Urol Res (1990) 18:227–231

Urological Research

Effects of papaverine on human isolated bladder muscle

L. Hertle[1] and H. Nawrath[2]

[1] Department of Urology, University of Bochum, Bochum and [2] Department of Pharmacology, University of Mainz, Mainz, FRG

Accepted: June 30, 1989

Summary. Papaverine is a non-specific smooth muscle relaxant and is thought to act at a site beyond the receptor sites on the cell membrane. In this study the relaxing properties of papaverine were tested in isolated muscle strips from the human bladder dome. In carbachol-induced contractions papaverine, even in high concentrations of 10^{-4} mol/l had virtually no effects on peak tension generation, whereas the fading was accelerated and the steady state tension at 30 min. was reduced by about 54%. In contrast, high potassium-induced contractions were relaxed by papaverine in a concentration-dependent way; a concentration of papaverine of 10^{-4} mol/l produced full relaxation. These findings might possibly be explained if it is assumed that papaverine blocks calcium ion channels in the cell membrane. However, the observation that rather high concentrations of papaverine were necessary to fully relax high potassium contractions and the fact, that papaverine affects cellular cAMP levels separate this drug from more selective calcium channel blockers. The calcium movements responsible for the peak tension generation in carbachol-induced contractions are obviously not affected by papaverine. Although papaverine had little effect on carbachol-induced contractions in vitro it cannot be excluded that the drug is effective in diseases were non-cholinergic mechanisms are involved.

Key words: Papaverine – Human bladder muscle

Papaverine, the main benzylisoquinoline alkaloid of opium, reduces the contractions of smooth muscles produced by a wide variety of stimulants. Its site of action appears to be beyond the receptor site on the cell membrane, and although the exact molecular mechanism is uncertain, there is evidence that papaverine may act by inhibiting the cyclic nucleotide phosphodiesterase activity in the cell.

Papaverine has also been shown to have profound effects on calcium mobilization during contraction. Bolton [1] suggested that papaverine could inhibit calcium ion channels in the cell membrane. Huddard et al. [9] found, that papaverine-induced inhibition of high potassium responses was related to an inhibition of calcium uptake and associated calcium efflux in rat vas deferens and bladder.

It is generally assumed that drugs which induce relaxation of smooth muscles may be of clinical importance in some urological disorders; such drugs are ideed widely used, for example in the therapy of unstable bladders or to facilitate the passage of ureteral stones. Recently, papaverine has been shown to be very effective e.g. in relaxing smooth muscle of the corpus cavernosum penis, thereby inducing erection in impotent men [14]. The purpose of this study was to determine the relaxing properties of papaverine in isolated muscle strips from the human detrusor and to evaluate its potential clinical importance.

Materials and methods

General

Detrusor specimen from the dome of the bladder were taken from 7 patients (6 male, 1 female) with a mean age of 58 years (range 49 to 68), who were undergoing radical cystectomy because of bladder carcinoma. None of these patients had received radiation therapy or chemotherapy prior to operation and all patients had sterile urine cultures. All tissue specimen were taken from a microscopically normal part of the detrusor. Immediately after removal of the bladder, the tissue specimen (1 to 2 from each patient) were immersed in cold (4°C) Tyrode solution. The serosal and mucosal layers were carefully dissected away and up to 16 muscle pieces approximately 10 by 3 to 4 mm weighing 50 to 100 mg were excised from each specimen. Some of the preparations were used within 2–4 h after the operation, while some were stored at 4°C for up to 24 h. No qualitative or quantitative differences in response were found between muscle strips which were used within 4 h after cystectomy and those which were used 24 h later.

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

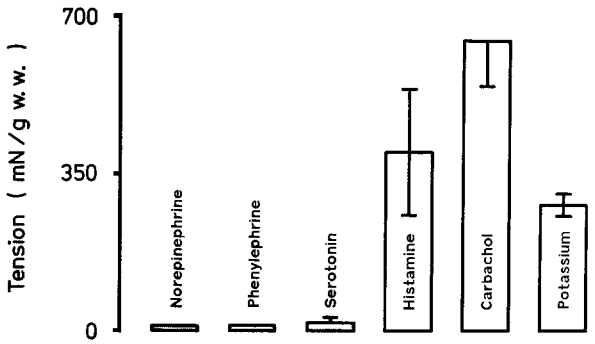

Fig. 1. Effects of norepinephrine ($n = 8$, 2 patients), phenylephrine ($n = 24$, 6 patients), serotonin ($n = 8$, 2 patients), histamine ($n = 12$, 3 patients), carbachol ($n = 24$, 6 patients) in concentrations of 10^{-4} mol/l each and effects of 85 mmol/l extracellular potassium ($n = 32$, 7 patients) on peak tension of muscle strips of human bladder dome. Means ± SEM (w.w. = wet weight)

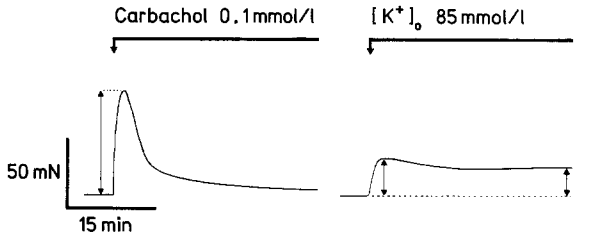

Fig. 2. Original recording of effects of carbachol 10^{-4} mol/l and 85 mmol/l extracellular potassium concentration on tension of muscle strips of human bladder dome ($CaCl_2$: 1.8 mmol/l). Carbachol-induced contractions were transient in nature, whereas stimulation by high potassium produced a stable plateau following an initial tension peak

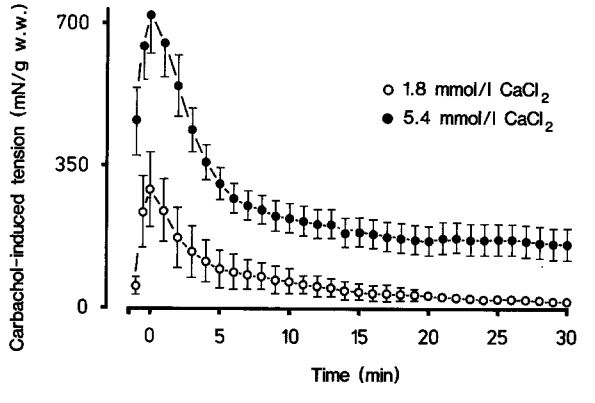

Fig. 3. Time course of effects of carbachol 10^{-4} mol/l on tension of isolated human bladder muscle at two different extracellular calcium concentrations. Open circles: 1.8 mmol/l $CaCl_2$ ($n = 4$, 2 patients), closed circles: 5.4 mmol/l $CaCl_2$ ($n = 20$, 7 patients). Means ± SEM (w.w. = wet weight)

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. An inverval of at least an hour was allowed for equilibration, after which experiments were performed.

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, $CaCl_2$ 5.4 and glucose 5.5. The pH of the solution was 7.2. The Tyrode solution used for potassium-induced contractions contained 85 mmol/l KCl and 57.3 mmol/l NaCl; the other ingredients remained the same as above. The stock solutions of norepinephrine, phenylephrine, serotonin, histamine, carbachol and papaverine were prepared in distilled water. From these stock solutions, desired concentrations of the drugs were prepared in Tyrode solution and added in appropriate volume into the muscle bath to give the desired concentrations. The pH was maintained at 7.2 ± 0.1. The experimental arrangement permitted a rapid exchange (1 to 2 s) of solutions.

Drugs

The following drugs were used: carbamoyl choline hydrochloride (carbachol, Merck), histamine 2-hydrochloride (Serva), L-norepinephrine bitartrate (Serva), L-phenylephrine hydrochloride (Boehringer), serotonin creatinine sulfate (Merck) and papaverine hydrochloride (Serva).

At the end of each experiment the preparations were blotted with filter paper for 90 s under constant pressure (280 gm) and weighed. Concentrations given are the final concentrations of drugs in the organ bath in mol/l. The EC_{50} value of papaverine was determined graphically, taking into account 2 points on the steep portion of each individual concentration-response curve, and geometric mean values were calculated. Results are expressed as means ± standard error of the means (SEM).

Results

Norepinephrine, phenylephrine and serotonin in concentrations of 10^{-4} mol/l had only very small effects on tension of isolated human bladder dome preparations. In contrast, histamine (10^{-4} mol/l), carbachol (10^{-4} mol/l) and high extracellular potassium concentrations (85 mmol/l) 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. The concentration of carbachol chosen was based on previous studies by Fovaeus et al. [5]; it represents a maximal concentration according to concentration-response relationships obtained in human bladder muscle strips.

The response to carbachol was biphasic and transient in nature (original recording is shown in Fig. 2). After an initial rapid increase in tension, which peaked, tension continuously decreased in a second phase. The decrease in tension returned to baseline level within 20 min, when extra-cellular calcium concentration was 1.8 mmol/l (Fig. 3). At a calcium concentration of 5.4 mmol/l carbachol produced a significantly higher increase in peak tension and a more sustained plateu in the fading phase (Fig. 3).

Depolarization of the detrusor muscle strips with an extracellular potassium concentration of 85 mmol/l induced a rapid increase in tension, which peaked and was followed by partial relaxation and sustained contraction (original recording is shown in Fig. 2). The sustained

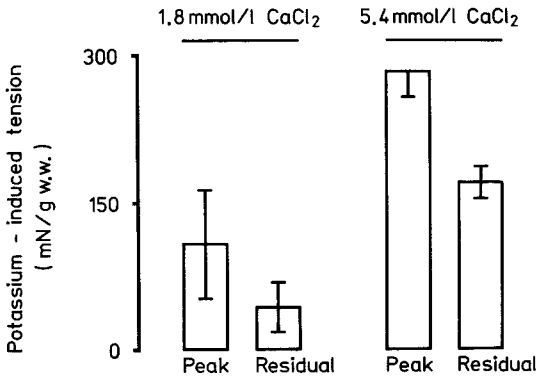

Fig. 4. Comparison of effects of 85 mmol/l extracellular potassium on peak and residual (plateau) tension of isolated human bladder muscle at extracellular calcium concentrations of 1.8 mmol/l ($n = 4$, 2 patients) or 5.4 mmol/l ($n = 32$, 7 patients). Means ± SEM (w.w. = wet weight)

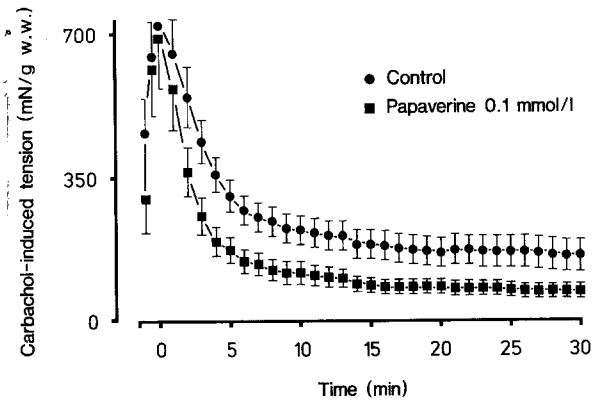

Fig. 6. Time course of effects of papaverine 100 µmol/l on carbachol-induced tension of isolated human bladder muscle. Papaverine had virtually no effect on peak tension induced by carbacol 10^{-4} mol/l Fading was accelerated and steady state tension at 30 min was depressed by about 54%. Circles: control ($n = 20$, 7 patients), squares: papaverine ($n = 7$, patients). Means ± SEM (w.w = wet weight)

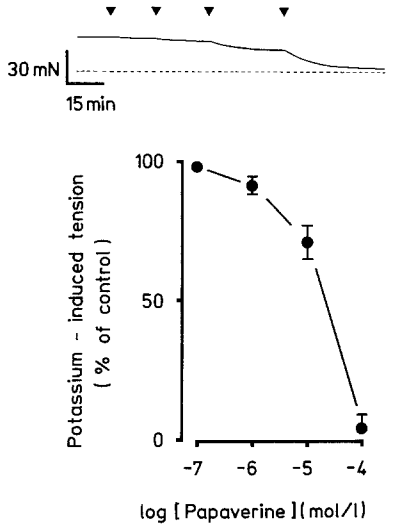

Fig. 5. Effects of papaverine on residual (plateau) tension induced by 85 mmol/l extracellular potassium. Original recording and concentration-response curve. Means ± SEM ($n = 12$, 3 patients)

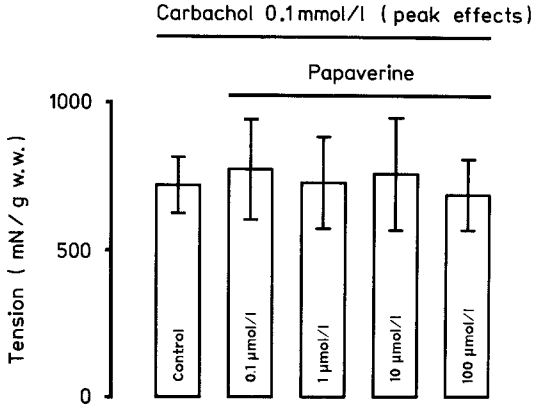

Fig. 7. Effects of various cencentrations of papaverine (10^{-7}-10^{-4} mol/l) on peak tension induced by carbachol 10^{-4} mol/l. Means ± SEM ($n = 7$ in each group, 7 patients, w.w. = wet weight)

contraction remained stable for several hours and varied by less than approximately ± 15%. Figure 4 presents a comparison of the effects of 85 mmol/l extracellular potassium concentration on peak and residual tension at extracellular calcium concentrations of 1.8 or 5.4 mmol/l. The figure clearly shows the dependence of the potassium-induced tension on the extracellular calcium concentration.

Figure 5 displays the relaxing effects of papaverine in high potassium-induced contractions. The muscle strips were activated with an extracellular potassium concentration of 85 mmol/l at a calcium concentration of 5.4 mmol/l. After the contraction had stabilized papaverine was added cumulatively, allowing time between additions for stabilization of relaxation. The inhibition of depolarization-induced contraction was measured as a function of drug concentration. The values are given as percentages of inhibition of equilibrium contraction (control) after depolarization. A concentration of papaverine of 10^{-7} mol/l was the threshold for relaxing activity, and the potassium-induced activation was completely antagonized at a concentration of 10^{-4} mol/l. The concentration of papaverine producing half maximum relaxation was 2.1×10^{-5} mol/l.

The effects of papaverine on carbachol-induced contractions were also studied at extracellular calcium concentrations of 5.4 mmol/l. In the control group ($n = 20$, 7 patients) muscle strips were activated with carbachol 10^{-4} mol/l. In the test group ($n = 7$ in each group from 7 patients) the muscle strips were preincubated for 60 min with papaverine in concentrations of 10^{-7} to 10^{-4} mol/l. Peak tension and time course of tension generation were evaluated under control and test conditions Figure 6 presents the time course of the effects of papaverine of 10^{-4}

mol/l on carbachol-induced contractions. The concentrations-response relationships of papaverine on peak tension of detrusor strips activated by carbachol are displayed in Fig. 7. Both figures clearly show that papaverine, even in high concentrations of 10^{-4} mol/l, had no significant effects on peak tension generation. However, the fading of the carbachol-induced peak response was accelerated by papaverine. The steady state tension at 30 min which amounted to 24 ± 5% peak tension under control conditions was reduced by papaverine 10^{-4} mol/l by about 54%.

Discussion

In the present study we found that contractions induced by carbachol and high extracellular potassium in human isolated bladder muscle were differently affected by papaverine. In carbachol-induced contractions papaverine, even in high concentrations of 10^{-4} mol/l had virtually no effects on peak tension generation, whereas the fading was accelerated and the steady state tension at 30 min. was reduced by about 54%. In contrast, high potassium-induced contractions were relaxed by papaverine in a concentration-dependent way; a concentration of papaverine of 10^{-4} mol/l produced full relaxation.

In our experiments carbachol and high extracellular potassium proved to be the two most prominent means of activation of bladder smooth muscle. It is well established that carbachol and high potassium differ in the way that they induce contractions: carbachol acts by binding to its specific muscarinic receptors on the smooth muscle membrane and high potassium acts by depolarization of the cell membrane which is not related to any specific receptor activation [1]. From our studies utilizing different extracellular calcium concentrations it can be concluded that both types of activation utilize at least in part influx of calcium from the extracellular space, as it has also been shown by several other authors [5, 6, 11, 12]. However, the details of the calcium movements which are responsible for carbachol- or acetylcholine-induced contractions of human bladder smooth muscle are unknown. At least three mechanisms may contribute to a rise in free intracellular calcium concentration: calcium entry through voltage-sensitive channels or receptor-operated channels and release of calcium from intracellular stores. Evidence for the existence of at least two of these pathways in bladder smooth muscle has been provided by several authors [5, 12]. The mechanisms involved in the rapid fading of carbachol-induced contractions are also not yet understood [2]. Either it reflects a reduction of the sensitivity of the muscarinic receptors or a decrease of the intracellular free calcium concentration by some yet undefined mechanisms.

Papaverine is a non-specific smooth muscle relaxant and is thought to act at a site distal to the cholinergic receptor site. However, the precise mode of its action is unknown. One possible mechanism of smooth muscle relaxation by papaverine is related to cyclic adenosine 3'5'-monophosphate (cyclic AMP). Pöch et al. [13] first reported an inhibition of cyclic AMP-breakdown in vitro by a number of spasmolytic drugs. An increase in cyclic AMP is supposed to lead to an enhanced calcium binding to membrane and intracellular storage sites, thereby reducing influx and/or release of calcium. A rise in cyclic AMP may be induced by an inhibition of phosphodiesterase, which degrades cyclic AMP to 5'-adenosine monophosphate (5'AMP). Papaverine has been described as inhibiting cAMP phosphodiesterase activity of smooth muscle, causing a rise in cAMP levels (for review see [1]).

An alternative mechanism of smooth muscle relaxation was proposed by Grün et al. [7] and Fleckenstein et al. [4] for verapamil and some other drugs with relaxing properties. Inhibition of transmembrane calcium influx, first observed with verapamil and D600 in the heart [3], was suggested to be the mechanism of action of these drugs.

Many of papaverine's actions might possibly be explained if it is assumed that it blocks calcium ion channels in the cell membrane [1]. In many smooth muscles papaverine reduces tension induced by high extracelular potassium concentrations and inhibits ^{45}Ca uptake from the preparations when stimulated by high potassium depolarization (for review see [1, 9]. Contractions induced by high extracellular potassium concentrations are easily blocked by low concentrations of calcium channel blockers, suggesting that the continuing entry of calcium through the membrane is necessary to keep the contractile machinery activated [8]. The data presented here confirm the earlier postulate that papaverine inhibits calcium influx [1, 9]. However, the observation that rather high concentrations of papaverine were necessary to fully relax high potassium contractions and the fact that papaverine affects cellular cAMP levels separate this drug from more selective calcium channel blockers.

The mechanisms responsible for the peak tension generation in carbachol-induced contractions are obviously not affected by papaverine even in high concentrations. On the other hand, papaverine accelerates the fading and reduces the steady state tension of carbachol-induced contractions, suggesting some effects on the not yet defined calcium movements in that phase of the carbachol-induced contraction. However, so little is unknown about the cellular mechanism of papaverine's action that at present this study cannot add substantial information to the understanding of the calcium movements during carbachol-induced contractions.

In order to establish if papaverine is useful clinically double blind studies should be performed. Although papaverine had little effects on carbachol-induced contractions it cannot be excluded that the drug is effective in diseases where non-cholinergic mechanisms are involved.

References

1. Bolton TB (1979) Mechanism of action of transmitters and other substances on smooth muscle. Physiol Rev 59:606
2. Brading A (1987) Physiology of bladder smooth muscle. In: Torrens M, Morrison JFB (eds) The physiology of the lower urinary tract. Springer, Berlin Heidelberg New York, p161

3. Fleckenstein A, Döring HJ, Kammermeier H (1968) Einfluß von Beta-Rezeptorenblockern and verwandten Substanzen auf Erregung, Kontraktion und Energiestoffwechsel der Myokardfaser. Klin Wochenschr 46:343
4. Fleckenstein A, Grün G, Tritthart H, Byon K, Harding P (1971) Uterus-Relaxation durch hochaktive Ca-antagonistische Hemmstoffe der elektromechanischen Koppelung wie Isoptin (Verapamil, Iproveratril), Substanz D600 und Segontin (Prenylamin). Klin Wochenschr 49:32
5. Fovaeus M, Andersson K-E, Batra S, Morgan E, Sjögren C (1987) Effects of calcium channel blockers and Bay K 8644 on contractions induced by muscarinic receptor stimulation of isolated bladder muscle from rabbit and man. J Urol 137:798
6. Forman A, Andersson K-E, Henriksson L, Rud T, Ulmsten U (1978) Effects of nifedipine on the smooth muscle of the human urinary tract in vitro and in vivo. Acta Pharmacol Toxicol 43:111
7. Grün G, Fleckenstein A, Tritthart H (1969) Electromechanische Entkoppelung durch muskulotrope Relaxatien der Uterusmuskulatur. Naunyn Schmiedebergs Arch Pharmacol 264:239
8. Högestätt ED, Andersson K-E (1984) Mechanism behind the biphasic contractile response to potassium depolarization in isolated rat cerebral arteries. J Pharmacol Exp Ther 228:187
9. Huddart H, Langton PD, Saad KHM (1984) Inhibition by papaverine of calcium movements and tension in the smooth muscles of rat vas deferens and urinary bladder. J Physiol 349:183
10. Kukovetz WR, Pöch G (1970) Inhibition of cyclic-3' -5' -nucleotide-phosphodiesterase as a possible mode of action of papaverine and similarly acting drugs. Naunyn Schmiedebergs Arch Pharmacol 267:189
11. Malkowicz SB, Wein AJ, Ruggieri MR, Levin RM (1987) Comparison of calcium antagonist properties of antispasmotic agents. J Urol 138:667
12. Mostwin JL (1985) Receptor operated intracellular calcium stores in the smooth muscle of the guinea pig bladder. J Urol 133:900
13. Pöch G, Juan H, Kukovetz WR (1969) Einfluß von herz- und gefäßwirksamen Substanzen auf die Aktivität der Phosphodiesterase. Naunyn Schmiedebergs Arch Pharmacol 264:293
14. Virag R (1982) Intracavernous injection of papaverine for erectile failure. Lancet II:983

Dr. Lothar Hertle
Urologische Klinik
Ruhr-Universität Bochum
Klinikum Marienhospital
Widumer Strasse 8
D-4690 Herne
Federal Republic of Germany