Fluoxetine Dilates Isolated Small Cerebral Arteries of Rats and Attenuates Constrictions to Serotonin, Norepinephrine, and a Voltage-Dependent Ca$^{2+}$ Channel Opener

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Background and Purpose—Recent clinical observations question that the antidepressant effect of fluoxetine (Prozac) can be explained solely with serotonin reuptake inhibition in the central nervous system. We hypothesized that fluoxetine affects the tone of vessels and thereby modulates cerebral blood flow.

Methods—A small branch of rat anterior cerebral artery (195±15 μm in diameter at 80 mm Hg perfusion pressure) was isolated, cannulated, and pressurized (at 80 mm Hg), and changes in diameter were measured by videomicroscopy.

Results—Fluoxetine dilated small cerebral arteries with an EC$_{50}$ of 7.7±1.0×10$^{-6}$ mol/L, a response that was not affected by removal of the endothelium or application of 4-aminopyridine (an inhibitor of aminopyridine-sensitive K$^+$ channels), glibenclamide (an inhibitor of ATP-sensitive K$^+$ channels), or tetraethylammonium (a nonspecific inhibitor of K$^+$ channels). The presence of fluoxetine (10$^{-6}$ to 3×10$^{-5}$ mol/L) significantly attenuated constrictions to serotonin (10$^{-9}$ to 10$^{-5}$ mol/L) and norepinephrine (10$^{-9}$ to 10$^{-7}$ mol/L). Increasing concentrations of Bay K 8644 (a voltage-dependent Ca$^{2+}$ channel opener, 10$^{-10}$ to 10$^{-6}$ mol/L) elicited contractions, which were markedly reduced by 2×10$^{-6}$ and 10$^{-5}$ mol/L fluoxetine, whereas 3×10$^{-5}$ mol/L fluoxetine practically abolished the responses.

Conclusions—Fluoxetine elicits substantial dilation of isolated small cerebral arteries, a response that is not mediated by endothelium-derived dilator factors or activation of K$^+$ channels. The finding that fluoxetine inhibits constrictor responses to Ca$^{2+}$ channel opener, as well as serotonin and norepinephrine, suggests that fluoxetine interferes with the Ca$^{2+}$ signaling mechanisms in the vascular smooth muscle. We speculate that fluoxetine increases cerebral blood flow in vivo, which contributes to its previously described beneficial actions in the treatment of mental disorders. (Stroke. 1999;30:1949-1954.)

Key Words: calcium channels ■ cerebral arteries ■ dilation ■ fluoxetine ■ smooth muscle

Previous studies suggest that 5-hydroxytryptaminergic (serotonergic) pathways in the central nervous system are involved in the pathogenesis of human depression. It is thought that by blocking 5-hydroxytryptamine (5-HT) reuptake from nerve terminals, newer antidepressant drugs, such as fluoxetine (Prozac), enhance 5-serotonergic transmission, which is regarded as the primary therapeutic action of these compounds.

Several recent studies suggested that fluoxetine has additional effects that are apparently not related to the inhibition of the neuronal 5-HT reuptake. For example, in neuronal tissue, fluoxetine has been reported to antagonize nicotinic acetylcholine and 5-HT$^3$ receptors and to block sodium and voltage-dependent potassium channels. Moreover, fluoxetine was reported to inhibit both voltage-dependent and 5-HT–induced increases in [Ca$^{2+}$]$_i$ and human cortical neurons. Fluoxetine has also been shown to affect the function of smooth muscle. It antagonized 5-HT–induced contraction of isolated rat intestinal preparation, affected potassium channels in isolated intestinal smooth muscle cells, and inhibited the high potassium–induced contraction of uterine preparations. In isolated pulmonary arterial smooth muscle cells, fluoxetine inhibited the 5-HT–induced [Ca$^{2+}$]$_i$ increase, suggesting that fluoxetine interferes with the generation of vascular tone. However, the possible effects of fluoxetine on the function of cerebral arteries is not known, and their possible contribution to the therapeutic action of fluoxetine has not been considered.

Thus, in the present study we aimed to characterize the effects of fluoxetine on the myogenic tone of small cerebral arteries of rats. To avoid the masking effect of neural and hormonal regulation of the vascular tone, experiments were conducted on isolated rat cerebral arteries. To elucidate the underlying mechanisms responsible for the vascular action of fluoxetine, the possible roles of endothelium, opening of K$^+$ channels, and their possible contribution to the therapeutic action of fluoxetine has not been considered.

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channels, inhibition of voltage-dependent Ca\(^{2+}\) channels, and the responses to 5-HT and norepinephrine were assessed.

**Materials and Methods**

**Isolation of Arterioles**

Experiments were conducted on isolated rat small cerebral arteries (\(\approx 195\ \mu m\) in diameter at 80 mm Hg). The methods for isolation of vessels have been described in detail previously. The procedures followed were in accord with institutional guidelines. Male Wistar rats (weighing 350\(\pm\)30 g) were anesthetized with pentobarbital (75 mg/kg IP). The brain was removed immediately after decapitation and placed in a silicone-lined Petri dish containing cold (0°C to 4°C) physiological saline (PS) solution composed of (in mmol/L) NaCl 110, KCl 5.0, CaCl\(_2\) 2.5, MgSO\(_4\) 1.0, KH\(_2\)PO\(_4\) 1.0, dextrose 10.0, and NaHCO\(_3\) 24.0 and was equilibrated with a gas mixture of 10% O\(_2\) and 5% CO\(_2\), balanced with nitrogen, at pH 7.4. With microsurgical instruments and an operating microscope, a 1.5-mm segment of a first daughter branch of the anterior cerebral artery running superficially was isolated and transferred into an organ chamber containing 2 glass micropipettes filled with PS solution. One artery was used from each rat. The vessel chamber (15 mL) was continuously supplied with PS solution from a reservoir at a rate of 40 mL/min. After the vessel had been mounted on the proximal micropipette and secured with sutures, the perfusion pressure was raised to 80 mm Hg to clear the debris from the lumen. Then the other end of the vessel was mounted on the distal pipette. Both micropipettes were connected with silicone tubing to an adjustable PS solution reservoir. Pressure on both sides was measured by an electromanometer. The perfusion pressure was slowly increased to 80 mm Hg. The temperature was set at 37°C by a temperature controller (Grant Instruments). The vessel was allowed to equilibrate for approximately 1 hour.

**Experimental Protocols**

Only those vessels that developed spontaneous tone in response to perfusion pressure were used, and thus, no vasoactive agent was added to the PS solution to establish arterial tone. After the equilibration period, the diameter of vessels was measured at 80 mm Hg perfusion pressure under zero-flow conditions. At the conclusion of each experiment, the suffusion solution was changed to a Ca\(^{2+}\)-free PS solution that contained sodium nitroprusside (SNP, 10\(^{-4}\) mol/L) and EGTA (1.0 mmol/L). The vessel was incubated for 10 minutes, and the passive diameter at 80 mm Hg pressure was used. The diameter was measured with a microangiometer and recorded with a chart recorder.

Responses to increasing concentrations of fluoxetine (10\(^{-7}\) to 3\(\times\)10\(^{-3}\) mol/L) were obtained before and after removal of endothelium or in the presence of ATP-dependent K\(^{+}\) channel inhibitor glibenclamide (10\(^{-4}\) mol/L, a nonspecific inhibitor of various K\(^{+}\) channels), or 10\(^{-4}\) mol/L, 4-aminoypyridine (4-AP, an inhibitor of aminoxypridine-sensitive K\(^{+}\) channels). The endothelium of arteries was removed by perfusion of the vessel with air for \(\approx\)1 minute at a perfusion pressure of 20 mm Hg as described previously. The artery was then perfused with PS solution to clear the debris. The perfusion pressure was then raised to 80 mm Hg for 30 minutes to establish a stable tone. The efficacy of endothelial denudation was ascertained by arterial responses to acetylecholine (10\(^{-7}\) mol/L, an endothelium-dependent agent) and SNP (10\(^{-4}\) mol/L, an endothelium-independent agent) before and after the administration of the air bolus. The infusion of air resulted in loss of function of the endothelium, as indicated by the absence of dilation to acetylecholine, whereas dilation to SNP remained intact.

In a second series of experiments, responses of vessels to cumulative doses 5-HT (10\(^{-7}\) to 10\(^{-2}\) mol/L) and norepinephrine (10\(^{-7}\) to 10\(^{-2}\) mol/L) were obtained. The vessel was then incubated with fluoxetine (10\(^{-5}\), 2\(\times\)10\(^{-5}\), 5\(\times\)10\(^{-5}\), 10\(^{-4}\), or 3\(\times\)10\(^{-3}\) mol/L) for 5 minutes, and vasoactive responses were reassessed. In separate experiments, responses to 5-HT were blocked in the presence or absence of ketanserin (10\(^{-6}\) mol/L), a known 5-HT\(_{1A}\) receptor antagonist. Also, after removal of the endothelium, changes in diameter to cumulative doses of Bay K 8644 (an opener of voltage-dependent Ca\(^{2+}\) channels, 10\(^{-10}\) to 10\(^{-6}\) mol/L) were assessed before and after preincubation with fluoxetine (2\(\times\)10\(^{-6}\), 5\(\times\)10\(^{-6}\), and 10\(^{-5}\) mol/L) for 5 minutes. All drugs were added to the vessel chamber, and final concentrations are reported. After responses to each drug subsided, the system was flushed with PS solution.

**Materials**

Fluoxetine was obtained from Research Biochemicals International Co. All other salts and chemicals were obtained from Sigma-Aldrich Co and were prepared on the day of the experiment. A 10\(^{-2}\) mol/L stock of glibenclamide was prepared by dissolving the substance in 30% wt/vol (2-hydroxypropyl)-(\(\tilde{\alpha}\)-cyclodextrin) (Cyclolab R&D Ltd), adding a small volume of 40% vol/vol ethanol, and dissolving in buffer. Fluoxetine was dissolved in PS solution. Bay K 8644 was dissolved in ethanol and protected from light; experiments were carried out in the dark. The vehicle did not have vasoactive effects.

**Statistical Analysis**

Dilations were expressed as a percentage of the maximal dilation of the vessel, defined as the passive diameter at 80 mm Hg perfusion pressure in Ca\(^{2+}\)-free medium containing 10\(^{-4}\) mol/L EGTA and 10\(^{-4}\) mol/L SNP. Constrictions were expressed as a percentage of the baseline diameter. The 50% effective concentrations (EC\(_{50}\)) were calculated from the logititmic regressions of the cumulative dose-response curves of vasoactive agents. In the graphs, the arithmetic mean\(\pm\)SEM values were used. Statistical analyses were performed by ANOVA for repeated measures followed by Tukey’s post hoc test or Student’s t test. A value of \(P<0.05\) was considered statistically significant.

**Results**

Rat small cerebral arteries developed a spontaneous myogenic tone in response to an increase in the perfusion pressure to 80 mm Hg without the use of any vasoactive agents. The active inner diameter of arterioles was 195\(\pm\)15 \(\mu m\). The passive diameter of arterioles obtained in the same conditions but in the absence of Ca\(^{2+}\) (see Methods) was 270\(\pm\)17 \(\mu m\).

**Responses to Fluoxetine: Role of Endothelium**

In a dose-dependent manner, fluoxetine (10\(^{-6}\) to 5\(\times\)10\(^{-5}\) mol/L) elicited substantial dilations in arteries, with an EC\(_{50}\) of 7.7\(\pm\)1.0\(\times\)10\(^{-6}\) mol/L (Figure 1A). We have also found that removal of the endothelium had no significant effect on the dilation of arteries to fluoxetine (EC\(_{50}\)=6.9\(\pm\)0.8\(\times\)10\(^{-6}\) mol/L, \(P=NS\); Figure 1A).

**Role of Potassium Channels**

Next, we tested the possible role of K\(^{+}\) channels in the vasodilator action of fluoxetine. Fluoxetine-induced dilations of arteries did not change significantly (EC\(_{50}\)=6.3\(\pm\)1.5\(\times\)10\(^{-6}\) mol/L, EC\(_{50}\)=7.8\(\pm\)1.7\(\times\)10\(^{-6}\) mol/L, and EC\(_{50}\)=6.8\(\pm\)1.1\(\times\)10\(^{-6}\) mol/L, respectively; Figure 1B) after preincubation and in the presence of the ATP-sensitive K\(^{+}\) channel inhibitor glibenclamide (10\(^{-3}\) mol/L, 4-AP (10\(^{-4}\) mol/L, or TEA (3\(\times\)10\(^{-3}\) mol/L).

**Responses to 5-HT and Norepinephrine**

5-HT constricted the arteries in a concentration-dependent manner, with an EC\(_{50}\) of 5.6\(\pm\)2.3\(\times\)10\(^{-7}\) mol/L (Figure 2). In the presence of ketanserin (10\(^{-6}\) mol/L), a 5-HT receptor antagonist, 5-HT-induced constrictions were abolished (data not shown). Fluoxetine (10\(^{-6}\), 2\(\times\)10\(^{-6}\), and 10\(^{-5}\) mol/L)
significantly ($P < 0.01$) reduced constrictor responses to 5-HT (Figure 2), whereas $3 \times 10^{-5}$ mol/L fluoxetine essentially abolished the response.

In control conditions, norepinephrine constricted arteries in a concentration-dependent manner with an EC$_{50}$ of $4.25 \pm 0.9 \times 10^{-7}$ mol/L (Figure 3). Increasing doses of fluoxetine ($2 \times 10^{-6}$ and $3 \times 10^{-6}$) significantly ($P < 0.01$) reduced responses to norepinephrine, whereas $10^{-5}$ mol/L completely abolished the response.

Responses to Bay K 8644
We tested the arterial responses to Bay K 8644, a voltage-dependent Ca$^{2+}$ channel opener. Bay K 8644 ($10^{-10}$ to $10^{-6}$ mol/L) constricted arteries in a concentration-dependent manner with an EC$_{50}$ of $5.6 \pm 2.1 \times 10^{-9}$ mol/L (Figure 4). Constrictions to Bay K 8644 were significantly ($P < 0.01$) reduced in the presence of $2 \times 10^{-6}$ or $10^{-5}$ mol/L fluoxetine (Figure 4), whereas $3 \times 10^{-5}$ mol/L completely abolished the response.

Discussion
This study demonstrates for the first time that fluoxetine, a serotonin reuptake inhibitor, dilates isolated rat small cerebral arteries. This dilation is not mediated by 4-AP–, TEA-, or glibenclamide-sensitive K$^+$ channels or endothelium-derived dilator factors. Fluoxetine antagonized arterial constrictions to 5-HT and norepinephrine and had a substantial inhibitory effect on constrictions elicited by opening of voltage-dependent Ca$^{2+}$ channels.

Fluoxetine Dilates Small Cerebral Arteries
In the present study, fluoxetine elicited a substantial concentration-dependent vasodilation in rat cerebral arteries (Figure 1). The vessels were isolated; thus, the effects of fluoxetine were induced by local factors intrinsic to the arterial wall. The concentrations of fluoxetine, which have a substantial cerebrovascular effect, are close to the upper range of therapeutic plasma concentrations (0.15 to 1.5 $\mu$mol/L$)^{16,17}$ Previous studies reported that similar concentrations of norfluoxetine, the active metabolite of fluoxetine, are also present in the plasma of...
fluoxetine-treated patients. In addition, recent data indicate that fluoxetine can be accumulated in the tissues. During chronic fluoxetine treatment, a concentration of fluoxetine 20 times higher than that in plasma has been detected in human brain, a finding that also indicates that fluoxetine effectively crosses the blood-brain barrier. Collectively, these findings suggest that during clinical treatments, the concentration of fluoxetine in the brain can reach levels that can potentially affect cerebral vascular tone and hence blood flow. Further studies are needed, however, to clarify the effect of fluoxetine on cerebral blood flow in patients with major depression. Nevertheless, our findings also suggest that the mechanisms of the medicinal actions of fluoxetine are more complicated than currently believed.

Possible Mechanisms of Action

Because there was no previous knowledge regarding the cerebrovascular effects of fluoxetine, we aimed to characterize the possible endothelial and smooth muscle mechanisms.

First, we aimed to elucidate whether the vasodilation to fluoxetine is mediated by release of factors from the endothelium. Removal of the endothelium, however, did not affect the fluoxetine-induced dilation (Figure 1). Therefore, we conclude that fluoxetine exerts its effect directly on the arterial smooth muscle.

Next, we hypothesized that opening of vascular K+ channels may be responsible for the dilation of small cerebral arteries to fluoxetine. Thus, we tested arterial responses to fluoxetine in the presence of glibenclamide (an inhibitor of ATP-sensitive K+ channels), 4-AP, or TEA (which inhibits several vascular K+ channels). However, the inhibition of these K+ channels did not significantly affect the vasodilator action of fluoxetine (Figure 2).

Fluoxetine is known to interfere with 5-HT receptors and/or signal transduction pathways in the central nervous system. Although 5-HT has a complex action on the cerebral vessels, the effect of fluoxetine on responses of isolated cerebral arteries to 5-HT has not been clarified. In the present study, we confirmed previous findings showing that fluoxetine inhibited constric-
tions to 5-HT. We found that fluoxetine inhibited constric-
tions to 5-HT in a dose-dependent manner (Figure 2). Interestingly, fluoxetine inhibited 5-HT–induced contractions of rat intestinal preparations as well. Previous studies demonstrated that other 5-HT uptake inhibitors, femoxetine and paroxetine, also antagonized 5-HT–induced contractions of isolated cat cerebral arteries at similar concentrations (from 3 x 10^-7 mol/L). On the basis of these findings and those of Ni and Miledi showing that fluoxetine is a competitive and reversible antagonist of 5-HT/x receptors, one would conclude that fluoxetine inhibits 5-HT–induced contractions by inhibiting 5-HT receptors on cerebral arteries. However, the finding that fluoxetine inhibited arterial constriction to norepinephrine also suggests that fluoxetine is not selective for 5-HT receptors and may affect postreceptor sites as well.

Previous studies established that after the stimulation of vascular 5-HT2 and adrenergic receptors, an increase in [Ca2+]i takes place in the vascular smooth muscle, due at least in part to the opening of voltage-dependent Ca2+ channels. Therefore, it was logical to hypothesize that fluoxetine may attenuate 5-HT– and norepinephrine-mediated responses by

Figure 3. Changes in diameter of isolated rat small cerebral arteries in response to cumulative doses of norepinephrine in the absence (control) or presence of 2 x 10^-6 mol/L, 3 x 10^-6 mol/L, or 10^-5 mol/L fluoxetine. Each point represents the mean±SEM of 4 to 6 separate experiments.

Figure 4. Changes in diameter of isolated rat small cerebral arteries in response to cumulative doses of Bay K 8644 in the absence (control) or presence of 2 x 10^-6 mol/L, 10^-5 mol/L, or 3 x 10^-5 mol/L fluoxetine. Each point represents the mean±SEM of 4 to 6 separate experiments.
inhibiting the influx of extracellular Ca^{2+}. This hypothesis is supported by previous in vitro findings that fluoxetine at a similar concentration (10^{-6} mol/L) markedly attenuated the 5-HT–induced rise in [Ca^{2+}]] in rat pulmonary arterial smooth muscle and human neuronal cells.9 Thus, the next logical step was to test the effect of fluoxetine on arterial responses to Ca^{2+} channel activation.

In the present study, small cerebral arteries developed spontaneous myogenic constriction in response to an increase in intraluminal pressure. Pressure-induced myogenic response is known to depend primarily on Ca^{2+} influx through voltage-dependent Ca^{2+} channels in the vascular smooth muscle cells.14,28,29 Thus, the finding that fluoxetine dilated cerebral arteries (inhibited myogenic tone) in an endothelium-independent manner suggests that fluoxetine interferes with Ca^{2+} signaling mechanisms. To further support this contention, we tested the effects of fluoxetine on cerebral arterial constriction elicited by Bay K 8644, an opener of voltage-dependent Ca^{2+} channels. We found that fluoxetine, in a concentration-dependent manner, inhibited the constriction evoked by the pharmacological stimulation of voltage-dependent Ca^{2+} channels. Thus, it is likely that fluoxetine inhibits Ca^{2+} entry by inhibiting voltage-dependent Ca^{2+} channels in vascular smooth muscle cells, thereby reducing the tone developed to pressure, ie, eliciting dilation. Previous studies support this hypothesis by demonstrating that fluoxetine antagonized voltage-dependent Ca^{2+} channels in the neuronal tissue.7,8 Fluoxetine also reduced the high potassium–induced contractions of uterine preparations,12 which may also suggest a role for inhibition of voltage-dependent Ca^{2+} channels in the smooth muscle. It is interesting to note that tricyclic antidepressants also seem to inhibit voltage-dependent Ca^{2+} channels in neural tissue,30,31 ventricular myocytes,32 and vascular smooth muscle33,34 and that several Ca^{2+} channel antagonists used to treat hypertension, especially dihydropyridine derivatives, were found to exert certain antidepressant effects.35 Nevertheless, future studies should further determine the possible involvement of inhibition of vascular and/or neural Ca^{2+} channels in the antidepressant action of fluoxetine.

In conclusion, we found that fluoxetine dilates small cerebral arteries. This response is not mediated by potassium channels or endothelium-derived dilator factors. The findings that fluoxetine inhibits not only responses to 5-HT and noradrenaline but also constriction to a Ca^{2+} channel opener suggest that fluoxetine interferes with the Ca^{2+} signaling mechanisms in the vascular smooth muscle of small cerebral arteries. The dilation of cerebral vessels to fluoxetine (Prozac) can result in an increase in cerebral blood flow in vivo, which may have clinical importance, because considerable data support the existence of impaired regional cerebral blood flow in major depression.36,37 Thus, the augmentation of the cerebral blood flow could contribute to the therapeutic action of fluoxetine.

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References

Fluoxetine (Prozac) is widely used as an antidepressant in treating patients. The action of fluoxetine is presumably linked to its inhibition of central nervous system neuronal uptake of serotonin, thus making serotonin more available in the brain. Although serotonin is a chemical that affects mood, it is also a potent vasoconstrictor in the cerebral circulation. Despite extensive studies of the clinical pharmacological effects of the drug, the effect of fluoxetine on cerebral circulation remains essentially unexplored. In the accompanying article, Ungvari and coworkers demonstrated that fluoxetine causes dose-dependent vasodilation in the isolated branch of anterior cerebral arteries of rats. They provided the first evidence that fluoxetine diminishes the vasoconstrictor action of serotonin, norepinephrine, and Ca²⁺ channel openers. They concluded that fluoxetine interferes with the Ca²⁺ signaling mechanisms in the vascular smooth muscle. Findings from this in vitro study represent an important initial step in understanding the role of fluoxetine in the cerebral circulation.

Obviously, the vascular effect of fluoxetine needs to be further assessed in in vivo animal models. The anterior cerebral artery in rat is a conduit vessel, and its responses may not reflect those of the smaller downstream arterioles. Therefore, it is not certain that the dilator effect of fluoxetine on large vessels necessarily translates into improved blood flow in the brain in vivo. Another issue is that clinically, a low dose (20 mg/d) of fluoxetine is generally recommended in treating depressed patients. Whether or not this therapeutic dosage could consistently raise the plasma concentration of fluoxetine in patients to micromolar levels remains to be tested. On the basis of the findings of this study, below this level, fluoxetine seems to exert little or no cerebrovascular effect. In humans, it was reported that peak plasma concentrations of fluoxetine can reach \( \approx 1 \) \( \mu \)mol/L after a single oral 40-mg dose. Thus, it is not clear that at the usual therapeutic doses, fluoxetine would have significant cerebrovascular effects.

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