Membrane Electrical Mechanism of Basilar Artery Constriction and Pial Artery Dilation by Norepinephrine

DAVID R. HARDER, PETER W. ABEL, AND KENT HERMSMEYER

SUMMARY To study the mechanism by which norepinephrine acts on vascular muscle cell membrane, we recorded membrane potential with intracellular microelectrodes in isolated cat basilar and pial arteries. On addition of norepinephrine concentrations less than 1 μM, pial arteries hyperpolarized and relaxed while basilar arteries depolarized and contracted. Relaxation and hyperpolarization of the pial arteries occurred without the need for addition of any other drug, which indicates the relaxation of spontaneous tone. The relaxation and hyperpolarization could be completely blocked by addition of propranolol before exposure to norepinephrine. The depolarization and contraction of both basilar and pial arteries was blocked by the previous exposure to phentolamine. Electrical spikes were not found spontaneously, but could be induced in both arteries by tetraethylammonium and subsequent addition of norepinephrine, blockable by phentolamine. We conclude that membrane property differences between basilar and pial arteries result in qualitatively different effects of norepinephrine. Circ Res 49: 1237-1242, 1981

THE RICH adrenergic innervation of cerebral blood vessels (Nielsen and Owman, 1967; Edvinsson et al., 1973) might suggest strong vasoconstriction during nerve stimulation because there is a good correlation between density of adrenergic innervation and responsiveness to nerve stimulation in the peripheral circulation (Mayer et al., 1968). However, stimulation of cervical sympathetic ganglia has little effect on cerebral blood flow in most species (Alm and Bill, 1973; Heistad et al., 1977, 1978). In addition, stimulation of nerves in isolated cerebral blood vessels produces much less constriction than nerve stimulation of peripheral arteries (Bevan et al., 1975). Thus, there is evidence that sympathetic nerves are unimportant in regulation of cerebral blood flow under normal conditions. However, sympathetic stimulation attenuates the increase in cerebral blood flow which occurs during severe hypertension (Heistad et al., 1978).

Responses to norepinephrine (NE) are also markedly less in isolated cerebral arteries than in arteries from other peripheral vascular beds (Shibata, 1977; Toda and Fujita, 1973). Rabbit small pial arteries demonstrate only a weak contractile response to high concentrations of NE applied in vitro (Duckles and Bevan, 1976, 1979). Using an open skull preparation, Wahl et al. (1972) demonstrated pial artery constriction only at very high doses (0.5 mM) of NE. Furthermore, both Raper et al. (1972) and Wei et al. (1975) found that cat pial arteries <100 μM were unresponsive to NE, whereas Kuschinsky and Wahl (1975) found equal responses of large and small pial arterioles. Large cerebral arteries, such as the basilar, also exhibit a smaller contractile response to NE (Bevan et al., 1980).

The only report of electrical properties of cerebral vascular muscle in the literature suggests that membrane properties are different from those found in peripheral arteries (Harder, 1980). Higher potassium conductance, a greater electrogenic ion transport contribution to membrane potential, and spontaneous electrical spiking induced by serotonin were found in the middle cerebral artery (Harder, 1980). The present studies were designed to determine the membrane electrical correlates of norepinephrine responses in large (basilar) and small (pial) arteries of the cat, in order to ascertain whether different membrane depolarization mechanisms might contribute to the unusual norepinephrine contractile responses. As we intended to study cellular mechanisms of the direct effects of NE on cerebral arterial muscle uncomplicated by adrenergic nerve ending uptake or release of NE, adrenergic nerve endings were destroyed in vitro by 6-hydroxydopamine (Aprigliano and Hermansmeyer, 1975).

Methods

Twenty-one mongrel cats (2-3 kg) were anesthetized with a combination of ketamine HCl (25 mg/kg) and xylazine (2.5 mg/kg), im, and the ascending aorta was cannulated through the left ventricle.

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Dr. Abel is the recipient of Postdoctoral Fellowship HL 08757. Dr. Hermansmeyer is the recipient of Research Career Development Award HL 00707. This research was supported by Grants HL 24007, HL 14388, and HL 16328 from the National Institutes of Health.

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Received October 10, 1980; accepted for publication July 15, 1981.

Decisions with regard to this paper were made by Drs. Brian F. Hoffman and Robert M. Berne.
After the vena cava had been opened and the abdominal aorta clamped, the cranial vasculature was perfused with isotonic solution for mammals (ISM) (composition in mM: NaCl, 130; NaHCO3, 16; Na2HPO4, 0.5; KCl, 4.7; CaCl2, 1.8; MgCl2, 0.4; MgSO4, 0.4; Hepes, 13; dextrose, 5.5; and CaNa2 ethylendiaminetetraacetic acid, 0.029) for 20 minutes to remove blood, after which the brain was removed. Pial and posterior basilar arterial segments were removed and stored overnight in ISM at 4°C. Pial artery diameters ranging from 153 to 287 μm were measured after dissection from the brain with a Wild M5 dissecting microscope (50×). The arterial segments were mounted in a non-recirculating organ bath maintained at 37°C and were continuously suffused via a Gilson roller pump with ISM gassed with 95% O2-5% CO2 at a pH of 7.4. In all arteries, adrenergic nerve endings were eliminated by 10 minutes of exposure to 300 μg/ml 6-hydroxydopamine (6-OHDA) in glutathione in vitro, with protection against stimulation by released NE by 1 μM phentolamine (Aprigliano and Hermans-meyer, 1976).

Glass microelectrodes filled with acidified 3 M KCl (pH 2), with resistances of 30-80 megohms and tip potentials of less than 7 mV, were used for measurements of membrane potential (E<sub>m</sub>). All impalements were from the adventitial surface of the arterial segments. The recording preamplifier was a Dagan model 8500, or a WPI model 701, with capacitance compensation and an internal bridge Meyer, 1976).

Membrane electrical effects of norepinephrine (NE)

The mean values of resting (unstimulated) E<sub>m</sub> of both the posterior basilar and pial arteries are summarized in Table 1. The mean resting E<sub>m</sub> in muscle cells of basilar arteries was -62 ± 0.7 mV, and of pial arteries was -52 ± 2.3 mV. The 10 mV difference in E<sub>m</sub> between basilar and pial arteries is statistically significant.

Addition of NE to the basilar arteries resulted in a dose-dependent depolarization, with initial effects observed at 30 nM and reaching maximum depolarization at 10 μM (Fig. 1). Phentolamine (1 μM) significantly reduced the NE depolarization in basilar arteries (Fig. 2; Table 1). Conversely, depolarization of basilar arteries occurred at lower concentrations of NE in the presence of 1 μM propranolol (Fig. 3), but the maximum level of depolarization was unchanged (Figs. 2 and 3).

In marked contrast, pial arterial muscle cells hyperpolarized in concentrations of NE from 30 nM to 1 μM (Figs. 1 and 2). Higher concentrations of NE, from 3 μM to 1 mM, produced dose-dependent depolarization of pial arteries (Fig. 1). Phentolamine (1 μM) did not alter the NE-induced hyperpolarization (Fig. 2; Table 1). However, propranolol (1 μM) prevented the NE hyperpolarization (Fig. 2; Table 1). Therefore, the predominate action of NE at concentrations below 1 μM appeared to be membrane hyperpolarization mediated via β-receptors.

### Table 1: Membrane Potential Changes during Stimulation

<table>
<thead>
<tr>
<th>Group*</th>
<th>E&lt;sub&gt;m&lt;/sub&gt; (mV) ± s.e.†</th>
<th>n‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basilar</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-62 ± 0.7</td>
<td>5, 23</td>
</tr>
<tr>
<td>NE</td>
<td>-33 ± 1.0§</td>
<td>5, 20</td>
</tr>
<tr>
<td>NE + phentolamine</td>
<td>-57 ± 1.3</td>
<td>4, 7</td>
</tr>
<tr>
<td>NE + propranolol</td>
<td>-32 ± 0.8§</td>
<td>4, 6</td>
</tr>
<tr>
<td>5-HT</td>
<td>-41 ± 0.9§</td>
<td>4, 7</td>
</tr>
<tr>
<td></td>
<td>Pial</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-52 ± 2.3</td>
<td>5, 26</td>
</tr>
<tr>
<td>NE</td>
<td>-51 ± 1.9§</td>
<td>4, 7</td>
</tr>
<tr>
<td>NE + phentolamine</td>
<td>-62 ± 1.7§</td>
<td>4, 6</td>
</tr>
<tr>
<td>NE + propranolol</td>
<td>-47 ± 1.3</td>
<td>4, 8</td>
</tr>
<tr>
<td>5-HT</td>
<td>-40 ± 1.2§</td>
<td>4, 9</td>
</tr>
</tbody>
</table>

* All drug concentrations are 1 μM.
† Values represent maximum drug effect.
‡ Number of arteries, number of impalements.
§ Significant difference from control P < 0.05.
Membrane potential ($E_m$) of muscle cells from large (>170 μm o.d) pial (closed circles and solid line) and basilar (open circles and dashed line) arteries are shown as a function of norepinephrine (NE) concentration. NE always depolarized basilar arteries, whereas pial arteries hyperpolarized at low and depolarized at high concentrations. The vertical bars through each point represent the standard error for 8–26 impalements from at least 5 arterial segments.

Electrical Spike Generation

In basilar arteries, the addition of 1 mM tetraethylammonium (TEA) had no effect on $E_m$ and did not cause spike generation, spontaneously or in response to electrical stimulation. Addition of 1 μM NE to basilar arteries treated with 1 mM TEA induced depolarization and spontaneous electrical spike activity which could be blocked by 1 μM phentolamine.

Membrane potential ($E_m$) of muscle cells from basilar arteries was a function of norepinephrine (NE) concentration before (closed circles and solid line) and after (open circle and dashed line) pretreatment with 1 μM propranolol, which caused a shift to the left. The vertical bars represent the standard error for the number of cells indicated at each point from at least 4 arterial segments.
Likewise, in pial arteries, 2 mM TEA alone did not alter $E_m$ or allow spike generation, spontaneously or in response to electrical stimulation. Addition of 1 $\mu$m NE to pial arteries treated with 2 mM TEA hyperpolarized the muscle cells without causing electrical spikes; however, when pial arteries were pre-treated with 1 $\mu$m propranolol and 2 mM TEA, NE (1 $\mu$m) depolarized and induced myogenic electrical spike activity (Fig. 4). No spikes could be induced in the presence of only 1 mM TEA, leading us to use 2 mM.

**Tension Measurements**

The basilar artery mean dose-response curve is presented in Figure 5. Increases in tension began at 10 nM NE, reached a plateau between 1 and 3 $\mu$m NE, and then a maximum at 100 $\mu$m. The geometrical mean NE EC$_{50}$ in the basilar artery was 5.1 $\mu$m. The mean maximum response to NE was 43% of the maximum response to 5-HT (Table 2).

In pial arteries, NE produced a dose-related relaxation of baseline tension from 0.01 to 1 $\mu$m NE of both larger (diameter $>$170 $\mu$m) and smaller (diameter $<$170 $\mu$m) size (Fig. 5). Relaxation was characterized by a rapid decrease in tension followed by a plateau (Fig. 6). The relaxation was blocked by pretreatment with 1 $\mu$m propranolol. Higher concentrations of NE from 3 $\mu$m to 1 mM produced significant contractions in all six of the larger (diameter $>$170 $\mu$m) pial arteries. The mean maximum NE contraction in large pial arteries was 36% of the maximum response to 5-HT. In contrast, four small (diameter $<$170 $\mu$m) pial arteries generated little tension in response to doses of NE from 3 $\mu$m to 1 mM. The mean maximum NE response of small pial arteries was only 8% of the maximum 5-HT response. However, the tension maximums of small and large pial arteries to 5-HT (100 $\mu$m) were respectively large and not different (2.9 vs. 2.7 kilodynes/mm$^2$) demonstrating responsiveness (Table 2).

Tension correlated well with $E_m$ even in pial arteries ($r = 0.98$) where opposite low and high NE concentration responses were consistently recorded. The regulation of tension appeared to be controlled primarily by $E_m$ in normally polarized cerebral arterial muscle cells.

**Figure 4** Pial artery spikes in TEA could be induced by NE only after $\beta$-receptor blockade with propranolol (Pro). Before Pro, 1 $\mu$m NE caused hyperpolarization (A). After 15 minutes in Pro, 1 $\mu$m NE depolarized and induced spiking (B). This artery was continuously suffused with 2 mM TEA beginning 15 minutes before A. Exposure to NE begins at arrow and continues through each record. Voltage and time calibrations in B also apply to A.

**Figure 5** Mean dose-response curve to NE of basilar (triangles, $n = 6$) and large (open circles, $n = 6$) and small (closed circles, $n = 4$) pial arteries, showed striking differences. Basilar arteries were contracted by all NE concentrations. In both large and small pial arteries, a relaxation from baseline tension (without stimulation by 5-HT or addition of any other drug) was observed at low doses of NE. Doses of NE greater than 3 $\mu$m produced contractions that were significantly greater in large than in small pial arteries. The ability of small pial arteries to contract was shown separately by large 5-HT contractions. The vertical bars through each point indicate the standard errors. EC$_{50}$ values and absolute tension are given in Table 2.
Discussion

The results of this study demonstrate marked differences in responses to adrenergic stimulation between basilar and pial arteries. Low NE concentrations depolarized and contracted basilar arteries, but hyperpolarized and relaxed pial arteries. Adrenergic nerve endings could not have interfered with these clearly different responses because all had been sympathectomized by 6-OHDA. We found a striking correlation between NE-induced changes in membrane potential (Em) and tension in cerebral vascular muscle, as small hyperpolarizations resulted in significant relaxation of pial arteries, even in the absence of induced tension.

These findings support the idea that Em is the major control point for regulating the active state of vascular muscle (Hermsmeyer, 1971; Haeusler, 1978; Harder, 1981; Hermsmeyer et al., 1981), with a change of only 1–2 mV producing significant tension changes. These results show that changes in Em were closely coupled to changes in tension, even at low NE concentrations. In pial artery, a hyperpolarization of 4 mV resulted in the maximum reduction in tension. In basilar artery, a 7-mV depolarization resulted in 18% of maximum tension development. Thus, in this study, a close correlation of electrical and mechanical events to adrenergic receptor stimulation in cerebral arteries was demonstrated over a wide range of concentrations.

However, at very high concentrations of NE, the close relationship between Em and tension was not maintained in basilar arteries. Concentrations of NE above 30 μM produced increasing tension in the basilar artery with hyperpolarization, rather than depolarization. Duckles and Bevan (1976) have reported a two-component NE contractile dose-response curve in the rabbit basilar artery, and suggested a different form of α-adrenergic receptor (Bevan et al., 1980). Edvinsson and Owman (1974) have also reported high dosage contractile activation in cat middle cerebral artery by phenylephrine when α-receptors were completely blocked with phenoxybenzamine. The present study has demonstrated a two-component dose-response curve for both tension and Em measurements in the basilar artery. At concentrations of NE less than 10 μM, tension and Em appear to be coupled through NE stimulation of α-receptors. However, at NE concentrations greater than 10 μM, a dissociation of Em and tension is seen. This dissociation appears to be due to contractile action of NE unrelated to α-receptor activation. The uncoupling of Em and tension at high concentrations of NE may also be related to an increase in K+ conductance, due to NE-induced increase in cytoplasmic Ca2+ (Haeusler, 1978; Hermsmeyer, 1980).

In pial arteries, NE produced hyperpolarization and relaxation by β-receptor stimulation in the absence of exogenously induced tone. Similar observations were made by Ito et al. (1979) regarding the porcine coronary artery and by Winquist and Bevan (1979) regarding the rabbit facial vein. Relaxation of unstimulated vessels is unusual in isolated blood vessels, where induced tone is normally necessary in experiments designed to study relaxation of vascular muscle (Bevan et al., 1980). Our results show that NE can directly dilate some cerebral arteries in the absence of induced tone.

These studies have demonstrated differences in responses to adrenergic stimulation between basilar and pial arteries of the cat. These findings may explain, in part, the ineffectiveness of sympathetic stimulation in altering cerebral blood flow (Heistad et al., 1977), since adrenergic activation may con-
strict some cerebral arteries, while dilating or having no effect on others.

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doi: 10.1161/01.RES.49.6.1237

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