Inhibitory Effect of Lomerizine, a Prophylactic Drug for Migraines, on Serotonin-Induced Contraction of the Basilar Artery

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Abstract. We examined the effects of lomerizine on serotonin (5-hydroxytryptamine, 5-HT)–induced contraction of the basilar artery and compared them with those of nifedipine. Although both lomerizine and nifedipine completely blocked K+-induced vasoconstriction, 5-HT–induced vasoconstriction was more strongly inhibited by lomerizine than nifedipine. A 5-HT2A antagonist inhibited the 5-HT–induced vasoconstriction, but a 5-HT1B antagonist did not. Lomerizine, but not nifedipine, suppressed 5-HT–induced Ca2+ release in 5-HT2A–expressing HEK293 cells. Moreover, neither antagonist affected ATP-induced Ca2+ release. These results suggest that lomerizine may inhibit not only voltage-dependent Ca2+ channels but also 5-HT2A receptors and so inhibit 5-HT–induced contraction in the basilar artery.

Keywords: lomerizine, serotonin (5-hydroxytryptamine), basilar artery

Migraines are improved by the use of triptan, a selective serotonin (5-hydroxytryptamine, 5-HT)–receptor agonist (5-HT1B/1D). However, the use of triptan in basilar-type migraines (BM) is restricted since it is possible that the neurologic symptoms of BM are associated with vasoconstriction and so triptan could increase the risk of brain infarction. Therefore, prophylactic agents, including lomerizine and verapamil, are very important for the treatment of BM.

Lomerizine, a voltage-dependent Ca2+-channel blocker, is used as the first-line prophylactic drug for migraines in Japan (1). Verapamil, another type of voltage-dependent Ca2+-channel blocker, is also used as a prophylactic drug for migraines (2, 3). On the other hand, nifedipine, a dihydropyridine Ca2+-channel blocker, did not show a clinical response as a prophylactic drug for migraines (4, 5). It is widely accepted that the first stage of migraine with aura, including BM, is related to the reduction of cerebral blood flow in response to 5-HT (6). The voltage-dependent L-type Ca2+ channel is known to mediate 5-HT–induced contraction of the basilar artery (7). However, we could not find any report showing the effects of lomerizine on 5-HT–induced contraction of the basilar artery.

The present study was undertaken to characterize the effects of lomerizine on 5-HT–induced contraction of the basilar artery and to compare the effects of verapamil and nifedipine.

All animal experiments were approved by the Experimental Animal Committee of Showa University. Male Wistar rats (250 – 300 g; Saitama Experimental Animal Supply, Inc., Saitama) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). The basilar artery was carefully isolated from the brain with the aid of a dissecting microscope, and cleaned of fat and adhering connective tissues. In protocols examining responsiveness in the absence of the vascular endothelium, the endothelial cells were destroyed by rubbing the intimal surface gently with a stainless steel wire (40 μm in diameter) inserted through the lumen. Blood clots in the lumen were flushed out with ice-cold physiological saline solution of the following composition: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM NaHCO3, 10 mM HEPES, and 11.1 mM HCl.
glucose.

Total RNA was extracted from whole rat brains and the basilar artery by a modified guanidinium isothiocyanate method using TRIzol Reagent (Invitrogen Co., Ltd., Carlsbad, CA, USA). Reverse transcription and PCR amplification from 0.1 μg of total RNA were performed using *Tth* DNA polymerase (RT-PCR Quick Master Mix; Toyobo Co., Osaka). The primer pair used...
for the amplification of 5-HT<sub>2A</sub> (NM_017254) was 5'-TGCCCTGTCCATGTTAACA-3' and 5'-AAAGACGACATCCAGTTAATCCA-3', and that for 5-HT<sub>1B</sub> (NM_022225) was and 5'-TCCGGGTCTCCTGTA CGT-3' and 5'-GGCGTCTGAGACTCGCACTT-3'. The thermocycler was programmed to perform an initial cycle consisting of denaturation at 90°C for 30 s, reverse transcription at 60°C for 30 min, and denaturation at 94°C for 1 min, followed by 35 – 40 cycles of denaturation at 94°C for 30 s and annealing/extension at 60°C for 30 s and 72°C for 1 min. To control for the amounts of total RNA, parallel RT-PCR of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was performed as a reference, using the following primer pair: 5'-TCCACCACCTGTTGCTGTA-3' and 5'-ACCACGTCCATGCCATCAC-3'. PCR products were electrophoresed on a 3% Agarose S (Wako Pure Chemicals, Osaka) gel containing ethidium bromide and visualized by UV-induced fluorescence.

HEK293 cells (RIKEN Cell Bank, Tsukuba) were transfected with the recombinant plasmid pTriEx4neo-5HT<sub>2A</sub>-mEYFP as described previously (8). Transfection was carried out using the SuperFect Transfection Reagent (QIAGEN, Hilden, Germany). After transfection, the cells were incubated in culture medium for 3 days. The culture medium was replaced with fresh culture medium containing 500 μg/ml G418, and thereafter, the medium was changed every 3 days for 10 days. The surviving cells were cloned and cultured.

The intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]) was measured using fura-2 as a Ca<sup>2+</sup> indicator, as previously described (9). Cells on coverslips were loaded with fura-2 by incubation in culture medium containing 5 μM fura-2/AM (Dojindo Laboratories, Kumamoto) and 10% FBS at 37°C for 40 min. Then, the fura-2-loaded cells were washed with HEPES-buffered saline (HBS) containing 107 mM NaCl, 6 mM KCl, 1.2 mM MgSO<sub>4</sub>, 2 mM CaCl<sub>2</sub>, 11.5 mM glucose, and 20 mM HEPES, adjusted to pH 7.4 with NaOH. The Ca<sup>2+</sup>-free solution contained 0.5 mM EGTA but no added CaCl<sub>2</sub>. Fluorescence images of the cells were recorded and analyzed with a video analysis system (Meta Fluora; Nippon Ropper, Tokyo). The fura-2 fluorescence at an emission wavelength of 510 nm (bandwidth: 50 nm) was observed at room temperature by exciting fura-2 alternately at 340 (bandwidth: 10 nm) and 380 nm (bandwidth: 13 nm). Pairs of 340 and 380 nm fluorescence images were captured every 10 s, and the 340/380 nm ratio was calculated after subtracting the background fluorescence. Data from all measurements are expressed as a 340/380 nm fluorescence ratio. All reagents were dissolved in distilled water or dimethyl sulfoxide to their final concentrations in HBS or Ca<sup>2+</sup>-free HBS and applied to the
cells by perfusion.

Data are presented as means ± S.E.M. of n observations. The statistical significance of observed differences was determined by analysis of variance followed by Bonferroni’s method. Differences between means were considered significant when \( P < 0.05 \).

High K\(^+\) (Fig. 1A), 5-HT (Fig. 1C), and vasopressin (Fig. 1E) induced monophasic contraction in the rat basilar artery in a concentration-dependent manner (Fig. 1). K\(^+\)-induced vasoconstriction was completely inhibited by lomerizine, verapamil, and nifedipine at 1 \( \mu \)M (Fig. 1: A and B). Although the vasoconstriction caused in response to 5-HT was reduced by nifedipine to 52% of the control value, lomerizine and verapamil attenuated it more strongly (Fig. 1: C and D). Moreover, with regard to the vasoconstriction that occurred in response to vasopressin, nifedipine had weaker potency than lomerizine and verapamil (Fig. 1: E and F). Flunarizine, which has a similar structure to lomerizine, is known to reduce the number of receptor-operated Ca\(^{2+}\) channels (10). Verapamil also inhibits receptor-operated Ca\(^{2+}\) channels such as the canonical transient receptor potential sub-type 3 (TRPC3) (11). The basilar artery expressed TRPC3, TRPC4, TRPC5, and TRPC6 mRNA, but not TRPC1 or TRPC7 mRNA (data not shown).

Fig. 3. Effects of the IP\(_3\)-receptor antagonist, phospholipase C inhibitor, and voltage-dependent Ca\(^{2+}\)-channel blockers on Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores. Cytosolic Ca\(^{2+}\) was measured using fura-2 as a Ca\(^{2+}\) indicator. In A, 5-HT (10 nM) was added to 5-HT\(_{2A}\)-expressing HEK293 cells after pretreatment with lomerizine (1 and 10 \( \mu \)M) for 60 min. In B, ATP (100 \( \mu \)M) was added to wild-type HEK293 cells after pretreatment with lomerizine (10 \( \mu \)M) for 60 min. In C and D, the effect of APB (50 \( \mu \)M, pretreatment for 30 min), U73122 (10 \( \mu \)M, pretreatment for 10 min), ketanserin (100 nM, pretreatment for 10 min), and voltage-dependent Ca\(^{2+}\)-channel blockers (lomerizine, 0.1 – 10 \( \mu \)M, pretreatment for 60 min; verapamil, 0.1 – 10 \( \mu \)M, pretreatment for 10 min; nifedipine, 10 \( \mu \)M, pretreatment for 10 min) on the peak levels of 5-HT (10 nM) or ATP (100 \( \mu \)M)-induced Ca\(^{2+}\) release were measured in individual cells. The ratios shown in panels C and D indicate the absolute changes of ratio intensity (\( \Delta \) ratio (340/380 nm) = peak ratio − resting ratio). The results are shown as the means ± S.E.M. of 32 – 87 cells. *Significant changes in comparison to the control group (\( P < 0.05 \)).
Fujishima et al. (14) reported that lomerizine inhibited specific \[^{3}H\]spiperone binding to 5-HT\(_1\) receptors in a competitive manner, but exhibited negligible affinity for radioligand binding to other 5-HT receptor subtypes such as 5-HT\(_1A\), 5-HT\(_1B\), 5-HT\(_1C\), and 5-HT\(_3\) in the rat cortical membrane. Verapamil also has antagonistic effects on the 5-HT\(_1A\) receptor (14, 15), but nifedipine does not have these effects (15). To investigate the inhibitory effects of lomerizine on Ca\(^{2+}\) release from Ca\(^{2+}\) stores, we employed a pharmacological approach. In wild type HEK293 cells, we did not observe any 5-HT–induced Ca\(^{2+}\) release in the absence of extracellular Ca\(^{2+}\). Treatment of wild type HEK293 cells with ATP also induced Ca\(^{2+}\) release from their intracellular Ca\(^{2+}\) stores. As shown in Fig. 3, 2-aminoethoxydiphenyl borate (APB, 100 \(\mu\)M), an antagonist of the IP\(_3\) receptor, and U73122 (1 \(\mu\)M), an inhibitor of phospholipase C (PLC), blocked the Ca\(^{2+}\) release induced by 5-HT (Fig. 3C) or ATP (Fig. 3D). Thus, Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores in response to 5-HT or ATP is mediated by the PLC-IP\(_3\) pathway. Treatment with lomerizine or verapamil, but not nifedipine, reduced 5-HT–induced Ca\(^{2+}\) release in 5-HT\(_{2A}\)–expressing HEK293 cells transiently increased the [Ca\(^{2+}\)]\(_i\) in the absence of extracellular Ca\(^{2+}\). On the other hand, these Ca\(^{2+}\) channel blockers did not have an inhibitory effect on ATP-induced Ca\(^{2+}\) release (Fig. 3: B and D). Therefore, lomerizine and verapamil seem to reduce 5-HT–induced Ca\(^{2+}\) release by blocking the 5-HT\(_{2A}\) receptor. Fujishima et al. (14) reported that lomerizine and verapamil reduce the 5-HT–induced increase in [Ca\(^{2+}\)]\(_i\) in platelets, which have 5-HT\(_{2A}\) receptors. Since platelets are known to lack voltage-dependent Ca\(^{2+}\) channels, lomerizine may inhibit the 5-HT\(_{2A}\) receptor. Therefore, the prophylactic effects of lomerizine and verapamil for migraines might be related to their antagonistic effects not only as a voltage-dependent Ca\(^{2+}\)–channel blockers but also as 5-HT\(_{2A}\)–receptor antagonists.

Our findings indicated that lomerizine and verapamil might ameliorate the basilar-type of migraine by preventing 5-HT–induced contraction in the basilar artery via the antagonistic effects against the voltage-dependent Ca\(^{2+}\) channels and the 5-HT\(_{2A}\) receptor.

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References