Phenothiazine-Mediated Protection of the Blood-Brain Barrier During Acute Hypertension

Evidence for a modification of the endothelial cell membrane

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SUMMARY The phenothiazine dixyrazine (5 mg · kg⁻¹ i.v.) had minimal, transient hypotensive effects but significantly reduced the leakage of ¹²⁵I labelled serum albumin in conscious rats subjected to acute hypertension provoked by i.v. adrenaline or bicuculline. By contrast, dixyrazine did not protect the blood-brain barrier during osmotic stress induced by intracarotid infusion of 2 M urea.

The diameters of pial arteries and veins were continuously measured with a multichannel videoangiometer through a closed cranial window in anesthetized rats before and after i.v. injection of dixyrazine (5 mg · kg⁻¹). No change in vessel diameter was observed except for a transient autoregulatory dilatation of arteries in response to a slight transitory decrease in blood pressure.

It is concluded that dixyrazine probably protects the blood-brain barrier during mechanical stress by modifying the endothelial cell membrane.

SOME DRUGS known to inhibit endo- or exocytosis in various membranes have been found to decrease the protein extravasation in the brain during acute hypertension,¹-⁶ and it has been postulated that the protection is mediated via inhibition of pinocytosis in cerebral endothelial cells. Among drugs shown to reduce protein extravasation during acute hypertension are some phenothiazines.⁶ Since drugs can also reduce protein leakage in this experimental model by enhancing the cerebrovascular tone,¹ it is important to rule out possible vasocostrictory effects of phenothiazines before conclusions as to an effect on the endothelial cell membrane can be drawn. In the present study we have investigated the effect of a phenothiazine, dixyrazine,⁹ on blood pressure, protein leakage over the blood-brain barrier during acute hypertension and on pial vessel diameter. Since it has been suggested that the mechanism behind hypertensive and osmotic opening of the blood-brain barrier may be the same (see Discussion), we likewise studied any dixyrazine effects on osmotic opening of the blood-brain barrier.

Materials and Methods

Male Sprague-Dawley rats (200-225 g) were used. The numbers of animals in each group are shown in the tables.

Studies on Albumin Extravasation During Acute Hypertension

Indwelling catheters were inserted under methohexitol anesthesia (Brietal®, 50 mg · kg⁻¹) into the abdominal aorta via the left femoral artery and the right jugular vein. The free ends of the catheters were exteriorized on the back of the neck. Two days later the aortic catheter was connected to a transducer and mean arterial pressure (MAP) recorded in conscious unrestrained rats. Arterial PCO₂, PO₂ and pH were measured. ¹²⁵I labelled human serum albumin (HSA, 100 μCi · kg⁻¹) and 0.5 ml 2 % Evans blue were given intravenously (i.v.) as indicators of the function of the blood-brain barrier. Evans blue binds to serum albumin in vivo and enables a macroscopic evaluation of albumin leakage. In addition, the colouring of the blood simplifies the recognition of satisfactory removal of blood by perfusion (see below). After administration of the tracers, the blood pressure was abruptly increased by i.v. injection of adrenaline (20 μg · kg⁻¹) or bicuculline (1.2 mg · kg⁻¹). Bicuculline induces epileptic seizures, and the hemodynamic consequences, that is an abrupt increase in blood pressure combined with pronounced cerebral vasodilatation,¹⁰ enhance the cerebrovascular permeability to albumin.¹¹ It is known that i.v. administration of adrenaline or bicuculline in the doses used in the present study leads to easily detected albumin leakage in the brain in conscious rats.¹² Half of the rats were pretreated with dixyrazine (Esucos®, USB, 5 mg · kg⁻¹ i.v.) 15-20 min before the administration of the hypertensive drugs. The dose dixyrazine was chosen for being roughly equivalent to the doses of some phenothiazines used in an earlier study.⁴ Three minutes after the blood pressure had reached its maximum level — which occurred within 5-30 s after the injection of bicuculline or adrenaline — the rats were given 30 mg · kg⁻¹ pentobarbital i.v. and the brains were perfused with physiological saline through the left heart ventricle for one minute to remove the tracers from the blood vessels. Pentobarbital was given rather than potassium chloride because it is important to start perfusion while the heart is still beating in order to get optimal perfusion of the cerebral vessels and thus remove the intravascular radioactivity.
The brains were dissected as shown in table 2, the samples weighed and the radioactivity determined in a scintillation counter. The brain radioactivity was calculated as a ratio of the activity in brain versus blood, i.e. $100 \times (\text{CPM/mg brain tissue over CPM/mg blood})$. Statistical differences were evaluated with Wilcoxon's rank sum test.

### Osmotic Opening of the Blood-Brain Barrier

Catheters were inserted in the aorta and right jugular vein under methohexital anesthesia in the same way as in rats subjected to acute hypertension. In addition, one catheter was inserted in the right external carotid artery with the tip of the catheter close to the carotid bifurcation. The superior thyroid, occipital, and pterygopalatine arteries were ligated (cf Johansson et al.). All catheters were exteriorized on the back of the neck. Two days later 1.5 ml 2 M urea were infused for 30 seconds through the carotid catheter during continuous recording of MAP. Evans blue and albumin were used as tracers in the same doses as above, and controls were compared to rats pretreated with dixyrazine 5 ml • kg$^{-1}$ or 15 mg • kg$^{-1}$. The lower dose was given 15–20 min before the infusion of urea (that is the same interval as in the hypertension experiments). Because of larger effects on blood pressure with higher doses (see results), the interval had to be extended to 40 min in this group. The radioactivity was determined in the right and left hemisphere and related to the activity in the blood as described above.

### Determination of Pial Vessel Diameter

Six rats initially anesthetized with 30 mg • kg$^{-1}$ sodium pentobarbital (Nembutal) were tracheotomized, immobilized with 0.5 mg • kg$^{-1}$ tubocurarine chloride, and ventilated with a 3:1 mixture of N$_2$O:O$_2$ in a small animal respirator (Harvard). Both femoral arteries and one femoral vein were cannulated for continuous recording of MAP. Evans blue and IHSA were used as tracers in the same doses as above, and controls were compared to rats pretreated with dixyrazine 5 ml • kg$^{-1}$ or 15 mg • kg$^{-1}$. The lower dose was given 15–20 min before the infusion of urea (that is the same interval as in the hypertension experiments). Because of larger effects on blood pressure with higher doses (see results), the interval had to be extended to 40 min in this group. The radioactivity was determined in the right and left hemisphere and related to the activity in the blood as described above.

### Table 1. Mean Arterial Pressure (MAP) Before and After the Induction of Acute Hypertension by i.v. Injection of Adrenaline (20 μg/kg$^{-1}$) or Bicuculline (1.2 mg/kg$^{-1}$) in Conscious Unrestrained Rats Pretreated with Dicyrazine (5 mg/kg$^{-1}$) and in Controls. Arterial P0$_2$, P0$_2$ and pH Prior to the Elevation of Blood Pressure

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Initial MAP mm Hg</th>
<th>Maximum MAP mm Hg</th>
<th>P0$_2$ kPa</th>
<th>P0$_2$ kPa</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicuculline</td>
<td></td>
<td></td>
<td>117 ± 5</td>
<td>115 ± 5</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>117 ± 5</td>
<td>187 ± 5</td>
<td>11.5 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>7.35 ± 0.01</td>
</tr>
<tr>
<td>Dixyrazine</td>
<td>115 ± 5</td>
<td>176 ± 4</td>
<td>11.6 ± 0.2</td>
<td>4.6 ± 0.2</td>
<td>7.37 ± 0.02</td>
</tr>
<tr>
<td>Adrenaline</td>
<td></td>
<td></td>
<td>116 ± 2</td>
<td>119 ± 2</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>116 ± 2</td>
<td>189 ± 5</td>
<td>11.7 ± 0.2</td>
<td>4.4 ± 0.1</td>
<td>7.37 ± 0.02</td>
</tr>
<tr>
<td>Dixyrazine</td>
<td>112 ± 5</td>
<td>174 ± 5</td>
<td>11.9 ± 0.2</td>
<td>4.7 ± 0.2</td>
<td>7.36 ± 0.01</td>
</tr>
</tbody>
</table>

Mean values ± SEM. n = 8 in all groups.

### Results

#### Albumin Extravasation in Acute Hypertension

Dicyrazine, 5 mg • kg$^{-1}$, induced a slight transitory decrease in blood pressure (10–20 mm Hg). At the time of induction of acute hypertension 15–20 min later the pressure had returned to the initial level. MAP before and after injection of the hypertensive drugs, as well as PaO$_2$, PaCO$_2$ and pH, is given in table 1. Maximum MAP was slightly but not significantly lower in dicyrazine-treated rats. There was no difference as to the abruptness or duration of the blood pressure response. As seen in table 2 dicyrazine reduced the radioactivity in the brain which corresponded to the macroscopic appearance of Evans blue albumin extravasation.

#### Albumin Extravasation in Osmotic Opening of the Blood-Brain Barrier

There was no difference in arterial blood gas parameters between the experimental groups. As in the preceding group 5 mg • kg$^{-1}$ dicyrazine induced a transitory decrease in blood pressure. Dicyraine 15 mg • kg$^{-1}$ lowered the blood pressure 40–65 mm Hg and at the time of urea infusion 40 min later MAP was 100 ± 1 mm Hg compared to 120 ± 2 for controls and 114 ± 2 for rats given the lower dose dicyrazine (p < 0.01 and p < 0.05 respectively). During the infusion of urea MAP rapidly increased to a maximum level 8–12 s
after the start of the infusion. This initial rise was followed by a marked fall and then a second rise in MAP, see figure 1 which gives the mean values for the groups. Maximum MAP during the first rise in pressure was significantly lower in rats pretreated with dixyrazine 15 mg·kg\(^{-1}\) (144 ± 6 mm Hg) than in controls (175 ± 4 mm Hg; \(p < 0.01\)). At no other times were there any statistically significant differences between the groups.

Some muscular twitchings were observed in most rats during the infusion of urea. When the injection ended there was no abnormal behaviour. There was no significant difference in albumin extravasation in the brain between the groups (table 3).

**Determination of Pial Vessel Diameter**

Thirty-four arteries (diameter 20–69 µm) and 19 veins (diameter 34–141 µm) were continuously observed and the diameters recorded. \(\text{PaCO}_2\) was kept between 4.0 and 5.0 kPa and \(\text{PaO}_2 > 14 \text{ kPa}\). The initial MAP was 104 ± 7 mm Hg. As in conscious rats, dixyrazine induced a transitory decrease in MAP (19 ± 11 mm Hg). A corresponding transient autoregulatory dilatation of pial arteries was recorded (\(p < 0.0005\)). An individual experiment is shown in figure 2. Figure 3 demonstrates graphically the changes of arterial and venous vessel diameters as well as MAP during 10 minutes after injection of dixyrazine. The slight venous dilatation was not significant. Both MAP and vessel diameter returned to about initial levels within 5 minutes. Thus, there was no indication of any direct effect of dixyrazine on cerebrovascular tone.

**Discussion**

The present results are in agreement with earlier work showing that phenothiazines can protect the blood-brain barrier during acute hypertension. The

![Figure 1](http://stroke.ahajournals.org/)

**FIGURE 1.** Mean arterial pressure (MAP) during and after infusion of 1.5 ml 2 M urea solution into the internal carotid artery (30s) in rats with and without pretreatment with dixyrazine.
TABLE 3. Radioactivity (\(^{125}\text{I}\) Labelled Serum Albumin) in Rat Brain After Osmotic Opening of the Blood-brain Barrier by Injection of 2 M Urea into the Right Internal Carotid Artery in Control Rats and Rats Pretreated with Dixyrazine (5 mg-kg\(^{-1}\) or 15 mg-kg\(^{-1}\)).

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>n</th>
<th>Telencephalon dx</th>
<th>Diencephalon dx</th>
<th>Telencephalon sin</th>
<th>Diencephalon sin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>0.94 ± 0.27</td>
<td>1.10 ± 0.48</td>
<td>0.10 ± 0.01</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Dixyrazine 5 mg-kg(^{-1})</td>
<td>8</td>
<td>0.75 ± 0.17</td>
<td>1.12 ± 0.35</td>
<td>0.10 ± 0.02</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>Dixyrazine 15 mg-kg(^{-1})</td>
<td>5</td>
<td>1.29 ± 0.35</td>
<td>1.42 ± 0.41</td>
<td>0.35 ± 0.09</td>
<td>0.41 ± 0.11</td>
</tr>
</tbody>
</table>

The radioactivity is given as \(\text{cpm/mg brain tissue} \times \text{100. Mean values} \pm \text{SEM.}

The fact that dixyrazine reduced albumin leakage also in rats given bicuculline supports the hypothesis that the drug acts on the endothelial cell membrane because drugs which increase the cerebrovascular tone do not prevent albumin extravasation in this experimental model. Thus, indomethacin, which markedly enhances the cerebrovascular tone in conscious animals,\(^{16, 17}\) protects the blood-brain barrier during acute hypertension \(\text{per se}\) but has no effect on albumin leakage in the bicuculline model,\(^{18}\) probably because the metabolically induced vasodilatation overrules the constrictory effect of the drug. The fact that dixyrazine did not enhance pial arterial tone further supports the hypothesis that the protective effect of phenothiazine during acute hypertension is not due to vasoconstriction.

The question whether the entry of macromolecules into the brain in acute hypertension is transendothelial or interendothelial has been much debated. Most recent reports seem to indicate that the passage is predominantly transendothelial (for references see Johansson\(^{6}\)) and there is evidence that tight junctions are highly resistant to mechanical trauma.\(^{19}\) The influence of drugs on the blood-brain barrier opening seems to favour the hypothesis of transendothelial passage, although to our knowledge, the possibility of pharmacological effects on tight junctions has not been investigated and thus cannot be ruled out. It has recently been questioned whether pinocytotic vesicles in mesenteric vessels are in fact freely moving entities transferring substances from the luminal to the abluminal side of the endothelial cells or are part of cluster-like invaginations from the cell surface.\(^{20, 21}\) Hansson et al\(^{22}\) reported the presence of channels in cerebral vessels during acute hypertension and pointed out that vesicles filled with tracer substance in endothelial cells may either be interpreted as pinocytotic vesicles or regarded as being part of a channel system as earlier shown in muscle capillaries.\(^{23}\) Evidence for the occurrence of transendothelial channels in cerebral vessels during acute hypertension has also been presented by Nag et al\(^{24}\) and Auer.\(^{8}\)

The hypothesis that tight junctions are opened during osmotic dysfunction of the blood-brain barrier\(^{25}\) has recently been questioned.\(^{19, 26, 27}\) Since hyperosmolar solutions induce vasodilatation and dilated vessels have an increased vulnerability to high intraluminal pressure it has been suggested that stretching of the vessel wall is of pathogenetic importance as in acute hypertension. The absence of a protective effect of dixyrazine on the osmotic blood-brain barrier opening does not support the hypothesis that the leakage is exclusively due to hemodynamic events but suggests that hyperosmolar solutions have some additional
effect on endothelial cells. This does not rule out transendothelial passage since formation of pinocytic vesicles seems to be a common reaction to cerebral trauma of various types (see Westergaard). In conclusion, the present study shows that dixyrazine protects the blood-brain barrier during a hypertensive insult. Since dixyrazine has no constrictory effect on pial vessels the protection is probably mediated via a change in the endothelial cell membrane.

Acknowledgement

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18. Johansson BB: Indomethacin and cerebrovascular permeability
Nitroglycerin Induced Hypotension Will Maintain CBF in Hypertensive Rats

WILLIAM E. HOFFMAN, PH.D., RONALD F. ALBRECHT, M.D., AND DAVID J. MILETICH, PH.D.

SUMMARY Cerebrovascular effects of intravenous (iv) nitroglycerin (NTG) infusions were tested in four month old spontaneously hypertensive rats (SHR) and Wistar Kyoto controls (WKY). Cerebral blood flow (CBF) changes were measured during iv NTG infusion in ventilated, halothane anesthetized rats using radioactive microspheres. In control WKY rats given isotonic saline infusions instead of NTG, blood pressure and CBF did not change over 3 microsphere injections. When blood pressure was decreased to 65 and then 45 torr with iv NTG infusions, CBF was maintained or increased in both SHR and WKY. There was no difference in response between SHR and WKY. These results support other reports that NTG has direct cerebrovasodilating effects, and indicate that this action will maintain adequate CBF in hypertensive as well as normotensive subjects to pressures below 50 torr.

DURING CHRONIC arterial hypertension there is an impairment of cerebral autoregulation. In normotensive subjects cerebral blood flow (CBF) may be maintained down to mean blood pressures of 60–70 torr, but CBF may decrease at pressures above 100 torr in hypertensive subjects. Impaired cerebral autoregulation has also been identified in spontaneously hypertensive rats (SHR). It is often desirable and necessary to control hypertension during intraoperative periods and to induce hypotension to reduce blood loss. Reductions in blood pressure increases the risk of cerebral ischemia in hypertensives more than in normotensive subjects. One possible solution to this problem is the use of a hypotensive drug which also has direct cerebrovasodilating effects. Nitroglycerin (NTG) is a drug which has been suggested for use in hypotensive anesthesia. This drug also has been reported to reverse cerebrovasospasm.

Although cerebrovasodilatation has been reported during NTG induced hypotension, other reports have indicated that NTG treatment increases intracranial pressure and may decrease CBF. In these experiments the cerebrovascular effects of NTG induced hypotension were studied in anesthetized spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto controls (WKY).

Methods

Surgery

Male SHR and WKY, (4 months old) were used in these experiments. One experiment was performed in the morning and one in the afternoon. Rats from each of the 3 groups, sham treated WKY (n=13), NTG treated WKY (n=10), NTG treated SHR (n=12), were tested in a randomized order. All rats were implanted with PE50 femoral artery and vein catheters and a left ventricle catheter implanted via the right carotid artery under 1.5% halothane anesthesia according to previously described methods. A tube was inserted into the trachea and used for artificial ventilation. Respiratory rate was 48 min⁻¹ and tidal volume was adjusted between 3 to 4 ml using a Harvard small animal respirator in order to obtain an arterial pCO₂.
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B B Johansson, L M Auer and L E Linder

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