Responsiveness of Isolated Cerebral and Peripheral Arteries to Serotonin, Norepinephrine, and Transmural Electrical Stimulation

By Noboru Toda and Yuxo Fujita

ABSTRACT

Spirally cut strips of cerebral and peripheral arteries from dogs were used for comparing the vasoconstricting effect of serotonin, norepinephrine, K⁺, and transmural electrical stimulation. Sensitivity of cerebral (basilar, posterior cerebral, and middle cerebral) arterial strips to serotonin was markedly greater than that to norepinephrine with respect to the median effective concentration (ED₃₀) and the maximum response. Contractile responses of isolated human cerebral arteries to serotonin and norepinephrine were similar to those observed in the dog arteries. In contrast, proximal and distal strips from superior mesenteric arteries and strips from renal arteries were more sensitive to norepinephrine than they were to serotonin. Mean values of contractions caused by 5 × 10⁻⁷ M serotonin relative to those caused by 30 mM K⁺ in cerebral, internal carotid, external carotid, common carotid, and superior mesenteric arteries were in a descending order, whereas those for norepinephrine were in an ascending order. These studies demonstrate that a gradual transition occurs from characteristic responses seen in mesenteric arteries (high sensitivity to norepinephrine, low sensitivity to serotonin) to those seen in cerebral arteries (high sensitivity to serotonin, low sensitivity to norepinephrine). Transmural stimulation did not produce contractions of cerebral and internal carotid arteries, but contractions were produced in external carotid, common carotid, and superior mesenteric arteries. It appears that sympathetic nerves cannot play an important role in the regulation of vascular tone in large cerebral arteries.

KEY WORDS  neurogenic control  spirally cut arterial strips  carotid arteries  human

different sensitivity to drugs  mesenteric arteries  dog

Numerous studies have suggested that cerebral and peripheral vessels respond differently to vasoconstricting agents and sympathetic nerve stimulation. In particular, Bohr et al. (1) have demonstrated that there are marked differences in the sensitivity of isolated dog cerebral and peripheral arteries (0.2–0.3 mm, o.d.) to epinephrine, norepinephrine, serotonin, angiotensin, and vasopressin but not to potassium (K⁺). With respect to intracranial arteries, Nielsen and Owman (2) have reported different constricting effects of epinephrine, norepinephrine, isoproterenol, serotonin, histamine, and acetylcholine on isolated feline middle cerebral arteries.

Light microscopic, fluorescence histochemical, and electron microscopic studies have clearly demonstrated the existence of cerebral sympathetic innervation (3–11), and total cerebral blood flow is reduced by electrical stimulation of cervical sympathetic nerves (12–18). The site of vasoconstriction, however, is controversial. Kobayashi et al. (16) and Wahl et al. (19), using a microscopic technique, have observed constriction of pial vessels following stimulation of sympathetic nerves and external application of norepinephrine. In contrast, Raper et al. (20) did not observe such vasoconstriction. The effect of sympathetic stimulation on large blood vessels has not been determined in situ or in vitro.

The present study was designed to analyze quantitatively the responsiveness of isolated dog cerebral and peripheral arteries to serotonin, norepinephrine, K⁺, and transmural neural stimulation. Data were also obtained from isolated human cerebral arteries.

Methods

Mongrel dogs of both sexes, weighing 8–15 kg, were used. The dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) and killed by bleeding from the common carotid arteries. The brain and the internal carotid (0.5–1 mm, o.d.), external carotid (1.5–
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3 mm), common carotid (2.5–4 mm), superior mesenteric (2.5–4 mm), distal mesenteric (0.5–1 mm), and renal (1.5–2 mm) arteries were isolated. Basilar, posterior cerebral, and middle cerebral arteries (0.3–0.7 mm) were removed from the brain. The vessels were spirally cut into strips approximately 25 mm long. The spiral strips were fixed vertically between hooks in a 20-ml bath containing the nutrient solution. The upper end of the strip was connected to the lever arm of a force-displacement transducer (Nihonkoden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 g in the smaller vessels (cerebral, internal carotid, and distal mesenteric arteries) and to 2 g in the larger vessels. The bathing solution was bubbled with a mixture of 95% O2-5% CO2 and was maintained at 37 ± 0.5°C. The pH of the solution was 7.2–7.4. Constituents of the solution were as follows (mM): Na+ 162.1, K+ 5.4, Ca2+ 2.2, Cl- 157.0, HCO3- 14.9, and dextrose 5.6. Osmotic adjustment was not made when K+ was added. Before the experiments were commenced, the preparations were allowed to equilibrate for 90–120 minutes in the bathing medium. During the equilibration period, the solution was replaced every 20–30 minutes.

Human cerebral arteries (basilar, intracranial internal carotid, and posterior cerebral arteries) were obtained during autopsy within 10 hours after death. The patients, a 29-year-old male, a 55-year-old male, and a 14-year-old female, died from brain tumors (glioblastoma, metastatic brain tumor, and glioblastoma, respectively). These arteries (1–2 mm, o.d.) were cut spirally into strips, fixed between hooks under a resting tension of 2 g, and placed in bathing medium. Drugs used were serotonin creatinine sulfate, dl-norepinephrine hydrochloride, phentolamine mesylate (Ciba-Geigy), methysergide (Sandoz), and tetrodotoxin (Sankyo). Serotonin and norepinephrine were added directly to the bathing medium in cumulative concentrations. Contractile responses to the amines were displayed on an ink-writing oscillograph (Sanei Sokki Co., Tokyo, Japan). Absolute values of the tension induced by the amines and K+ were compared in different vessels. Dose-response relationships for serotonin and norepinephrine were determined in two series of experiments done on alternate days, and the relationship for K+ was always determined in a third series. Effects of transmural electrical stimulation were studied immediately before the response to norepinephrine was obtained and also at the end of some experiments. Preparations were repeatedly washed with fresh nutrient solution and were allowed to relax to their initial level of tension. Results shown in the text, tables, and figures are expressed as means ± se. Comparisons of the results were made using Student's t-test.

For studies on the responsiveness to transmural electrical stimulation, the arterial strips were placed between a pair of stimulating electrodes as described in earlier reports (21, 22). A train of 0.3-msec rectangular pulses of supramaximal intensity (about 80 v) at frequencies of 5, 20, and 100/sec for a period of 40, 10, and 2 seconds, respectively, was used for stimulating intramural nerve elements in the vascular wall.

Electrical pulses were provided by an electronic stimulator (type WSE-3R, Nihonkoden Kogyo Co.). The concentrations of exogenous norepinephrine required to cause contractions equivalent to those caused by transmural neural stimulation were estimated from the dose-response curves for norepinephrine obtained in the same preparations.

Results

CONTRACTILE RESPONSES OF CEREBRAL AND MESENTERIC ARTERIES TO STIMULATORY AGENTS

Twenty-eight cerebral arteries from 28 dogs were used: 19 were basilar arteries, 7 were posterior cerebral arteries, and 2 were middle cerebral arteries. These different arteries showed similar responsiveness to the stimulatory agents.

Serotonin in concentrations ranging from 2.5 × 10^-8 to 1.25 × 10^-6M caused a dose-dependent increase in the tension of cerebral arterial strips (Fig. 1). An additional increase in the concentration up to 5 × 10^-5M resulted in a relaxation in 8 of 11 preparations. Dose-related contractions were also produced by norepinephrine in concentrations ranging from 10^-7 to 10^-6M. However, the maximum

![Figure 1: Contractile responses of Isolated dog cerebral arteries to serotonin, norepinephrine, and K+. Eleven cerebral arteries (7 basilar, 3 posterior cerebral, and 1 middle cerebral) were tested. Vertical bars represent ± se. Figures in parentheses indicate the number of preparations tested.](http://circres.ahajournals.org/Downloaded_from)
tension developed in response to $1.25 \times 10^{-8} \text{M}$ serotonin (460 ± 100 mg, $N = 11$) was markedly greater than that developed in response to $10^{-5} \text{M}$ norepinephrine (210 ± 60 mg, $N = 9$). The median effective concentration ($E_D_{50}$) of the two drugs also differed: it was $4.3 \times 10^{-8} \pm 1.0 \times 10^{-8} \text{M}$ for serotonin and $8.2 \times 10^{-7} = 1.3 \times 10^{-6} \text{M}$ for norepinephrine. Contrasting responses of a basilar artery to serotonin and norepinephrine are demonstrated in Figure 2.

Five cerebral arterial preparations from three humans were used for comparing stimulating effects of serotonin and norepinephrine. The results are summarized in Figure 3. The contractile response to serotonin was much greater than that to norepinephrine, as observed in the dog cerebral arteries. The mean value of the maximum contraction produced by $5 \times 10^{-6} \text{M}$ serotonin relative to that produced by $30 \text{ mM} K^+$ was 0.85 ± 0.08 ($N = 5$), and the mean value of the contraction produced by $10^{-5} \text{M}$ norepinephrine relative to that produced by $K^+$ was 0.26 ± 0.07 ($N = 5$). Figure 4 shows typical records from a human artery; automatic contractions were produced by the highest concentration of serotonin in two (internal carotid and basilar arteries) of the five preparations.

Effects of the stimulatory agents in superior mesenteric arteries contrasted to those in cerebral arteries. In concentrations between $5 \times 10^{-8}$ and $5 \times 10^{-7} \text{M}$, serotonin and norepinephrine caused similar contractions of mesenteric arteries. However, in concentrations higher than $10^{-5} \text{M}$, the contractile response to serotonin was apparently less than the response to norepinephrine. The maximum tension induced by $5 \times 10^{-6} \text{M}$ serotonin was $1.24 \pm 0.33 \text{ g} (N = 7)$, whereas that induced by $5 \times 10^{-5} \text{M}$ norepinephrine was $5.67 \pm 0.83 \text{ g} (N = 7)$. Similar findings were obtained in six renal arterial strips: the mean values of the maximum tension induced by serotonin and norepinephrine were $1.34 \pm 0.35 \text{ g}$ and $5.97 \pm 0.61 \text{ g}$, respectively.

**COMPARISON OF THE EFFECTS OF STIMULATORY AGENTS IN CEREBRAL, CAROTID, AND MESENTERIC ARTERIES**

To investigate regional differences in susceptibility to serotonin and norepinephrine, the responsiveness to these amines was compared in cerebral, internal carotid, external carotid, and common carotid arteries from the same dogs. The results are summarized in Table 1. The tension induced by $5 \times 10^{-6} \text{M}$ serotonin was greatest in the common carotid arteries, and smallest in the cerebral and internal carotid arteries. Contractility in response to $5 \times 10^{-6} \text{M}$ norepinephrine was in the order of mesenteric artery > common carotid artery > external carotid artery > internal carotid artery.
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Ser 1 2 3 4 5 E 7

NE I 2 3 4 56

KCl 5mM 10mM 20mM 25mM

**FIGURE 4**

Contractions induced by serotonin, norepinephrine, and K⁺ in a basilar arterial strip (human). Concentrations of serotonin and norepinephrine indicated by the numbers 1–6 are the same as those in Figure 2. The concentration of serotonin indicated by 7 is 2.5 x 10⁻⁶. Time scale = 1 minute.

cerebral artery. Similar differences in contractility of these vessels were observed in response to K⁺.

It has been demonstrated that K⁺ produces approximately equal contractions in cerebral and peripheral arteries (1). In the present study, phentolamine in a concentration (10⁻⁶M) sufficient to markedly attenuate norepinephrine-induced contractions did not cause relaxation in two basilar and three mesenteric arterial strips previously contracted by 30 mM K⁺. In one mesenteric and two basilar arterial strips, 30 mM K⁺ caused approximately equal contractions before and after administration of 10⁻⁶M phentolamine. Similar results were obtained with 10⁻⁶M methysergide in one basilar and two mesenteric arteries. Thus, it appears that contractile responses to K⁺ are not the result of actions involving specific alpha receptors and serotonin receptors but are more likely dependent on the extent of development of the contractile machinery in vessels. Accordingly, tension increments induced by serotonin and norepinephrine were expressed as values relative to the tension induced by 30 mM K⁺. The results are presented in Table 1. Mean values for the contraction caused by serotonin relative to that caused by K⁺ were in the order of cerebral artery > internal carotid artery > external carotid artery > common carotid artery > distal mesenteric artery > proximal mesenteric artery, whereas those for the contraction caused by norepinephrine relative to that caused by K⁺ were in the inverse order.

**CONTRACTILE RESPONSES TO TRANSMURAL ELECTRICAL STIMULATION**

Transmural electrical stimulation at frequencies of 5, 20, and 100/sec caused a transient, frequency-dependent increase in the tension of external carotid, common carotid, and superior mesenteric arterial strips. The pulse duration was 0.3 msec. In five external carotid, four common carotid, and four mesenteric arteries it was confirmed at the end of experiments that the contractile response to the electrical stimulation was abolished by treatment with 10⁻⁷M tetrodotoxin. The results are shown in

<table>
<thead>
<tr>
<th>Artery</th>
<th>N</th>
<th>Response to serotonin (g)</th>
<th>Serotonin/K⁺</th>
<th>Response to norepinephrine (g)</th>
<th>Norepinephrine/K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral</td>
<td>17</td>
<td>0.38 ± 0.10</td>
<td>1.13 ± 0.13</td>
<td>0.11 ± 0.02</td>
<td>0.33 ± 0.08</td>
</tr>
<tr>
<td>Internal carotid</td>
<td>17</td>
<td>0.39 ± 0.07</td>
<td>0.95 ± 0.14</td>
<td>0.21 ± 0.06</td>
<td>0.46 ± 0.06</td>
</tr>
<tr>
<td>External carotid</td>
<td>17</td>
<td>1.68 ± 0.19</td>
<td>0.91 ± 0.07</td>
<td>1.37 ± 0.17</td>
<td>0.73 ± 0.07*</td>
</tr>
<tr>
<td>Common carotid</td>
<td>17</td>
<td>2.83 ± 0.29</td>
<td>0.84 ± 0.08*</td>
<td>3.08 ± 0.31</td>
<td>0.90 ± 0.06*</td>
</tr>
<tr>
<td>Distal mesenteric</td>
<td>7</td>
<td>0.62 ± 0.17</td>
<td>0.35 ± 0.13*</td>
<td>1.48 ± 0.38</td>
<td>1.22 ± 0.16*</td>
</tr>
<tr>
<td>Superior mesenteric</td>
<td>9</td>
<td>1.24 ± 0.33</td>
<td>0.36 ± 0.15*</td>
<td>5.07 ± 0.83</td>
<td>1.39 ± 0.12*</td>
</tr>
</tbody>
</table>

All values are means ± se. The 17 cerebral arteries consisted of 12 basilar, 1 middle cerebral, and 4 posterior cerebral arteries.

*Significantly different (P < 0.01) from values obtained in cerebral arteries.

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Table 2. Mean values of the contractile response at 5/sec were not significantly different in these arterial strips. The greatest response to the stimulation at 20 and 100/sec was seen in superior mesenteric arteries, and the smallest response occurred in external carotid arteries. However, the mean values of concentration of exogenous norepinephrine required to produce contractions equivalent to those caused by transmural stimulation were in the order of common carotid artery > mesenteric artery > external carotid artery. The differences were not significant.

Cerebral and internal carotid arteries never did respond to transmural electrical stimulation under the same conditions as those used for external carotid, common carotid, and mesenteric arteries. However, when the pulse duration was increased to 1 msec or longer, the stimulation produced slowly developing contractions, which were not influenced by treatment with tetrodotoxin (10^-7 to 5 x 10^-5M) or phentolamine (10^-6M). In four basilar arterial strips soaked in control medium, the mean values of contractions induced by stimulation with pulses 3 msec long at 5 and 20/sec were 44 ± 17 mg and 67 ± 24 mg, respectively, whereas the values were 53 ± 24 mg and 65 ± 26 mg, respectively, after 10–20 minutes exposure to 3 x 10^-7M tetrodotoxin.

**Discussion**

The present study revealed that the susceptibility to serotonin of spirally cut strips of dog cerebral arteries, including basilar, posterior cerebral, and middle cerebral arteries, was markedly greater than the susceptibility to norepinephrine with respect to both the ED50 and the maximum response. Similar differences in the sensitivity to these amines were also demonstrated in human cerebral arteries. These findings confirm the preliminary results obtained by Bohr et al. (1) in dog cerebral arteries and by Nielsen and Owman (2) in cat middle cerebral arteries. In contrast the contractile responses to norepinephrine of superior mesenteric and renal arteries were appreciably greater than those to serotonin. Different susceptibility to serotonin and norepinephrine of cerebral and peripheral arteries does not seem to be associated with differences in the diameter of the arteries that were used, since dog distal mesenteric and intrarenal arteries with outside diameter of 0.5–1 mm responded to the amines in a similar manner to the proximal arteries.

Studies of regional differences in the sensitivity to serotonin and norepinephrine of isolated vessels from the same dogs provided evidence that a gradual transition occurs from the characteristic responses seen in mesenteric arteries (high sensitivity to norepinephrine, low sensitivity to serotonin) to those seen in cerebral arteries (high sensitivity to serotonin, low sensitivity to norepinephrine). Mean values of the contraction caused by serotonin relative to that caused by K+ in cerebral, internal carotid, external carotid, common carotid, and mesenteric arteries were in a descending order, whereas those for norepinephrine were in an
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ascending order. It appears that the distribution of alpha receptors and serotonin receptors or their sensitivity to the amines are different in these cerebral and peripheral arteries.

Serotonin was by far the most potent vasoconstricting drug of the test drugs studied under the same experimental conditions in cerebral arterial strips; the other test drugs included histamine, angiotensin, epinephrine (unpublished data), and norepinephrine. These results are consistent with those obtained by Nielsen and Owman (2) in feline middle cerebral arteries. Furthermore, it has been demonstrated that serotonin administered topically (23) or intra-arterially (24) causes constriction of large cerebral arteries. Serotonin is present in blood platelets in concentrated form (25, 26), and during clotting there is a release of serotonin (27-29). Thus, it has been suggested that this amine when released can be involved in the post-hemorrhage vasospasm produced in relatively large cerebral arteries (23, 30). Findings obtained in the present study support this hypothesis.

The possible existence of sympathetic control of large cerebral arteries has been postulated by Raper et al. (20), since neurogenic control could not be obtained in cerebral small arteries and arterioles. In the present study, however, transmural electrical stimulation with a short pulse duration (0.3 msec) applied to isolated large cerebral and internal carotid arteries failed to produce contractions, whereas stimulation of external carotid, common carotid, and superior mesenteric arteries under the same experimental conditions caused contractions. It has been shown that transmural stimulation of this pulse duration causes contractions and a sharp rise in the overflow of labeled norepinephrine (31) and that the contractile response is attenuated by alpha-receptor blocking agents, adrenergic neuron blocking agents, tetrodotoxin, and removal of calcium from the bathing medium (22, 32-34). Transmural neural stimulation applied to cerebral arterial strips does not seem to liberate norepinephrine in concentrations sufficient to induce contractions. This inability of cerebral arteries to contract is probably associated with a decreased sensitivity to norepinephrine, but a decreased release of norepinephrine cannot be ruled out. Thus, it appears that sympathetic nerves cannot play an important role in the regulation of vascular tone in large cerebral arteries. It is possible that the responsiveness to norepinephrine and the release of the amine are reduced to a greater extent in cerebral arteries by trauma and gross alteration in environment than they are in the other arteries used.

Contractile responses of external carotid, common carotid, and superior mesenteric arteries to transmural electrical stimulation at a frequency of 5/sec were similar, but those at frequencies of 20 and 100/sec were greatest in mesenteric arteries and smallest in external carotid arteries. However, the concentrations of externally applied norepinephrine required to produce contractions equivalent to those caused by transmural stimulation at all stimulation frequencies did not significantly differ in these arteries (about $3 \times 10^{-7}$M at 5/sec and 5-8 $\times 10^{-7}$M at 20/sec). The reason for the different responses at the higher frequencies is not known, but it may be related to differences in the distribution and the density of nerves innervating the vascular wall and in the neuromuscular distance. Therefore, to clarify the mechanism, additional studies are needed on the quantitative determination of norepinephrine released by nerve endings (35), the estimation of effective concentrations of norepinephrine at the neuroeffector junction (36), and the elimination of norepinephrine in these arteries.

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References


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