Endothelium-Dependent Relaxation of Human Basilar Arteries

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We studied the effects of acetylcholine and calcium ionophore A23187 on human basilar artery rings. Among 11 arteries, both agents produced significant relaxations in five, A23187 but not acetylcholine caused a response in six, and neither agent was effective in four. After rubbing off the endothelium, the relaxations induced by both agents were significantly attenuated. Indomethacin (a cyclooxygenase inhibitor) and AA861 (a specific inhibitor of 5-lipoxygenase) did not but SKF525A (an inhibitor of cytochrome P450-dependent monoxygenase) did significantly inhibit the acetylcholine-induced relaxation in human basilar arteries. On the other hand, AA861 inhibited while neither indomethacin nor SKF525A had any effect on the A23187-induced relaxation. Our results suggest that different mechanisms may be involved in acetylcholine and A23187-induced relaxations in human basilar arteries. (Stroke 1989;20:1208–1211)

The endothelium plays an obligatory role in the relaxation of arteries in response to substances such as acetylcholine (ACh) and calcium ionophore A23187.1–2 It has been suggested that endothelium-dependent relaxation of canine basilar arteries by A23187 may be mediated by noncyclooxygenase metabolite(s).3 Both ACh and A23187 also stimulate prostacyclin synthesis in endothelial cells, which involves deesterification of arachidonic acid4–6; consequently, ACh and A23187 may also promote arachidonic acid metabolism in endothelial cells. Thus, ACh- and A23187-induced relaxations are likely to be accompanied by and may be due in part to the formation of metabolites from arachidonic acid and other polyunsaturated fatty acids.

Recent studies have suggested that the release of endothelium-derived relaxing factor (EDRF) can be inhibited by a potent inhibitor of phospholipase A2.7 Moreover, the peptidoleukotrienes LTC4 and LTD4 possess the capacity to relax some arterial rings in an endothelium-dependent manner.8,9 It has also been demonstrated that the decrease in tone in response to ACh and arachidonic acid is attenuated in the presence of SKF525A, an inhibitor of cytochrome P450-dependent monoxygenase.10–13 These observations indicate that lipoxygenase and/or cytochrome P450-dependent monoxygenase derivatives of arachidonic acid should be included among those substances known to produce vasomotor relaxation in an endothelium-dependent manner.

To our knowledge, there have been few reports regarding EDRF induced by ACh or A23187 in human basilar arteries. Our present study was undertaken to demonstrate this possibility and to investigate the effects of indomethacin, AA861 (a specific inhibitor of 5-lipoxygenase),14 and SKF525A on ACh- and A23187-induced relaxation in human basilar arteries.

Materials and Methods

Human basilar arteries were obtained from 11 bodies at autopsy ≤3 hours after death. The cases are summarized in Table 1.

ACh chloride, papaverine hydrochloride, and indomethacin were obtained from Wako Chemical Industries (Osaka, Japan), AA861 from Takeda Industries (Osaka, Japan), SKF525A from Smith Kline & French Laboratories (Philadelphia, Pennsylvania), prostaglandin (PG) F2α from Ono Company (Osaka, Japan), and A23187 from Calbiochem-Behring (La Jolla, California). A23187 and AA861 were dissolved in dimethylsulfoxide, the final concentration of which had no effect on the contractile response to agonists.

The experiments were carried out as previously described.3 Human basilar arteries were cut into 2-mm-long rings, and an optimal resting tension of 1.09 g was applied to the rings. The basic experimental protocol involved a 20-minute control period during which the rings were contracted with the approximate ED50 of PGF2α in the presence of each
TABLE 1. Summary of Cases and Relaxation Responses of Basilar Artery Rings to Acetylcholine and A23187

<table>
<thead>
<tr>
<th>Case/age/sex</th>
<th>Cause of death</th>
<th>n</th>
<th>Maximum relaxation of rings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2/M</td>
<td>Pneumonia</td>
<td>5</td>
<td>50.35±4.32</td>
</tr>
<tr>
<td>2/3/M</td>
<td>Heart failure</td>
<td>4</td>
<td>52.34±3.55</td>
</tr>
<tr>
<td>3/20/M</td>
<td>Brain tumor</td>
<td>5</td>
<td>17.12±5.32</td>
</tr>
<tr>
<td>4/46/M</td>
<td>Aneurysm of aorta</td>
<td>5</td>
<td>10.76±6.35</td>
</tr>
<tr>
<td>5/73/M</td>
<td>Renal failure</td>
<td>5</td>
<td>22.88±4.01</td>
</tr>
<tr>
<td>6/76/F</td>
<td>Liver cirrhosis</td>
<td>4</td>
<td>21.03±6.34</td>
</tr>
<tr>
<td>7/5/F</td>
<td>Traffic accident</td>
<td>5</td>
<td>55.41±8.76</td>
</tr>
<tr>
<td>8/30/M</td>
<td>Head injury</td>
<td>5</td>
<td>15.41±10.01</td>
</tr>
<tr>
<td>9/40/F</td>
<td>Heart attack</td>
<td>5</td>
<td>11.33±7.63</td>
</tr>
<tr>
<td>10/8/F</td>
<td>Heart failure</td>
<td>5</td>
<td>53.25±11.48</td>
</tr>
<tr>
<td>11/3/M</td>
<td>Traffic accident</td>
<td>5</td>
<td>55.44±15.77</td>
</tr>
</tbody>
</table>

blocking agent (1.5×10⁻⁵ M indomethacin, 10⁻⁵ M AA861, and 3×10⁻⁵ M SKF525A) and an experimental period during which the rings were relaxed by the cumulative addition of ACh and A23187. The EDSO was obtained from a plot of percentage response vs. logarithm of the agonist concentration and was expressed as the negative logarithm (pD₂). At the end of each experiment, 10⁻⁴ M papaverine was added to attain maximum relaxation. Endothelial cells were then rubbed from the human basilar arteries according to the method previously described, and the experiment was repeated. Mean±SD relaxation relative to the papaverine-induced maximum are presented in the text, tables, and figures. The results were compared using Student’s t test; p≤0.05 was considered significant.

Results

One of the problems involved in the use of human vessels is that of postmortem changes in vascular function. For this reason, we used human basilar arteries removed from bodies ≤3 hours after death. Remarkable individual variations were noted in the maximum relaxations to ACh and A23187 of human basilar artery rings precontracted with 10⁻⁶ M PGF₂α. Both ACh and A23187 relaxed the basilar artery rings of Cases 1, 2, 7, 10, and 11 by >50%; neither agent was effective in the rings of Cases 3, 6, 8, or 9; and only A23187 was effective in the rings of Cases 4 and 5 (Table 1). Rubbing off the endothelium significantly attenuated both ACh- and A23187-induced relaxations in the human basilar artery rings (Figure 1).

The dose–response curve for ACh was shifted to the right (Figure 2, left) and the maximum relaxation to ACh was significantly reduced (Table 2) in rings pretreated with AA861; pD₂ was significantly different from control (Table 2). Pretreatment with indomethacin and SKF525A did not affect A23187-induced relaxation.

Discussion

Although ACh did not produce endothelium-dependent relaxation of canine basilar artery rings, ACh did induce significant relaxation in human basilar artery rings. Thus, significant species differences exist in the responses of basilar arteries to ACh. The differences may be due to characteristics of the muscarinic cholinergic receptors in cerebral arteries. On the other hand, A23187 induces relaxation in these arteries by the same mechanism, namely Ca²⁺ influx via the plasma membrane. A23187 is reported to be approximately 10–30 times more potent than ACh in producing endothelium-dependent relaxation of rabbit aortas. Moreover, in the presence of the full relaxing activity of A23187 (10⁻⁴ M), no relaxation by any other agent can be demonstrated.

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

**Figure 1.** Left: Dose–response curves showing mean±SD relaxation to acetylcholine (ACh) in rings of human basilar artery with (●, n=15) and without (○, n=12) endothelium. Right: Dose–response curves showing relaxation to A23187. *p<0.05, †p<0.01 different from rings with endothelium at same concentration.
ACh produces concentration-related relaxations in nonatherosclerotic human coronary arteries.18,19 In atherosclerotic human coronary arteries, endothelium-dependent relaxations are abolished by ACh, are partly suppressed by substance P and histamine, and are amaranth preserved by A23187.19 However, in hypercholesterolemic rabbits, the inhibition of ACh- and A23187-induced relaxation of cerebral arteries is correlated with fatty streak lesion formation.20 Thus, it is likely that both ACh- and A23187-induced relaxations are reduced by atherosclerosis in human basilar arteries. In the case of human umbilical blood vessels, the endothelium releases an EDRF in response to histamine, acting via H1 receptors, but not in response to ACh.21 Thus, it seems that the ability of in vitro vascular preparations to show endothelium-mediated effects varies widely with the blood vessel under study. In our study, both ACh and A23187 were effective in relaxing the basilar arteries of five of 11 cases. Substantial individual variation in the responses of human basilar arteries to ACh and A23187 may be due to alterations in endothelial cell function. Possible explanations for such alterations are 1) postmortem changes, 2) atherosclerotic changes, 3) cause of death, 4) medications administered before death, and 5) aging. Lusher and Vanhoutte22 reported that the endothelium of human arteries is very labile and soon after death may lose its capacity to release and/or produce EDRF(s) in response to ACh. The authors also suggested that autopsy specimens may be unsuitable for studying endothelium-dependent responses in human blood vessels.22

We demonstrated that AA861 significantly inhibits A23187-induced relaxation in human basilar arteries, and we suggest that the relaxation response to A23187 is mediated by lipoxygenase metabolite(s). Recent studies have suggested that 15-lipoxygenase products of arachidonic acid relax cat cerebral arteries23 and rat aorta,24 mesenteric, and pulmonary arteries.25 The 5-lipoxygenase metabolites LTC4 and LTD4 have been found to relax canine superior mesenteric and renal artery rings in an endothelium-dependent manner.8,9 It has also been demonstrated that LTD4-induced relaxation depends on the release of an EDRF which, in a manner similar to that of ACh, subsequently decreases vasomotor tone via activation of guanylate cyclase and generation of cyclic guanosine monophosphate.26 In addition, Toda27 reported the inhibitory effect of AA861 on endothelium-dependent relaxation induced by histamine in monkey coronary arteries and suggested that the AA861-induced inhibition derived mainly from other than an antioxidant action, probably from the specific inhibition of lipoxygenase.

On the other hand, SKF525A significantly inhibited ACh-induced relaxation without affecting A23187-induced relaxation. These results are consistent with findings that SKF525A inhibits ACh, but not A23187-induced endothelium-dependent relaxation in rabbit aortas.13 Since the cytochrome P450-dependent monooxygenase system is located primarily in the endothelium,28 ACh-induced relaxation may be mediated by a cytochrome P450-dependent monooxygenase metabolite. However, it has been demonstrated that higher concentrations of SKF525A (30–200 μM) cause transient endothelium-dependent relaxation of rabbit aorta rings and the release of nitric oxide and prostacyclin from endothelial cells.13 Since the threshold concentration of SKF525A producing prostacyclin is 20–30 μM,12,29

### Table 2. Effects of Indomethacin, AA861, and SKF525A on Acetylcholine- and A23187-Induced Relaxation of Precontracted Human Basilar Artery Rings

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>n</th>
<th>Acetylcholine Maximum %</th>
<th>pD2</th>
<th>A23187 Maximum %</th>
<th>pD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (control)</td>
<td>15</td>
<td>51.50±10.10</td>
<td>7.27±0.25</td>
<td>94.21±4.56</td>
<td>8.31±0.25</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>12</td>
<td>56.20±7.86</td>
<td>7.30±0.31</td>
<td>96.05±6.20</td>
<td>8.33±0.21</td>
</tr>
<tr>
<td>AA861</td>
<td>10</td>
<td>52.05±10.21</td>
<td>6.79±0.21</td>
<td>49.50±12.01*</td>
<td>7.78±0.11*</td>
</tr>
<tr>
<td>SKF525A</td>
<td>8</td>
<td>19.51±5.00*</td>
<td>6.20±0.18*</td>
<td>86.13±16.50</td>
<td>8.25±0.31</td>
</tr>
</tbody>
</table>

*p<0.01 different from control.  
pD2, negative logarithm. Data are mean±SD.
prostacyclin production by SKF525A in our present study seems to be minimal. It has recently been demonstrated that EDRF is nitric oxide and that its biosynthetic precursor is L-arginine. It is very likely that in most circumstances nitric oxide is the major EDRF. It has also been demonstrated that the inhibition of arachidonic acid release from membrane phospholipid pools does not attenuate endothelium-dependent relaxation in rat aortas nor the release and/or response of EDRF in cultured cells. However, ACh-induced relaxation in cerebral arteries may not be mediated by nitric oxide. Our preliminary study demonstrated that melittin, an activator of phospholipase A2, causes endothelium-dependent relaxation in canine basilar arteries and that melittin-induced relaxation is significantly inhibited by pretreatment with AA861. Therefore, the possibility that EDRF is lipid-like in nature or controlled by an arachidonic acid metabolite must continue to receive attention, especially in cerebral arteries.

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