Effect of an Acute Increase of the Intravascular Pressure on the Blood-Brain Barrier
A Comparison between Conscious and Anesthetized Rats
BARBRO B. JOHANSSON, M.D.

SUMMARY Conscious rats and rats under nitrous oxide anesthesia were subjected to blood pressure elevations by injection of epinephrine, bicuculline and amphetamine. Mean arterial pressure was measured from a chronic indwelling cannula in the aorta in awake rats. The protein leakage in the brains was studied using Evans blue and 125I-HSA. Conscious animals developed less blood-brain barrier dysfunction than anesthetized ones. The largest difference was obtained with amphetamine and the smallest with epinephrine. Possible explanations to the results are discussed.

IT HAS BEEN SHOWN in many studies that acute hypertension can increase the cerebral vascular permeability to protein in anesthetized animals (Johansson). In conscious rats blood-brain barrier (BBB) dysfunction has been observed in renal hypertensive rats in connection with abrupt blood pressure elevations and in angiotensin-induced acute hypertension. No comparison between the degree of extravasation in conscious and anesthetized animals has so far been reported. As some preliminary observations indicated there was a difference between conscious and anesthetized rats, the aim of the present study was to compare the pattern and degree of extravasation after a blood pressure increase induced by epinephrine, bicuculline or amphetamine. In addition to the forced dilatation that may occur in acute hypertension per se, bicuculline and amphetamine give rise to a marked vasodilatation which increases the vessel wall tension and thus the vulnerability to mechanical stress. Extensive and consistent patterns of protein extravasation are observed in the brains and the protein leakage can be completely prevented if the blood pressure is kept low.

Material and Methods

A. Conscious Rats

Male Sprague-Dawley rats (200-300 g) were used. Mean arterial pressure (MAP) was measured directly from a chronic indwelling cannula in the aorta. The cannula was implanted under barbiturate anesthesia 2 or 3 days prior to the hypertension experiments. The cannula was positioned in the right external jugular vein. Two ml of a 2 percent solution of Evans blue (EB) in saline were injected i.v. except in the amphetamine group which received EB only. After arterial blood gas analyses, epinephrine (20 μg · kg⁻¹), bicuculline (1.2 mg · kg⁻¹) or d,l-amphetamine (2.5 mg · kg⁻¹) was administered i.v. Three minutes later the rat was anesthetized with pentobarbital i.v. and the brain perfused with saline in situ for 30 seconds to rinse blood from the cerebral vessels. Predetermined parts were subjected to scintillation counting and the extravasation of 125I-HSA was expressed as 100 · (counts · min⁻¹ · mg⁻¹ brain tissue)/(counts · min⁻¹ · mg⁻¹ blood). The blood sample was taken immediately before starting the perfusion through the heart.

B. Anesthetized Rats

Anesthesia was induced with diethyl ether. A femoral artery and a femoral vein were cannulated, the animals were tracheotomized, immobilized with suxamethonium chloride and artificially ventilated with a mixture of 30% O₂ and 70% N₂O. The experiments were thereafter performed in the same way as in the conscious rats, i.e. the same tracers and drugs were used and perfusion through the heart was started 3 minutes after the injection of epinephrine, bicuculline or amphetamine. As preliminary experiments showed that the pressure response to epinephrine was less in the anesthetized rats, probably because the initial MAP was higher than in conscious rats, a small dose of dihydralazine (0.1-0.2 mg, sufficient to lower the blood pressure 15-25 mm Hg), was given to the rats before the injection of epinephrine. The electroencephalogram (EEG) was recorded from scalp electrodes in rats that received bicuculline. Statistical differences were evaluated with the Wilcoxon rank sum test.

Results

Initial and maximum MAP are given in table 1. The pressure increase was slightly more abrupt in conscious than in anesthetized rats after epinephrine and bicuculline, whereas no consistent difference was seen after amphetamine which in both groups resulted in a more gradual pressure increase. In conscious rats the blood pressure response to amphetamine varied considerably. (The 6 rats presented in table 1 were selected from a total of 15 rats because the pressure in-
crease exceeded 40 mm Hg). \(P_{CO_2}\) was \(\geq 13.3 \text{kPa}\) (100 mm Hg) in all anesthetized rats and slightly lower in awake animals. \(P_{CO_2}\) before the induction of a pressure increase is shown in Table 1.

Bicuculline induced seizure activity in the EEG in the anesthetized rats and generalized convulsions in the conscious rats within a few seconds after the drug administration. Convulsions were also seen in one conscious rat after epinephrine administration.

Evans Blue-Albumin (EBA) Extravasation

All conscious and anesthetized rats given epinephrine showed areas of extravasation but to a smaller degree in the former group. In 2 conscious rats the protein leakage was very slight, i.e. only a few blue dots were seen. Extravasation could be observed in all areas of the brain, and in 3 of the anesthetized rats it was particularly prominent in the cerebellum. In the bicuculline groups all rats showed protein leakage although to a considerably less extent in awake rats in spite of a higher blood pressure increase in this group. The brains from 5 of 6 awake rats given amphetamine showed no extravasation at all and in one brain 2 small blue spots were observed in the frontal cortex. In contrast, all anesthetized rats showed the typical pattern of protein extravasation seen after amphetamine administration, i.e. leakage predominantly in anterior and lateral parts of cerebral cortex.

**125IHSA Leakage**

The correspondence between the degree of EBA and 125IHSA extravasation in the brains was good. The results of 125IHSA determination in rats given epinephrine and bicuculline are given in tables 2 and 3. The difference in epinephrine-induced extravasation between conscious and anesthetized rats was statistically significant only for the cerebellum.

**Discussion**

The results imply that conscious rats are less prone to develop pressure-induced BBB disturbances than rats anesthetized with nitrous oxide. In the amphetamine groups one possible explanation would be that conscious rats hyperventilate when given amphetamine (\(P_{CO_2}\) 3.5-4.1 kPa). Hyperventilation has been shown to diminish the BBB dysfunction both in hypertension per se\(^8\) and after amphetamine administration.\(^9\) However, the mild degree of hypercapnia would not be expected to completely prevent extravasation, nor can changes in blood gases explain the difference between conscious and anesthetized rats subjected to repeated arterial blood gas determinations of \(P_{CO_2}\) increased to 6.67-7.17 kPa in ventilated rats. Thus, in anesthetized and ventilated rats \(P_{CO_2}\) decreases in the early stage of bicuculline-induced epileptic seizures\(^10\) whereas it increases markedly in conscious rats. (In 3 additional conscious rats subjected to repeated arterial blood gas determinations of \(P_{CO_2}\) increased to 6.67-7.17 kPa after 60 seconds and to 10.61-11.92 kPa after 2 minutes).

**Table 1** Mean Arterial Pressure (MAP) Before and After a Blood Pressure Increase Induced by Epinephrine (80 \(\mu\)g \(\cdot\) kg\(^{-1}\)), Bicuculline (1.2 \(mg\) \(\cdot\) kg\(^{-1}\)) or \(d, l\)-amphetamine (2.5 \(mg\) \(\cdot\) kg\(^{-1}\)) in Awake and in Anesthetized Rats. \(P_{CO_2}\) Before Induction of Hypertension. Mean Values \(\pm\) SEM.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>N</th>
<th>Initial MAP (mm Hg)</th>
<th>Maximum MAP (mm Hg)</th>
<th>(\Delta)MAP</th>
<th>(P_{CO_2}) (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine, awake</td>
<td>6</td>
<td>128 (\pm) 4</td>
<td>203 (\pm) 4</td>
<td>75 (\pm) 4</td>
<td>4.4 (\pm) 0.1</td>
</tr>
<tr>
<td>Epinephrine, (N_2O/O_2)</td>
<td>9</td>
<td>121 (\pm) 6</td>
<td>194 (\pm) 1</td>
<td>73 (\pm) 6</td>
<td>4.5 (\pm) 0.1</td>
</tr>
<tr>
<td>Bicuculline, awake</td>
<td>10</td>
<td>117 (\pm) 5</td>
<td>189 (\pm) 3</td>
<td>72 (\pm) 5</td>
<td>4.5 (\pm) 0.1</td>
</tr>
<tr>
<td>Bicuculline, (N_2O/O_2)</td>
<td>9</td>
<td>142 (\pm) 2</td>
<td>185 (\pm) 3</td>
<td>43 (\pm) 4</td>
<td>4.7 (\pm) 0.1</td>
</tr>
<tr>
<td>(d,l)-amphetamine, awake</td>
<td>6</td>
<td>123 (\pm) 4</td>
<td>183 (\pm) 6</td>
<td>61 (\pm) 8</td>
<td>4.6 (\pm) 0.2</td>
</tr>
<tr>
<td>(d,l)-amphetamine, (N_2O/O_2)</td>
<td>6</td>
<td>135 (\pm) 5</td>
<td>185 (\pm) 6</td>
<td>50 (\pm) 6</td>
<td>4.8 (\pm) 0.1</td>
</tr>
</tbody>
</table>

**Table 2** 125IHSA Leakage in the Brains from Awake and Anesthetized Rats After Epinephrine-induced Acute Hypertension (80 \(\mu\)g \(\cdot\) kg\(^{-1}\)). The Tracer Content Is Expressed as 100 \(\cdot\) (counts \(\cdot\) min\(^{-1}\) \(\cdot\) mg\(^{-1}\) brain tissue)/(counts \(\cdot\) min\(^{-1}\) \(\cdot\) mg\(^{-1}\) blood). Mean Values \(\pm\) SEM.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>N</th>
<th>Cerebral cortex</th>
<th>Thalamus + Mesencephalon</th>
<th>Pons + Medulla obl.</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake</td>
<td>6</td>
<td>0.22 (\pm) 0.02</td>
<td>0.30 (\pm) 0.04</td>
<td>0.31 (\pm) 0.06</td>
<td>0.27 (\pm) 0.03*</td>
</tr>
<tr>
<td>(N_2O/O_2)</td>
<td>9</td>
<td>0.45 (\pm) 0.12</td>
<td>0.37 (\pm) 0.12</td>
<td>0.34 (\pm) 0.08</td>
<td>1.11 (\pm) 0.55</td>
</tr>
<tr>
<td>Control ((N_2O/O_2) no epinephrine)</td>
<td>5</td>
<td>0.03 (\pm) 0.001</td>
<td>0.03 (\pm) 0.002</td>
<td>0.04 (\pm) 0.004</td>
<td></td>
</tr>
</tbody>
</table>

* \(p <0.05\) for difference from anesthetized rats.

**Table 3** 125IHSA Leakage in the Brains from Conscious and Anesthetized Rats Subjected to an Abrupt Blood Pressure Increase Induced by Bicuculline (1.2 \(mg\) \(\cdot\) kg\(^{-1}\)). The Tracer Content is Expressed as 100 \(\cdot\) (counts \(\cdot\) min\(^{-1}\) \(\cdot\) mg\(^{-1}\) brain tissue)/(counts \(\cdot\) min\(^{-1}\) \(\cdot\) mg\(^{-1}\) blood). Mean Values \(\pm\) SEM.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>N</th>
<th>Cerebral cortex</th>
<th>Thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake</td>
<td>10</td>
<td>0.19 (\pm) 0.02*</td>
<td>0.41 (\pm) 0.04*</td>
</tr>
<tr>
<td>(N_2O/O_2)</td>
<td>9</td>
<td>0.64 (\pm) 0.14</td>
<td>1.07 (\pm) 0.33</td>
</tr>
</tbody>
</table>

* \(p <0.05\) and ** \(p <0.01\) for differences from anesthetized rate.
pathetic stimulation of the cervical sympathetic chain can prevent the BBB dysfunction and flow increase in anesthetized cats during acute hypertension. This has been confirmed by MacKenzie et al. in studies on baboons. Sympathetic stimulation can also diminish protein extravasation in rats given amphetamine or bicuculline. Thus, a difference in sympathetic tone between anesthetized and conscious animals could be one possible explanation for the present finding. However, preliminary experiments indicate that cervical sympathectomy does not, to any substantial degree, increase the protein leakage in conscious rats (Hardebo, Johansson and Edvinsson, unpublished observations). Further studies are in progress to analyze the effect of the peripheral sympathetic system as well as to evaluate the possible role of the central noradrenergic system proposed by Raichle et al. to take part in the regulation of the cerebral blood flow and vascular permeability.

References

Effect of an acute increase of the intravascular pressure on the blood-brain barrier: a comparison between conscious and anesthetized rats.

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