Effect of Intracarotid Hyperosmolar Mannitol on Cerebral Cortical Arterioles — A Morphometric Study

DAVID W. BECK, M.D., MICHAEL N. HART, M.D.,* AND KAREN E. HANSEN, B.SC.*

SUMMARY The morphometric response of cerebral cortical arterioles to intracarotid hyperosmolar mannitol was assessed utilizing a technique of rapid freezing of rat brain tissue in vivo.

A significant decrease in the wall to lumen (W/L) ratio was noted in the small parenchymal arterioles (20–49 μ) (p < .0001) ipsilateral to the mannitol infusion. Larger arterioles showed a decreased W/L ratio but this change was not significant. Control infusion of 0.9% NaCl resulted in no change in arteriolar caliper.

These results indicate that intracarotid hyperosmolar mannitol causes a direct vasodilatory response in small cerebral cortical arterioles.

INTRACAROTID INFUSION of hyperosmolar solutions (e.g. urea or mannitol) has been used as a method to transiently open the blood brain barrier to proteins and water-soluble compounds.1 It has been shown that intracarotid infusion of these solutions causes a transient increase in cerebral blood flow, presumably due to a direct vasodilatory effect.2,3 Although pial arterial and arteriolar diameters are influenced by local changes in perivascular osmolality4 and are dilated with intracarotid infusion of hyperosmolar agents,5 the changes in cerebral parenchymal vessels with hyperosmolar solutions has not been directly investigated because of the inability to assess the in vivo physiologic state of these small vessels. The present study utilizes a technique of rapid freezing of rat brain tissue in vivo to assess morphometric changes of brain parenchymal vessels in response to intracarotid infusion of hyperosmolar mannitol.

Methods

Experiments were performed on 16 Sprague-Dawley male rats weighing between 200 and 220 grams. The rats were anesthetized with pentobarbital (50 mg per 100 grams IP), intubated, paralyzed with intravenous pancuronium bromide (0.5 mg per kg), and ventilated artificially. Depth of anesthesia was sufficient that no physiologic response to pain was noted. Arterial pCO₂, pH and PO₂ were measured periodically during each experiment. The arterial blood pressure was recorded continuously from a catheter placed in the descending aorta via the right femoral artery. A right external carotid artery catheter was placed after distal ligation of the right external carotid artery for infusion of 1.4 M mannitol in 0.9% NaCl or 0.9% NaCl alone. Body temperature was maintained between 37 and 38°C. Following placement of catheters, the animals were secured in a head holder and bilateral 1 × 1 cm craniectomies were performed using a dental drill with a round burr. Irrigation was used throughout the drilling process to prevent heating of the cortex. Great care was taken to avoid interrupting the dura that is translucent and paper-thin in the rat. For determination of parenchymal vessel morphometry, the rats were divided into two groups. In nine rats, 1.4 M mannitol in 0.9% NaCl was infused into the internal carotid artery via the right external carotid artery at a rate of .08 cc per second for 30 seconds. At this rate, the right cerebral cortex showed extravasation of Evans Blue-albumin if injected intravenously following the mannitol infusion. In seven rats 0.9% NaCl alone was infused. Immediately following the infusion the brain was frozen in situ using isopentane cooled at −150°C in liquid nitrogen. The animal was then removed from the head holder and the head was immersed directly into liquid nitrogen. The frozen brain was chiseled out of the remaining skull, being careful not to allow the brain to thaw by constantly pouring liquid nitrogen over it. When the brain was free it was wrapped in foil and placed in a cryostat to equilibrate thermally (−18 to −22°C). The areas of cortex in the cranial “windows” were used. Six μm sections were cut parallel to the brain surface, blown dry, and desiccated for several hours. The sections were then stained with a Hematoxylin and Eosin (H & E) method to distinguish and outline the arterioles. The medial thickness and external diameters of the arterioles were measured with an ocular ruler on a light microscope and calculations were completed using an appropriate objective conversion factor. The wall (W) to lumen (L) ratio was thus determined. All arterioles in each section that retained a circular contour were measured and several histologic sections were taken from each window. The cranial windows were made directly opposite each other and therefore the same region of brain was investigated in each animal. The arterioles in the cranial windows ipsilateral to the infusion of mannitol or 0.9% NaCl were then compared to the arterioles in the contralateral windows.

All data are expressed as the mean ± SEM and were analyzed utilizing analyses of variances. Values of p ≤ 0.05 were considered significant.
Results

The arterial \( pCO_2 \) remained constant in each rat with a mean \( pCO_2 \) of 34 ± 2.0 (SEM) mmHg, and \( pO_2 \) was 140 ± 5 (SEM) mmHg. The mean arterial blood pressure was 100 mmHg and did not change greater than 10 mmHg during infusion of either mannitol or NaCl.

In 20–49 \( \mu \) sized arterioles the W/L ratio was 11% less than controls. (\( p < .0001 \).) In the 50–180 \( \mu \) arterioles a slight decrease in the W/L ratio was also noted but this change was not significant. Infusion of 0.9% NaCl alone resulted in no change in the W/L ratio in ipsilateral arterioles compared to the arterioles in the contralateral hemisphere. Assuming that cross sectional area does not change during changes in vessel caliper, a 10% decrease in W/L ratio of arterioles 20–50 \( \mu \) in diameter is equal to a vessel dilation of approximately 5%. Based on changes in vascular resistance being inversely proportional to radius\(^4 \) (CVR \( \alpha^{-1} \)), a dilation of 5% in arterioles of this size would produce a 20–25% decrease in vascular resistance. Thus, infusion of mannitol resulted in significant dilation of the small parenchymal arterioles compared to the equivalent sized arterioles in the contralateral hemisphere.

Discussion

It has been shown that cerebral vessels can be retained in a physiologic state by rapid freezing followed by freeze-substitution.\(^10 \) Rapid freezing alone has been compared with freezing followed by freeze-substitution and found to give identical morphometric results, yet it is far less time consuming (unpublished observations). The vessels measured in this study were arranged and analyzed as previously described.\(^10,11 \)

Infusion of hyperosmolar solution into the internal carotid artery as a means of reversibly disrupting the blood brain barrier has become a potentially useful clinical procedure.\(^12 \) The mechanism of disruption is still unknown although it has been speculated that the hyperosmolar agent causes shrinkage of endothelium, and dilation of the vessel resulting in the opening of tight junctions.\(^13-15 \) Other evidence has been shown that the mechanism may be due to increased pinocytosis and intraendothelial channel formation.\(^16 \)

Hyperosmolar agents have been shown to increase cerebral blood flow following intracarotid infusion thus providing indirect evidence that mannitol does have a direct vasodilatory effect on cerebral vessels.\(^5,6 \) In this study we have shown that infusion of hyperosmolar mannitol into the internal carotid artery does have a vasodilatory effect on the small parenchymal arterioles.

The mechanism of the vasodilation is not known. Pial arterial and arteriolar diameters have been shown to increase with increasing perivascular osmolarity, suggesting that osmolarity per se can influence vessel diameter.\(^7 \) In vivo studies on other vascular beds report similar findings.\(^17,18 \) Isolated arterial preparations examining the influence of hyperosmolar mannitol on vascular smooth muscle contractile responses suggest that mannitol produces vasodilation by mechanisms dissimilar from that of other vasodilatory substances, and suggest that other mechanisms in addition to hyperosmolarity may be involved in the vasodilatory effect.\(^5 \)

Systolic blood pressure changes have been observed following carotid infusion of hyperosmolar solution.\(^5,6 \) and it is possible that the increase in cerebral blood flow represents an inability of the brain to autoregulate. However, the changes in aortic pressure followed changes in cerebral blood flow\(^6 \) and in our study aortic pressure did not change by the time the brains were frozen, yet vasodilation had occurred. This would suggest a direct vasodilatory effect.

Cerebral blood flow increases have been observed with intravenous mannitol as well as intraarterial.\(^19,20 \) The mechanism of the increase of cerebral blood flow may be due to a direct vasodilatory effect similar to that seen with intraarterial mannitol. However, a profound vasoconstriction of pial arteries has been demonstrated with intravenous mannitol accompanied by a decrease in blood viscosity.\(^21 \) This suggests that the blood viscosity changes may be the reason for the increased cerebral blood flow and that the pial artery vasoconstriction is an autoregulatory response. It would appear then that the mechanism for increased cerebral blood flow following intravenous mannitol is different than that seen with intracarotid mannitol.

In conclusion we have demonstrated that intracarotid mannitol causes a significant dilatation of small parenchymal arterioles. These results suggest that the transient increase in cerebral blood flow following intracarotid mannitol infusion is due in part to a direct vasodilatory effect of the mannitol on the brain parenchymal vessels. The results also suggest that small intraparenchymal arterioles may be important as resistance vessels.

Acknowledgment

The authors wish to thank James C. Tomer for his help with statistical analysis.

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**Table 1** Effect of Intracarotid Mannitol Infusion on Cerebral Cortical Arteriolar Caliber

<table>
<thead>
<tr>
<th>Mannitol Infusion (9 Rats)</th>
<th>Ipsilateral hemisphere</th>
<th>Contralateral hemisphere (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel size</td>
<td>X W/L</td>
<td>SEM</td>
</tr>
<tr>
<td>20–49 ( \mu )</td>
<td>.275*</td>
<td>.007</td>
</tr>
<tr>
<td>50–180 ( \mu )</td>
<td>.185</td>
<td>.006</td>
</tr>
</tbody>
</table>

*\( p \leq .0001 \)

**Table 2** Effect of Intracarotid 0.9% NaCl Infusion of Cerebral Cortical Arteriolar Caliber

<table>
<thead>
<tr>
<th>Vessel size</th>
<th>X W/L</th>
<th>SEM</th>
<th>n</th>
<th>X W/L</th>
<th>SEM</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–49 ( \mu )</td>
<td>.306</td>
<td>.006</td>
<td>195</td>
<td>.297</td>
<td>.007</td>
<td>171</td>
</tr>
<tr>
<td>50–176 ( \mu )</td>
<td>.184</td>
<td>.007</td>
<td>73</td>
<td>.174</td>
<td>.007</td>
<td>80</td>
</tr>
</tbody>
</table>

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Rapid Freezing and Frozen Sectioning as a Means Of Preserving Brain Vessel Morphometric Characteristics

MICHAEL NOEL HART, M.D., AND KAREN E. HANSEN, B.S.

SUMMARY A study was performed for the purpose of determining whether ordinary frozen sections of brain could preserve in vivo parenchymal arteriolar morphometric characteristics. The results showed that flash-freezing followed by frozen sectioning is just as reliable a means of preserving brain arteriolar morphometric characteristics as the time consuming process of flash-freezing followed by freeze substitution.

STROKES OF THE PHYSIOLOGIC PROPERTIES of blood vessels such as blood flow and resistance would ideally be combined with morphometric studies of the vessels' caliber if such studies were possible. Thus, form could readily be reconciled with function. However, it is very difficult to "capture" or fix a vessel in vitro in the exact morphologic state that it held in vivo.

In previous publications we have shown the inadequacy of formaldehyde fixation as a means of preserving basilar artery morphometry and meningeal vessel morphometry. In contrast, rapid freezing followed by freeze substitution is an excellent method of preserving meningeal vessel morphometry. Freeze substitution, however, is tedious and time consuming and only a few vessels can be analyzed from each animal.

Consequently we undertook the following study to determine if rapid freezing followed by ordinary frozen sectioning is as efficacious and reliable as freeze substitution for morphometric preservation.
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