± 6.7, 28.8 ± 5.5 and 3.0 ± 0.8 ml/min/100g respectively. Subsequent infusion of NE in these animals caused no significant alteration in f<sub>IA</sub> or CMRO<sub>2</sub>. However, the f<sub>H/A</sub> index showed a constrictor response (Wilcoxon rank sum test p < 0.05). The alterations in blood flow were significantly different from the control alterations (table). Thus, adrenergic denervation with 6-OHDA produced some constrictor responses to NE with no alteration in CMRO<sub>2</sub>.

**Discussion**

The present results show that the control response to the infused doses of NE is vasodilator. This increased flow is associated with a small increase in CMRO<sub>2</sub> and therefore may be partly due to an NE-induced increase in cerebral metabolism and subsequent vasodilatation. Other workers<sup>1</sup> have demonstrated that 0.05 µg/min/kg, when infused after opening of the blood-brain barrier with hypertonic urea, causes approximately a 30% increase in CMRO<sub>2</sub> and a 45% increase in blood flow. The control dilatation recorded in the present experiments show that, with 10 to 20 times the dose of NE and with no manipulation of the blood-brain barrier, the NE (1.10 µg/min/kg) produces a 10% increase in CMRO<sub>2</sub> and a 34% (f<sub>H/A</sub>) to 48% (f<sub>IA</sub>) increase in flow. As this increase in flow-per-unit change in metabolism is much greater than that previously reported for NE when it can cross the blood-brain barrier<sup>7</sup>, or when injected intraventricularly,<sup>8</sup> the probability is that at the present NE concentrations an active vasodilatation was induced. Our previous study<sup>1</sup> supports this idea as the dilation was blocked by a beta-adrenoreceptor antagonist.

The present results suggest that the enzymes catechol-o-methyl transferase and monoamine oxidase together with the neuronal uptake of NE are collectively responsible for preserving the dilator response to these doses of NE and preventing vasoconstriction. Our hypothesis is that the rapid degradation and uptake of NE prevents the amine from reaching sufficient concentration in the tunica media to activate alpha-receptors. MAO inhibition abolished the control dilatation while COMT inhibition and denervation produced a vasoconstrictor response to the NE.

The location of COMT within the cell cytoplasm of vascular smooth muscle cells<sup>5</sup> is important in the control of extraneuronal uptake of catecholamines. The catecholamines are transported across the smooth muscle cell wall and degraded by the cytoplasmic COMT enzyme. This system acts as a "site of loss" of the catecholamine from the biophase (the region of extracellular space around the membrane adrenergic receptors<sup>26</sup>). In the general systemic circulation this extraneuronal uptake and metabolism system has more affinity for epinephrine than norepinephrine.<sup>17</sup> Thus inhibition of COMT has been shown to potentiate the effects of epinephrine but not norepinephrine in the cardiovascular system.<sup>16</sup> This potentiation is presumed to be as a result of accumulation of unmetabolized amine intracellularly from where it diffuses out again to react with receptors. Thus, from a given dose of applied amine more becomes available at the receptor sites. Blockade of COMT in vivo has also been shown to have little or no effect on the magnitude of the pressor response to intravenous catecholamines.<sup>17, 18</sup> However, the COMT mechanism is still considered to be important in the disposition of NA in the tunica media of blood vessels.<sup>19</sup> Although there is little alteration in the magnitude of an NE vascular response with COMT inhibition, it seems clear that prolongation of the time required for relaxation occurs.<sup>19</sup> The present work suggests that this COMT mechanism is more important in the cerebral circulation than these other systemic vascular beds, and that the presence of a high COMT concentration may be essential to prevent cerebrovascular constriction in periods of stress.

Monoamine oxidase has been localized in blood vessels in the endothelium<sup>4</sup> and within the nerve terminals.<sup>20</sup> The MAO activity is mainly associated with the innervation in the general systemic circulation and is thought to play an important role in the regulation of the neuronal catecholamine uptake processes.<sup>20</sup> MAO blockade causes little or no potentiation of the response to exogenous NE.<sup>18</sup> The cerebrovascular localization of MAO in the endothelium plays an important role in the blood-brain barrier.<sup>5</sup> Amines are degraded by this barrier before moving from blood to tunica media and we have demonstrated that this MAO is important in limiting the access of 5-hydroxytryptamine (5-HT)<sup>21</sup> to cerebrovascular smooth muscle. The present study has, however, indicated that the MAO is not as important a barrier to NE as it is for 5-HT. It is probable that the endothelial and medial enzymes are responsible for limiting the access of blood borne NE to the receptors, while neuronal uptake limits that of endogenous NE or
perivascular NE. Such a role has previously been suggested for these perivascular adrenergic nerves and it has also been shown that MAO inhibition causes a pressor response (instead of a dilator response) to intraventricular administration of NE. The present evidence suggests that the perivascular innervation may be important in limiting the cerebrovascular NE concentration when the NE is applied from the luminal surface. However, this influence is not as important as the COMT enzyme.

The constrictor responses of CBF to NE were generally accompanied by a decrease in CMRO₂. This is opposite to the control response of a slight increase in CMRO₂. Such a graded neuronal response to NE has been described and has been attributed to recruitment of neuronal alpha-receptors (producing excitation) or recruitment of beta-receptors (producing inhibition). However, the present alterations in CMRO₂ were generally non-significant, and thus the major influence of NE in the present study seems to be directly on the vascular smooth muscle.

The doses of U-0521 and tranylcyromine used in this study were sufficient to cause an increase in brain CMRO₂, indicating that they have an influence on brain function as well as a cerebrovascular action. The central action was small but resulted in an increased baseline blood flow. The use of a lower concentration may have eliminated these baseline effects and allowed the cerebral vasculature only to be studied. The 6-OHDA dose produced similar non-significant changes. This dose was previously shown to result in adrenergic denervation with almost no detectable adrenergic fluorescence on baboon cerebral blood vessels. Thus, the doses of the 3 drugs were considered optimal for maximal effect on the blood vessels with minimum disruption of the brain function. It is, however, possible that manipulation of the doses would have produced a different order of importance for COMT, MAO and neuronal uptake in modifying the cerebrovascular response to NE.

The alterations reported in response to NE in the present study are unlikely to be due to deterioration in the viability of our animals over the course of the experiment. Such a deterioration would have been accompanied by a decrease in baseline CBF with alterations in the EEG pattern. However, such a possibility must be kept in view. The present evidence suggests that the cerebrovascular uptake and degradation mechanisms may be efficient. This remains to be demonstrated by established in vitro techniques. This hypothesis may have clinical importance in providing a possible explanation for the cerebral vasospasm seen after trauma or subarachnoid hemorrhage when uptake mechanisms may be inhibited) and for the vasoconstriction seen in the prodromal phase of migraine (where latent defects in uptake of amines may be swamped).

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References

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