Increased Binding of Norepinephrine by Nerves to Cerebral Blood Vessels: Evidence from the Effects of Reserpine on Nerves to Cerebral and Extracerebral Blood Vessels

BY WILLIAM I. ROSENBLUM, M.D.

Abstract: Increased Binding of Norepinephrine by Nerves to Cerebral Blood Vessels: Evidence from the Effects of Reserpine on Nerves to Cerebral and Extracerebral Blood Vessels

It has been proposed by others that adrenergic nerves to cerebral blood vessels bind norepinephrine more avidly than do nerves to vessels outside the brain. This suggestion is supported by the present data which show that in the rat intraperitoneal reserpine depletes norepinephrine less readily from nerves to cerebral blood vessels than from nerves to extracerebral blood vessels. Alternate hypotheses to explain our data are contradicted by available evidence, except for the hypothesis of unequal distribution of reserpine between perivascular nerves in various locations. No evidence has been located to favor the latter hypothesis. The postulate of increased norepinephrine binding by nerves to cerebral vessels not only explains the present data, but also can account for the surprisingly small responses of cerebral vessels to exogenous norepinephrine or to sympathetic stimulation.

Additional Key Words
- catecholamines
- fluorescence histochemistry
- cerebral circulation
- adrenergic nerves
- paraformaldehyde technique
- cerebral blood flow
- sympathetic nerves

The vessels on the surface of the brain are richly innervated by adrenergic nerves, yet the function of these nerves remains an enigma, principally because their stimulation results in either no vascular response, or in a response much smaller than that produced in other vascular beds by neurogenic stimulation. Recently, Nielsen and Owman suggested that the nerves to cerebral vessels might bind unusually large amounts of exogenous norepinephrine, and this binding might account for unexpectedly small responses to exogenous neurotransmitter, since the latter would be taken up by the nerves before it could arrive at cerebrovascular smooth muscle in sufficiently high concentration to cause a large contractile response. Although their suggestion was made with particular reference to exogenous transmitter, similar reasoning might also apply to the small responses often obtained after stimulation of sympathetic nerves to cerebral vessels. That is, increased binding or enhanced reuptake of endogenous norepinephrine might produce minimal release of this neurotransmitter or reduce the amount of norepinephrine arriving at the vascular smooth muscle. Shortly after the publication of Nielsen and Owman, we observed that a given dose of reserpine would often completely deplete norepinephrine from perivascular nerves outside the brain, while leaving residual norepinephrine in the nerves to cerebral vessels. The reverse (depletion of norepinephrine from cerebral vascular nerves with retention of norepinephrine in perivascular nerves outside the brain) was never observed. Since these observations support the hypothesis of diminished release or enhanced binding or reuptake of norepinephrine by cerebrovascular nerves, it was thought worthwhile to systematize our observations. The resulting data are presented below.

Methods
Male rats of the Holtzman strain (Holtzman Laboratories, Madison, Wisconsin) were used. The formaldehyde vapor technique was used to detect...
norepinephrine. Rats were decapitated and vessels were immediately excised and placed in Krebs-Henseleit solution (pH 7.4) until all specimens from a given animal had been taken. Cerebral vessels were obtained from the circle of Willis and branches of the circle which had not yet penetrated the brain. Extracerebral vessels were the portal vein and the branches of the femoral artery. They were then mounted on cover slips as whole mounts and dried overnight at less than 0.5 torr and room temperature (24 to 26°C), after which they were placed in vapors of paraformaldehyde at 80°C for one hour and subsequently mounted in paraffin oil.

Semiquantitative studies were performed by employing a grating monochromator (Farrand Optical Corporation) placed over the ocular tube of the microscope. The intensity of fluorescence was analyzed at the emission peak for each specimen (approximately 495 μ) and recorded on a photometer. No attempt was made to convert photometer readings into absolute amounts of norepinephrine (see Discussion). The monochromator employs a pinhole mask, which at the magnification used for readings (400 X) enabled one to limit examination to a circular field approximately 4 μ in diameter. The portion of the nerve examined was placed in the center of the field. The field was sometimes somewhat wider than the nerve. Whether wider or not, after every reading the field was shifted so that only the background immediately adjacent to the nerve was read. The background was then subtracted from the original reading to give a final value. Three such values were obtained from each whole mount. Portions of nerves to be examined with the monochromator were selected at random from each whole mount. To avoid bias in the selection of spots, all slides were examined without knowing whether they were from experimental or control preparations. It also should be stressed that the semiquantitative studies are only ancillary to the first portion of the investigation. In the first part of the study no quantitative measurements were needed because absolutely no norepinephrine could be detected anywhere in the nerves to extracerebral vessels. Preliminary observations made 15 to 20 hours after intraperitoneal injection of reserpine showed that in rats weighing 250 to 375 gm, 0.50 mg/kg of reserpine was required to reliably eliminate fluorescence from nerves to the circle of Willis or its branches. Thereafter, 11 consecutive rats weighing 250 to 356 gm were examined using 0.25 mg/kg. One or two experimental animals were examined on a given day, and on the same day a control, nontreated rat was examined also.

In addition another set of experiments was performed in which rats were sacrificed only six hours after reserpine. Semiquantitative evaluation of fluorescence intensity was performed as mentioned above. On each day one control rat and one reserpinized rat was sacrificed. The experiment was repeated for a total of three days with a total of 24 whole mounts from reserpinized rats (12 cerebral and 12 femoral) and 24 whole mounts from unreserpinized rats being examined over the three-day period.

**Results**

In the experiment where the treated rats were observed 15 to 20 hours after the intraperitoneal injection of reserpine, 50 whole mounts made from the femoral branches of the 11 experimental rats did not show a single fluorescent nerve fiber, while on the cerebral vessels, fluorescent fibers were seen in 15 of 18 preparations (table 1). All 11 rats had fluorescent fibers on at least one preparation of cerebral vessels. Control rats always showed fluorescence (i.e., norepinephrine) in both the nerves to cerebral vessels and the nerves to femoral branches. Since in the experimental group a much greater number of femoral branch preparations were examined than cerebral preparations (table 1), the chances of finding fluorescent nerves on femoral branch vessels were increased. Thus, our failure to find such nerves on femoral vessels is even more impressive.

In addition to examining femoral and cerebral vessels, nine mounts of portal vein were examined in five of the 11 experimental rats. Like the branches of the femoral artery, these preparations of an extracerebral vessel also failed to show fluorescent nerve fibers (table 1), though these were readily visible in control preparations from rats that did not receive reserpine.

In the rats sacrificed only six hours after receiving reserpine, fluorescent nerves were found on both the cerebral and extracerebral vessels. However, fluorescence was brighter on nerves to cerebral vessels than on nerves to branches of the femoral artery (table 2).

Another way of looking at these semiquantitative data is to compare the residual fluorescence in nerves to cerebral vessels with the residual fluorescence in nerves to femoral branches. This can be done by expressing the residual fluorescence in vessels from reserpinized rats as a percentage of the fluorescence obtained in vessels from nonreserpinized controls. Table 3 shows that on each of the three repetitions of the experiment, residual fluorescence of nerves to cerebral vessels was greater than it was in nerves from femoral branches.

**TABLE 1**

<table>
<thead>
<tr>
<th>Effect of Reserpine* on Norepinephrine in Perivascular Nerves</th>
<th>Number of preparations</th>
<th>Number showing fluorescence of nerves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral vessels</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Femoral branches</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Portal vein</td>
<td>9</td>
<td>0</td>
</tr>
</tbody>
</table>

*0.25 mg/kg intraperitoneally. Rats sacrificed 15 to 20 hours after injection.
Reduction in Norepinephrine Six Hours After Reserpine*

<table>
<thead>
<tr>
<th></th>
<th>Repetition 1</th>
<th>Repetition 2</th>
<th>Repetition 3</th>
<th>Three exp combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>F</td>
<td>B</td>
<td>F</td>
</tr>
<tr>
<td>No reserpine</td>
<td>0.20</td>
<td>0.16</td>
<td>0.10</td>
<td>0.33</td>
</tr>
<tr>
<td>Reserpine</td>
<td>0.13</td>
<td>0.02</td>
<td>0.07</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*0.25 mg/kg intraperitoneally.
+B = cerebral arteries, +F = femoral branches.
Values in table represent photometer readings of norepinephrine fluorescence expressed as nerve readings minus background (see Methods).

Discussion

The data indicate that after administration of reserpine to the rat, nerves to the cerebral blood vessels are depleted of their norepinephrine to a lesser extent than are nerves to extracerebral vessels. These findings support the hypothesis that cerebral perivascular nerves may bind norepinephrine more avidly than do nerves to extracerebral vessels. We have also considered other possibilities.

We wondered whether unrecognized variables peculiar to the femoral preparations and affecting visibility might contribute to the appearance of greater loss of fluorescence from nerves to femoral branches. However, nerves in portal vein preparations also were more readily depleted of fluorescence than nerves to cerebral vessels, and, in addition, the level of fluorescence in femoral branch preparations from control, nonreserpinized rats was actually higher than the level of fluorescence in nerves to cerebral vessels. This is indicated by data in table 3, and also by more extensive data in which fluorescence values from cerebral vascular nerves in 11 rats averaged 0.12 ± 0.04. Hence, our results cannot be ascribed to mechanical optical factors interfering with visibility of nerves to femoral branches.

Since in control preparations femoral nerve fluorescence is, in general, higher than that in control preparations from cerebral vessels, we feel that the direction of our semiquantitative data is not being artifactually determined by quenching phenomena at high concentrations of norepinephrine. Quenching, if it occurs, would be greatest in the femoral branch preparations, since these are more brightly fluorescent to begin with, and hence would make it more difficult rather than easier to demonstrate depletion of norepinephrine from the femoral branch nerves. However, the possibility of quenching, together with the technical difficulties in making norepinephrine standards that actually are relevant to whole mounts of tissue, have led us to apply the term semiquantitative rather than quantitative to our photometer data, and thus we have made no attempt to translate our photometer readings into absolute amounts of norepinephrine.

We also wondered whether our results with reserpine were caused by less reserpine arriving at nerves to cerebral vessels than was arriving at nerves to extracerebral vessels. Such a possibility has not been ruled out, and technical problems have made it impossible for us to carry out in vitro experiments with reserpine, which might obviate the possibility of unequal drug distribution following intraperitoneal injection. It might be that with respect to reserpine, the blood-brain, or blood-CSF or blood-nerve barrier limits the amount of reserpine reaching perivascular cerebral nerves. However, reserpine crosses the blood-brain barrier, as is evident from its well-known pharmacological action on central neurotransmitters. Thus, if there are barriers to its intracranial distribution, they are relative rather than absolute. Moreover, there is also a blood-peripheral nerve barrier which restricts the entry of many substances into peripheral nerve. We are unaware of data in the literature that would suggest greater penetrance of reserpine into the perivascular nerves of peripheral blood vessels as compared with penetrance into the perivascular nerves of the brain.

Finally, our results might be explained if cerebral vessels had a much denser innervation than extracerebral vessels. A given amount of reserpine passing out of the cerebrovascular bed might then be

<table>
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<tbody>
<tr>
<td></td>
<td>B</td>
<td>F</td>
<td>B</td>
<td>F</td>
</tr>
<tr>
<td>65</td>
<td>12</td>
<td>70</td>
<td>6</td>
<td>57</td>
</tr>
</tbody>
</table>

*All legends same as for table 2.

**TABLE 3**

Residual Fluorescence as Percent of Nonreserpinized Control*

**TABLE 2**

Reduction in Norepinephrine Six Hours After Reserpine*
INCREASED BINDING OF NOREPINEPHRINE

diluted through capture by a relatively large number of perivascular nerves, with the result that the concentration of reserpine and its effect per nerve would be reduced in comparison with the concentration of reserpine and its effect in nerves to either the femoral or portal vessels. Indeed Nielsen and Owman may have envisaged such a mechanism in postulating excessive uptake of norepinephrine by nerves to cerebral vessels, since these authors also have stated that vessels on the surface of the brain have a richer sympathetic innervation than vessels to any other organ. However, we have not been able to convince ourselves that the innervation of cerebral vessels is unusually rich, and published quantitative data to substantiate such a claim appear to be lacking. Moreover, excessive innervation of cerebral vessels cannot explain the relative unresponsiveness of these vessels to sympathetic stimulation. In contrast, this latter phenomenon, together with our present data on the effects of reserpine, as well as the data of Owman and Nielsen and the increased uptake of norepinephrine suggested by them to explain their data, could all be handled by postulating increased binding and uptake of norepinephrine by a normal number of perivascular nerves to cerebral vessels. According to this hypothesis, exogenous norepinephrine would be bound by these nerves with unusual rapidity and endogenous norepinephrine would be released with difficulty or recaptured with unusual dispatch after either sympathetic stimulation or exposure to reserpine.

References

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