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European Journal of Pharmacology 372 (1999) 103–107

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Effects of paxilline on K^+ channels in rat mesenteric arterial cells

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Received 29 October 1998; received in revised form 15 March 1999; accepted 16 March 1999

Abstract

The effects of paxilline, a mycotoxin, on whole-cell outward currents from freshly isolated cells of the rat mesenteric artery were studied. Paxilline inhibited a component of the outward current that was also sensitive to iberiotoxin. Inhibition could be observed at a concentration of 10 nM and complete inhibition of the iberiotoxin-sensitive current was achieved at 300 nM. The inhibition could be described by a single site of interaction with a K_i of 35.7 nM. Paxilline had no effect on the component of the current that was sensitive to 4-aminopyridine. It is concluded that paxilline is a potent inhibitor of large conductance Ca^{2+}-activated K^+ currents in vascular smooth muscle cells. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Paxilline; K^+ channel, Ca^{2+}-activated; Smooth muscle, vascular; Iberiotoxin

1. Introduction

Paxilline is an indole diterpene produced by fungi. This mycotoxin causes tremors in animals when fed on contaminated grains (Cole and Cox, 1981). Recent studies indicate inhibition of large-conductance Ca^{2+}-activated K^+ channels may contribute to the pharmacological action of paxilline. In fact, paxilline is among the most potent non-peptidyl blockers of these channels (Knaus et al., 1994; Sanchez and McManus, 1996).

Paxilline effectively blocks single channel activities of large-conductance Ca^{2+}-activated K^+ channels in inside-out patches from cultured bovine aortic smooth muscle cells without affecting the single channel conductance (Knaus et al., 1994). The site of block by paxilline on the channel is distinct from the charybdotoxin site. Binding studies with charybdotoxin showed that it does not compete with but rather enhances charybdotoxin binding allosterically to vascular membranes (Knaus et al., 1994).

In the present study, we examined the effect of paxilline on K^+ currents in single vascular smooth muscle cells freshly isolated from rat mesenteric arteries. Similar to most other arterial cells, depolarization induces two types of outward currents in these cells. The delayed rectifier current is activated at a lower threshold and can be blocked by 4-aminopyridine. At more positive potentials, a larger charybdotoxin- and iberiotoxin-sensitive outward current mediated by large-conductance Ca^{2+}-activated K^+ channels is also activated (Beech and Bolton, 1989; Bolzon et al., 1993). In the present study, the specificity of action of paxilline on these two types of K^+ currents in intact rat mesenteric arterial cells was determined using whole-cell recording technique.

2. Materials and methods

2.1. Cell isolation

Single cells were isolated from mesenteric arteries of male Wistar rats (10–12 weeks old, Charles River) with a technique similar to that developed in our laboratory (Bolzon and Cheung, 1989). The arteries were treated with a Ca^{2+}-free HEPES buffer containing 0.02% collagenase, (Sigma, St. Louis, USA), 0.1% papain (Sigma) and 4 mM dithithreitol (Sigma) for 2 h. Most of the single cells isolated were relaxed and contracted in response to stimulation by stimulants such as high K^+ and noradrenaline. Only relaxed cells were used in the experiments. The average length of these cells were 72.2 ± 4.2 μm and their average diameter was 7.7 ± 0.3 μm ($n = 26$).

2.2. Electrophysiology

Recordings from freshly isolated cells were made in the conventional whole-cell recording mode with an Ax-

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PII: S0014-2999(99)00188-0

opatch-1D amplifier (Axon Instuments, Foster City, USA). Patch pipettes were pulled from borosilicate glass with a Flaming–Brown P-80 puller (Sutter Instruments, Novato, USA). Data acquisition and analysis were performed using pClamp 5.5 and 6.1 software (Axon Instruments, USA). Series resistance and cell capacitance were electronically compensated. Leak currents were relatively small and were not subtracted from the records. Most studies were carried out at a holding potential of −80 mV. In a few experiments, a holding potential of −40 mV was used to inactivate delayed rectifier currents (Beech and Bolton, 1989; Bolzon et al., 1993). Signals were digitalized at 3–5 kHz and filtered at 2 kHz with an eight-pole Bessel filter. Figures and curve-fitting were made with SigmaPlot 5.0 software (Jandel Scientific, San Rafael, USA). For construction of current–voltage curves, currents were normalized to current density (current/cell capacitance) to take into consideration variations in current with cell size. To

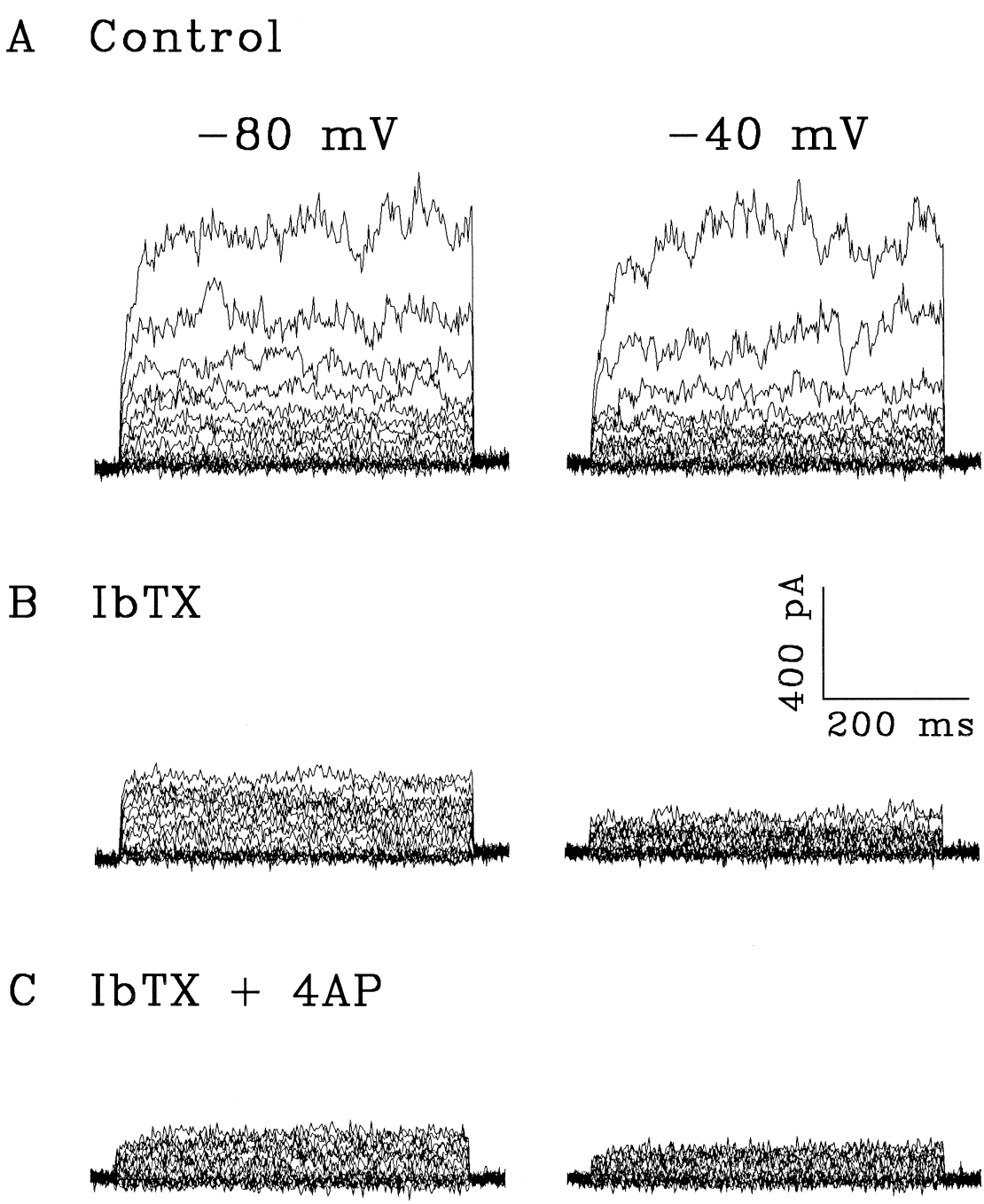

Fig. 1. Whole-cell currents from a rat mesenteric arterial cell elicited by depolarizing pulses from holding potentials of −80 mV and −40 mV. (A) Control. (B) In the presence of iberiotoxin (IbTX, 150 nM). (C) In the presence of IbTX and 4-aminopyridine (4AP, 2 mM). Test pulses from −80 mV to +60 mV.

obtain a more accurate measure of the currents, signals from 3–4 consecutive sweeps were signal-averaged.

The ionic composition of the extracellular solution was (in mM): NaCl 137, KCl 5.5, $CaCl_2$ 1.8, $MgCl_2$ 1.0, KH_2PO_4 0.4, $NaHCO_3$ 4.2, glucose 5.6, and pH adjusted to 7.4 with 10 mM HEPES. Nifedipine (1 μM) was also included to block voltage-sensitive Ca^{2+} channels. The intracellular solution consisted of (in mM): KCl 150, $MgCl_2$ 1.0, Na_2 ATP 1.0, and pH adjusted to 7.2 with HEPES. Free Ca^{2+} concentration was adjusted to 155 nM with EGTA (5 mM). Paxilline, iberiotoxin, nifedipine, and 4-aminopyridine were obtained from Sigma were introduced by adding to the perfusate. Recordings were made only after the currents had reached a stable level with each addition of the agent. All experiments were performed at room temperature.

The data were plotted as means ± S.E.M. Student's *t*-test was used for statistical analysis of the data. The significance level was set at $P < 0.05$.

3. Results

3.1. Whole-cell currents in rat mesenteric arterial cells

At a holding potential of −80 mV, depolarization pulses elicited outward currents comprising of two distinguishable components. At a threshold of about −40 mV, a

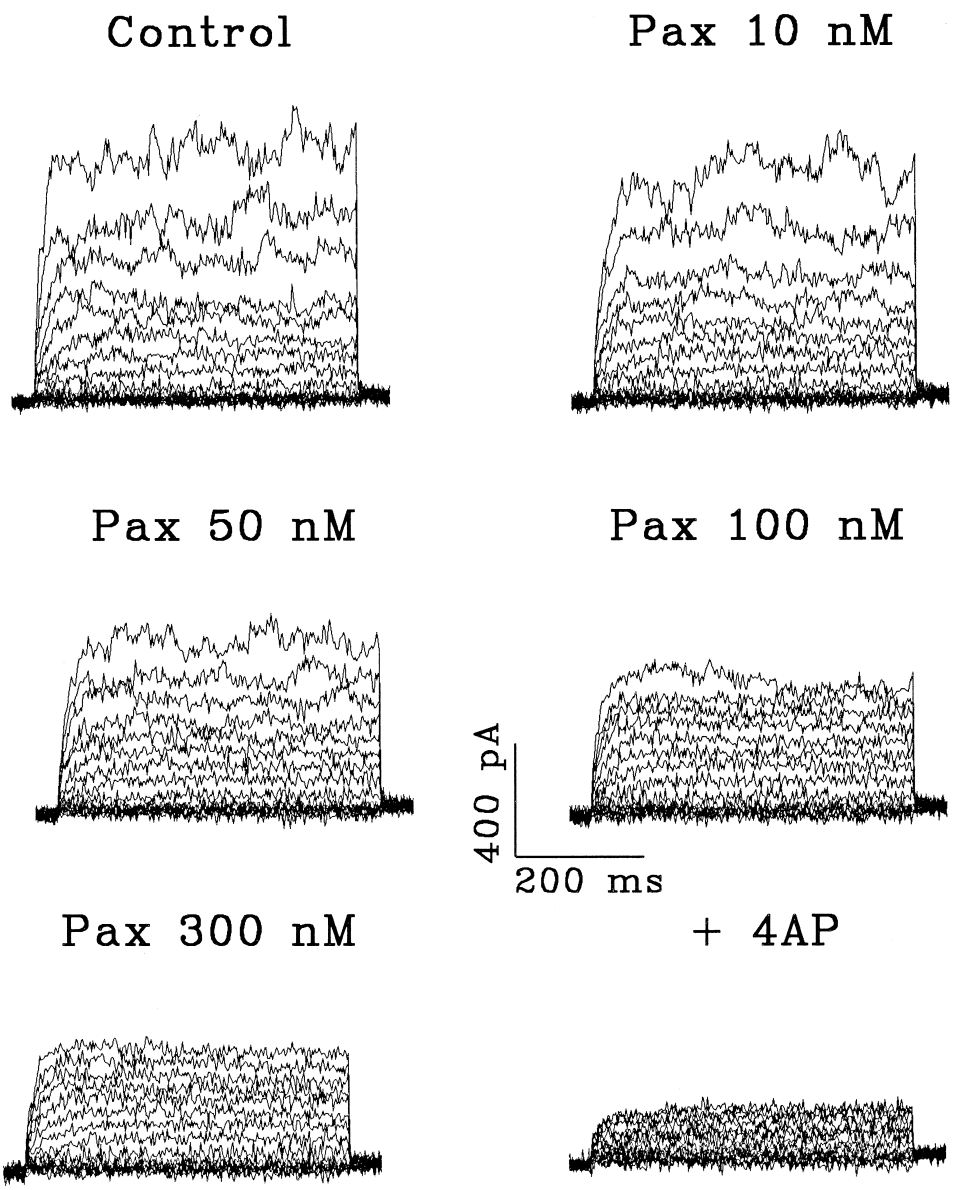

Fig. 2. Effects of various concentrations of paxilline and 4-aminopyridine (4AP, 2 mM) on whole-cell currents from a rat mesenteric arterial cell. Holding potential = −80 mV. Test pulses from −80 mV to +60 mV.

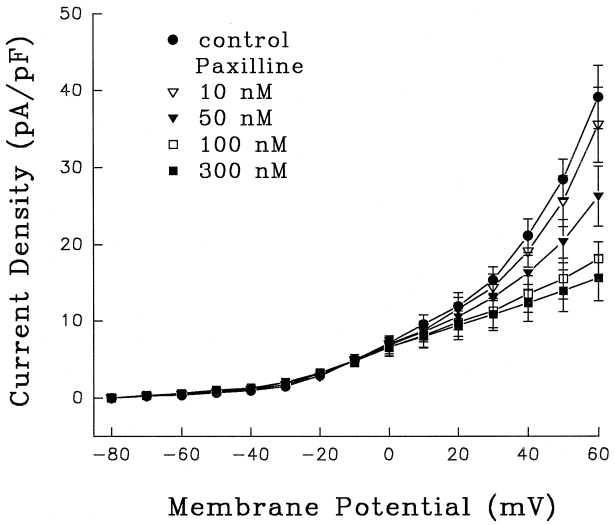

Fig. 3. Concentration-dependent inhibition of whole-cell outward currents by paxilline at various test potentials. $n = 4–6$.

small sustained outward current began to emerge. At potentials positive to 0 mV, there was a more pronounced increase in current amplitude with depolarization. The currents also became oscillatory. This latter component was abolished by the addition of iberiotoxin (100–150 nM), a specific blocker of large conductance Ca^{2+}-activated K^+ channels (Fig. 1). The remaining low-threshold component was inhibited by 4-aminopyridine (2 mM), a blocker of delayed rectifier channels. The 4-aminopyridine sensitive component could also be significantly inactivated by holding the cells at a more positive potential of −40 mV. The iberiotoxin-sensitive currents were not significantly altered by holding the membrane potential at −40 mV (Fig. 1).

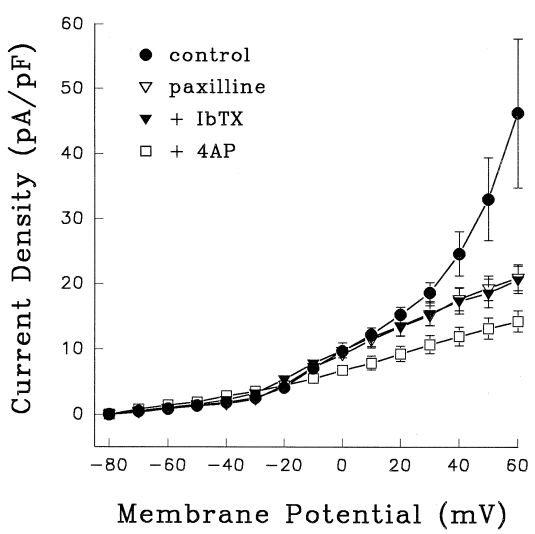

Fig. 4. Effects of paxilline, iberiotoxin, and 4-aminopyridine on whole-cell currents in rat mesenteric arterial cells. Addition of iberiotoxin (IbTX, 150 nM) did not induce further inhibition of the currents than those achieved with paxilline (300 nM). 4-Aminopyridine (4AP, 2 mM) was effective in reducing significantly the remaining currents. $n = 4–6$.

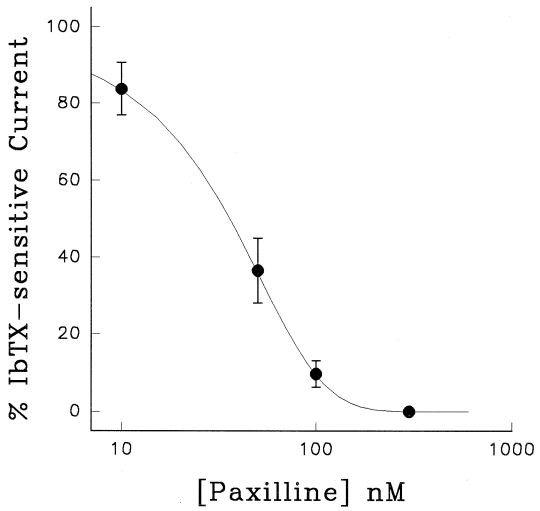

Fig. 5. Concentration-dependent inhibition of the iberiotoxin (IbTX)-sensitive component of the current by paxilline. The data was fitted by a four parameter logistic function with a K_i of 35.7 nM. $n = 4–6$.

3.2. Effects of paxilline

Paxilline inhibited the outward currents in a concentration-dependent manner (Fig. 2). Depression of the currents could be observed at a concentration of 10 nM and maximal inhibition was achieved at 300 nM of paxilline. At a test potential of +60 mV, the current density was reduced significantly from a control value of 54.1 ± 18.2 pA/pF to 20.9 ± 2.0 pA/pF by paxilline (300 nM). Paxilline inhibited only currents positive to 0 mV and the low-threshold currents were not affected (Fig. 3). The paxilline-insensitive currents were reduced significantly by 4-aminopyridine (2 mM) (Figs. 2 and 4). Thus the current density at +60 mV was further reduced to 14.2 ± 1.6 pA/pF by the addition of 4-aminopyridine. The pattern and the level of inhibition by paxilline was similar to that of iberiotoxin. Addition of iberiotoxin (150 nM) would not cause further reduction of the currents in the presence of paxilline (300 nM) (Fig. 4). The concentration-dependent inhibition of iberiotoxin-sensitive current by paxilline was best fitted by a single site of interaction with a K_i of 35.7 nM (Fig. 5).

4. Discussion

Paxilline is a tremorgenic mycotoxin that is recognized as a potent non-peptidyl blocker of large conductance Ca^{2+}-activated K^+ channels. It does not compete with charybdotoxin for binding sites on vascular smooth muscle membranes. Instead, it increases the affinity of charybdotoxin to its binding site allosterically without changes in receptor density. Therefore, paxillin acts on a site on the Ca^{2+}-activated K^+ channel that is distinct from that of charybdotoxin (Knaus et al., 1994). Patch clamp recordings from excised inside-out patches from cultured bovine

aortic smooth muscle cells showed that 10 nM of paxilline could inhibit single Ca^{2+}-activated K^+ channel activities by about 44–70% when applied to the cytoplasmic side of the membrane (Knaus et al., 1994).

In the present study, we examined the effect of paxilline on whole-cell currents in intact cells of the rat mesenteric artery. This is different from the original study of Knaus et al. (1994) in which excised patches of vascular membranes were used and paxilline was applied to the cytoplasmic side of the membrane. Another major difference is that we used a free cytoplasmic Ca^{2+} concentration of 155 nM, as compared to 10 μM in the other studies (Knaus et al., 1994; Gribkoff et al., 1996). This Ca^{2+} concentration is similar to the normal resting level found in vascular smooth muscle cells. Even with stimulation causing maximum contraction in isolated cells or in intact vascular smooth muscle, the maximum Ca^{2+} in vascular cells seldom exceeds 1 μM (Defeo and Morgan, 1985; Alexander and Cheung, 1994).

We compared the effectiveness of paxilline to iberiotoxin, which is the most potent and specific inhibitor of large-conductance Ca^{2+}-activated K^+ channels. Paxilline applied to the external surface of the cells inhibited a component of the outward currents that was also sensitive to iberiotoxin. The concentration range of paxilline required to block the Ca^{2+}-activated K^+ channel currents was similar to that required of iberiotoxin, confirming that it is a potent blocker when applied to intact vascular smooth muscle cells. The only other study on the effect of paxilline on intact cells was on currents of cloned human Ca^{2+}-activated K^+ channels expressed in *Xenopus laevis* oocytes. Paxilline was not completely effective in inhibiting iberiotoxin-sensitive currents in the oocytes. The maximum inhibition was about 80% even with concentrations up to 10 μM (Gribkoff et al., 1996). The concentration-dependent inhibition of iberiotoxin-sensitive cloned human Ca^{2+}-activated K^+ channel current by paxilline was characterized by two components, implicating the presence of two binding sites. In the present study, paxilline was effective in inhibiting iberiotoxin-sensitive currents completely in rat mesenteric arterial cells. Furthermore, paxilline = s inhibition of iberiotoxin-sensitive current can be well described by a single site of interaction with a K_i of 35.7 nM.

The effect of paxilline on delayed rectifier currents could also be tested with the use of intact cells. The delayed rectifier currents in vascular smooth muscle cells are characterized by their sensitivity to 4-aminopyridine and inactivation at more positive potentials (Beech and Bolton, 1989; Bolzon et al., 1993). Iberiotoxin had no effect on delayed rectifier currents in freshly isolated rat mesenteric arterial cells. Similarly, paxilline had no effect on delayed rectifier currents in the rat mesenteric arterial cells. Paxilline therefore confers a level of potency and specificity on large conductance Ca^{2+}-activated K^+ channels in intact vascular smooth muscle cells comparable to those of the peptidyl toxin iberiotoxin.

Acknowledgements

This work was supported by the Heart and Stroke Foundation of Ontario.

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