Cerebral circulation and norepinephrine: relevance of the blood-brain barrier

ERIC T. MACKENZIE, JAMES MCCULLOCH, MAUREEN O'KEANE, JOHN D. PICKARD, AND A. MURRAY HARPER
Wellcome Surgical Research Institute, University of Glasgow, Glasgow G61 1QH, Scotland

The sympathetic innervation of the cerebral circulation is currently a matter of some debate, as evidenced from the diversity of opinions in a number of recent reviews (20, 29, 33, 37). Equivocal results ranging from cerebral vasodilation, through no effect, to cerebral vasoconstriction have been reported.

A sympathetic innervation of the cerebral circulation has been demonstrated in various histochemical and electron microscopic studies, although it would appear that it is only the larger, extraparenchymal arteries that receive this nerve supply (7, 23, 34). Both in vitro preparations and in vivo studies in which the application of norepinephrine would have no systemic effects (24, 40) have shown that norepinephrine constricts cerebrovascular smooth muscle, but only at high concentrations. Autoradiographic (1, 18), inert gas (8, 26), and other direct (38) techniques for cerebral blood flow measurement have revealed that norepinephrine, or cervical sympathetic nerve stimulation, will effect only a small decrease in resting cerebral blood flow on the order of 5–10%.

The minimal reactivity of the cerebral vasculature to circulating norepinephrine might be due to the inability of this amine to cross the blood-brain barrier (25). Therefore, two series of experiments were carried out in anesthetized baboons to test this hypothesis. Cerebral perfusion and metabolism were measured in the following series of experiments.

In the first series, norepinephrine was injected into a lateral cerebral ventricle. In a separate series of experiments, norepinephrine was infused into one internal carotid artery after the bolus injection of a hypertonic urea solution. This procedure is known to effect a transient disruption of the blood-brain barrier because of osmotic shrinkage of the cerebral endothelial cells (31).

MATERIALS AND METHODS

Young, healthy baboons (Papio anubis and Papio cynocephalus), weighing between 10 and 30 kg, were sedated with 12–20 mg phencyclidine. Subsequent to the intravenous injection of thiopentone sodium (7.5 mg/kg) they were intubated and connected to a positive-pressure ventilator delivering 75% N₂O and 25% O₂ in open circuit. Phencyclidine (2–4 mg, im) was administered half-hourly, and suxamethonium (50 mg, im) was administered at the same time in order to control ventilation. The minute volume of the ventilator was adjusted as necessary in order to maintain normocapnia (PaCO₂ close to 40 mmHg). The oxygen tension of arterial blood (PaO₂) was always greater than 100 mmHg. Body temperature was maintained around 37°C with infrared heating lamps.

Catheters were introduced into the abdominal aorta and the inferior vena cava (through the femoral vessels) to allow the measurement of mean arterial blood pressure (MABP), and the infusion of saline, respectively. Another catheter was inserted into one linguofacial artery, and the remaining branches of the external carotid artery were ligated on that side. The ipsilateral scalp and temporal muscle were resected. Through a small bur hole, a fine catheter was inserted into the superior sagittal sinus for the withdrawal of cerebral venous blood and the craniotomy was sealed.

Regional cerebral blood flow (rCBF) was determined by the intracarotid injection of the radioactive inert gas, ¹³³Xenon. The isotope, dissolved in saline and warmed to 37°C was injected through the linguofacial catheter. The scintillation detector was mounted over the denuded calvarium in the parietal region, and the cerebral
blood flow (CBF) calculated from the height/area equation (16).

At each CBF determination, both arterial and cerebral venous blood samples were taken and PCO₂, pH, and PO₂ measured on a direct-reading electrode system (Corning). Blood oxygen saturation was also measured using a hemoreflector (Kipp & Zonen) and the blood hemoglobin content determined colorimetrically (Pye Unicam) because facilities for direct determination of oxygen content were not available. Arterial and cerebral venous blood glucose concentrations were measured by a standard enzymatic assay. The cerebral oxygen consumption (CMRO₂) was calculated from the product of the CBF and the arteriovenous oxygen content difference, and the cerebral glucose uptake (CMRGlc) from the product of the CBF and arteriovenous blood glucose difference.

Intraventricular norepinephrine. In seven animals a fine catheter was inserted into the anterior horn of the lateral ventricle on the same side as that of the CBF estimation. Norepinephrine bitartrate (40 μg/kg, as salt) was injected into the lateral ventricle after at least three base-line determinations of CBF, CMRO₂, and CMRGlc. Norepinephrine was dissolved in 0.1 ml mock cerebrospinal fluid (CSF) immediately prior to use and was then flushed in with a further dose of 0.05 ml mock CSF. Intracranial pressure was measured through the ventricular catheter. Norepinephrine was injected on a total of 10 occasions in seven animals, and repeated measurements of all the observed variables were made at approximately 20-min intervals. On seven separate occasions the effects of a 0.15-ml injection of mock CSF alone were examined as a control.

Intracarotid norepinephrine after hypertonic urea. A total of 11 animals were used in this study. In six animals the effects of intracarotid norepinephrine infusions on cerebral blood flow and metabolism were studied after osmotic disruption of the blood-brain barrier. In three of these animals the effect of intracarotid norepinephrine alone was first examined, and in the other three animals the effect of intracarotid hypertonic urea alone was first investigated.

The barrier was opened by a modification of the method of Rapoport et al. (31). Between 7 and 10 ml of hypertonic urea (2 osM) was injected into one carotid artery, through the lingual artery catheter, over a period of 15 s. Care was taken to ensure that the pressure within the cannula, during injection, was not more than 10 mmHg above the MABP in each animal. The norepinephrine infusion (50 ng/kg-min) was started immediately after the urea injection.

The CBF estimation was made after the MABP had returned to resting values (3–5 min after the urea injection) since a biphasic response of arterial pressure was usually noted. Urea alone occasionally effected an initial, though variable, moderate hypotension, which was always followed by a moderate hypertension (as seen in Fig. 1). Bradycardia, effected by the urea infusion, was more sustained and returned to normal over a 20-min period.

As controls, in six animals the effects of CBF, CMRO₂, and CMRGlc of hypertonic urea by itself were studied. In three animals the effects of an intracarotid norepinephrine infusion after hypertonic urea were later studied, in one animal the effects of an intracarotid norepinephrine infusion alone were first studied, and in two animals the effects of hypertonic urea only were examined. In a total of six animals the cerebral vascular and metabolic effects of intracarotid norepinephrine infusion were studied prior to osmotic opening of the blood-brain barrier. In three animals the effects of norepinephrine after hypertonic urea were later examined, in one animal the effects of hypertonic urea alone were later examined, and in two animals the effects of intracarotid norepinephrine only were studied.

The statistical comparison used in these experiments was the Student paired-t test.

RESULTS

Intraventricular norepinephrine. In the seven animals in which 0.15 ml mock CSF alone was injected into a lateral ventricle, no significant changes in PCO₂, CMRO₂, CMRGlc, CBF, or MABP were noted (Table 1). Estimations of these variables were made up to 60 min after the intraventricular injection of mock CSF. The pH of the mock CSF (7.15) was adjusted to the pH of the mock CSF containing norepinephrine (6.9) in three of these animals.

FIG. 1. Effects of intracarotid hypertonic urea (10 ml, 2 osM) on mean arterial blood pressure (MABP) and heart rate (HR) in 1 animal. Time base is shown in center of figure. Interval between each dash is 1 min. Measurement of cerebral blood flow (CBF) was made 4 min after urea when MABP had returned to base-line values.
TABLE 1. Effects of intraventricular mock CSF

<table>
<thead>
<tr>
<th></th>
<th>Base Line</th>
<th>CSF*</th>
<th>Diff ± SE</th>
<th>t, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{aCO_2}$, mmHg</td>
<td>39.1</td>
<td>38.9</td>
<td>-0.3 ± 0.3</td>
<td>0.122</td>
</tr>
<tr>
<td>CMRO$_2$, ml/100 g · min</td>
<td>2.76</td>
<td>2.69</td>
<td>-0.07 ± 0.10</td>
<td>0.762</td>
</tr>
<tr>
<td>CMR$_{Glc}$, mg/100 g · min</td>
<td>6.70</td>
<td>6.26</td>
<td>-0.43 ± 0.49</td>
<td>0.967</td>
</tr>
<tr>
<td>CBF, ml/100 g · min</td>
<td>47</td>
<td>47</td>
<td>0 ± 1</td>
<td>0.000</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>82</td>
<td>81</td>
<td>-1 ± 1</td>
<td>1.081</td>
</tr>
</tbody>
</table>

The data are from seven animals. * 0.15 ml mock CSF.

FIG. 2. Effect of intraventricular norepinephrine (NA) on cerebral blood flow (CBF) in 7 animals. Bars represent 1 SE of mean. Each flow determination in 60-min period after administration is significantly increased from baseline value ($P < .05$).

In contrast, the intraventricular injection of 40 μg/kg norepinephrine, dissolved in 0.1 ml mock CSF, affected significant increases in CBF (Fig. 2). Cerebral oxygen consumption was increased ($P < 0.05$) from 2.78 ± 0.10 to a maximum of 3.44 ± 0.42 ml/100 g · min (mean ± SE); and cerebral glucose uptake from 4.21 ± 0.42 to 10.65 ± 2.96 mg/100 g · min ($P < 0.05$). These increases in metabolism accompanied the period of increased cerebral blood flow subsequent to intraventricular norepinephrine. Neither $P_{aCO_2}$ nor MABP differed significantly from their respective mean baseline values of 39.7 ± 0.4 mmHg and 96 ± 3 mmHg (means ± 1 SE) subsequent to intraventricular norepinephrine.

Intracarotid norepinephrine after hypertonic urea. The intracarotid infusion of norepinephrine (50 ng/kg per min) by itself did not significantly alter $P_{aCO_2}$, CMR$_{Glc}$, or CBF in six animals. Systemic arterial pressure rose slightly to 110%, and oxygen consumption by the brain decreased to 96% of baseline values (Table 2).

The effects of the intracarotid bolus injection of hypertonic urea alone are shown in Table 3. There was no significant difference in any of the measurements made. Confirmation of barrier disruption was obtained in two ways. In two animals the intravenous injection of penicillin G (1 g) gave rise to high voltage paroxysmal activity on the electroencephalogram (EEG) only after the disruption of the blood-brain barrier with hypertonic urea. Penicillin is normally incapable of crossing the blood-brain barrier and has been used experimentally to detect alterations in the blood-brain barrier (19).

In other animals 3 ml/kg of a 2.5% solution of Evans blue was injected intravenously prior to the administration of the hypertonic urea. When the animals were sacrificed the brains were found to have been stained heavily with Evans blue. This dye binds to plasma proteins so that cerebral tissue is stained only when there is an alteration in the permeability of the blood-brain barrier.

The bolus injection of hypertonic urea was combined with the intracarotid infusion of 50 ng/kg · min norepinephrine in six animals. Arterial $P_{aCO_2}$ was unaltered but a slight pressor response was noted, as was seen with norepinephrine alone (Table 4). However, cerebral blood flow, and oxygen and glucose consumption by the brain were all significantly elevated. The percentage changes from baseline values in the three conditions studied (norepinephrine alone, hypertonic urea alone, and norepinephrine after hypertonic urea) are shown in Fig. 3.

TABLE 2. Effects of intracarotid norepinephrine infusion

<table>
<thead>
<tr>
<th></th>
<th>Base Line</th>
<th>NE, 50 ng/ kg · min</th>
<th>Diff ± SE</th>
<th>t, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{aCO_2}$, mmHg</td>
<td>39.7</td>
<td>39.9 ± 0.2 ± 1.4</td>
<td>0.169</td>
<td>4.0</td>
</tr>
<tr>
<td>CMR$_{O_2}$, ml/100 g · min</td>
<td>3.34</td>
<td>3.20 ± 0.14 ± 0.05</td>
<td>7.036</td>
<td>0.02</td>
</tr>
<tr>
<td>CMR$_{Glc}$, mg/100 g · min</td>
<td>5.38</td>
<td>5.63 ± 0.25 ± 0.37</td>
<td>0.834</td>
<td>0.02</td>
</tr>
<tr>
<td>CBF, ml/100 g · min</td>
<td>54</td>
<td>55 ± 1 ± 2</td>
<td>1.000</td>
<td>0.02</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>90</td>
<td>95 ± 5 ± 5</td>
<td>0.974</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The data are from six animals.

TABLE 3. Effects of intracarotid hypertonic urea

<table>
<thead>
<tr>
<th></th>
<th>Base Line</th>
<th>Urea*</th>
<th>Diff ± SE</th>
<th>t, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{aCO_2}$, mmHg</td>
<td>39.6</td>
<td>38.8 ± 0.9 ± 0.7</td>
<td>1.478</td>
<td>0.96</td>
</tr>
<tr>
<td>CMR$_{O_2}$, ml/100 g · min</td>
<td>3.23</td>
<td>3.19 ± 0.04 ± 0.18</td>
<td>0.252</td>
<td>0.96</td>
</tr>
<tr>
<td>CMR$_{Glc}$, mg/100 g · min</td>
<td>5.37</td>
<td>5.68 ± 0.33 ± 0.4</td>
<td>0.834</td>
<td>0.96</td>
</tr>
<tr>
<td>CBF, ml/100 g · min</td>
<td>55</td>
<td>52 ± 3 ± 3</td>
<td>1.000</td>
<td>0.96</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>90</td>
<td>95 ± 5 ± 5</td>
<td>0.974</td>
<td>0.96</td>
</tr>
</tbody>
</table>

The data are from six animals. * 7–10 ml of 2 osM urea solution over 15 s.

TABLE 4. Effects of intracarotid norepinephrine after hypertonic urea

<table>
<thead>
<tr>
<th></th>
<th>Base Line</th>
<th>NE + Urea*</th>
<th>Diff ± SE</th>
<th>t, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{aCO_2}$, mmHg</td>
<td>39.9</td>
<td>39.9 ± 0.1 ± 0.1</td>
<td>0.986</td>
<td>0.96</td>
</tr>
<tr>
<td>CMR$_{O_2}$, ml/100 g · min</td>
<td>3.70</td>
<td>4.48 ± 0.79 ± 0.11</td>
<td>7.036</td>
<td>0.96</td>
</tr>
<tr>
<td>CMR$_{Glc}$, g/100 g · min</td>
<td>6.39</td>
<td>11.22 ± 4.84 ± 1.67</td>
<td>3.763</td>
<td>0.02</td>
</tr>
<tr>
<td>CBF, ml/100 g · min</td>
<td>53</td>
<td>79 ± 26 ± 7</td>
<td>4.031</td>
<td>0.02</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>90</td>
<td>97 ± 6 ± 2</td>
<td>0.900</td>
<td>0.96</td>
</tr>
</tbody>
</table>

The data are from six animals. * Norepinephrine, 50 ng/kg · min, after the bolus injection of 7–10 ml of 2 osM urea solution.
The intraventricular administration of high doses of norepinephrine gave rise to an increase in CBF accompanied by increases in oxygen and glucose consumption by the brain. Likewise, the intracarotid infusion of norepinephrine increased cerebral blood flow, and oxygen and glucose consumption after transient disruption of the blood-brain barrier with hypertonic urea. These responses could be explained by the ability of norepinephrine to bypass, and produce effects beyond, the blood-brain barrier under the experimental conditions that were studied.

Nature of blood-brain barrier to monoamines. Oldendorf (25) measured the brain uptake of various amines, including norepinephrine, against titrated water (3H2O), which is a relatively freely diffusible test substance. When the brain uptake of 3H2O was termed 100%, then the uptake of labeled norepinephrine was very small. It would appear that, under normal conditions, there is an effective barrier mechanism that is capable of preventing the entry of systemic norepinephrine into the cerebral interstitial fluid.

Fluorescent microscopy techniques have helped to localize this blood-brain barrier mechanism for monoamines. The endothelial cells of the cerebral capillary walls contain both dopa decarboxylase and monoamine oxidase that together impede the passage of monoamines. The endothelial cells of the cerebral capillary that were studied.

Intracarotid noradrenaline following hypertonic urea. In the series of six animals used as controls for the effect of urea alone in this investigation, there was neither an increase in cerebral blood flow nor in the cerebral rate of glucose metabolism. Both autoregulation and the response of the cerebral circulation to $P_{aCO_2}$ are intact following barrier disruption by hypertonic urea in the baboon (27).
Rapoport et al. (31) have shown that the intracarotid infusion of concentrated urea solutions opened the blood-brain barrier in a reversible manner. They found that 5 ml of 2 osM urea, when injected into the carotid artery, caused a breakdown of the blood-brain barrier in rabbits, as demonstrated by the extravasation of Evans blue. However, within 30 min of the administration of urea no further Evans blue extravasation could be detected, which indicated that the blood-brain barrier had resealed. These workers suggested that hypertonic solutions would open the barrier by shrinking the endothelial cells and, hence, opening the junctions between them. This hypothesis was supported by an independent electron microscope study where the cerebrovascular endothelium was examined after perfusion of the brain with hyperosmolar agents (36).

The time interval reported by Rapoport et al. (31) for blood-brain barrier opening, after intracarotid hyperosmolar urea, was very similar to that seen in the present investigation. Subsequent to the urea treatment noradrenaline infusion usually increased cerebral blood flow and metabolism for only one determination of blood flow (i.e., for up to 20 min after urea).

Barrier lesions induced by techniques other than the hyperosmolar method also influence the passage of norepinephrine. Both mercuric chloride and cold injury increase brain uptake of catecholamines, although irreversibly damaging the blood-brain barrier (14). Thus it is probable on a priori grounds that intracarotid norepinephrine after hyperosmolar urea, like intraventricular norepinephrine, is capable of bypassing the blood-brain barrier. This argument has been substantiated recently. The passage of norepinephrine into cortical neurons and intraparenchymal endothelial cells has been visualized directly, by the Falck-Hillarp technique, after the intracarotid administration of hypertonic urea in the rat (Hardebo, Edvinsson, MacKenzie, and Owman, unpublished results).

Mechanism of action of norepinephrine. Throughout the body norepinephrine has an almost universal α-adrenergic vasoconstrictor action. Although α-adrenergic constriction has been induced in isolated cerebral vessels (24) and in pial vessels in vivo (40), the sensitivity of cerebral vessels to norepinephrine is low when compared to peripheral vessels. This low sensitivity and the protection afforded by the blood-brain barrier are possible explanations as to why little or no effect on cerebral blood flow has been noted previously with norepinephrine infusions (8, 11, 26).

Norepinephrine effected a dilation of the cerebral circulation whether delivered by intraventricular injection or after osmotic disruption, in both instances bypassing the blood-brain barrier. Since Wahl and his co-workers (39) reported that vascular β-receptors are of minor or no importance in the regulation of pial artery resistance, it is unlikely that the increase in flow, noted with norepinephrine, was the result of a β-adrenergic vascular mechanism.

In our investigations, the effects of norepinephrine on cerebral blood flow could not be dissociated from its effects on oxygen and glucose consumption by the brain. This finding raises the possibility that the responses observed, after circumvention of the blood-brain barrier by norepinephrine, could be the result of stimulation of norepinephrine of cerebral carbohydrate metabolism. The catecholamines are capable of stimulating oxygen and glucose consumption in almost every type of tissue examined (15). A number of in vitro studies have demonstrated that the catecholamines, including norepinephrine, can stimulate the production of cyclic 3',5'-AMP in brain tissue in many species (17, 22).

However, with the methods employed in this study, it is not possible to say whether the stimulation of cerebral metabolism by norepinephrine is a direct phenomenon, or whether it is secondary to synaptic events within the brain (19, 35). It is of interest to compare the current findings with those studies that indicate that the central norepinephrine systems are of importance in the electrocortical aspects of wakefulness, and in the arousal phenomenon (21).

In conclusion, the object of these studies was to determine the cerebral circulatory effects of norepinephrine after the blood-brain barrier had been bypassed. In neither of the two experimental series studied was any decrease in cerebral blood flow associated with the administration of norepinephrine. Once norepinephrine gains access to the cerebral interstitial fluid it would appear that the dominant circulatory response is vasodilation, this being accompanied by increased oxygen and glucose utilization by the brain. If any adrenoreceptors, mediating vasoconstriction, exist on the cerebral arteriolar smooth muscle, then the effects of stimulating these adrenoreceptors are not evident. The physiological significance of the increases in cerebral blood flow and metabolism, brought about by norepinephrine, remain to be determined.

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Address requests for reprints to E. T. MacKenzie, Wellcome Surgical Research Institute, Univ. of Glasgow, Garscube Estate, Bearsden Road, Bearsden, Glasgow, G61 1QH, Scotland.

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