Role of Tyrosine Kinase in Serotonin-Induced Constriction of the Basilar Artery In Vivo

Takanari Kitazono, MD; Setsuro Ibayashi, MD; Tetsuhiko Nagao, MD; Tomoko Kagiyama, MD; Jiro Kitayama, MD; Masatoshi Fujishima, MD

Background and Purpose—Serotonin is one of the most potent constrictors of cerebral blood vessels and is implicated in several pathological conditions, including migraine and cerebral ischemia. Recent evidence has suggested that tyrosine kinase is involved in vasconstrictor responses. The objective of this study was to test the hypothesis that activation of tyrosine kinase contributes to serotonin-induced constriction of the basilar artery in vivo.

Methods—Using a cranial window in anesthetized Sprague-Dawley rats, we examined effects of inhibitors of tyrosine kinase and tyrosine phosphatase on constrictor responses of the basilar artery to serotonin in vivo.

Results—Serotonin (10⁻⁸, 10⁻⁷, and 10⁻⁶ mol/L) produced constriction of the basilar artery by 12±2%, 27±2%, and 37±3%, respectively. Genistein (3×10⁻⁶ mol/L), an inhibitor of tyrosine kinase, did not affect baseline diameter of the basilar artery but attenuated serotonin-induced vasoconstriction (P<.05 versus control responses). Daidzein, an inactive analogue of genistein, did not affect serotonin-induced constriction of the basilar artery. Tyrphostin 47 (10⁻⁵ mol/L), another inhibitor of tyrosine kinase, also attenuated serotonin-induced vasoconstriction, and tyrphostin 63, an inactive analogue of tyrphostin 47, did not affect the vasoconstriction. Sodium orthovanadate (10⁻³ mol/L), an inhibitor of tyrosine phosphatase, enhanced serotonin-induced vasoconstriction. Phorbol 12,13-dibutyrate, a direct activator of protein kinase C, also caused constriction of the basilar artery, which was not affected by genistein or sodium orthovanadate.

Conclusions—These results suggest that serotonin-induced constriction of the basilar artery is mediated, at least in part, by activation of tyrosine kinase in vivo.

Key Words: cerebral arteries • genistein • protein kinase C • tyrphostin

Cerebral blood vessels are richly innervated by serotonergic nerve fibers. Although serotonin causes dilatation of small cerebral arterioles, it has potent constrictive actions on large cerebral arteries such as basilar artery. It is also suggested that serotonin has an important role in several pathological conditions including migraine, cerebral vasospasm, and cerebral ischemia. Murray et al have found a role of calcium and protein kinase C in serotonin-induced constriction of the basilar artery. However, the precise mechanism by which serotonin produces constriction of the basilar artery is not fully understood.

Activity of tyrosine kinase appears to be an important determinant of cell growth and oncogenesis. Recent evidence has suggested that the activity of tyrosine kinase has a major influence on the contractility of vascular smooth muscle in situ. There are no data, however, regarding the role of tyrosine kinase in constrictor responses of cerebral arteries in vivo. Because vascular responses in vivo may not be as those in vitro, it is valuable to study the mechanisms of vascular responses in vivo. Thus, the goal of the present study was to test the hypothesis that serotonin is mediated by activation of tyrosine kinase in vivo. For this purpose, we tested the effects of test the hypothesis that constriction of the basilar artery in vitro.5,7–10 There are no data, however, regarding the role of tyrosine kinase in vivo.

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was bubbled continuously with 5% CO$_2$ and 95% N$_2$. Cerebrospinal fluid sampled from the cranial window had a pH of 7.42 ± 0.01, a PCO$_2$ of 36 ± 1 mm Hg, and a P0$_2$ of 109 ± 3 mm Hg. The diameter of the blood vessel was measured with a microscope equipped with a television camera coupled to an autowidth analyzer (C3161, Hamamatsu Photonics K.K.).

After a craniotomy was prepared, pH, PCO$_2$, and P0$_2$ of arterial blood were adjusted by changing rate and volume of the respirator and the oxygen content of inspiratory air. Arterial blood gas monitored during the experiments had a pH of 7.44 ± 0.01, a PCO$_2$ of 37 ± 1 mm Hg, and a P0$_2$ of 119 ± 6 mm Hg.

**Experimental Protocol**

We examined responses of the basilar artery to topical application of serotonin (10$^{-6}$ to 10$^{-4}$ mol/L). Serotonin was mixed in artificial cerebrospinal fluid and suffused over the craniotomy for 5 minutes. Diameters of the basilar artery were measured immediately before and during the last minute of application of the agonist. We used two different inhibitors of tyrosine kinase: genistein, tyrphostin 47. To show specificity of these inhibitors, we used daidzein, an inactive analogue of genistein, and tyrphostin 63, an inactive analogue of tyrphostin 47. We also used sodium orthovanadate, an inhibitor of tyrosine phosphatase. Genistein, daidzein, and tyrphostins were dissolved in dimethyl sulfoxide (DMSO). The maximum final concentration of DMSO was 0.1% in these experiments. The concentration of DMSO did not cause any significant changes in diameter of the basilar artery (data not shown). Sodium orthovanadate was dissolved in saline. Inhibitors were suffused starting from 15 minutes before and during application of the agonist. Topical application of these agents did not cause any changes in systemic arterial pressure (data not shown).

We next examined responses of the basilar artery to phorbol 12,13-dibutyrate (PDBu) (10$^{-7}$ to 10$^{-5}$ mol/L), a direct activator of protein kinase C. PDBu was mixed in DMSO and suffused over the craniotomy for 15 minutes. Because phorbol esters are metabolized slowly, they persist in the cell membrane for long periods of time. For this reason, PDBu was applied to the basilar artery only once during an experiment, either in the absence or the presence of inhibitors. The maximum final concentration of DMSO was 0.1% in these experiments. The concentration of DMSO did not cause any significant changes in diameter of the basilar artery (data not shown).

**Statistical Analysis**

All values were expressed as mean ± SEM. One-way repeated-measures ANOVA was used to compare concentration–dependent responses to vasoconstrictors. Two-way repeated-measures ANOVA was used to compare responses under control conditions and during interventions. When a significant F value was found, post hoc analysis was made with Wilcoxon’s test for responses to serotonin and Mann–Whitney’s U test for responses to PDBu. A value of P < 0.05 was considered significant.

**Results**

**Effects of Genistein on Serotonin-Induced Vasoconstriction**

Under control conditions, the diameter of the basilar artery was 255 ± 5 μm (n = 36). Topical application of serotonin (10$^{-8}$, 10$^{-7}$, and 10$^{-6}$ mol/L) produced constriction of the basilar artery by 12 ± 2%, 27 ± 2%, and 37 ± 3%, respectively (Fig 1). Serotonin–induced vasoconstriction was reproducible, since there was no significant attenuation of the response during repeated application of serotonin (n = 6, data not shown). Genistein (3 × 10$^{-7}$ mol/L), an inhibitor of tyrosine kinase, had no effect on baseline diameter of the basilar artery but attenuated serotonin–induced vasoconstriction (Fig 1). In the presence of 3 × 10$^{-6}$ mol/L genistein, serotonin (10$^{-8}$ and 10$^{-7}$ mol/L) produced constriction of the artery by 3 ± 1% and 15 ± 2%, respectively (P < 0.05 versus control responses, Fig 1).

**Effects of Sodium Orthovanadate on Serotonin-Induced Vasoconstriction**

We next tested effects of sodium orthovanadate, an inhibitor of tyrosine phosphatase, on serotonin–induced constriction of the basilar artery. Under control conditions, serotonin (10$^{-8}$, 10$^{-7}$, and 10$^{-6}$ mol/L) produced constriction of the basilar
artery by 6±1%, 21±2%, and 33±3%, respectively (Fig 3). In the presence of 10⁻⁵ mol/L sodium orthovanadate, serotonin (10⁻⁵ and 10⁻⁷ mol/L) caused vasoconstriction by 13±2% and 31±5%, respectively (P<.05 versus control responses) (Fig 3). Serotonin (10⁻⁶ mol/L) caused constriction of the basilar artery by 38±3% in the presence of sodium orthovanadate, which was not different from control responses.

**Effects of Genistein and Sodium Orthovanadate on PDBu-Induced Vasoconstriction.**

Topical application of PDBu (10⁻⁸, 3×10⁻⁷, and 10⁻⁶ mol/L) produced constriction of the basilar artery by 12±2%, 21±3%, and 30±4%, respectively. Neither genistein nor sodium orthovanadate affected PDBu-induced vasoconstriction (Table). Thus, constriction of the basilar artery to activation of protein kinase C is not mediated by activation of tyrosine kinase in vivo.

**Discussion**

The major new finding in the present study is that constriction of rat basilar artery in response to serotonin is mediated, at least in part, by activation of tyrosine kinase in vivo. Because PDBu-induced vasoconstriction is not affected by genistein or sodium orthovanadate, activation of tyrosine kinase may not be involved in protein kinase C–dependent constriction of the basilar artery in vivo.

The first evidence regarding the role of tyrosine kinase in vasocontractile responses was based on the observation that epidermal growth factor (EGF) produces contractile responses as well as growth of vascular muscle. It is well known that the activity of tyrosine kinase plays a role in EGF-induced vasocontractile responses. Tyrosine kinase, coupled to GTP-binding protein, is mediated in part by activation of tyrosine kinase in EGF-induced vasoconstriction. Recently, it has also been reported that tyrosine kinase plays a role in EGF-induced vasoconstriction. Toma et al have shown that contractile responses of rat mesenteric arteries to norepinephrine, whose receptors do not contain the activity of tyrosine kinase and are coupled to GTP-binding protein, are mediated in part by activation of tyrosine kinase in vitro. Abebe et al have also reported that activation of tyrosine kinase is involved in norepinephrine-induced contraction of rat aorta in vitro. Thus, the activity of tyrosine kinase may be one of the major regulators of vasocontractile responses.

In the present study we have found, using a cranial window technique, that inhibition of tyrosine kinase markedly attenuates serotonin-induced constriction of the basilar artery and sodium orthovanadate, an inhibitor of tyrosine phosphatase, conversely enhances the vasoconstriction. Thus, activation of tyrosine kinase may also contribute to serotonin-induced constriction of the basilar artery in vivo. This is the first report thus far to show the presence of the activity of tyrosine kinase in cerebral blood vessels in vivo and to show the role of the kinase in contractile responses of the basilar artery to agonists.

A major concern regarding the findings mentioned above might be specificity of the inhibitors. In the present study both 3×10⁻⁶ mol/L genistein and 10⁻⁵ mol/L tyrphostin 47 had good inhibitory effects on the vasoconstriction, and these effects were not different from control responses. However, changes in diameter of the basilar artery were measured in response to phorbol 12,13-dibutyrate (PDBu) (10⁻⁸ to 10⁻⁶ mol/L) under control conditions and in the presence of genistein (3×10⁻⁶ mol/L) and sodium orthovanadate (10⁻⁵ mol/L). Baseline diameters under control conditions and in the presence of genistein and sodium orthovanadate were 240±10, 238±14, and 237±4 μm, respectively. Values are mean±SEM, expressed as percentages (n=6).

**Table**

<table>
<thead>
<tr>
<th>PDBu (log mol/L)</th>
<th>−8</th>
<th>−7.5</th>
<th>−7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−12±2</td>
<td>−21±3</td>
<td>−30±4</td>
</tr>
<tr>
<td>Genistein</td>
<td>−10±2</td>
<td>−20±4</td>
<td>−27±4</td>
</tr>
<tr>
<td>Sodium orthovanadate</td>
<td>−15±3</td>
<td>−20±3</td>
<td>−29±5</td>
</tr>
</tbody>
</table>

Changes in diameter of the basilar artery were measured in response to photobol 12,13-dibutyrate (PDBu) (10⁻⁸ to 10⁻⁶ mol/L) under control conditions and in the presence of genistein (3×10⁻⁶ mol/L) and sodium orthovanadate (10⁻⁵ mol/L). Baseline diameters under control conditions and in the presence of genistein and sodium orthovanadate were 240±10, 238±14, and 237±4 μm, respectively. Values are mean±SEM, expressed as percentages (n=6).

**Figure 2.** Effects of tyrphostin 47 on serotonin-induced vasoconstriction. Changes in diameter of the basilar artery were measured in response to serotonin (10⁻⁸ to 10⁻⁶ mol/L) under control conditions and in the presence of tyrphostin 47 (10⁻⁵ mol/L). Baseline diameters in the absence and the presence of tyrphostin 47 were 244±10 and 239±7 μm, respectively. Values are mean±SEM (n=6). *P<.05 vs control response.

**Figure 3.** Effects of sodium orthovanadate on serotonin-induced vasoconstriction. Changes in diameter of the basilar artery were measured in response to serotonin (10⁻⁸ to 10⁻⁶ mol/L) under control conditions and in the presence of sodium orthovanadate (1×10⁻⁵ mol/L). Baseline diameters in the absence and the presence of sodium orthovanadate were 247±6 and 238±9 μm, respectively. Values are mean±SEM (n=6). *P<.05 vs control response.
concentrations are very close to half-maximum concentrations for inhibition of tyrosine kinase.11,12 Moreover, daidzein and tyrophostin 63, inactive analogues of genistein14 and tyrophostin 47,13 did not affect serotonin-induced vasoconstriction. Thus, the inhibitory effects of genistein and tyrophostin 47 are likely to be specific for tyrosine kinase. The finding that genistein did not affect PDBu-induced constriction of the basilar artery may also support our interpretation that the inhibitory action of genistein and tyrophostin 47 on vasoconstriction may be specific for tyrosine kinase. The concentration of sodium orthovanadate (10−5 mol/L) is also very close to half-maximum concentration for inhibition of tyrosine phosphatase.15 The finding that the concentration of sodium orthovanadate did not affect PDBu-induced vasoconstriction may also support the interpretation that the inhibitory effects of sodium orthovanadate may not be nonspecific.

Serotonin appears to activate phospholipase C through GTP-binding protein and thereby produces inositol 1,4,5-trisphosphate and diacylglycerol.20 Inositol 1,4,5-trisphosphate increases cytoplasmic Ca2+ level by means of activation of intracellular Ca2+ stores, and diacylglycerol activates protein kinase C.16,21 It is reported that serotonin-induced constriction of the basilar artery is mediated in part by activation of protein kinase C in vivo.2,3 Thus, we next tested the role of tyrosine kinase in constriction of the basilar artery produced by activation of protein kinase C. PDBu, a direct activator of protein kinase C,16 produced constriction of the basilar artery, which was not affected by genistein or sodium orthovanadate. Thus, PDBu-induced constriction of the basilar artery may not be mediated by activation of tyrosine kinase in vivo. The findings are similar to the recent studies of noncerebral blood vessels.5,8 It is reported that tyrosine kinase inhibitors attenuate agonist-induced increase in the cytoplasmic Ca2+ level of vascular muscle.3,22 Thus, it may be possible that tyrosine kinase has a role in calcium signaling of the basilar arterial muscle in vivo.

Masumoto et al10 have reported that activation of tyrosine kinase is involved in pressure-induced contraction of rat cerebral artery in vitro. Thus, it may be possible that inhibition of tyrosine kinase affected the resting (myogenic) tone of the basilar artery. In the present study, however, neither genistein nor tyrophostin 47 affected the baseline diameter of the basilar artery. Activation of tyrosine kinase appears to be involved in nitric oxide production of vascular endothelial cells.23 Thus, inhibition of tyrosine kinase may have attenuated dilator responses as well as constrictor responses of the basilar artery and thereby masked the inhibitory effects of tyrosine kinase inhibitors on myogenic tone under control conditions. Another possibility may be that some compensatory mechanisms may have counteracted the inhibitory actions of genistein and tyrophostin 47 on myogenic tone under control conditions in vivo.

In summary, activation of tyrosine kinase may be involved in constrictor responses of rat basilar artery to serotonin in vivo. Protein kinase C–dependent constriction of the basilar artery may not be mediated by activation of tyrosine kinase.

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References

Although it is well known that serotonin (5-hydroxytryptamine) is a potent constrictor of large cerebral arteries, the mechanism that mediates the constriction has not been fully defined. This study provides evidence that tyrosine kinases play an important role in serotonin-induced constriction of the basilar artery.

Tyrosine kinases are thought to be a major signal transduction system in a variety of cells, including vascular muscle. For example, several effects of angiotensin II on vascular muscle, including contraction, appear to be mediated by tyrosine kinases. The study presented here by Kitazono et al supports this concept by providing pharmacological evidence that constriction of the basilar artery in response to serotonin in vivo is dependent on activation of tyrosine kinases. The conclusion supports previous work that implicated a role for these kinases in contraction of cerebral arteries in response to other stimuli in vitro.

Serotonin has been implicated in cerebral vascular pathophysiology, including conditions involving intravascular activation of platelets. Serotonin-induced contraction of large cerebral arteries is enhanced under pathophysiological conditions, including chronic hypertension, atherosclerosis, and subarachnoid hemorrhage. Because activation of tyrosine kinases appears to be an important mechanism of constriction of large cerebral arteries under normal conditions, it is tempting to speculate that enhanced activity of tyrosine kinases may contribute to augmented vasoconstrictor effects of serotonin under pathophysiological conditions. This study contributes to our understanding of signaling events in cerebral vascular muscle and may help provide insight into management of cerebral vascular disorders, including vasospasm.

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