ELSEVIER

European Journal of Pharmacology 335 (1997) 23–30

ejp

Effect of cromakalim on the purinergic and cholinergic transmission in the rat detrusor muscle

Cinzia Boselli, Linda Bianchi, Enzo Grana *

Istituto di Farmacologia, Facoltà di Farmacia, Università di Pavia, Viale Taramelli, 14, I-27100 Pavia, Italy

Received 14 April 1997; revised 23 June 1997; accepted 4 July 1997

Abstract

Contraction of the rat detrusor muscle is mediated by cholinergic and purinergic mechanisms. The present study was carried out to look at the influence of cromakalim, compared with atropine, suramin and nifedipine on the contractile response evoked by single shock and exogenous agonists (carbachol and ATP) in rat urinary bladder. Cromakalim was able to inhibit only partially the response to carbachol and profoundly affected the response to exogenous ATP. Atropine suppressed the response to carbachol and was inactive versus ATP. Suramin was inactive versus carbachol and was able to antagonize the response to ATP. Nifedipine proved to be a non-competitive antagonist versus carbachol ($pD_2 = 7.66 \pm 0.05$) and deeply affected the response to ATP. Cromakalim inhibited only partially the first, purinergic, phase of the electrically evoked response but was able to inhibit in a concentration-dependent manner the second, cholinergic, phase ($\log IC_{50} = 6.87 \pm 0.05$). Nifedipine blocked both the phases. Atropine blocked partially only the second phase. Suramin inhibited the first phase but, at least partially, also the second one. The combination of atropine and suramin enhanced the inhibition of the second phase. The antagonistic effect of suramin on the second phase could indicate an overlap of the purinergic and cholinergic components. The comparison between pre- and postjunctional effects indicates that cromakalim acts on purinergic transmission predominantly postjunctionally. On the contrary, the action on cholinergic transmission seems to occur mainly at prejunctional level. This conclusion can be relevant in view of the claimed importance of K^+ channel openers in the treatment of urinary disorders. © 1997 Elsevier Science B.V.

Keywords: Detrusor muscle, rat; Purinergic transmission; Cholinergic transmission; Cromakalim; Atropine; Suramin; Nifedipine

1. Introduction

Bladder contraction is mediated by both cholinergic and nonadrenergic, noncholinergic (NANC) mechanisms. Muscarinic receptor stimulation is responsible for the main, cholinergic, part of bladder emptying and ATP may be the main NANC excitatory transmitter (Andersson, 1993, for review).

Recently, clinical applications of the K^+ channel openers have been proposed as a novel approach to treating urinary bladder disorders. K^+ channel openers have been reported to hyperpolarize smooth muscle cells and, therefore, might be effective in the treatment of bladder instability by directly inhibiting the excitability of detrusor smooth muscle, although other mechanisms can not be excluded. Cromakalim and other K^+ channel openers have been quite extensively studied as concerns their action on the detrusor muscle (Andersson, 1993, for review; Zhou et al., 1995), but there is a paucity of informations concerning a possible prejunctional action of cromakalim in the bladder. Recently we have demonstrated that in rat vas deferens cromakalim inhibits the release of ATP (Grana et al., 1997). Some authors demonstrated that cromakalim had inhibitory effects on cholinergic and NANC neuroeffector transmission in non-vascular smooth muscle preparations like guinea-pig trachea (Burka et al., 1991) and in guinea-pig airways in vivo (Ichinose and Barnes, 1990).

In the present study we investigated the possible prejunctional inhibitory effect of cromakalim on cholinergic and NANC neuroeffector transmission in electrically stimulated isolated preparations of detrusor muscle of rat. Research has been extended to study the action of suramin and atropine on the response induced by a single shock and to investigate the effects of these antagonists and cromakalim on the contraction produced by carbachol and ATP.

* Corresponding author. Tel.: (39-382) 507-395; Fax: (39-382) 507-405; e-mail: egrana@ipv36.unipv.it

In view of the fact that cromakalim produces smooth muscle hyperpolarization and reduces the probability of the opening of voltage-sensitive Ca^{2+} channels (Cook and Quast, 1990) the effect of nifedipine, an L-type Ca^{2+} channel blocker, was also investigated.

2. Materials and methods

2.1. Isolated rat urinary bladder preparations

Male Wistar-Nossan rats (250–350 g body weight) were killed by CO_2 asphyxiation and exsanguination. Rat urinary bladder was quickly removed and placed in oxygenated (95% O_2–5% CO_2) Krebs–Henseleit physiological solution of the following composition (mM): NaCl 118; KCl 5.6; $CaCl_2$ 2.5; $MgSO_4$ 1.19; $NaHPO_4$ 1.3; $NaHCO_3$ 25, EDTA 0.003 and D-glucose 10. Longitudinal strips of the extratrigonal portion of the detrusor muscle (8–10 mm long and 1–1.5 mm wide) were dissected and placed in heated (37°C ± 0.5) 20 ml organ baths. The strip was tied at one end to the organ bath across two platinum electrodes; the other end was connected to a Mangoni isometric transducer under a resting tension of 1 g. Tissues were equilibrated for at least 45 min with bath fluid changes every 10 min.

2.2. Experiments involving exogenous spasmogens

After equilibration period each tissue was challenged with a submaximal concentration of carbachol equal to 1×10^{-5} M. This single concentration of carbachol was left in contact with the tissue for about 30 s and only the phasic component of the contractile response was considered. The administration of carbachol was repeated twice or three times up to the attainment of a reproducible response.

Then cumulative concentration–response curves to carbachol were obtained by dosing at 0.5 log unit intervals. Higher concentrations of agonist were added to the organ bath after the response to the previous concentration had reached a plateau.

After two reproducible cumulative concentration–response curves were obtained, the tissue was left to equilibrate with a fixed concentration of compound under investigation for 20 min (atropine, cromakalim or nifedipine) or 40 min (suramin). Then the cumulative concentration–response curve to carbachol in the presence of a given concentration of compound under investigation or vehicle was obtained. Only one concentration of each compound was tested on each tissue. The responses were expressed as percentage of the maximal contraction obtained in the cumulative concentration–response curve constructed in the absence of the test compound.

A different protocol was used to study the effects of compounds under investigation (atropine, cromakalim, nifedipine and suramin) on the contraction induced by ATP. Preliminary experiments demonstrated that 1×10^{-4} M ATP produced a very weak contraction. Therefore 3×10^{-4} M ATP was used to study the effects of the compounds under investigation on exogenous ATP. No desensitization occurred with a rest interval of at least 30 min between two following administrations and the contractile response to 3×10^{-4} M ATP was reproducible.

After two identical responses had been elicited the effect of the test compound was investigated. A given concentration of compound or vehicle was added to the organ bath and was left in contact for 20 min (atropine, cromakalim and nifedipine) or 40 min (suramin) before testing the agonist. Only one agonist and only one concentration of each compound under examination was tested on each preparation.

When glibenclamide was tested the protocol was as follows. After two reproducible control responses were obtained, glibenclamide (3×10^{-6} M) was allowed to equilibrate for 20 min and then a concentration of cromakalim was added to the organ bath for 20 min. Thereafter the response to ATP (3×10^{-4} M) was tested.

Results are expressed as percentage of inhibition exerted by each compound under investigation of the control response to exogenous ATP.

2.3. Experiments involving transmural stimulation

Each tissue was challenged with a reference concentration of carbachol (1×10^{-5} M) as reported above. Then strips of the extratrigonal portion of the detrusor muscle were stimulated with a single stimulus (field stimulation, square waves, threshold voltage + 100%, 1 ms duration) to produce isometric contractions. Preliminary experiments indicated that a 2 ms width stimulus did not produce any increase of the biphasic response to single-pulse field stimulation (data not shown) and that no desensitization of the 1 ms width stimulus occurred with a rest interval of at least 20 min; the response was reproducible over a period of 3 h. After two reproducible control responses to single-pulse field stimulation were obtained, each tissue was equilibrated with a fixed concentration of test compound alone or in combination before measurement of responses to a further single-pulse field stimulation. Preliminary experiments indicated that the maximum effect was reached within 20 min contact time for atropine, cromakalim and nifedipine and within 40 min for suramin.

Only one concentration of each compound was tested on each tissue. Responses are expressed as percentage of inhibition of the pre-drug(s) response.

2.4. Drugs

The following drugs were used: (−)-noradrenaline bitartrate (Sigma), atropine sulphate monohydrate (Koch-Light), suramin sodium (a generous gift from Dr. A.

Faggiotto, Bayer), cromakalim (Sigma), ATP disodium (Sigma), nifedipine (Sigma), glibenclamide (Sigma). Carbachol (10^{-2} M), suramin (10^{-2} M) and atropine (10^{-2} M) were dissolved once a week, the stock solutions were kept frozen and dilutions were made daily in double-distilled water. ATP (10^{-2} M) was dissolved daily in double-distilled water. Cromakalim (10^{-2} M) was dissolved in ethanol 70% (v/v) and dilutions were made daily in double-distilled water. Nifedipine (10^{-3} M) was dissolved in ethanol 95% (v/v). Dilutions were made daily in double-distilled water. Because nifedipine is highly unstable if exposed to daylight or to ordinary laboratory light, all experiments involving the use of nifedipine were conducted in a laboratory illuminated solely with sodium light.

Glibenclamide (10^{-3} M) was dissolved in dimethylsulfoxide (final concentration of solvent less than 0.5% and without effect).

2.5. Statistics

For atropine linear regression was calculated and slope analyzed with respect to the differences from unity using 95% confidence limits according to Tallarida and Murray (1987). $pA_2 \pm$ S.E.M. was estimated by Schild analysis (Arunlakshana and Schild, 1959), fitting linear regression by least squares and verifying parallelism before calculating dose-ratios (Tallarida and Murray, 1987).

For nifedipine $pD_2 \pm$ S.E.M. was calculated graphically by Van Rossum (1963) analysis.

For cromakalim $-\log IC_{50} \pm$ S.E.M. was calculated with GraphPadPrism (a data analysis package, San Diego, CA).

Results are expressed as mean values $\pm$ S.E.M. Statistical analysis of the results was performed using the Student's *t*-test for paired or unpaired data. $P < 0.05$ was taken as the significance criterion.

3. Results

Preliminary experiments pointed out that more than 60% of the tissues showed spontaneous activity characterized by intermittent twitches with height on average equal to $3.87 \pm 0.59\%$ ($n = 23$, data not shown) of the phasic contractile response elicited by carbachol (1×10^{-5} M), i.e., the concentration of carbachol challenged as single dose at the beginning of each experiment which produced a contraction equal to 2.95 ± 0.24 g ($n = 23$, data not shown).

3.1. Action of exogenous spasmogens and of test compounds on the extratrigonal portion of rat urinary bladder

Fig. 1 shows the concentration–response curves to carbachol in absence and in presence of suramin (3×10^{-4} M, contact time 40 min).

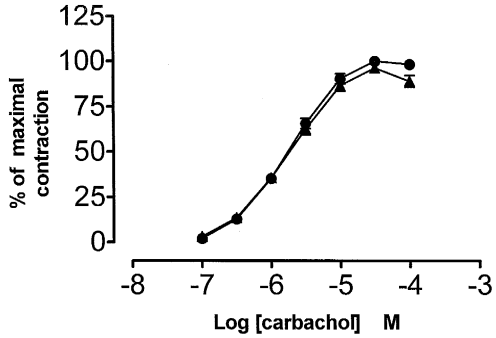

Fig. 1. Mean concentration–response curves for carbachol in the absence (filled circle, $n = 4$), and presence of suramin 3×10^{-4} M (filled triangle, $n = 4$). Bars represent S.E.M..

The P_{2x} purinoceptor antagonist suramin resulted devoid of any inhibitory effect.

In Fig. 2 are shown the concentration–response curves to carbachol in absence and in presence of cromakalim (3×10^{-6}–1×10^{-5}–3×10^{-5} M, contact time 20 min). Cromakalim at concentration 3×10^{-6} M and 1×10^{-5} M produced a concentration-dependent but modest shift to the right with significant ($P < 0.05$) depression of the maximal effect of the concentration–response curve to carbachol by $9.39 \pm 4.12\%$ and $21.32 \pm 2.17\%$, respectively (Fig. 2). The highest concentration of cromakalim tested (3×10^{-5} M) produced no further inhibitory effect as depicted in Fig. 2. It is relevant to notice that cromakalim starting from 3×10^{-6} M after 3 min of contact time was able to inhibit the spontaneous activity, when present.

Nifedipine at concentrations of 1×10^{-8} M, 3×10^{-8} M and 1×10^{-7} M significantly ($P < 0.05$) flattened in a concentration-dependent fashion the concentration–response curve to carbachol; the maximum response was depressed by $20.46 \pm 2.20\%$, $62.36 \pm 6.09\%$ and $83.62 \pm 2.83\%$, respectively (Fig. 3), with a pD_2 of 7.66 ± 0.05. Nifedipine at all the concentrations tested in the present

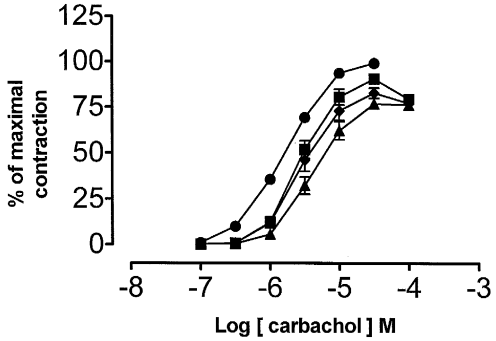

Fig. 2. Mean concentration–response curves for carbachol in the absence (filled circle, $n = 21$), and presence of cromakalim 3×10^{-6} M (filled square, $n = 6$), 1×10^{-5} M (filled triangle, $n = 8$) 3×10^{-5} M (filled diamond, $n = 7$). Bars represent S.E.M., where not visible the vertical bars lie within the sign.

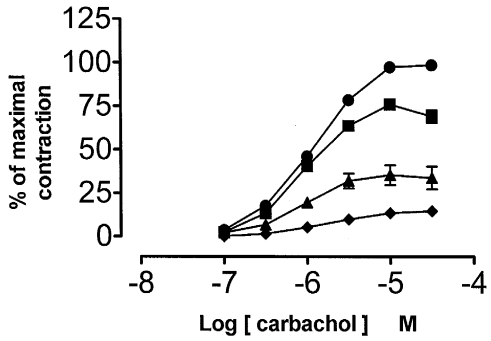

Fig. 3. Mean concentration–response curves for carbachol in the absence (filled circle, $n = 21$), and presence of nifedipine 1×10^{-8} M (filled square, $n = 8$), 3×10^{-8} M (filled triangle, $n = 6$), 1×10^{-7} M (filled diamond, $n = 7$). Bars represent S.E.M., where not visible the vertical bars lie within the sign.

study was able to completely suppress the spontaneous activity when occurred.

Concentration–response curves to carbachol in the absence and presence of increasing concentrations of atropine (1×10^{-9}–3×10^{-9}–1×10^{-8}–3×10^{-8} M) are reported in Fig. 4. The resulting Schild regression was linear and the pA_2 value of 9.45 ± 0.02 was obtained from a Schild plot constrained to unity (Mackay, 1978).

Then the effects of suramin, cromakalim, nifedipine and atropine were analyzed on the responses due to purinoceptor activation using ATP as agonist.

The response to 1×10^{-4} M ATP, a concentration that produced a contraction similar to that induced by endogenous ATP, as described below, consisted of a phasic contraction amounting to $7.14 \pm 1.55\%$ of the response elicited by 1×10^{-5} M carbachol. It was often difficult to distinguish clearly the ATP-induced contraction from the basal spontaneous spikes. Therefore, a concentration of ATP 3-fold higher was used to test all the compounds under investigation. ATP (3×10^{-4} M) produced a monophasic contraction equal to $16.24 \pm 1.66\%$ of the

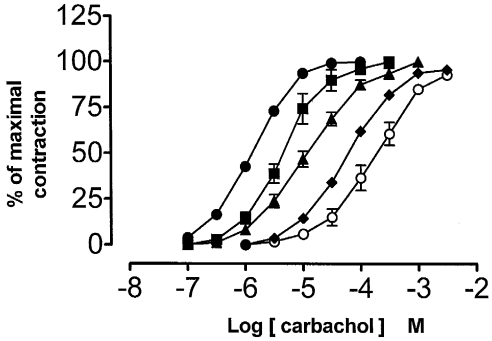

Fig. 4. Mean concentration–response curves for carbachol in the absence (filled circle, $n = 20$), and presence of atropine 1×10^{-9} M (filled square, $n = 5$), 3×10^{-9} M (filled triangle, $n = 5$), 1×10^{-8} M (filled diamond, $n = 5$), 3×10^{-8} M (open circle, $n = 5$). Bars represent S.E.M., where not visible the vertical bars lie within the sign.

Table 1
Antagonism by cromakalim, suramin, atropine and nifedipine on the response to ATP on the extratrigonal portion of rat urinary bladder

	ATP 3×10^{-4} M	n
Cromakalim 1×10^{-5} M	66.54 ± 1.98 [a]	6
Suramin 3×10^{-4} M	80.14 ± 10.02 [a]	5
Atropine 1×10^{-7} M	3.88 ± 9.91	4
Nifedipine 1×10^{-6} M	77.55 ± 4.68 [a]	7

Results are expressed as the percentage of inhibition (mean ± S.E.M.) of the response to ATP in the absence (control) of the test compounds.
[a] Student's t-test for paired data, $P < 0.05$.

contraction produced by 1×10^{-5} M carbachol. The reproducibility of the contractile response to ATP was good (data not shown) using intervals of 20 min between two consecutive administrations.

Atropine (1×10^{-7} M) left the response to exogenous ATP practically unaffected (Table 1). Suramin 3×10^{-4} M profoundly affected the contractile response to ATP 3×10^{-4} M (Table 1). Cromakalim 1×10^{-5} M inhibited by $66.54 \pm 1.98\%$ the response to exogenous ATP (Table 1) and the ATP sensitive K^+channel inhibitor glibenclamide (3×10^{-6} M) was able to almost completely counteract this inhibitory action of cromakalim (data not shown). The L-type Ca^{2+} channel blocker nifedipine 1×10^{-6} M inhibited the contractile response produced by ATP at the same extent as suramin (Table 1).

3.2. Transmural stimulation of extratrigonal portion of rat urinary bladder

The mechanical response of the extratrigonal portion of rat urinary bladder to single-pulse field stimulation was generally biphasic. It consisted of an early phase (phase I) peaking at about 0.5 s followed by a second slower phase (phase II) peaking at about 3 s (Fig. 5A). Mean absolute values of early and late components of the electrically induced contraction were 0.21 ± 0.03 g and 0.25 ± 0.04 g ($n = 12$), respectively.

Only when the tissues exhibited spontaneous activity was a third component observed, on the descending arm of the second phase of the contraction, at about 5 s after the start of the contractile response to single-pulse field stimulation (Fig. 5B).

Suramin (3×10^{-5} M) left unaffected both the phases of the response to single-pulse field stimulation but at the highest concentration used in this study (3×10^{-4} M) it markedly inhibited the first phase and severely affected the second one (Table 2). Atropine at a concentration of 1×10^{-7} M was devoid of any action on the first phase and depressed by $61.98 \pm 6.74\%$ the second one (Table 2). A concentration of atropine 30-fold higher (3×10^{-6} M) caused no further inhibition of either phase. The combination of suramin (3×10^{-4} M) and atropine (1×10^{-7} M) abolished, to the same extent as suramin alone, the first

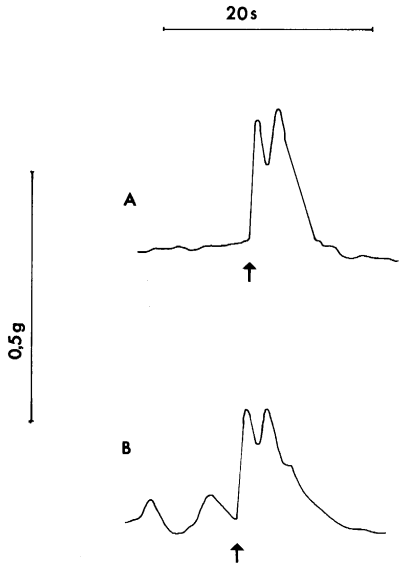

Fig. 5. Typical tracings showing the early (phase I) and late (phase II) components of the response induced by single-pulse field stimulation in the rat urinary bladder (A). A third delayed component can be observed only in tissue with spontaneous activity (B). Arrows indicate the point at which electrical stimulation was applied.

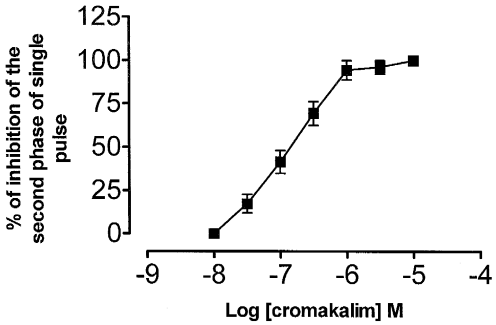

Fig. 6. Inhibitory effect of cromakalim on the second phase of the response (filled square) to single-pulse field stimulation in the rat urinary bladder. Each point is the mean ± S.E.M. of 6–9 observations; where not visible the vertical bars lie within the sign.

phase but the inhibition of the second component was significantly ($P < 0.05$) increased (Table 2).

Increasing concentrations of cromakalim (from 1×10^{-8} M to 1×10^{-5} M) resulted in a concentration-dependent inhibition of the second phase of the response to single-pulse field stimulation ($-\log IC_{50} = 6.87 \pm 0.03$) with a maximum inhibition of 100% (Fig. 6). The first phase of the response was affected only partially and in a non-linear way as reported in Table 2.

Nifedipine (from 1×10^{-7} M to 1×10^{-6} M) caused a concentration-dependent inhibition of both the phases and the highest concentration (1×10^{-6} M) was almost able to abolish the electrically induced contraction (Table 2).

As reported above, when the tissues showed spontaneous activity a third delayed phase was present. This phase was abolished by all the concentrations of nifedipine tested and by cromakalim from 3×10^{-7} M (data not shown).

4. Discussion

The main aim of this work was to study the action of cromakalim on cholinergic and purinergic transmission at pre- and postjunctional level in rat urinary bladder. The action of cromakalim was compared with that of nifedip-

Table 2
Antagonism by cromakalim, atropine, suramin and nifedipine alone or in combination on the response to single-pulse field stimulation on the extratrigonal portion of rat urinary bladder

	phase I	phase II	*n*
Cromakalim 1×10^{-8} M	0.51 ± 0.51	0	6
Cromakalim 3×10^{-8} M	4.18 ± 1.84	17.28 ± 5.43 [a]	9
Cromakalim 1×10^{-7} M	18.96 ± 8.46 [a]	41.55 ± 6.64 [a]	9
Cromakalim 3×10^{-7} M	15.47 ± 9.84 [a]	69.42 ± 6.84 [a]	6
Cromakalim 1×10^{-6} M	18.28 ± 10.75 [a]	94.45 ± 5.55 [a]	6
Cromakalim 3×10^{-6} M	41.12 ± 10.53 [a]	96.14 ± 3.86 [a]	8
Cromakalim 1×10^{-5} M	22.62 ± 3.41 [a]	100 [a]	9
Atropine 1×10^{-7} M	7.13 ± 4.96	61.98 ± 6.74 [a]	7
Atropine 3×10^{-6} M	5.17 ± 2.44	45.71 ± 2.71 [a]	7
Suramin 3×10^{-5} M	0	0	4
Suramin 3×10^{-4} M	72.62 ± 7.01 [a]	55.19 ± 10.64 [a]	6
Nifedipine 1×10^{-7} M	39.24 ± 7.44 [a]	58.11 ± 11.55 [a]	7
Nifedipine 3×10^{-7} M	78.05 ± 2.51 [a]	96.85 ± 1.99 [a]	6
Nifedipine 1×10^{-6} M	94.60 ± 1.95 [a]	100 [a]	6
Atropine 1×10^{-7} M + suramin 3×10^{-4} M	75.50 ± 3.58 [a]	89.78 ± 1.60 [a]	7

Results are expressed as the percentage of inhibition (mean ± S.E.M.) of the response to single-pulse field stimulation in the absence (control) of the test compound(s).

[a] Student's *t*-test for paired data, $P < 0.05$.

ine, suramin and atropine. In the current literature there is a relative shortage of information about many aspects of this problem: anyhow, when a comparison is practicable our data generally confirmed and extended previous observations. As concerns the action of compounds under examination on the contraction induced by carbachol, suramin resulted devoid of any action as demonstrated by Hoyle et al. (1990) in the guinea-pig urinary bladder. Nifedipine seemed to behave as noncompetitive antagonist versus carbachol: the proportion of the contraction that Bo and Burnstock (1990) described as nifedipine-resistant, if present, was very small. Atropine, as expected, behaved as competitive antagonist. Cromakalim could be described as a dualistic antagonist (Ariëns, 1964) in view of the fact that it caused a rightward shift of the concentration–response curve of carbachol with a concomitant reduction of the maximal response: such a definition is strongly hindered because both the effects were limited and both did not enhance when the concentration of cromakalim was increased. Zhou et al. (1995) demonstrated that cromakalim partially inhibited the contractile response to acetylcholine in rat urinary bladder, but in the guinea-pig urinary bladder cromakalim was not able to prevent the contractile response to carbachol (Foster et al., 1989). There is much information about the spontaneous activity of the rat urinary bladder that we found in about 60% of preparations (Bath et al., 1989; Luheshi and Zar, 1990; Edwards et al., 1991; Parija et al., 1991; Zhou et al., 1995). Cromakalim was able to inhibit this activity.

When the action of the compounds under investigations on the contraction induced by ATP was considered, the data reported in this study about the inhibitory action exerted by nifedipine agreed with those presented in the literature, although they were obtained under different experimental conditions (Bath et al., 1989; Bo and Burnstock, 1990; Katsuragi et al., 1990). Suramin, as demonstrated by Hoyle et al. (1990) and Usune et al. (1996) in the guinea-pig urinary bladder, blocked the response to ATP, while atropine was practically inactive, as previously reported by Maggi (1991). A relevant antagonistic action unreported so far can be ascribed to cromakalim. The involvement of ATP-sensitive K^+ channels was confirmed by the ability of glibenclamide to reverse completely this inhibitory effect of cromakalim. This result disagreed with the lack of any inhibitory action of cromakalim on ATP-induced contraction, as shown in our previous work on the rat vas deferens (Grana et al., 1997). The greater involvement of the ATP-sensitive K^+ channels in the purinergic response of urinary bladder than in the vas deferens level is, so far, without any dependable explanation.

The analysis of the results obtained, evaluating the action of the compounds under investigation on the response induced by electrical stimulation led to the following considerations.

First of all, we confirmed that the response to single-pulse field stimulation of the rat detrusor muscle was biphasic. It consisted of an 'early' NANC, putative purinergic, component (phase I) and of a 'late' formally cholinergic one (phase II) (Maggi et al., 1985; Maggi, 1991; Parija et al., 1991; Luheshi and Zar, 1990). In preparations with great spontaneous activity a third unclassified component could be evidenced as reported by Parija et al. (1991). This third component was abolished by cromakalim and nifedipine at the same concentrations that were able to abolish the spontaneous activity.

Atropine, as expected (Maggi, 1991), left practically unaffected phase I and inhibited about 60% of phase II at concentrations at which the response to carbachol was abolished.

The results reported in the present study showed that suramin (3×10^{-4} M) markedly but not completely depressed the first phase and impaired the second one. It should be noticed that suramin has never been previously used as antagonist of the neurogenic response in rat urinary bladder but its action on phase I was easily understandable on the basis of the results obtained with other antagonists of the purinergic response (Parija et al., 1991; Maggi, 1991; Luheshi and Zar, 1990). The combination of atropine and suramin almost completely abolished phase II but did not modify phase I. The partial inhibition of the first phase by suramin alone or with atropine could suggest that phase I is mainly purinergic, but ATP may not be the only NANC transmitter. The presence in this tissue of another so far unknown neurotransmitter coreleased with ATP is supported by the findings of Choo and Mitchelson (1980), Luheshi and Zar (1990) and Creed and Tulloch (1991). The activation of neuronal 5-HT_4 receptors could be taken into account (Tonini and Candura, 1996). On the other hand, the incomplete inhibition of phase I by suramin could also be due to the presence in this tissue of suramin-insensitive purinergic receptors as described by Von Kügelgen et al. (1989) and Bailey and Hourani (1994) for other preparations (i.e., rat, mouse and guinea-pig vas deferens).

Less understandable was the action of suramin on phase II, which seemed to be prevailingly but not exclusively cholinergic. The partial inhibition of the second phase by suramin alone and the potentiation of the effect of atropine could suggest the presence of an overlap of the purinergic phase with the cholinergic one. A similar situation occurred in the prostatic portion of rat vas deferens as demonstrated in our previous work (Grana et al., 1997). Furthermore, another phenomenon complicating the response to single-pulse field stimulation in this tissue could occur: an ecto-ATPase blocking property of suramin reported in the rabbit isolated ear artery by Crack et al. (1994) and in the guinea-pig isolated urinary bladder by Hourani and Chown (1989). This could prolong ATP life sustaining phase I and making phase II less clear and probably the resultant of more than one phenomenon.

Nifedipine (from 1×10^{-7} M to 1×10^{-6} M) inhibited in a concentration-dependent fashion both the phases and

at the highest concentration used abolished both the responses induced by single-pulse field stimulation. It is important to notice that our data obtained in the rat detrusor muscle on the inhibitory action exerted by nifedipine on both phase I and phase II of the response to single-pulse field stimulation are in agreement with those reported in the literature by many authors (Bath et al., 1989; Bo and Burnstock, 1990; Maggi, 1991): the solely appreciable discrepancy was the total inhibition seen by us but not by all the quoted authors.

The experiments carried out studying the action of cromakalim put in evidence that this compound was able to inhibit only partially the purinergic component but showed a complete inhibitory action, strictly concentration dependent versus the cholinergic component which occurred at a concentration range relatively low ($-\log IC_{50} = 6.87 \pm 0.03$).

These results should be considered keeping in mind the results obtained with the exogenous agonists ATP and carbachol.

In the case of suramin, atropine and nifedipine the inhibitory actions seen on phase I and/or phase II should be considered largely due to postjunctional effects in view of the fact that these effects could be obtained also when exogenous stimulants were applied.

In the case of cromakalim the results are worthy of the highest attention. The inhibitory action of this compound on the purinergic phase of single-pulse field stimulation seemed to be modest and quite lower than that exerted on exogenous ATP: this led to exclude any relevant action of cromakalim on the release of ATP following electrical stimulation of the rat urinary bladder and this action is therefore widely explainable as postjunctional effect. It is important to put in evidence that in a previous work we succeeded to demonstrate that cromakalim inhibited the release of ATP in the rat vas deferens (Grana et al., 1997). Therefore one of the most relevant results presented in this work is the occurrence of a deep difference among mechanisms underlying the release of ATP when this is a co-transmitter in noradrenergic and cholinergic nerve endings, respectively.

Furthermore, the marked action of cromakalim on phase II that we demonstrated is of relevant importance because it is hardly ascribable only to the postjunctional effect that we found to be very modest; this effect could be probably explained with an action exerted also prejunctionally: this explanation could be in agreement with the findings reported by Ichinose and Barnes (1990) on guinea-pig airways and Zini et al. (1991) in the guinea-pig isolated small intestine.

The prejunctional, indirectly 'anticholinergic' effect of cromakalim might be of interest in light of the tremendous interest recently developed in the use of K^+ channel openers as a novel approach to treating urinary incontinence.

Acknowledgements

This work was supported by F.A.R. University of Pavia.

References

Andersson, K.E., 1993. Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. Pharmacol. Rev. 45, 253–308.

Ariëns, E.J., 1964. Drug-receptor interaction: Interaction of one or more drugs with different receptor systems. In: Molecular Pharmacology. Academic Press, New York, NY, pp. 287–393.

Arunlakshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48–58.

Bailey, S.J., Hourani, S.M.O., 1994. Differential effects of suramin on P_2 purinoceptors mediating contraction of the guinea pig vas deferens and urinary bladder. Br. J. Pharmacol. 112, 219–225.

Bath, M.B., Mishra, S.K., Raviprakash, V., 1989. Differential susceptibility of cholinergic and noncholinergic neurogenic responses to calcium channel blockers and low Ca^{2+} medium in rat urinary bladder. Br. J. Pharmacol. 96, 837–842.

Bo, X., Burnstock, G., 1990. The effects of Bay K 8644 and nifedipine on the responses of rat urinary bladder to electrical field stimulation, β,γ-methylene ATP and acetylcholine. Br. J. Pharmacol. 101, 494–498.

Burka, J.F., Berry, J.L., Foster, R.W., Small, R.C., Watt, A.J., 1991. Effects of cromakalim on neurally-mediated responses of guinea pig tracheal smooth muscle. Br. J. Pharmacol. 104, 263.

Choo, L.K., Mitchelson, F., 1980. The effect of indomethacin and adenosine 5′-triphosphate on the excitatory innervation of the rat urinary bladder. Can. J. Physiol. Pharmacol. 58, 1042–1048.

Cook, N.S., Quast, U., 1990. Potassium channel pharmacology. In: Cook, N.S. (Ed.), Potassium Channels. Ellis Horwood, Chichester, pp. 181–255.

Crack, B.E., Beukers, M.W., McKechnie, K.C.W., Ijzerman, A.P., Leff, P., 1994. Pharmacological analysis of ecto-ATPase inhibition: Evidence for combined enzyme inhibition and receptor antagonism in P_{2x}-purinoceptor ligands. Br. J. Pharmacol. 113, 1432–1438.

Creed, K.E., Tulloch, A.G.S., 1991. The effect of pelvic nerve stimulation and some drugs on the urethra and bladder of the dog. Br. J. Urol. 50, 398–405.

Edwards, G., Henshaw, M., Miller, M., Weston, A.H., 1991. Comparison of the effects of several potassium channel openers on rat urinary bladder and rat portal vein in vitro. Br. J. Pharmacol. 102, 679–686.

Foster, C.D., Fujii, K., Kingdom, J., Brading, A.F., 1989. The effect of cromakalim on the smooth muscle of the guinea pig urinary bladder. Br. J. Pharmacol. 97, 281–291.

Grana, E., Boselli, C., Bianchi, L., 1997. Cromakalim blocks the purinergic response evoked in rat vas deferens by single-pulse electrical stimulation. Eur. J. Pharmacol. 319, 57–64.

Hourani, S.M.O., Chown, J.A., 1989. The effect of some possible inhibitors of ectonucleotidases on the breakdown and pharmacological effects of ATP in the guinea pig urinary bladder. Gen. Pharmacol. 20, 413–416.

Hoyle, C.H.V., Knight, G.E., Burnstock, G., 1990. Suramin antagonizes responses to P_2-purinoceptor agonists and purinergic nerve stimulation in the guinea-pig urinary bladder and taenia coli. Br. J. Pharmacol. 99, 617–621.

Ichinose, M., Barnes, P.J., 1990. A potassium channel activator modulates both excitatory noncholinergic and cholinergic neurotransmission in guinea pig airways. J. Pharmacol. Exp. Ther. 252, 1207.

Katsuragi, T., Usune, S., Furukawa, T., 1990. Antagonism by nifedipine of contraction and Ca^{2+}-influx evoked by ATP in guinea pig urinary bladder. Br. J. Pharmacol. 100, 370–374.

Luheshi, G., Zar, A., 1990. Purinoceptor desensitization impairs but does not abolish the non-cholinergic motor transmission in rat isolated urinary bladder. Eur. J. Pharmacol. 185, 203–208.

Mackay, D., 1978. How should values of the pA_2 and affinity constants for pharmacological competitive antagonists be estimated?. J. Pharm. Pharmacol. 30, 312–313.

Maggi, C.A., 1991. Omega conotoxin and prejunctional modulation of the biphasic response of the rat isolated urinary bladder to single pulse electrical field stimulation. J. Auton. Pharmacol. 11, 295–304.

Maggi, C.A., Santiccioli, P., Meli, A., 1985. Pharmacological evidence for the existence of two components in the twitch response to field stimulation of detrusor strips from the rat urinary bladder. J. Auton. Pharmacol. 5, 221–230.

Parija, S.C., Raviprakash, V., Mishra, S.K., 1991. Adenosine- and α,β-methylene ATP-induced differential inhibition of cholinergic and non-cholinergic neurogenic responses in rat urinary bladder. Br. J. Pharmacol. 102, 396–400.

Tallarida, R.J., Murray, R.B., 1987. Manual of pharmacologic calculations with computer programs. Springer-Verlag, New York, NY.

Tonini, M., Candura, S.M., 1996. 5-HT_4 receptors agonists and bladder disorders. Trends Pharmacol. Sci. 17, 314.

Usune, S., Katsuragi, T., Furukawa, T., 1996. Effects of PPADS and suramin on contractions and cytoplasmic Ca^{2+} changes evoked by AP_4A, ATP and α,β-methylene ATP in guinea-pig urinary bladder. Br. J. Pharmacol. 117, 698–702.

Van Rossum, J.M., 1963. Cumulative dose–response curve. II. Technique for the making of dose–response curve in isolated organs and evaluation of drug parameters. Arch. Int. Pharmacodyn. Ther. 143, 299–330.

Von Kügelgen, I., Bultmann, R., Starke, K., 1989. Effect of suramin and α,β-methylene-ATP indicate noradrenaline-ATP cotransmission in the response of the mouse vas deferens to single and low frequency pulses. Naunyn-Schmiedeberg's Arch. Pharmacol. 340, 760.

Zini, S., Ben-Ari, Y., Ashford, M.L.J., 1991. Characterization of sulfonylurea receptors and the action of potassium channel openers on cholinergic neurotransmission in guinea pig isolated small intestine. J. Pharmacol. Exp. Ther. 259, 566–573.

Zhou, Q., Satake, N., Shibata, S., 1995. The inhibitory mechanisms of nicorandil in isolated rat urinary bladder and femoral artery. Eur. J. Pharmacol. 273, 153–159.