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Effects of papaverine on tension and ^{45}Ca-uptake in isolated urinary bladder

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Summary. Papaverine is believed to relax smooth muscle by reducing transmembrane calcium transport and cyclic nucleotide phosphodiesterase activity. The present study characterizes the different relaxing effects of papaverine on isolated muscle strips of rat bladder dome. Compared to histamine, norepinephrine and serotonin, carbachol and high potassium induced the most prominent contractions in rat bladder strips. For this reason both agents were used as stimulants. High-potassium-induced muscle contractions were reduced by a lower concentration of papaverine than carbachol-induced muscle concentrations. Compared to verapamil, papaverine, especially in low concentrations, was less potent on both kinds of induced muscle contractions. These tension responses correspond to a difference in ^{45}Ca uptake, suggesting a nonspecific blocking property of papaverine on transmembrane calcium channels. The β-sympathomimetic effects of isoprenaline on carbachol-induced contractions were not enhanced by verapamil. In contrast, papaverine increased this tension response of isoprenaline on carbachol- induced contraction. From these results it is possible that part of the papaverine action seems to be related to an intracellular mechanism probably to cAMP.

Key words: ^{45}Ca influx – Papaverine – Rat urinary bladder

In non-human bladders there is evidence for two types of excitatory innervation: one cholinergic, and the other non-adrenergic, non-cholinergic [3]. To reduce this excitatory innervation papaverine may be a substance of interest because it normally relaxes smooth muscles and reduces the contractile response to a variety of stimulant agents [2]. On the one hand, there is evidence that papaverine acts on transmembrane calcium transport in smooth muscle [2, 10]. On the other, the action of papaverine involves cAMP by inhibition of cyclic phosphodiesterase activity in smooth muscle [11, 12]. However, there is no consensus of opinion regarding the mechanism of papaverine-induced relaxation in bladder smooth muscle.

This study was designed to characterize the distinct effects of papaverine in isolated urinary bladder. In rats, the potency of papaverine on contractions induced by 85 mmol/l potassium or carbachol (10^{-4} mol/l) were compared to the effect of a calcium channel blocker (verapamil) and to a cAMP activator (isoprenaline) on smooth muscle tension. To provide details on calcium, calcium uptake was also measured.

Materials and methods

General

Adult Sprague-Dawley rats (165–200 g) were killed by a blow to the head. The bladder dome was excised immediately. In a dissection chamber containing warm, oxygenated Tyrode solution, the bladder dome was carefully dissected free from visible connective tissue and cut longitudinally into four parts.

Contractions

By tying both ends with fine silk sutures (5×0), the preparations were suspended in 10 ml organ baths containing Tyrode solution at 37°C gassed with 95% O_2 and 5% CO_2. The tension was measured under isometric conditions with inductive force displacement transducers and recorded on paper. The preload tension was adjusted to 10 mN. All muscle strips exhibited spontaneous phasic rhythmic changes in tension immediately after mounting in the organ bath and application of the preload tension. An interval of at least half an hour was allowed for equilibration, after which experiments were performed.

Contractions were produced by exposing the muscle strips to high concentrations of carbachol, histamine, norepinephrine, serotonin (10^{-4} mol/l each) and high potassium (85 mmol/l) Tyrode solution. The effects of the drugs on contractions induced by a high level of potassium (85 mmol/l) and carbachol (10^{-4} mol/l) were studied in muscle strips using two different protocols. In the first protocol, the cummulative concentration response relationships of papaverine (10^{-7} to 10^{-4} mol/l), verapamil (10^{-10} to 10^{-5} mol/l) and isoprenaline (10^{-10} to 10^{-5} mol/l) were obtained as follows. First, an initial contraction (control) was produced by using 85 mmol/l potassium Tyrode or carbachol (10^{-4} mol/l). After a wash-out

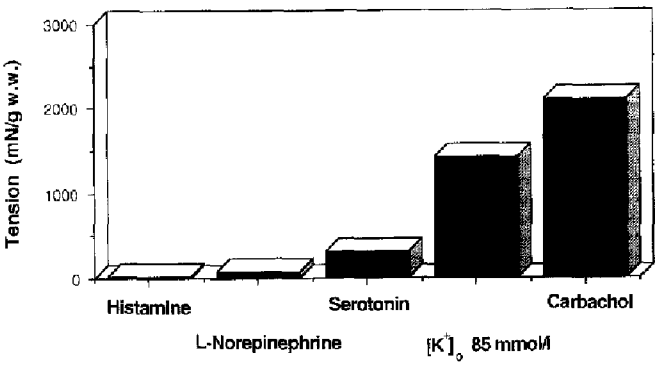

Fig. 1. Effects of histamine ($n = 4$), L-norepinephrine ($n = 4$), serotonin ($n = 4$) and carbachol ($n = 40$) in concentrations of 10^{-4} mol/l each and the effects of 85 mmol/l extracellular potassium ($n = 64$) on peak tension of muscle strips of rat bladder dome (means). (*w.w.*, wet weight)

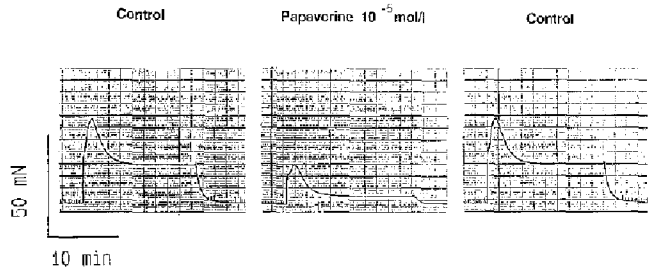

Fig. 2. Original recordings of effects of 85 mmol/l extracellular potassium concentration (control) and papaverine 10^{-5} mol/l on tension of muscle strips of rat bladder dome

period of 30 min, the muscle strips returned to their previous resting tension. Then the muscle strips were incubated with one of the three drugs at the lowest concentration for 30 min. Afterwards, a contraction was produced by high potassium or carbachol in the presence of the same drug concentration. Inhibition of the induced contraction was measured as a function of drug concentration. Further responses were obtained by increasing the drug concentration in logarithmic increments.

In a second protocol, muscle strips were contracted by high potassium or carbachol (10^{-4} mol/l) as noted. The plateau tension was allowed to stabilize. Then the muscle strips were incubated with increasing drug concentration as mentioned. The solutions of the bath remained unchanged. The reduction of the plateau phase was expressed as a function of the drug concentration.

Ca uptake

Parallel experiments were carried out to study the effects of papaverine, verapamil and isoprenaline on net ^{45}Ca uptake in high-potassium-activated muscle strips. Initially, the tissues were allowed to equilibrate for 60 min in an oxygenated Tyrode solution at 37°C. The muscle strips were then exposed for different periods (1 to 100 min) to normal Tyrode solution or 85 mmol/l KCl Tyrode. Both solutions were labelled with ^{45}Ca (0.1 ml/l l solutions). At the end of the exposure, the tissues were washed in physiological salt solution (PSS) at 0 °C three times for 5 min each to inhibit further ^{45}Ca influx. The muscle strips were blotted, weighed and incubated in 1 ml of tissue solubilizer (TS-1, Zinsser). A scintillation liquid (Quickszint 402, Zinsser) was added and vials analyzed for ^{45}Ca in a liquid scintillation counter (Packard, Model 544). The uptake of calcium was calculated from the ratio of radioactivity in the tissue, in the incubation medium, and the concentration of calcium in the Tyrode solution. The data were expressed as calcium uptake in micromole per gram tissue wet weight.

Only one concentration response curve for ^{45}Ca-uptake was obtained from each muscle strip.

Solutions

The Tyrode solution used was prepared with distilled deionized water and had the following composition (mmol/l): NaCl 136.9, KCl 5.4, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, $CaCl_2$ 1.8 and glucose 5.5. The pH of the solution was 7.2 to 7.4. The Tyrode solution used for potassium-induced contractions contained 85 mmol/l KCl and 57.3 mmol/l NaCl; other ingredients remained unchanged.

For removal of extracellular calcium, PSS was prepared (mmol/l): NaCl 140, KCl 4.6, EGTA 2.0, $MgCl_2$ 1.0, glucose 10.0 and HEPES 5.0. The pH was adjusted to 7.2 at 0°C using 0.1 mol/l of NaOH.

The stock solutions of carbachol, histamine, isoprenaline, norepinephrine, papaverine, serotonin and verapamil were prepared in double-distilled water. From these stock solutions, desired concentrations were prepared in Tyrode solution.

Drugs

The following drugs were used: ^{45}Ca (Du Pont), carbachol hydrochloride (Merck), histamine hydrocholoride (Merck), isoprenaline sulfate (Boehringer), L-norepinephrine bitarbrate (Serva), papaverine hydrochloride (Serva), phentolamine (Ciba Geigy), serotonin creatinine sulfate (Merck) and verapamil (Knoll).

Results are expressed as means ± standard error of the means (SEM). An overall statistical comparison of the independent mean values was based on the analysis of variance, Documenta Geigy, equations 623–625 [7]; this procedure was followed by standard *t*-statistics. A *P*-value of less than 0.05 was considered significant.

Results

Histamine, norepinephrine and serotonin in concentrations of 10^{-4} mol/l had only a small effect on the tension of the isolated muscle strips. In contrast, high potassium (85 mmol/l) and carbachol (10^{-4}) produced strong increases in tension, carbachol being the strongest stimulatory agent (Fig. 1). Therefore, carbachol and high potassium were used as stimulants to study the relaxing properties of papaverine, verapamil and isoprenaline.

The response to high potassium was biphasic and transient in nature (Fig. 2: original tracing). After an initial rapid increase in tension, which peaked, tension decreased in a second phase to a more sustained plateau. Both components of tension response were reduced or even abolished by papaverine (Figs. 2, 3). Incubation of muscles in reverse order showed identical results compared to the effects on plateau tension.

Application of carbachol also caused a biphasic tension response. However, in contrast to high potassium contractions numerous rapid phasic contractions were induced during the plateau phase (see Fig. 4, upper line: original tracing). The peak and plateau tension was reduced by papaverine (see Fig. 4, upper line: original

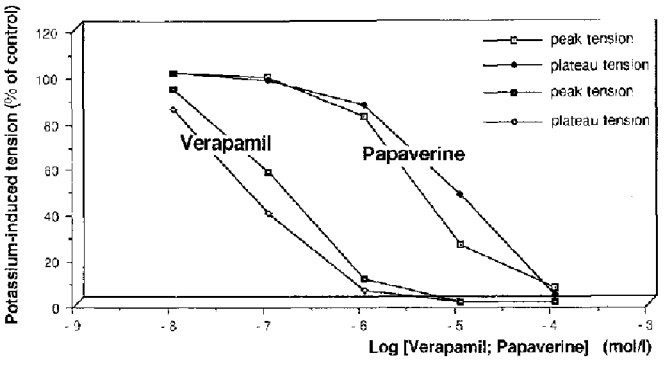

Fig. 3. Concentration response relationships of increasing papaverine and verapamil concentration on peak and plateau tension induced by 85 mmol/l extracellular potassium on rat bladder dome

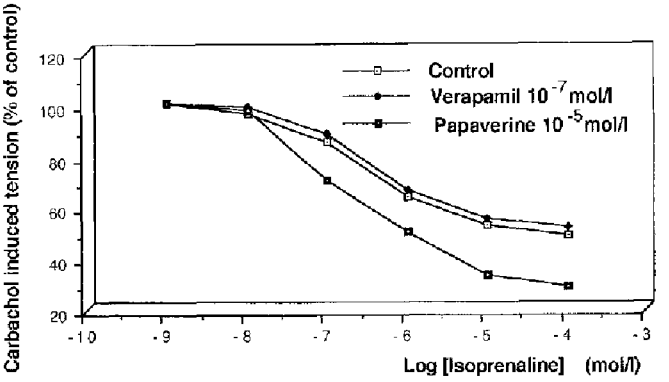

Fig. 5. Concentration-response relationships of increasing isoprenaline concentration (control), isoprenaline and papaverine 10^{-5} mol/l or isoprenaline and verapamil 10^{-7} mol/l on peak tension induced by carbachol 10^{-4} mol/l on rat bladder dome (means; $n = 4$–12)

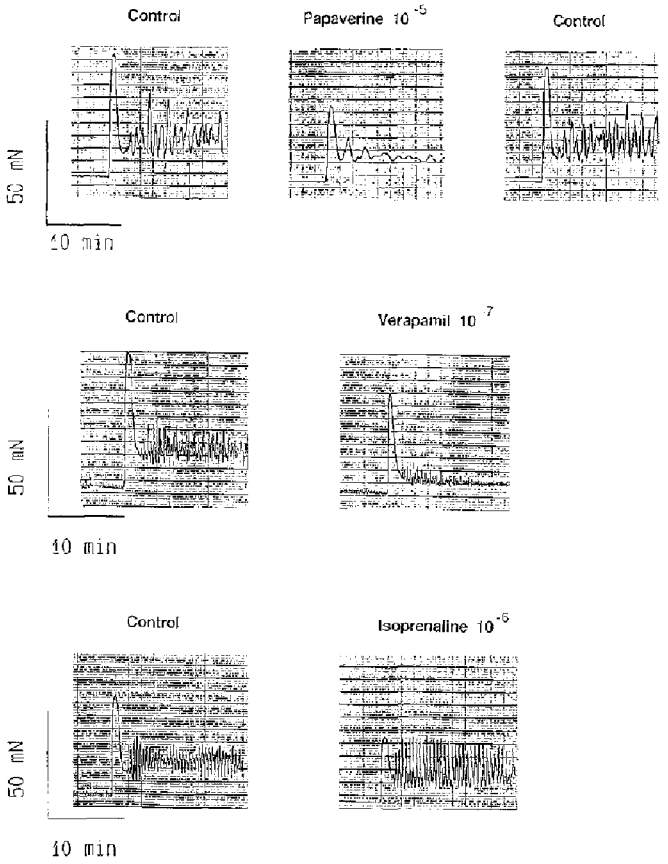

Fig. 4. Original recording of effects of carbachol 10^{-4} mol/l (control), papaverine 10^{-5} mol/l, verapamil 10^{-7} mol/l and isoprenaline 10^{-6} mol/l on tension of muscle strips of rat bladder dome

tracing). The concentration of papaverine-producing half maximum relaxation (EC_{50}) on peak tension was 0.16×10^{-4} mol/l. The effects of carbachol (10^{-4} mol/l) were completely antagonized at a papaverine concentration of 10^{-4} mol/l. Carbachol-induced muscle contractions were reduced less effectively by papaverine than by high-potassium-induced muscle contractions.

To evaluate the calcium-channel-blocking property of papaverine, the effects of verapamil (10^{-10} to 10^{-5} mol/l) were studied on high-potassium-induced contractions. Verapamil reduced the peak and plateau tension and abolished both components at a concentration of 10^{-5} mol/l (Fig. 3). Figure 4 demonstrates an example (middle trace) of the relaxing properties of verapamil on carbachol-induced contractions. The potency of verapamil on peak tension was similar ($EC_{50} = 0.18 \times 10^{-6}$ mol/l), as obtained by high-potassium-induced contractions. Compared with papaverine, low concentrations of verapamil were more potent in reducing carbachol and high-potassium-induced muscle contractions.

To study β-sympathomimetic effects on induced contractions, isoprenaline was applied to muscle strips. The plateau tension of high-potassium induced contractions was only diminished up to 80% (10^{-4} mol/l isoprenaline; EC_{50} 1.16×10^{-7} mol/l). The isoprenaline effects on carbachol-induced contractions are displayed in Fig. 4 (original tracing: bottom trace). An increase in spontaneous contraction amplitudes was observed during the plateau phase. In contrast, the peak tension was markedly reduced, but even high concentrations of isoprenaline (10^{-4} mol/l) inhibited only maximal 50% of peak tension (Fig. 5). To evaluate the additional effects of papaverine or verapamil, the isoprenaline concentration response relationships were repeated in the presence of a nearly half maximum concentration of papaverine (10^{-5} mol/l) or verapamil (10^{-4} mol/l). As Fig. 5 clearly shows, verapamil did not influence the isoprenaline response. In contrast, papaverine significantly ($P < 0.01$) reduced the peak tension.

The calcium uptake was measured to provide details of calcium movements in addition to the tension effects. Table 1 shows the time course of calcium uptake in unstimulated (normal Tyrode = control) and in muscle strips stimulated with high potassium. The uptake of calcium under control conditions markedly increased up to 30 min. Stimulation of the muscle strips with high potassium extended the calcium uptake over the control values at 100 min (Table 1).

Table 1. Time course of ^{45}Ca uptake (nmol/100 mg tissue wet weight) in muscle strips of rat bladder dome (means ± SEM; $n = 4$)

Minutes	1	3	10	30	100
Normal Tyrode	8.2 ± 2.4	20.8 ± 0.9	47.1 ± 1.6	77.8 ± 2.2	87.9 ± 13.8
High K + Tyrode	11.9 ± 0.8	18.8 ± 0.4	29.3 ± 1.9	64.2 ± 2.9	140.5 ± 1.1

Table 2. Concentration response relationships of papaverine, verapamil and isoprenaline on ^{45}Cap-uptake in muscle strips of rat bladder dome contracted by 85 mmol/l extracellular potassium after 100 min (means ± SEM).

	Control	10^{-8} mol/l	10^{-7} mol/l	10^{-6} mol/l	10^{-5} mol/l	10^{-4} mol/l
Papaverine ($n = 8$)	106.9 ± 1.0	-	101.1 ± 2.1	95.7 ± 1.6	96.6 ± 2.4	60.0 ± 0.6
Verapamil ($n = 8$)	111.0 ± 3.0	104.6 ± 1.3	91.6 ± 0.7	83.3 ± 0.9	69.1 ± 1.6	-
Isoprenalin ($n = 4$)	126.4 ± 3.7	124.3 ± 4.5	137.5 ± 0.2	125.1 ± 1.3	139.7 ± 0.8	-

The concentration response relationships of papaverine and verapamil on muscle strips conctracted by high potassium are shown in Table 2. Both drugs reduced the calcium uptake significantly (Table 2). Isoprenaline activation induced a small but nof significant rise in calcium uptake.

Discussion

In the present study it could be observed that high-potassium-induced muscle contractions were reduced by a lower concentration of papaverine than carbachol-induced muscle contractions. Compared to verapamil, papaverine, especially in low concentrations, was less potent for both kinds of induced muscle contractions. These differences in potency corresponded with a decrease of ^{45}Ca uptake. However, in contrast to verapamil, papaverine increased the relaxing effects of isoprenaline on carbachol-induced tension.

In general, the mechanical activity of smooth muscles is determined by the contraction of free intracellular calcium. Two integrated membrane systems, the cell membrane and the sacroplasmic reticulum are involved in the control of free intracellular calcium concentration [6]. Carbachol and high potassium were used, as they differ in the way they induce contractions. Carbachol binds to a specific muscarin receptor, and high potassium seems to exert its effects mainly because it reduces the potassium gradient across the cell membrane [2]. From these studies it can be assumed that both types of activation use in part the influx of calcium from the extracellular space, as mentioned for other mammalian tissue [1, 4, 8, 13]. The strong muscle contractions by carbachol reinforce the predominance of a cholinergic-mediated contractile response in rat urinary bladder. Earlier Cohen and Drey mentioned carbamylcholin, a cholinergic agonist, and high potassium as the two most prominent agents in inducing bladder muscle contractions in rats [9].

The main site of papaverin action appears to be the calcium-influx mechanism during prolonged potassium depolarization on the urinary bladder muscle of rats [10]. Earlier it was postulated that much of papaverine's action could possibly be explained if it were assumed that it blocks calcium channel in the cell membrane [3]. When muscle contraction was induced by a high potassium concentration, a dose-dependent decrease in muscle contraction by papaverine was observed. In addition, ^{45}Ca uptake was reduced. These results pertain to the action of papaverine on the cell membrane. However, compared to verapamil higher concentrations of papaverine were necessary to decrease ^{45}Ca uptake. Similar significant differences in the potency of the two drugs were observed on the degree of smooth muscle relaxation. From these findings it is suggested that the calcium-channel-blocking property of papaverine is fairly non-specific. High-potassium and carbachol-induced muscle contractions were reduced by papaverine by a variable degree in contrast to verapamil. Therefore, this papaverine effect does not seem to be related to a blockade of calcium channels. In human urinary bladder, this papaverine response is even more prominent. In high concentrations papaverine had virtually no effect on peak tension in carbachol-induced contractions [13].

Another approach to the analysis of papaverine action involves cAMP. Papaverine is thought to be a potent inhibitor of phosphodiesterase in urinary bladder [12]. Inhibition of this enzyme causes a rise in cAMP which mediates relaxation by several mechanisms [2]. In addition, cAMP production can be increased by β-receptor activation [5]. Isoprenaline was used for β-receptor stimulation in order to define the possible papaverine effects on cAMP. With reference to these experiments, a decrease in muscle contraction activity was observed but no decrease in ^{45}Ca uptake by isoprenaline. This refers to a sequestration of calcium into intracellular stores, possibly the sacroplasmic reticulum. It is suggested that the isoprenaline concentration response shift to the left by papaverine alone on carbachol-induced contractions is related to a further increase in cAMP.

In conclusion, papaverine action on the rat bladder dome seems to be related to a non-specific calcium channel blockade and a β-receptor-related effect on cAMP.

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