Inhibitory Effect of Lomerizine, a Diphenylpiperazine Ca\(^{2+}\)-Channel Blocker, on Ba\(^{2+}\) Current through Voltage-Gated Ca\(^{2+}\) Channels in PC12 Cells

Tomokazu Watanol, Hideaki Hara\(^2,\)* and Takayuki Sukamoto\(^2\)

\(^1\) Department of Pharmacology, New Drug Discovery Research Laboratory and \(^2\) Department of Pharmacology, New Drug R & D Laboratory, Kanebo Ltd., 1-5-90 Tomobuchi-cho, Miyakojima-ku, Osaka 534, Japan

Received July 8, 1997 Accepted August 22, 1997

ABSTRACT—We investigated the effect of lomerizine, an anti-migraine drug, on the Ba\(^{2+}\) current through voltage-gated Ca\(^{2+}\) channels in rat pheochromocytoma (PC12) cells using a whole-cell voltage-clamp technique. Lomerizine inhibited the Ba\(^{2+}\) current with an IC\(_{50}\) value of 1.9 \(\mu\)M. Lomerizine and nicardipine were \(>4\) times more potent than flunarizine, diltiazem, verapamil and dimetotiazine. The time course of inactivation induced by lomerizine was similar to that induced by nicardipine and flunarizine. These data indicate that lomerizine may inhibit the Ca\(^{2+}\) channel in a similar manner to nicardipine and flunarizine, and its potency is almost equal to that of nicardipine.

Keywords: Lomerizine, Voltage-gated Ca\(^{2+}\) channel, PC12 cell
CaCl$_2$ was replaced with BaCl$_2$ (10.8 mM) to obtain a large inward Ba$^{2+}$ current permeating through voltage-gated Ca$^{2+}$ channels. Compensation was made for series resistance, and electrical signals were filtered at 5 kHz. The inward current was activated by a depolarizing step to +10 mV from a holding potential of −60 mV. The experiments were performed at approximately 36°C using a TC$^{2}$ bipolar temperature controller (Biotechnics, Inc., Butler, PA, USA). The IC$_{50}$ value for each drug in inhibiting Ba$^{2+}$ current was calculated by Probit analysis.

Lomerizine 2HCl (Kanebo, Osaka); nicardipine HCl, flunarizine 2HCl, (±)-verapamil, diltiazem HCl (Sigma, St. Louis, MO, USA); and dimetotiazine mesylate (Shionogi, Osaka) were first dissolved in dimethyl sulfoxide (DMSO, Wako) and added to extracellular solutions so that the final concentration of DMSO was ≤0.1%.

Figure 1 shows typical records of the blocking effect of lomerizine and diltiazem on the Ba$^{2+}$ current through voltage-gated Ca$^{2+}$ channels in PC12 cells. The inhibition produced by lomerizine was time-dependent: it was first observed at 20 sec after the start of the superfusion, and a steady-state blockade was obtained by 60 sec after the start of the superfusion (Fig. 1A) at concentrations of 0.1−10 μM. This effect was similar to those produced by nicardipine, flunarizine and dimetotiazine (data not shown). On the other hand, the steady-state blocking effect of diltiazem reached a steady state level at 120 sec after the start of the superfusion at concentrations of 1−100 μM in most of the cells examined (Fig. 1B). The profile of the inhibition produced by verapamil was similar to that seen with diltiazem (data not shown). Therefore, for routine assessment of the typical inhibition produced by each drug and to derive the concentration-inhibition curves, the effects on the Ba$^{2+}$ current of lomerizine, nicardipine, flunarizine and dimetotiazine in concentrations of 0.1−10 μM were tested at 60 sec after the beginning of their superfusion, whereas the effects of diltiazem and verapamil in concentrations of 1−100 μM were tested at 120 sec.

Figure 2 shows typical examples of the inhibitory effects of lomerizine, nicardipine, flunarizine, diltiazem, verapamil and dimetotiazine on the Ba$^{2+}$ current. Current traces obtained just before (Control) and after the
Fig. 2. Effects of lomerizine, nicardipine, flunarizine, diltiazem, verapamil and dimetotiazine on Ba$^{2+}$ current in PC12 cells. The Ba$^{2+}$ current was activated by a 200-msec depolarizing step pulse from -60 mV to +10 mV every 10 sec. Panels for lomerizine (A), nicardipine (B), flunarizine (C) and dimetotiazine (F) show superimposed the current traces from just before (control) and 60 sec after the application of the doses shown. Those for diltiazem (D) and verapamil (E) show superimposed current traces from just before (control) and 120 sec after the application of the doses shown.

Superfusion of a given drug at various concentrations (between 0.1 and 100 μM) are superimposed. Each of the drugs concentration-dependently inhibited the Ba$^{2+}$ current. In a preliminary experiment, the current-voltage (I-V) relationship exhibited a bell-shape, peaking at +10 mV, as has been previously reported (9, 10); and lomeri-
zine did not affect the I-V relationship. Therefore, we investigated the time-dependency and concentration-response relationship with a depolarizing step to +10 mV.

Figure 3 shows the concentration-response relationship for these drugs. The IC<sub>50</sub> values (with 95% confidence limits) for lomerizine, nicardipine, flunarizine, diltiazem and verapamil were 1.9 (1.1-3.9), 2.0 (0.86-6.2), 7.9 (3.1-52), 13.0 (6.7-27) and 10.4 (5.1-21) μM, respectively. Dimetotiazine produced only an approximately 40% inhibition at a concentration of 10 μM (the highest concentration used). Lomerizine and nicardipine were both >4 times more potent than the other three calcium channel blockers and dimetotiazine.

We examined the effect of lomerizine on Ca<sup>2+</sup> channels in PC12 cells and compared the effect with those of four other calcium channel blockers and with that of dimetotiazine. PC12 cells have been used widely in studies requiring a neuron-like clone cell-line. The Ba<sup>2+</sup> current in PC12 cells was classified as an example of L-type Ca<sub>2+</sub> channel activity by Tsien et al. (11). Although undifferentiated PC12 cells express predominantly L-type channels, recently they have been reported to possess an N-type and ω-agatoxin-IVA-sensitive (P/Q-type) component, but not the T-type (12, 13). However, the experimental condition (−60 mV holding potential) in the present study is suitable for detecting L-type, but not N- or T-type (11), Ca<sub>2+</sub> channel activity. Lomerizine inhibits both L-type and T-type Ca<sup>2+</sup> currents in a hippocampal CA1 pyramidal single neuron (4) and does not have any affinity for N-type Ca<sup>2+</sup> channels in a [125I]ω-conotoxin binding study using rat cortical membranes (unpublished observation, H. Hara et al.).

In this experiment, lomerizine concentration-dependently inhibited high-voltage activated inward Ba<sup>2+</sup> currents. The time course (inhibition pattern) of the effect of lomerizine on Ba<sup>2+</sup> current was similar to those of nicardipine and flunarizine, but different from those of diltiazem and verapamil. Maximal steady state inhibition induced by lomerizine, nicardipine or flunarizine was observed faster than that induced by diltiazem or verapamil. These data indicate that the mode of action of lomerizine in inhibiting L-type Ca<sup>2+</sup> channels may be similar to that of nicardipine and flunarizine and different from that of diltiazem and verapamil.

Each of the 1,4-dihydropyridines studied here, diltiazem and verapamil, interacts with a specific receptor domain found on a large (about 165 kD) membrane-spanning protein that constitutes a substantial portion of the L-type, voltage-gated Ca<sup>2+</sup> channel (14). The 1,4-dihydropyridine receptor is located on the surface of the channel; on the other hand, the diltiazem and verapamil binding sites are located internally, deep within the channel. Lomerizine has been supposed to bind to the 1,4-dihydropyridine binding site from the results obtained by a [3H]nitrendipine binding study using synaptosome membranes in guinea pig cortex (2). These different locations of the binding sites of the drugs may explain the different time course of the inhibition of Ba<sup>2+</sup> current among the drugs tested in the present experiment.

The potencies of lomerizine and other calcium channel blockers tested in inhibiting the Ba<sup>2+</sup> currents through the high voltage-activated Ca<sub>2+</sub> channels were in the following order: lomerizine = nicardipine > flunarizine > verapamil > diltiazem > dimetotiazine. Although the order obtained with lomerizine and flunarizine are well-consistent with that obtained by a [3H]nitrendipine binding study (2), the inhibitory effect of nicardipine in this experiment was somewhat weaker than that expected from the binding study. Nicardipine was about 30 times more potent than lomerizine in inhibiting [3H]nitrendipine binding to synaptosome membranes in guinea pig cortex. Nicardipine has been reported to suppress the L-type Ca<sup>2+</sup> channel current found in PC12 cells with an IC<sub>50</sub> value of 2.7 μM (12). The IC<sub>50</sub> value for nicardipine in PC12 cells is consistent with that reported here (2 μM). The inhibitory effect of 1,4-dihydropyridine derivatives such as nicardipine on the Ba<sup>2+</sup> currents through the high-voltage activated Ca<sup>2+</sup> channels has been reported to be weaker in neuronal cells than in other cells such as cardiac cells and smooth muscle cells (15, 16). The effect
of lomerizine on the high-voltage activated Ca\(^{2+}\) channels has not yet been determined in cells other than neuronal cells. Therefore, further studies are needed to address whether the inhibitory effect of lomerizine on the Ca\(^{2+}\) channels found in neuronal cells is different from those found in peripheral cells.

Lomerizine has been reported to preferentially inhibit T-type Ca\(^{2+}\) channels rather than L-type Ca\(^{2+}\) channels in rat pyramidal cells (4). The profile of lomerizine resembled that of flunarizine (4). Furthermore, the effect of lomerizine on the Ba\(^{2+}\) currents in the present study was also similar to that of flunarizine. The similarity between lomerizine and flunarizine may be due to the common diphenylpiperazine structure.

Lomerizine has shown to reduce the number of migraine attacks in patients with migraine (5). Flunarizine and nicardipine have been reported to show better therapeutic effects than the placebo in double-blind and placebo-controlled clinical trials in migraine patients (17, 18). The beneficial effects of lomerizine in migraine patients are supposed to mainly result from the anti-vasoconstrictor effect and inhibitory effect on spreading depression by suppression of L-type Ca\(^{2+}\) channels in smooth muscle and neuronal cells, respectively (6). Taken together, the data in the present study and the above findings indicate that the mechanism of the anti-migraine effect of lomerizine may be similar to those of flunarizine and nicardipine from the viewpoint of the inhibitory effects on L-type Ca\(^{2+}\) channels.

Dimetotiazine, a classical anti-migraine drug with histamine H\(_1\) and serotonin 5-HT\(_2\)-receptor blocking effects, showed a weak inhibitory effect on the Ba\(^{2+}\) currents, like diltiazem and verapamil did, although the potency of dimetotiazine was approximately 10 times less than that of lomerizine. Therefore, we can not rule out the possibility that the mechanism of action of dimetotiazine on migraine is partly due to the suppression of L-type Ca\(^{2+}\) channels in smooth muscle and/or neuronal cell. Further studies are needed to clarify the mechanism.

Acknowledgments
The authors would like to thank Dr. Ken-ichi Nakazawa, Division of Pharmacology, National Institute of Health Sciences, for helpful discussions and Dr. Tomoyuki Ono, Department of Pharmacology, Fukushima Medical College, for helpful advice on the culture and serum. Furthermore, we are grateful to Dr. Koji Yamamoto, New Drug Discovery Research Lab., Kanbo Ltd., for his advice on the culture method.

REFERENCES