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Inhibitory effects of propiverine on rat and guinea-pig urinary bladder muscle

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Summary. In muscle strips isolated from guinea-pig and rat urinary bladder, propiverine (3–10 μM) inhibited carbachol-induced contractions in the presence of verapamil and Ca^{2+}-induced contractions in excess K^+ medium containing atropine, suggesting it has both anticholinergic and Ca^{2+} channel blocking actions.

The Ca^{2+} channel blocking action was also demonstrated by recording inward Ca^{2+} currents in single cells dispersed from both species. The inhibition of inward currents by propiverine was three times stronger in the rat than the guinea-pig, ID_{50} being 7 μM for rat and 21 μM for guinea-pig. The recovery of the current after washout was faster than that of mechanical inhibition. It is concluded that propiverine blocks not only muscarinic receptors, but also Ca^{2+} channels at similar concentrations.

Key words: Propiverine – Urinary bladder – Smooth muscle – Ca^{2+} channel blocker – Antimuscarinic action

Introduction

Propiverine hydrochloride (1-methyl-4-piperidyl diphenylpropoxyacetate hydrochloride, P-4) has been developed by VEB Apogepha, Dresden and clinically used to treat frequency of micturition (Dorschner et al. 1982). In muscle strips of guinea-pig urinary bladder, propiverine has been reported to inhibit contractions induced by acetylcholine and KCl (Vietinghoff and Hammer 1981; Haruno et al. 1989). These results have been interpreted to indicate that propiverine has both anticholinergic and Ca^{2+} channel blocking actions. The latter idea is based on the assumption that KCl-induced contractions are due to Ca^{2+} influx through voltage-gated channels. Since KCl-induced contractions are partially inhibited by atropine in the guinea-pig urinary bladder (Vietinghoff and Hammer 1981), it is possible that the inhibition of KCl-induced contraction by propiverine is not simply due to Ca^{2+} channel blockade but also to its anticholinergic action. In the present experiments, therefore, the direct effects of propiverine on inward Ca^{2+} currents were investigated mainly in single cells dispersed from the guinea-pig and rat urinary bladder using the whole-cell patch-clamp method.

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Methods

Guinea-pigs (250–350 g) and Wistar rats (200–250 g) of either sex were sacrificed by stunning and bleeding, and the urinary bladder was excised. Muscle strips (about 1.5×10 mm) were obtained longitudinally from the ventral wall of the bladder after removing the mucosa.

Two preparations, one from a rat and another from a guinea-pig, were mounted in a small organ bath (about 1 ml capacity) for simultaneous records of isometric tension with strain gauges and superfused at a constant rate of 3 ml/min with physiological solution prewarmed to 35 °C. The solution had the following composition (mM): NaCl 127, $KHCO_3$ 6, $CaCl_2$ 2.4, $MgCl_2$ 1.2, glucose 12, Tris buffer 10, pH adjusted to 7.4 with HCl. The solution was saturated with O_2.

For electrophysiological experiments, muscle strips were digested with collagenase to obtain single muscle cells. The dispersion and whole-cell recording method were essentially the same as those described by Inoue and Brading (1990). The dispersed cells were placed in a small chamber (0.3 ml) and superfused with physiological solution at a rate of 3 ml/min prewarmed to 32 °c. Membrane currents were recorded by the patch-clamp technique in the whole-cell configuration using a voltage-clamp amplifier (List, EPC-7). The bathing solution for the whole-cell clamp experiments contained (mM): NaCl 91, KCl 6, $CaCl_2$ 2.4, $MgCl_2$ 1.2, glucose 12, tetraethylammonium chloride 30, Hepes-buffer 10, pH being adjusted with NaOH to 7.4. The solution used to fill the patch pipettes contained (mM): Cs-aspartate 110, CsCl 20, $MgCl_2$ 1.6, $CaCl_2$ 1.2, EGTA 10, ATP 5, Hepes-buffer 10, pH 7.0.

Propiverine hydrochloride was supplied by Taiho Seiyaku (Japan). Other drugs were obtained from Sigma (St. Louis, USA).

Results

Muscle strips of the urinary bladder produced spontaneous irregular phasic contractions both in rat and guinea-pig, but the spontaneous activity often tended to cease

gradually with repeated applications of carbachol (CCh). CCh increased the amplitude of phasic contractions superimposed on a tonic contraction. Propiverine inhibited the CCh-induced contraction (Fig. 1), probably due partly to an anticholinergic effect and partly to a Ca^{2+} channel blocking action, as considered previously (Haruno et al. 1989). With cumulative application of CCh, relatively strong tachyphylaxis and irregular phasic activities made a quantitative analysis difficult. Therefore, the results shown in Fig. 1 were obtained with brief (20 sec) noncumulative applications of CCh at 10 min intervals.

In order to discriminate the anticholinergic action from the Ca^{2+} channel blocking action, the effects of propiverine were also studied in the presence of verapamil. The CCh response was reduced by verapamil (5 μM) to a similar extent in guinea-pig (30% ± 9 with 30 μM CCh, $n = 8$) and rat urinary bladder (33% ± 7, $n = 8$). The inhibition with propiverine (1 – 10 μM) was clearly observed in the presence of verapamil (5 μM), and no significant difference in potency was found between the two species (Fig. 1). These results suggest that propiverine has a significant anticholinergic action. Contractions induced by prostaglandin E_2 (10 – 100 nM) in the presence of verapamil (5 – 10 μM) were not significantly affected by propiverine (10 μM) (not shown).

Effects of propiverine on the contraction caused by Ca influx in excess K^+ medium were studied under the condition in which the muscarinic receptors were blocked by atropine. 60 mM K^+ produced phasic contractions followed by a tonic contracture. The phasic contraction was more prominent in guinea-pig and the slow component was relatively more dominant in rat urinary bladder. To eliminate the phasic activity, contractures were induced by Ca^{2+} readmission to Ca^{2+}-free 60 mM K^+ medium. The Ca^{2+}-induced contractures were partially (by 25 – 50% in both species) inhibited by atropine (1 μM), suggesting that acetylcholine released from intrinsic nerves contributed to the K-contracture. As shown in Fig. 2, in the presence of atropine (1 μM) which produced maximal inhibition on its own, propiverine (3 – 30 μM) strongly inhibited K^+-contractures, suggesting that propiverine has a Ca^{2+} channel blocking action in addition to an anticholinergic action. The inhibitory effect on K-contracture in the presence of atropine was slightly stronger in rat than guinea-pig. Only a partial recovery was observed, the contraction being about 50% of the control at 30 min after wash-out of 30 μM propiverine.

The direct action of propiverine on Ca^{2+} channels was studied by recording inward Ca^{2+} currents with the whole-cell clamp method. When the plasma membrane was depolarized from a holding potential of −80 mV, inward currents appeared at about −30 mV and reached the maximum between −10 and +10 mV. No clear difference in electrical properties was found between smooth muscles from rat and guinea-pig urinary bladders. Propiverine (30 μM) markedly reduced the inward current without shifting the threshold and the membrane potential at which the current reached the maximum (Fig. 3). The inhibition of the late phase of inward current was stronger than the early phase, so that the decay of inward current became faster.

Figure 4 shows concentration-inhibition curves for maximum inward currents obtained from 8 – 9 cells of the rat and guinea-pig urinary bladder. The ID_{50} of propiverine was 7 μM and 21 μM for rat and guinea-pig, respectively.

The time course of the drug action is shwon in Fig. 5. In both species, propiverine rapidly reduced inward currents evoked by depolarizing pulses to 0 mV applied every 15 sec and the recovery of the currents on wash-out was also quick.

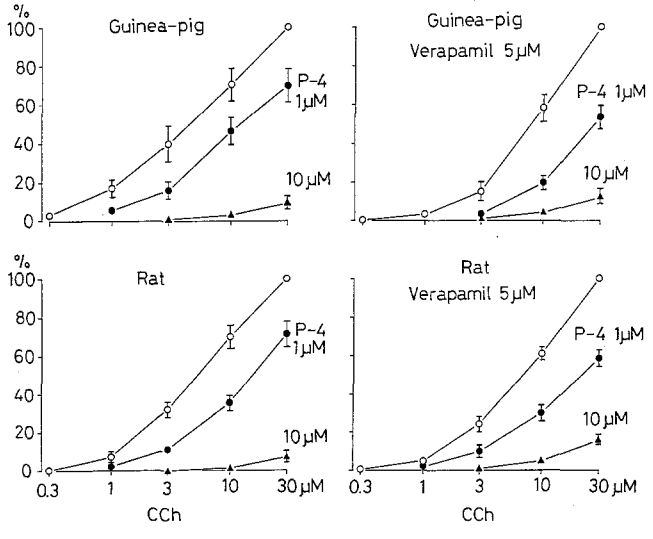

Fig. 1. Effects of propiverine (*P–4*) on contractions induced by carbachol (*CCh*) applied for 20 sec noncumulatively in muscle strips of guinea-pig (*upper*) and rat urinary bladder (*lower*). Right panels are obtained in the presence of verapamil (5 μM). Contractions produced by 30 μM CCh are taken as 100% and *vertical bars* indicate SEM ($n = 12$ from 3 animals)

Discussion

Propiverine has multiple actions, i.e. it blocks muscarinic receptors as well as Ca^{2+} channels in smooth muscles of

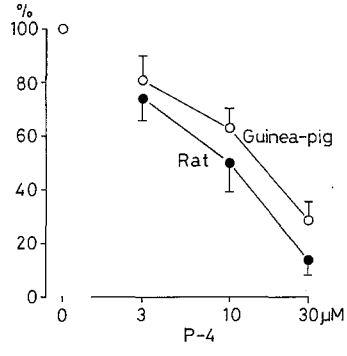

Fig. 2. Inhibitory effects of propiverine (*P–4*) on contractions induced by Ca^{2+} readmission in 60 mM K^+ medium containing atropine (1 μM) obtained from muscle strips of guinea-pig (*empty circle*) and rat urinary bladders (*filled circles*) ($n = 12$, from 3 animals). After 10 min exposure to Ca^{2+}-free 60 mM K^+ solution, 2.4 mM Ca^{2+} was applied for 5 min. Preparations were pretreated with P–4 for 10 min before Ca^{2+} readmission

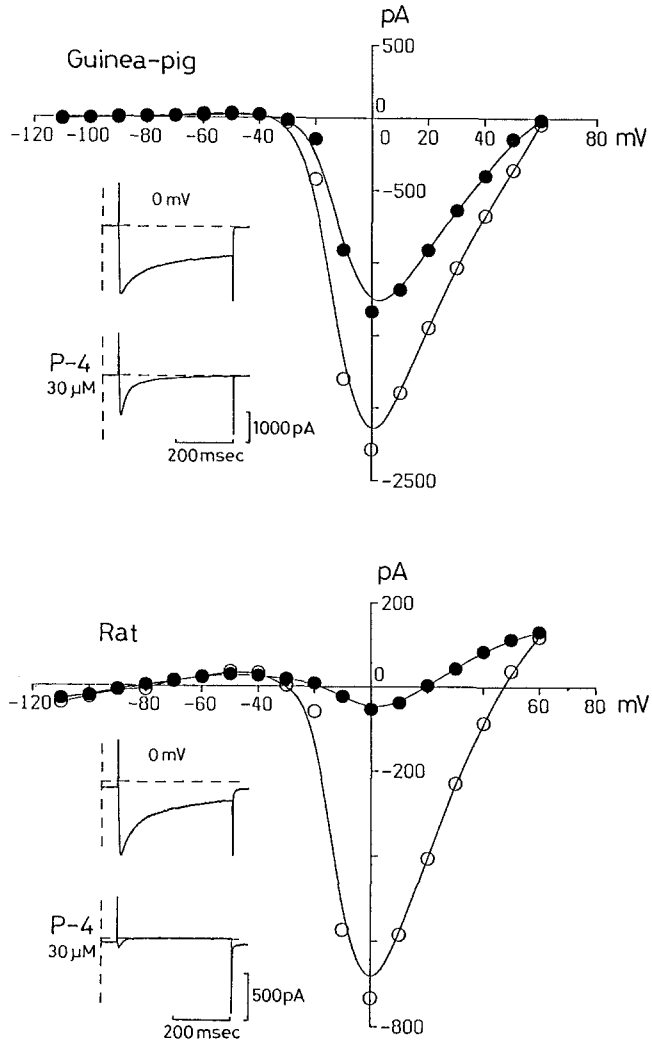

Fig. 3. Effects of propiverine on the voltage-current relationship at the time of peak inward currents recorded with the whole-cell patch-clamp method from single cells dispersed from the guinea-pig (*upper*) and rat urinary bladder (*lower record*). Currents were obtained by voltage pulses (400 ms) from a holding potential of −80 mV applied every 15 s. *Empty circles*, control; *filled circles*, in the presence of 30 μM propiverine (*P–4*). *Insets* show inward currents obtained at 0 mV before and after propiverine application

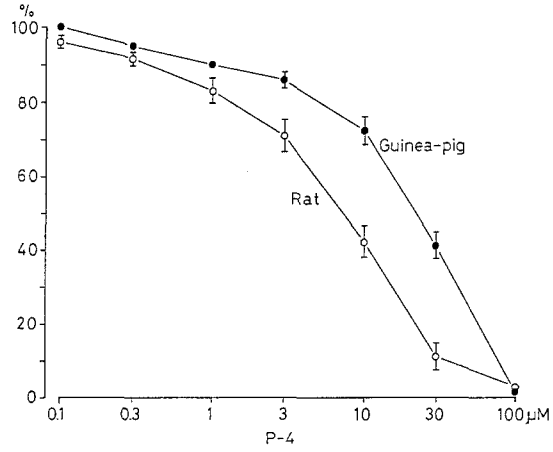

Fig. 4. Concentration-inhibition curve of propiverine (*P–4*) for peak inward curerents (holding potential: −80 mV) in single cells from rat and guinea-pig urinary bladder. Data from 8–9 cells were averaged after normalizing

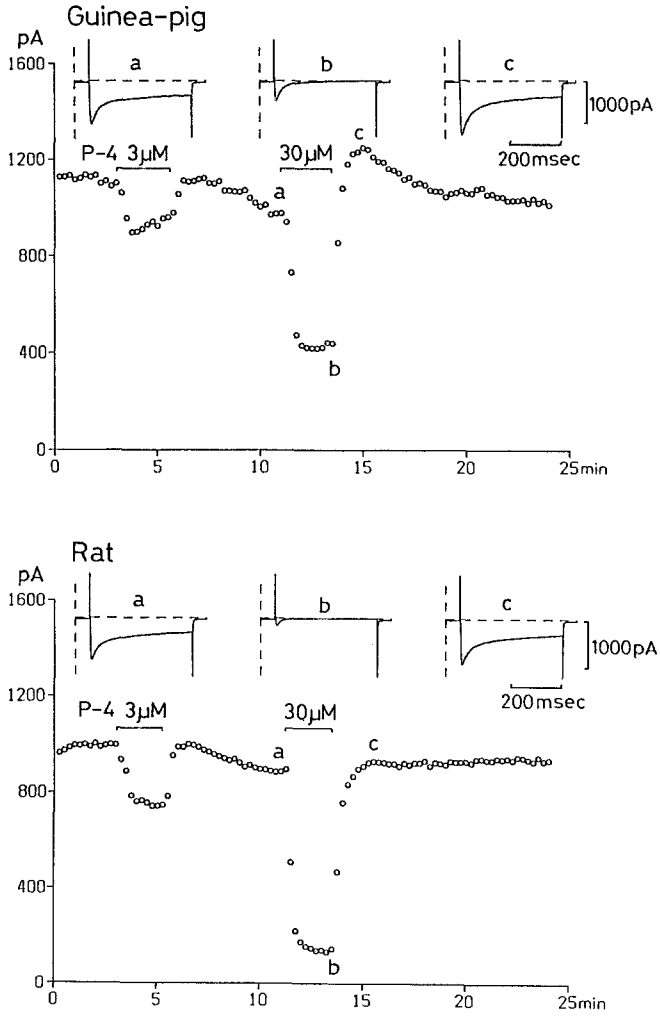

Fig. 5. Time course of the propiverine (*P–4*) effect on peak inward currents in guinea-pig (*upper*) and rat urinary bladder (*lower record*). Inward membrane currents were obtained with depolarizing pulses (400 ms) to 0 mV from a holding potential of −80 mV at an interval of 15 s. Propiverine (P–4, 3 and 30 μM) was applied as indicated. Records shown above correspond with those having the same letter before, during, and after application of 30 μM propiverine

the rat and guinea-pig urinary bladders, as previously reported for the guinea-pig urinary bladder (Vietinghoff and Hammer 1981; Haruno et al. 1989). Verapamil (5–10 μM) strongly reduces CCh-induced contractions, suggesting that CCh activates not only a receptor-mediated Ca^{2+} pathway that is verapamil resistant, but also a voltage-gated Ca^{2+} pathway that is susceptible to verapamil (Bolton 1979). The inhibition of CCh-induced contraction with verapamil is similar between guinea-pig and rat urinary bladder. In the presence of verapamil, the inhibition of CCh contraction by propiverine is probably mainly due to an anticholinergic action. There is no significant difference in the anticholinergic action between rat and guinea-pig urinary bladder. In the presence of verapamil (5 μM), CCh could still produce phasic contractions in many guinea-pig preparations, but the ionic

channels responsible for this contractions have not been studied in the present experiments.

Propiverine can inhibit Ca^{2+}-induced contractures in 60 mM K^+ medium containing atropine, probably by blocking voltage-dependent Ca^{2+} channels. This action was clearly demonstrated by observing inward Ca^{2+} currents with the whole-cell patch-clamp experiment. The properties of the inward Ca^{2+} currents are similar in rat and guinea-pig, and they are essentially the same as those previously reported (Klöckner and Isenberg 1985). Membrane potential dependency of activation and the time course of the inward currents suggest that the L-type channel is responsible for the current flow. In the guinea-pig urinary bladder, the IC_{50} of propiverine against the inward current is 21 μM which is very similar to that (18 μM) previously obtained against the contraction induced by 100 mM K^+ (Haruno et al. 1989). Propiverine is 3 times more potent in the rat than in the guinea-pig urinary bladder in inhibiting the inward current. This is also consistent with the result of mechanical recording that propiverine inhibits KCl-induced contraction in the presence of atropine more strongly in the rat than in the guinea-pig.

The recovery from the inhibition of inward currents by propiverine is quite fast, being completed in a few minutes. On the other hand, the recovery from the mechanical inhibition by propiverine is very slow, the recovery being only partial even after 30 min wash-out, not only for CCh-response in the presence of verapamil but also for Ca^{2+}-induced contracture in excess K^+ in the presence of atropine. This may suggest that propiverine is exerting some intracellular inhibitory action, in addition to anticholinergic and Ca-channel blocking actions. This possibility has, however, not been investigated in the present experiments.

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