

Na⁺-permeable channels induced by maitotoxin in guinea-pig single ventricular cells

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Abstract

The characteristics of maitotoxin-induced single channel currents were studied in guinea-pig single ventricular cells using the cell-attached or inside-out configuration of the patch clamp. When the patch electrode was filled with normal Tyrode solution containing 10 nM maitotoxin, elementary currents flowing through the single channel were observed in the cell-attached patch. The amplitude of the single channel current at the resting potential was 1.6 ± 0.1 pA. The current-voltage relation of the current was linear and the single channel conductance was 16.0 ± 0.9 pS. The distribution of open times was fitted by a single exponential function (decay time constant: 27 ms), while that of closed times was fitted by the sum of two exponential functions (decay time constants: 1.6 and 34 ms). When the electrode solution was filled with the Ca²⁺-free Tyrode solution, maitotoxin also induced single channel currents with parameters similar to those in the normal Tyrode solution. Under inside-out patch clamp conditions and in 150 mM Na⁺ solution on both sides of the patch membrane, maitotoxin also induced single channel currents. Choline⁺ could not substitute for Na⁺. These results indicate that maitotoxin induces single ionic channels irrespective of the presence or absence of Ca²⁺ and that the charge carrier of the single channel current is Na⁺ rather than Ca²⁺. The increase in Na⁺ permeability through maitotoxin-induced channels may be possibly responsible for its biological actions.

Keywords: Maitotoxin; Na⁺-permeable channel; Patch clamp; Ventricular cell, guinea-pig

1. Introduction

Maitotoxin isolated from a toxic marine dinoflagellate, *Gambierdiscus toxicus*, is one of the most potent marine toxins (Yokoyama et al., 1988). This toxin has diverse biological actions in a variety of preparations such as contraction of smooth muscle, skeletal muscle and cardiac muscle preparations, increase in neurotransmitter/hormone release and stimulation of phosphoinositide breakdown (Gusovsky and Daly, 1990, for review). Maitotoxin has been recognized to be a specific activator of Ca²⁺ channels because: (1) maitotoxin-induced contraction of muscle preparations is Ca²⁺-dependent, (2) maitotoxin causes a tetrodotoxin-insensitive increase in Ca²⁺ influx, (3) maitotoxin-induced stimulation of transmitter release is depen-

dent on Ca²⁺, and (4) most of the maitotoxin-induced actions are inhibited by inorganic and organic blockers of Ca²⁺ channels.

However, some maitotoxin-induced actions such as stimulation of phosphoinositide breakdown have been reported not to be mediated by Ca²⁺ channel activation. Several groups have reported that the ionic channels responsible for the actions of maitotoxin may be different from voltage-dependent Ca²⁺ channels (Kobayashi et al., 1987; Yoshii et al., 1987). Extracellular Na⁺-dependent neurotransmitter release and increase in Na⁺ uptake have also been reported (Pin et al., 1988; Sladeczek et al., 1988; Takahashi et al., 1982). Therefore, ionic mechanisms responsible for maitotoxin-induced events still remain controversial.

In the present study we examined mechanisms of maitotoxin-induced ion permeability using cell-attached and inside-out configuration of patch clamp and found that

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maitotoxin induces an ion channel which conducts the Na^+ as charge carrier.

2. Materials and methods

2.1. Preparation of guinea-pig single ventricular cells

Male guinea-pigs weighing 250–350 g were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and exsanguinated from the carotid arteries. The hearts were quickly dissected and mounted on the Langendorf apparatus to perfuse the coronary artery with oxygenated and pre-warmed (37°C) Tyrode solution of the following composition (mM): NaCl 136.9, KCl 5.4, CaCl_2 1.8, MgCl_2 0.5, glucose 10 and Hepes 5. The pH of the Tyrode solution was adjusted to 7.3 with NaOH. After the heart had been cleared of blood, the perfusate was switched to Ca^{2+} -free Tyrode solution containing 0.4 mg/ml collagenase (Type 1, Sigma, St. Louis, USA) and the solution was recirculated using a peristaltic pump for 25 min. Thereafter, the collagenase was washed with 100 ml of high K^+ -low Cl^- solution (storage solution). The composition of the storage solution was (mM): taurine, 10; oxalic acid, 14; glutamic acid, 80; KCl, 25; KH_2PO_4 , 10; *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes), 10; glucose, 11; ethyleneglycol-bis(β -aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), 0.5. The pH was adjusted to 7.2 by adding KOH (Isenberg and Klöckner,

1982). The preparation was stored in this solution at 4°C for the experiments.

2.2. Single channel recordings

Single channel recordings were made at room temperature (21–23°C) in the cell-attached or inside-out configuration of the patch clamp (Hamill et al., 1981) with a patch clamp amplifier (CEZ-2100, Nihon Kohden, Tokyo, Japan). Patch electrodes were fabricated from glass capillaries of 1.5 mm in outer diameter, using a double step microelectrode puller (PP-83, Narishige, Tokyo, Japan). To reduce the capacity transient and noise, the electrode tip was coated with Sylgard (Dow Corning, Midland, USA) as close to the tip as possible. After the tip was polished by heat, the electrode was filled with the solution containing 10 nM maitotoxin. The outer diameter of the electrode tip was about 1 μm and the electrode resistance ranged between 8 and 10 M Ω . After the electrode tip was applied to the cell surface, a negative pressure of 20–40 cm H_2O was applied to the interior of the electrode. Within a few seconds, a giga-ohm seal was established between the electrode tip and the cell surface. The patch membrane was excised from the cell to obtain the inside-out configuration of the patch clamp. The current signals were filtered at 5 kHz, digitized using a PCM recording system (RP-880, NF Circuit Design Block, Nagoya, Japan) at a sampling frequency of 57 kHz and stored on video cassette (NV 730, National, Japan) for later analysis.

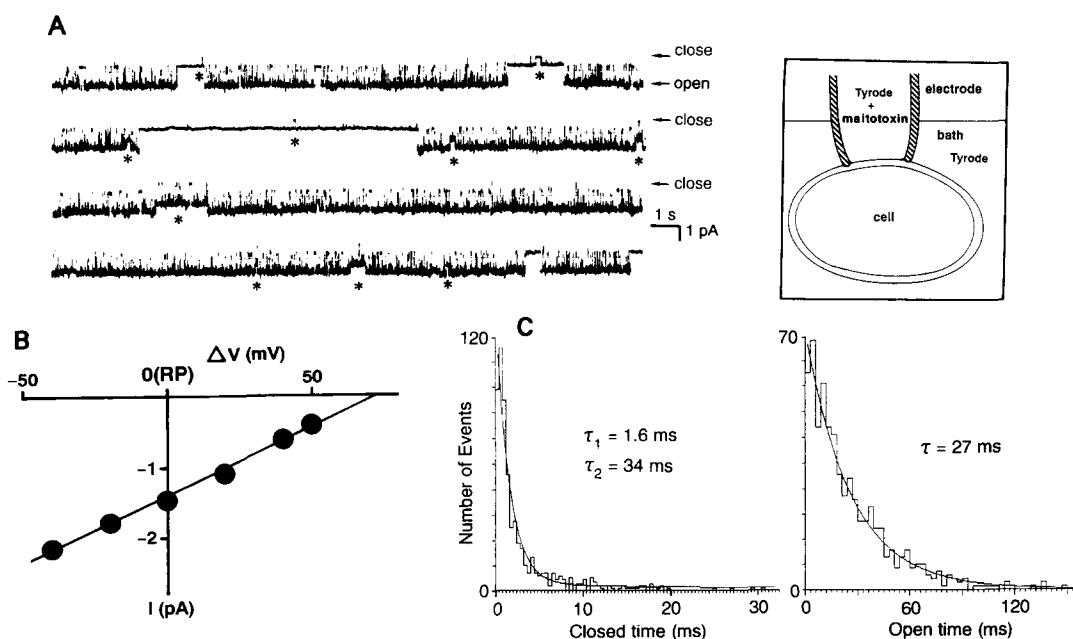


Fig. 1. Maitotoxin-induced single channel currents recorded from a single ventricular myocyte of guinea-pig using a patch electrode filled with normal Tyrode solution containing 10 nM maitotoxin. Currents were recorded in the cell-attached configuration at the resting potential. Inset: milieu inside and outside the patch electrode. (A) Continuous current recording from 2 min after the formation of a gigaohm seal (from top left to bottom right). *: small amplitude of current fluctuation was overlapped (see text for further explanation). (B) Current-voltage relationship. ΔV : deviation of the membrane potential from the resting potential (RP). (C) Distribution of closed and open times. Closed periods longer than 30 ms were not analyzed.

2.3. Single channel data analysis

The recorded signals were reproduced on a strip chart recorder (RJG-4128, Nihon Kohden) or digitized on the disc of a computer (IBM PS/2) using an analog-to-digital converter (TL-1 DMA interface, Axon Instruments, Foster City, USA) and analyzed by the program, pCLAMP 5.5 (Axon Instruments) on the computer. Experimental values are given as means \pm S.E.M.

2.4. Toxin

Maitotoxin was prepared by T. Yasumoto by the method described previously (Takahashi et al., 1982). Maitotoxin was dissolved as 1 μ M stock solution in distilled water and frozen until use.

3. Results

When the cell-attached configuration of the patch clamp was applied to a guinea-pig single ventricular myocyte with a patch electrode filled with normal Tyrode solution containing 10 nM maitotoxin, jumps of the membrane current were observed within 1–3 min after the formation of a gigaseal patch (Fig. 1). Fig. 1A shows a continuous recording of the membrane current. The current flowed inwardly when the patch membrane was held at the resting membrane potential (-83 ± 1 mV, $n = 6$) and fluctuated between the two levels. On the other hand, no current

jump was seen in maitotoxin-free patch membranes. These results suggest that the observed currents may flow through the ionic channels formed or induced by maitotoxin and that the current fluctuation may reflect the gating behavior of the channels.

Maitotoxin induced only one or two channels simultaneously in each patch membrane over a wide range of maitotoxin concentrations (3–100 nM) or electrode resistances (6–12 M Ω). The channel mainly stayed in its open state during which closing occurred in bursts. The open states were occasionally separated by a longer closed period of more than 100 ms.

The amplitude of the inward current was 1.6 ± 0.1 pA ($n = 5$) at the resting membrane potential. The current-voltage relationship of the single channel current is shown in Fig. 1B, where the amplitude of the single channel current was plotted against the membrane potential at which the current was recorded. The abscissa indicates the deviation (ΔV) from the resting membrane potential. The amplitude changed linearly in the deviation range from -40 to 50 mV. The single channel conductance was 16.0 ± 0.9 pS ($n = 5$). Fig. 1C shows closed and open time histograms during the bursts at the resting potential, in which closed periods longer than 30 ms were not analyzed. The distribution of the closed times was best fitted by the sum of two exponential functions (decay time constants: 1.6 and 34 ms), while that of open times was fitted by a single exponential function (decay time constant: 27 ms).

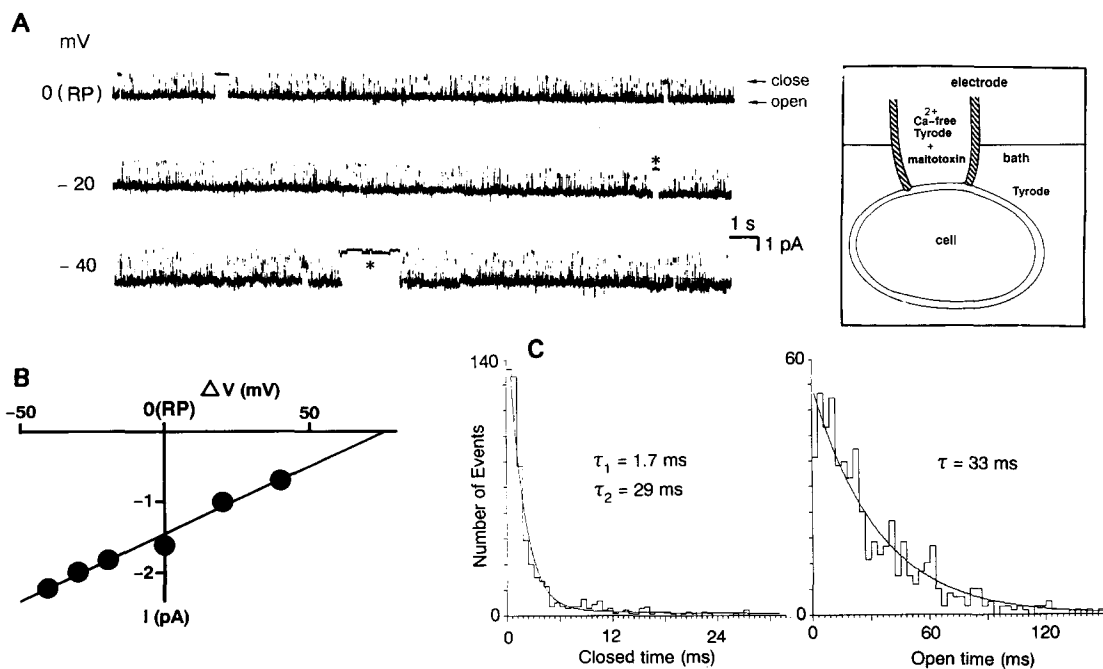


Fig. 2. Maitotoxin-induced single channel currents recorded in the cell-attached configuration. A patch electrode was filled with Ca^{2+} -free Tyrode solution containing 10 nM maitotoxin. Inset: milieu inside and outside the patch electrode. (A) Current recordings at various membrane potentials. The numbers on the left of each current trace indicate the deviation of the membrane potential from the resting potential. RP: resting membrane potential. *: small amplitude of current fluctuation. (B) Current-voltage relationship. (C) Distribution of closed and open times. Closed periods longer than 30 ms were not analyzed.

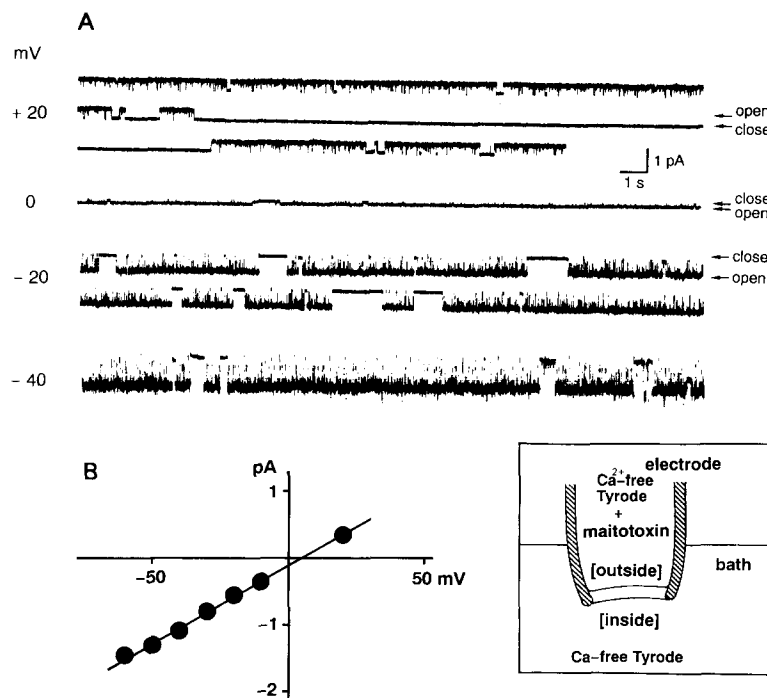


Fig. 3. Maitotoxin-induced single channel currents recorded in the inside-out configuration. A patch electrode was filled with Ca^{2+} -free Tyrode solution and 10 nM maitotoxin. The bath was perfused with Ca^{2+} -free Tyrode solution. (A) Current recordings at various membrane potentials. The numbers on the left of each current trace indicate the holding membrane potential. (B) Current-voltage relationship. Inset shows the milieu inside and outside the patch membrane.

Close inspection further revealed the occurrence of overlapping inward current with a smaller amplitude (0.7 ± 0.1 pA at the resting potential) which basically continued to flow during the recording period and occasionally stopped (asterisks in Fig. 1A). These small currents were not analyzed because of much less frequency of channel closing.

When the patch membrane was formed with the electrode filled with Ca^{2+} -free Tyrode solution containing 10 nM maitotoxin, single channel currents were also observed (Fig. 2). Fig. 2A shows representative current recordings at various membrane potentials. The parameters of the single channel current were not significantly different from those obtained in normal Tyrode solution: the amplitude of the inward current at the resting membrane potential was 1.6 ± 0.2 pA ($n = 4$); the slope of conductance was 19 ± 0.2 pS ($n = 4$); the time constants of the closed time were 1.7 ms and 29 ms; the time constant of the open time was 33 ms. Another type of current with a smaller amplitude was also seen (asterisks in Fig. 2A). Maitotoxin-induced single channel currents were observed in the inside-out configuration of the patch clamp (Fig. 3). In this case, both sides of the patch membrane were exposed to Ca^{2+} -free Tyrode solution (Fig. 3, inset). The direction of single channel currents flowing through the inside-out patch membrane was inward at a negative patch membrane potential and was outward at a positive membrane potential. The reversal potential was close to 0 mV and no rectification was seen.

To clarify which ion is the major charge carrier, the solutions inside and outside the membrane were first replaced with 150 mM NaCl solution (Fig. 4, inset). The

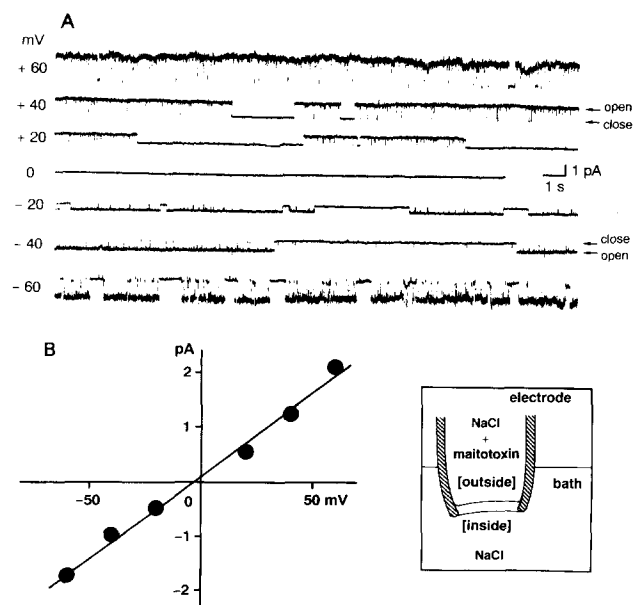


Fig. 4. Maitotoxin-induced single channel currents recorded using a patch electrode filled with 150 mM NaCl solution instead of Tyrode solution: inside-out configuration. The bath was perfused with 150 mM NaCl solution. (A) Current recordings at various membrane potentials. The numbers on the left of each current trace indicate the holding membrane potential. (B) Current-voltage relationship. Inset shows the milieu inside and outside the patch membrane.

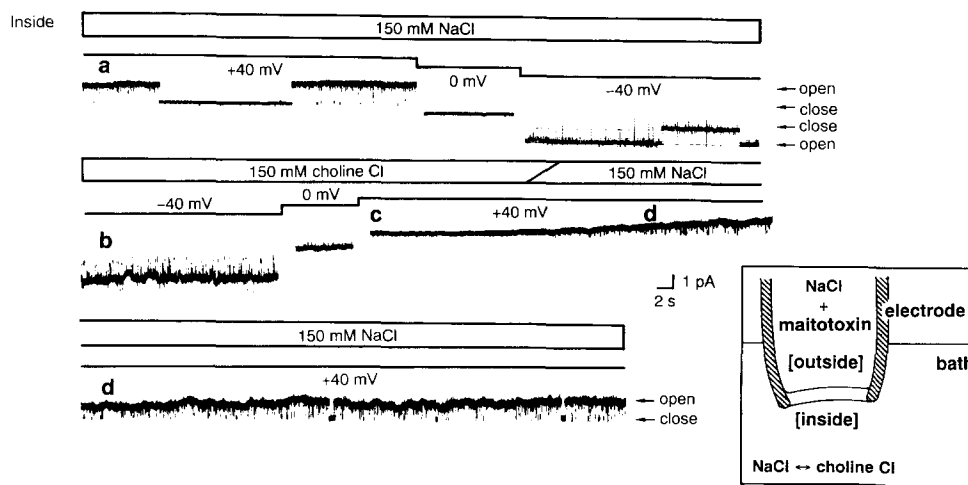


Fig. 5. Effects of choline⁺ substitution for Na⁺ in the bathing solution: inside-out configuration. A patch electrode was filled with 150 mM NaCl and 10 nM maitotoxin. The bath solution was exchanged from 150 mM NaCl solution (a) to 150 mM choline chloride solution (b and c) and then returned to the first 150 mM NaCl solution (d). The membrane potentials indicated above each current trace show the holding potentials of the patch membrane.

single channel current was produced by maitotoxin under this condition and its linear current-voltage relationship with slope conductance of 30 pS was observed in the range of membrane potentials of ± 60 mV (Fig. 4). When the bathing solution was then switched to Na⁺-free choline⁺-substituted solution (Fig. 5, inset), the channel current at a positive membrane potential was abolished (Fig. 5c). The inward current elicited at a negative membrane potential was not inhibited by choline⁺ substitution (Fig. 5b). This inhibition was reversible, resulting in the reappearance of the outward current after reperfusion with NaCl solution (Fig. 5d).

4. Discussion

The present study clearly demonstrates that maitotoxin induces single channel currents in the guinea-pig ventricular myocytes. The currents showed two different amplitudes of deflection at the same membrane potential, suggesting the possible occurrence of a substate of the single channel or the possible induction of two distinct channels, although detailed analysis was not done. Both currents were abolished under Na⁺-free conditions and the reversal potential was close to 0 mV when the Na⁺ concentrations inside and outside the patch membrane were equal. Therefore, it is likely that Na⁺ is the main charge carrier of the currents, although the ionic permeability must be clarified in further studies.

Previously, Kobayashi et al. (1987) reported that maitotoxin induced Ca²⁺- and Ba²⁺-permeable channel in the ventricular myocytes and that adrenaline enhanced the channel activity, therefore suggesting a modification of voltage-dependent Ca²⁺ channels by maitotoxin. In contrast, the currents observed in the present study was produced under Ca²⁺-free conditions and the slope conduc-

tance and kinetic parameters were completely different from those of the maitotoxin-modified Ca²⁺ channel reported. Further, the characteristics of existing channels which have been so far reported in the ventricular myocyte (Adams and Nonner, 1990; Fozzard et al., 1985; Irisawa, 1984; Nilius et al., 1985) differ from those of the presently observed currents. Therefore, it is likely that the observed channels are a unique and new type.

The Na⁺ permeability characteristics of the maitotoxin-induced channel demonstrated in the present study are reminiscent of those described in a report by Yoshii et al. (1987), in which they proposed that maitotoxin may create a pore which is permeable to Na⁺ and Cs⁺ in the neuroblastoma cell membrane.

We previously reported that a marine toxin, palytoxin, creates ion channels permeable to Na⁺ and some other cations (Muramatsu et al., 1988). In the case of palytoxin, more channels could be induced with higher concentrations of palytoxin, thus resulting in a staircase current jump in a single patch membrane. However, maitotoxin induced only one or two single channels over a wide range of electrode resistances (i.e. irrespective of patch membrane area) or in a wide range of maitotoxin concentrations. These findings may account for the smaller amplitude of the membrane depolarization produced by maitotoxin (Nishio et al., 1993) than that produced by palytoxin (Muramatsu et al., 1984) and other marine toxins such as ciguatoxin (Bidard et al., 1984) or brevetoxin (Huang et al., 1984). The findings also indicate that maitotoxin may be not an ionophore, but rather activate a channel or interact with a pre-existing transmembrane protein to form a channel.

Ca²⁺ dependence is a well-known aspect of maitotoxin-induced actions (Gusovsky and Daly, 1990). In addition, Na⁺ dependence has also been reported for maitotoxin-induced actions such as neurotransmitter re-

lease (Pin et al., 1988; Takahashi et al., 1982), increase in Na^+ uptake (Sladeczek et al., 1988) and membrane depolarization (Nishio et al., 1993). The Na^+ -permeable channels found in the present study may be involved in the Na^+ -dependent actions of maitotoxin.

In conclusion, the present study shows that maitotoxin can induce Na^+ -permeable channels in the guinea-pig ventricular myocytes. Maitotoxin seems to be a unique toxin which affects not only Ca^{2+} channel activities as reported previously (Gusovsky and Daly, 1990; Kobayashi et al., 1987) but also on Na^+ permeability (present study).

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