Brief Communication

Effects of Formaldehyde Fixation on Basilar Artery Caliber

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SUMMARY  The external diameters of rat and rabbit basilar arteries were determined before and after formaldehyde fixation in relaxed and drug constricted vessels. Fixation usually resulted in an immediate vessel constriction followed by partial relaxation after several hours. However, the results were inconstant from vessel to vessel and from area to area of the same vessel. It is concluded that morphologic as well as morphometric assessment of formaldehyde-fixed basilar arteries is unreliable.

AS MORE STUDIES are being performed on the phenomenon of vascular spasm, especially in cerebral arteries, it becomes imperative to develop reliable in vitro and in vivo methods of quantifying spasm. Spasm has been approached morphologically in man and in the experimental animal predominantly by angiography. This technique offers in vivo assessment of vascular spasm but also has limitations related to vessel size and presence of other luminal disease states. Many investigators have attempted to evaluate spasm in the sectioned, aldehyde-fixed vessel by both light and electron microscopy. Although lesions created by spasm may be morphologically assessed, the presence of, absence of, or degree of spasm is difficult to evaluate because spasm is a physiologic state of smooth muscle hypercontraction. The question must then be asked if any fixatives can capture or “freeze” a vessel in the relaxed or contracted configuration it was in at the moment the fixative was applied. The purpose of this study is to analyze the effects of formaldehyde fixation on vessel calibre in normal and constricted rat and rabbit basilar arteries.

Methods and Results

Fourteen rat and 9 rabbit basilar arteries (BA) were used in the study. The rats were killed with ether and the rabbits with intravenous pentobarbital sodium and the brains immediately removed. In most cases the basilar artery was left intact with the brain. Photographs were taken of each basilar artery immediately, after induced spasm (when performed) and after fixation with a 10 percent neutral buffered formaldehyde solution. A small ruler was placed adjacent to the artery and the external arterial diameters were measured with a micrometer in several different locations on each photo. (Results summarized in table).

Summary and Conclusions

Immersion and/or perfusion of rat and rabbit basilar arteries in 10 percent neutral buffered formaldehyde generally results in an immediate (10-30 min) mean vessel diameter reduction (contraction). A slight increase in rat mean BA diameter with formaldehyde immersion following drug constriction is an exception. Another exception is the generalized dilation of previously relaxed rabbit BA perfused at 50 mm Hg pressure, probably due to internal pressure. The overall vessel reaction following drugs, trauma and formalin in both rat and rabbits is inconstant and irregular. Some portions of the arteries actually dilate with formalin fixation while other areas constrict severely. Further, under the influence of formalin fixation, vessels tend to relax after more than an hour and may approach the original baseline diameter, even following previous drug constriction. Also noteworthy is the focal dilation or aneurysm formation which occurs following perfusion of an artery in the zone of previous trauma (pinch).

There have been only a few attempts to quantify the effects of fixation on vessel diameter. Wei et al. have fixed brain pial vessels by creating a pial window and overlaying the window with formalin while the animal is still alive. Thus, the vessels are kept patent by physiologic blood pressure from within while they are concomitantly fixed from their external surface. This results in post-fixation diameters which are nearly the same as the prefixation diameter. This technique is easily applied to the pial circulation but would not be applicable to most circulatory systems which are buried in parenchyma.

Mulvaney et al. mounted segments of rat mesenteric artery on a myograph and measured media thickness while mounted and again after fixation with 2.5 percent gluteraldehyde. They discovered very little change in medial thickness after fixation in hypertensive vessels but there was a 16.4 percent increase in
TABLE  Reactions of Basilar Arteries to Formaldehyde Fixation

<table>
<thead>
<tr>
<th>Model</th>
<th>No. of animals</th>
<th>Treatment and % change</th>
<th>% change after immersion fixation</th>
<th>% change after perfusion fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat BA in situ</td>
<td>6</td>
<td>0</td>
<td>-2 (15 min)</td>
<td>+13.6 (overnight)</td>
</tr>
<tr>
<td>Rat BA in situ</td>
<td>3</td>
<td>overlayed with 5HT or ergotamine -19.8</td>
<td>-18.6 (15 min)</td>
<td></td>
</tr>
<tr>
<td>Rat BA in situ</td>
<td>2</td>
<td>isopentane immersion +10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat BA dissected</td>
<td>3</td>
<td>0</td>
<td>-11.6 in length</td>
<td>+4 in diameter (15 min)</td>
</tr>
<tr>
<td>Rabbit BA in situ</td>
<td>3</td>
<td>0</td>
<td></td>
<td>+40 (15 min)</td>
</tr>
<tr>
<td>Rabbit BA in situ</td>
<td>3</td>
<td>overlayed with ergotamine -16.7</td>
<td>-20.7 (15 min)</td>
<td>-6.3 (60 min)</td>
</tr>
<tr>
<td>Rabbit BA in situ</td>
<td>3</td>
<td>pinch -13</td>
<td>-23 (15 min)</td>
<td></td>
</tr>
</tbody>
</table>

Key: (−) = constriction (+) = dilation (BA) = basilar artery.

mean media thickness after fixation in the control rats’ vessels. These results suggest that the control vessels constricted following fixation but that the hypertensive vessel walls remained essentially the same, perhaps because of greater rigidity inherent in those vessels with greater media mass. However, in both of these studies the mean vessel diameters might mask a wide variation in diameter from area to area, as brought out in our study.

We conclude that investigators should be extremely cautious in the interpretation of vessel constriction by either light or electron microscopy in fixed vessels. More precise studies of the effects of conventional fixatives (including gluteraldehyde) should be performed on brain parenchymal vessels.

**References**

5. Mulvany MJ, Hansen PK, Aalkjaer C: Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media, and an increased number of smooth muscle cell layers. Circ Res 43: 854-864, 1978
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