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The electrophysiological effects of tetraphenylphosphonium on vascular smooth muscle

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Abstract

The effect of the lipophilic quaternary ion, tetraphenylphosphonium, on membrane potential of segments of rat small mesenteric artery and on the current in single voltage-clamped smooth muscle cells from rabbit portal vein was studied. In rat small mesenteric artery, tetraphenylphosphonium (1–30 μM) caused membrane depolarization of approximately 23 mV and decreased or abolished the hyperpolarization induced by the K_{ATP} channel opener, levcromakalim (0.1–3 μM). In rabbit portal vein K^+ currents induced by levcromakalim (10 μM) or pinacidil (10 μM) were completely inhibited by tetraphenylphosphonium (IC_{50} 0.5 μM). The results show that tetraphenylphosphonium antagonizes the K_{ATP} current induced by K^+ channel openers in vascular smooth muscle possibly by acting on the K_{ATP} channel itself. © 1998 Elsevier Science B.V.

Keywords: Membrane potential; K^+ current; Quaternary ion; K^+ channel opener; Levcromakalim; Pinacidil; Tetraphenylphosphonium

1. Introduction

Recently, we found that lipophilic quaternary compounds, in particular tetraphenylphosphonium and tetraphenylarsonium, are two of the most potent antagonists of levcromakalim-mediated vasorelaxation responses identified so far (McPherson and Piekarska, 1994), having a potency similar to that of glibenclamide (Challinor and McPherson, 1993). Thus, in functional experiments on vascular smooth muscle, these compounds are able to antagonize levcromakalim responses with a calculated pK_B of approximately 7 (McPherson and Piekarska, 1994). The exact mode of interaction between the lipophilic quaternary ions and the K_{ATP} channel is not known, and in the present study we examined the electrophysiological effects of tetraphenylphosphonium on vascular smooth muscle cells. We performed two series of experiments. First in rat small mesenteric arteries, where we examined the gross electrophysiological effects of tetraphenylphosphonium and its interaction with levcromakalim, by monitoring whole cell membrane potential. In a second series of experiments, using rabbit portal vein, we utilized patch clamp techniques to examine directly the effect of tetraphenylphosphonium on K_{ATP} channel-mediated currents.

2. Methods

2.1. Isolation of the rat mesenteric artery and membrane potential recording

Wistar Kyoto (WKY) rats were killed by CO_2 asphyxia. The mesenteric bed was rapidly removed and placed in ice-cold Krebs' solution (composition in mM: NaCl 119, KCl 4.7, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, $CaCl_2$ 2.5, and glucose 11) gassed with 5% CO_2 in O_2; pH 7.4. A segment of rat small mesenteric artery, 2 mm in length, corresponding to a third- or fourth-order branch from the superior mesenteric artery, was mounted in a small vessel myograph as previously described (McPherson and Angus, 1991; McPherson, 1992). Vessels were allowed to equili-

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brate under zero force for 30 min. The intracellular membrane potential of a single smooth muscle cell was monitored in the vessel. A conventional glass electrode (1 mm blanks, World Precision Instruments, New Haven USA) filled with 0.5 M KCl (tip resistance approximately 100 MΩ) was used to impale a single smooth muscle cell. Membrane potential data were captured by the use of the data acquisition system CVMS (Version 2.0, WPI USA) for the IBM PC. Results in the text are given as the mean ± S.E.M. for experiments as specified.

2.2. Preparation of rabbit portal vein single smooth muscle cells suspension and whole-cell patch clamping

Adult, male New Zealand white rabbits (2–2.5 kg) were killed by injection of a lethal dose of sodium pentobarbitone. The main branch of the portal vein was removed and placed in physiological salt solution (PSS, composition in mM: NaCl 130, KCl 5, $MgCl_2$ 1.2, $CaCl_2$ 1.7, glucose 10, HEPES 10, pH 7.4 with NaOH). It was dissected free of fat and connective tissue, cut into small sections (~ 2 × 3 mm) and incubated for 10 min at 37°C, in low-Ca^{2+} (10 μM) and Mg^{2+}-free solution (composition in mM: NaCl 130, KCl 5, $CaCl_2$ 0.01, glucose 10, HEPES 10, pH 7.4 with NaOH). The tissue was then incubated (37°C) in the same salt solution with the addition of collagenase 1 mg/ml (type XI, Sigma) and 4 mg/ml papain (Sigma) for

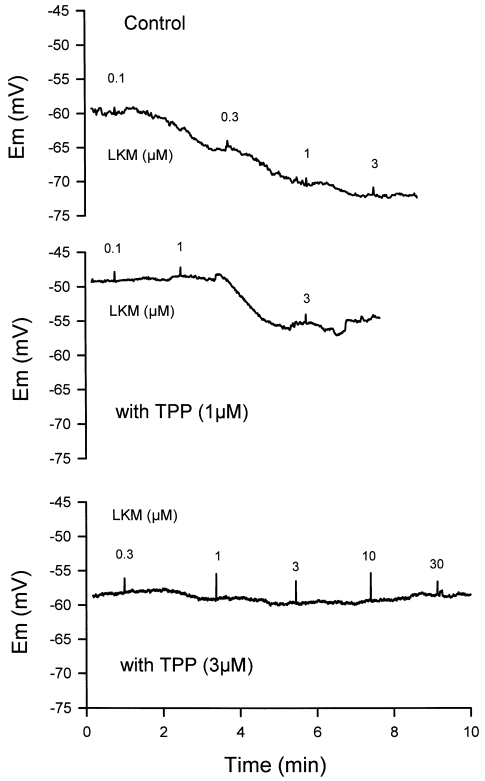

Fig. 2. The effect of levcromakalim (LKM, 0.1–30 μM) on resting membrane potential of rat mesenteric artery segment alone (top trace) and in the presence of 1 μM (middle trace) and 3 μM tetraphenylphosphonium (lower trace).

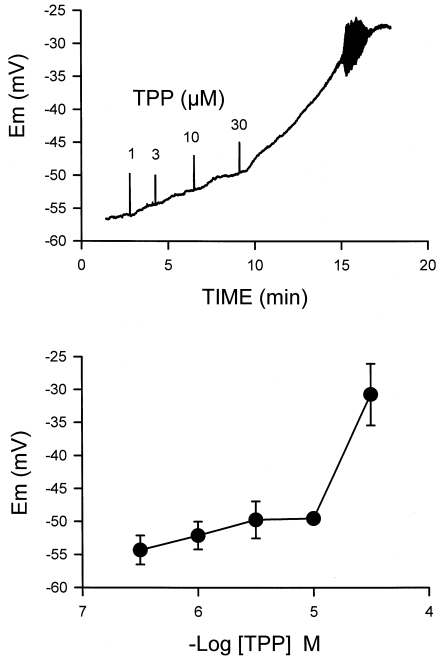

Fig. 1. (Top) The effect of tetraphenylphosphonium (1–30 μM) on resting membrane potential of a segment of rat mesenteric artery. (Bottom) Mean concentration–response curve to tetraphenylphosphonium on resting membrane potential (Mean data from 4 cells from 4 different vessels). Error bars are the S.E.M.

25–30 min. Next, it was washed with enzyme-free solution and triturated by sucking in and out of a wide bore, smooth-tipped pipette. The solution containing the best cells (assessed via visual inspection) was centrifuged, and the pellet was re-suspended in a quasi-physiological medium containing either 0.2 mM Ca^{2+} or Mg^{2+}. This suspension was placed on glass cover slips and the cells allowed to sink and attach to the glass. The cells were stored at 4°C and used for experiments within 10 h of separation. All experiments were performed at room temperature (22–25°C).

A conventional whole-cell (Hamill et al., 1981) method was used in these experiments. The patch amplifier was an RK300 (Biologic) and the voltages were viewed on a storage oscilloscope (Tektronix). Patch pipettes were made from Borosilicate glass (Plowden and Thompson) with an external diameter of 1.5 mm and internal diameter of 0.8 mm; they had resistance of 2–5 MΩ after fire-polishing. Data were captured on a 486 PC for analysis after digitization using CED interface and software.

2.2.1. Patch clamping solutions

High K^+ pipette solution comprised (mM): KCl 120, $MgCl_2$ 2, EGTA 5, glucose 10, HEPES 20, pH 7.4 with KOH. The bathing solution was PSS.

2.3. Drug actions

In all studies, drugs were added in a cumulative manner to the tissue. The effect of the drug was then followed until an apparent plateau response had been obtained and was maintained for 2–3 min, at which time the next concentration of drug was added.

2.4. Drugs

The following drugs were used: levcromakalim (Beecham, UK), pinacidil monohydrate (Leo, Denmark), tetraphenylphosphonium chloride (Sigma, USA). Levcromakalim and pinacidil were made up daily in 100% methanol as stock solutions and diluted in distilled water. Tetraphenylphosphonium was made up fresh in distilled water.

3. Results

3.1. Electrophysiological response to tetraphenylphosphonium of rat mesenteric artery segments

Smooth muscle cells of segments of the rat small mesenteric artery (diameter 306 ± 11 μm, $n = 4$ under 100 mmHg pressure) had a resting membrane potential of -54 ± 2 mV ($n = 4$ cells, 4 different vessels). Tetraphenylphosphonium (1–30 μM) caused concentration-dependent membrane depolarization to -31 ± 5 mV, a depolarization of approximately 23 mV ($n = 4$ cells, 4 different vessels) (see Fig. 1). At concentrations less than 30 μM, tetraphenylphosphonium caused relatively small depolarizations (less than 10 mV) while 30 μM tetraphenylphosphonium produced a much larger depolarization (Fig. 1).

In a separate series of experiments, levcromakalim (0.1–3 μM) in the absence of tetraphenylphosphonium, caused a concentration-dependent hyperpolarization of the resting membrane potential from -60 ± 1 mV to -77 ± 2 mV ($n = 4$ cells, 4 different vessels; Fig. 2), a hyperpolarization of approximately 17 mV. In the presence of 1 μM tetraphenylphosphonium, levcromakalim (0.1–3 μM) caused a concentration-dependent hyperpolarization to a maximum of only 8 mV (from -52 ± 4 to -60 ± 6; $p < 0.05$ compared with maximum response in the absence of tetraphenylphosphonium, Fig. 2). Similar experiments using 3 μM tetraphenylphosphonium showed that this concentration completely inhibited hyperpolarizing responses to levcromakalim (Fig. 2).

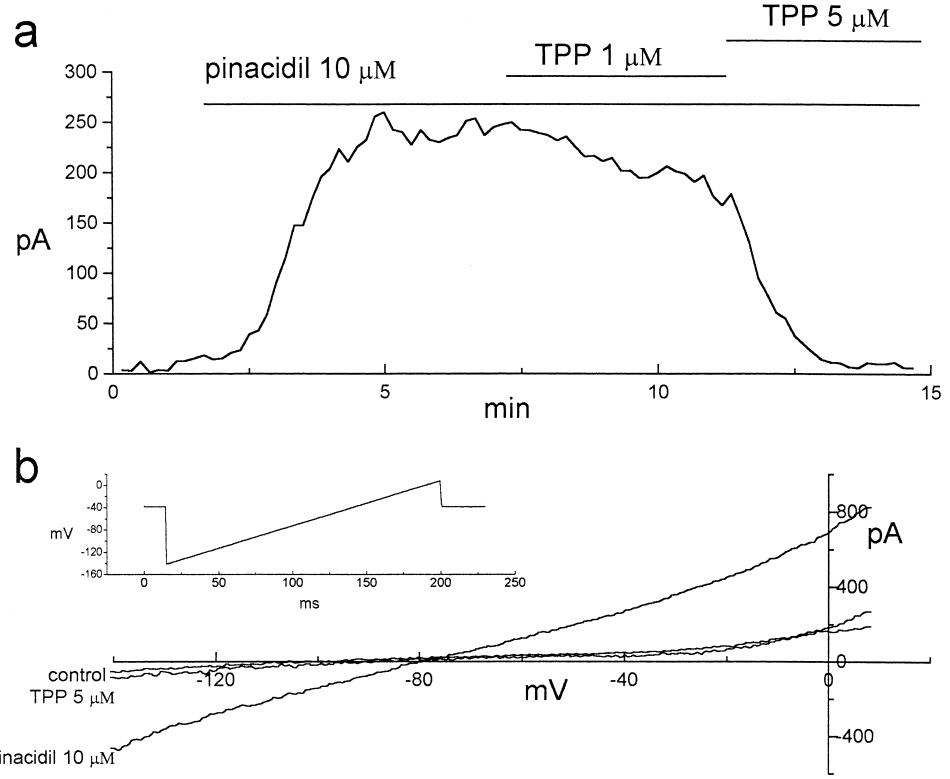

Fig. 3. Effects of tetraphenylphosphonium on outward current evoked by the potassium channel opener pinacidil. (a) Rabbit portal vein smooth muscle cell was held at -40 mV, and the holding current was sampled every 10 s. Pinacidil (10 μM) and tetraphenylphosphonium (1 and 5 μM) were applied via the bathing solution as indicated by bars. PSS was in the bath and high K^+ solution was in the pipette. (b) Current in response to linear ramp changes in membrane voltage (170 ms duration as shown in inset) from -140 mV to $+10$ mV. Current records were taken before, in the presence of pinacidil (10 μM) and with the addition of tetraphenylphosphonium (5 μM). The calculated potassium equilibrium potential was -83 mV.

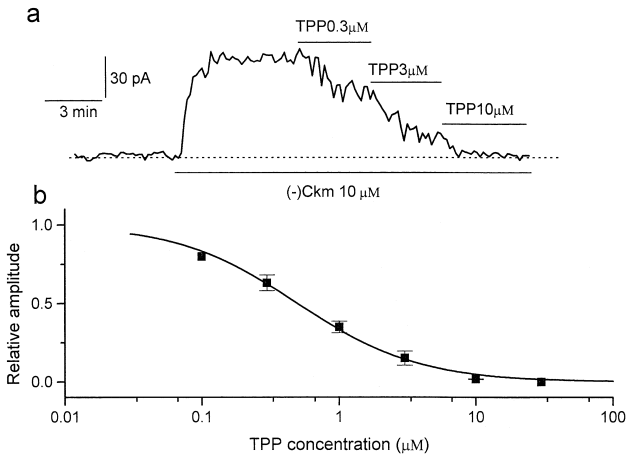

Fig. 4. (a) Effects of tetraphenylphosphonium (0.3–10 μM) on outward current evoked by levcromakalim ((−)Ckm, 10 μM) applied in the bathing solution to a single rabbit portal vein smooth muscle cell held at −40 mV. The holding current was sampled every 10 s and is plotted in the figure. Drugs were applied via the bathing solution as indicated. PSS was in the bath and high K solution was in the pipette. The broken line indicates zero current level. (b) Concentration–response relationship of tetraphenylphosphonium on current evoked by levcromakalim (10 μM). Values are means ± S.E.M. of 2–7 cells.

3.2. Effect of tetraphenylphosphonium on pinacidil and levcromakalim currents in rabbit portal vein smooth muscle cells

Whole-cell recordings were made from rabbit portal vein smooth muscle cells. The cells were held at −40 mV and the currents sampled every 10 s as shown in Fig. 3a and Fig. 4a. When 10 μM pinacidil (Fig. 3a) or 10 μM levcromakalim (Fig. 4) was applied in the bath solution, an outward current developed, which had a reversal potential around the K^+ equilibrium potential (−83 mV; Fig. 3b), suggesting that this outward current was a K^+ current.

The outward currents induced by both pinacidil and levcromakalim were inhibited by tetraphenylphosphonium in a concentration-dependent manner (Fig. 3a and Fig. 4). In four cells tested, 5 μM tetraphenylphosphonium inhibited 93 ± 7% of the current induced by pinacidil. Tetraphenylphosphonium (10 μM) completely abolished current induced by levcromakalim (Fig. 4a). The IC_{50} for tetraphenylphosphonium was approximately 0.5 μM (Fig. 4b). The effect of tetraphenylphosphonium developed relatively slowly, taking 2 to 3 min, and was not readily reversed by washing. Thus, in 4 cells in which the effect of washout was determined, the current returned to 31 ± 11% of the pre-tetraphenylphosphonium levels.

4. Discussion

Quaternary ions, such as tetraphenylphosphonium and tetraphenylarsonium, are highly lipophilic. They can delocalise their charge and passively equilibrate across the plasma membrane in accordance with the membrane potential across the cell wall. Because of this property, lipophilic cations have been used to measure the polarity of membrane potentials in biological systems (Saito et al., 1992). In previous studies, examining the actions of tetraphenylphosphonium and tetraphenylarsonium, we found that these two quaternary cations are also potent antagonists of levcromakalim-mediated vasorelaxation responses in the rat thoracic aorta (McPherson and Piekarska, 1994). They are two of the most potent antagonists of levcromakalim-mediated actions, having a potency similar to the sulphonylurea, glibenclamide ($pA_2 \sim 7.2$). More detailed studies were performed in this study to characterize the electrophysiological actions of tetraphenylphosphonium.

4.1. Effect of tetraphenylphosphonium and levcromakalim on membrane potential in rat small mesenteric artery

Previous studies by us (McPherson and Angus, 1991) using the rat small mesenteric artery have shown that K_{ATP} channel antagonists (the sulphonylurea glibenclamide and the imidazolidine, alinidine) cause a concentration-dependent depolarization of smooth muscle cells in this preparation. We have suggested that, in this tissue, K_{ATP} channels are spontaneously opened and hold the membrane potential approximately 10 mV more negative than the membrane potential in preparations in which K_{ATP} channels are closed. Consistent with this idea, tetraphenylphosphonium at concentrations expected to antagonize K_{ATP} channels (0.1–3 μM) also caused a small concentration-dependent depolarization of this magnitude. However, at higher concentrations (30 μM), much more marked depolarizing responses were observed. It is likely that tetraphenylphosphonium can interact with a number of different ion channels that could account for this phenomenon. In any event, studies using low concentrations of tetraphenylphosphonium (1 and 3 μM) showed that levcromakalim-mediated hyperpolarization responses were antagonized, suggesting that, at these concentrations, tetraphenylphosphonium interacts with the K_{ATP} channel opened by levcromakalim.

4.2. Effect of tetraphenylphosphonium on levcromakalim-induced current in rabbit portal vein smooth muscle cells

The present results clearly show that the outward K^+ currents induced by pinacidil and levcromakalim were inhibited by tetraphenylphosphonium in a concentration-dependent manner. Glibenclamide has been suggested to be a specific blocker of K_{ATP} channel. In previous work, we found glibenclamide inhibited outward whole-cell K^+ current induced by levcromakalim (10 μM) in rabbit portal vein cells with an IC_{50} of 0.2 μM (Beech et al., 1993), which is similar to the IC_{50} (0.5 μM) of te-

traphenylphosphonium we found in this study in a similar recording condition. Thus, tetraphenylphosphonium is a new, potent blocker of K_{ATP}. In a similar condition, tetraethylammonium, another quaternary ion, inhibited levcromakalim current with an IC_{50} of about 7 mM (Beech et al., 1993), three orders higher than the IC_{50} of tetraphenylphosphonium. Furthermore, the inhibitory effect of tetraethylammonium could be easily washed out, while that of tetraphenylphosphonium was difficult. We also found that tetraphenylphosphonium was much less potent in inhibiting voltage-dependent K^+ currents (delayed rectifier K^+ current IC_{50} approximately 30 μM, unpublished observations). These results indicate that tetraphenylphosphonium inhibits the currents by interaction with the K_{ATP} channel rather than by a non-specific effect on the cell membrane due to its highly lipophilic properties.

The results from this study show that the quaternary ion, tetraphenylphosphonium, is a potent blocker of vascular K_{ATP} channels. Apart from sulphonylureas such as glibenclamide, other compounds including imidazolidines (e.g., phentolamine and alinidine) also block K_{ATP} channels (reviewed by Challinor-Rogers and McPherson, 1994). However, the present study is the first to show that tetraphenylphosphonium, a quaternary ion, is able to block the K^+ current through K_{ATP} channel induced by levcromakalim or pinacidil. The precise mechanism by which tetraphenylphosphonium blocks the K_{ATP} channel is not clear from the present study, and further work is needed to understand more about the effect of tetraphenylphosphonium as well as properties of the K_{ATP} channel it regulates. It is likely, however, that tetraphenylphosphonium acts on the K_{ATP} channel itself to regulate channel activity.

Acknowledgements

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