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European Journal of Pharmacology 319 (1997) 57–64

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Cromakalim blocks the purinergic response evoked in rat vas deferens by single-pulse electrical stimulation

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Received 14 March 1996; revised 14 October 1996; accepted 15 October 1996

Abstract

The present study was carried out to look at the influence of the K^+ channel opener cromakalim, compared with suramin and prazosin, on the contractile response evoked by single-pulse field stimulation and exogenous agonists in epididymal and prostatic portions of rat vas deferens. In the epididymal portion suramin abolished the first phase of the response to single shock, prazosin deeply affected the second phase and a combination of both antagonists almost completely abolished both phases. Cromakalim was able to inhibit in a concentration-dependent manner the first purinergic phase ($pD_2 = 5.90 \pm 0.11$), leaving practically unaffected the second, adrenergic phase. This inhibitory effect of cromakalim on the electrically evoked response was counteracted by glibenclamide. Cromakalim and prazosin, but not suramin, affected the response to exogenous noradrenaline. Suramin but not cromakalim was able to antagonize responses to α,β-methylene-ATP. In the prostatic portion because of a less clear discrimination between adrenergic and purinergic phases of the electrically evoked response, the picture was less clear although the trend was identical. Cromakalim was not able to antagonize the response to ATP. It is concluded that in rat vas deferens cromakalim inhibits purinergic transmission by acting prejunctionally.

Keywords: Vas deferens, rat; Purinergic transmission; Noradrenergic transmission; Cromakalim; Prazosin; Suramin; Glibenclamide

1. Introduction

Cromakalim belongs to the group of drugs which are classified pharmacodynamically as potassium channel openers. Although the effects of these compounds in smooth muscles have been extensively studied (Cook and Quast, 1990), very little is known about their effects on autonomic nerve terminals. Inhibitory effects exhibited by cromakalim on the vascular responses to spinal cord stimulation have been reported to be exerted pre- rather than postjunctionally (Richer et al., 1990), but a study of noradrenergic transmission in rat isolated mesenteric artery has provided no evidence of a prejunctional inhibitory effect of cromakalim on transmitter noradrenaline release (Fabiani and Story, 1994). Recently Docherty and Brady (1995) have found that the K^+ channel blocker apamin does not affect electrically induced contractions in the prostatic portion of rat vas deferens. Evidence has accumulated to suggest that cromakalim inhibits cholinergic neuro-effector transmission in guinea-pig trachea (Burka et al., 1991) and ileum (Zini et al., 1991). Furthermore, it has been reported that cromakalim modulates non-adrenergic non-cholinergic neurotransmission in guinea-pig airways in vivo (Ichinose and Barnes, 1990) and in guinea-pig tracheal smooth muscle (Burka et al., 1991).

The present study was carried out to investigate possible effects of cromakalim on transmitter(s) released from adrenergic nerves such as noradrenaline and ATP (Burnstock, 1981; Hirst and Edwards, 1989; Starke et al., 1989; Stjärne, 1989). This possibility has been examined by studying the action of cromakalim on electrically induced contractions of the epididymal and prostatic portion of the rat isolated vas deferens. Research has been extended to study the action of suramin and prazosin on the biphasic response induced by a single shock and to investigate the effects of these antagonists and cromakalim on the contractions produced by exogenous noradrenaline and α,β-methylene-ATP or ATP.

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PII S0014-2999(96)00828-X

2. Materials and methods

2.1. Isolated rat vas deferens preparation

Male Wistar-Morini rats (250–400 g body weight) were killed by CO_2 asphyxiation and exsanguination. Vasa deferentia were 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 mM. Longitudinal segments (1.5–2 cm) beginning from the epididymal end of the vas deferens were dissected (only one segment per vas was used) and placed in heated (37 ± 0.5°C) 20-ml organ baths and 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 and challenged with a reference concentration of noradrenaline (1×10^{-5} M). Responses elicited by electrical stimulation or exogenous agonists are expressed as a percentage of the contraction elicited by the reference concentration of noradrenaline tested on each tissue as described above.

In some experiments the prostatic portion was used. In these cases, because the response to noradrenaline was very modest, the responses are expressed as percentages of the response obtained in the absence of the test drug.

2.2. Experiments involving transmural stimulation

Epididymal or prostatic portions of rat vas deferens were stimulated with a single stimulus (field stimulation, square waves, threshold voltage + 100%, 1 ms duration) to produce isometric contractions. Preliminary experiments indicated that no desensitization occurred with a rest interval of at least 10 min and that the response was reproducible over a period of 180 min. After two control responses to single-pulse field stimulation were obtained, each tissue was equilibrated with a fixed concentration of test compound alone or in combination for at least 20 min before measurement of responses to single-pulse field stimulation. Only one concentration of each compound was tested on each tissue. The compounds tested were the α_1-adrenoceptor antagonist prazosin, the P_{2X} purinoceptor antagonist suramin and the K^+ channel opener cromakalim.

When glibenclamide was tested the protocol was as follows. After two reproducible control responses were obtained, each concentration of glibenclamide tested 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 single-pulse field stimulation was tested. The smooth muscle cells were not being stimulated directly, since tetrodotoxin (1 μM, $n = 3$) abolished the electrically induced contraction, confirming that the contraction of the preparation induced by field stimulation is neurogenic.

2.3. Experiments involving exogenous spasmogens

First of all concentration-response curves of agonists were obtained in the epididymal portion by dosing at 0.5-log unit intervals in a non-cumulative manner. Each concentration of agonist (noradrenaline or α,β-methylene-ATP) was added to the organ bath at intervals of 30 min. Each concentration of agonist was left in contact with the tissue until the response had peaked (between 3–30 s) and then the agonist was immediately washed out. Only one concentration-response curve for noradrenaline or α,β-methylene-ATP was determined with each tissue.

Subsequently, the effects of various concentrations of compounds under investigation (cromakalim, suramin and prazosin) were studied on the contraction induced by a given concentration of agonist. Therefore noradrenaline (1×10^{-5} M) and α,β-methylene-ATP (1×10^{-6} or 1×10^{-5} M) for the epididymal portion or ATP (1×10^{-3} M) for the prostatic portion was added to the bath fluid and the response was allowed to reach a plateau, then the agonist was immediately washed out. In the prostatic portion the response to noradrenaline at the concentration used in the epididymal portion (1×10^{-5} M) was very poor (amounting to 0.06 ± 0.01 g, $n = 9$, data not shown); consequently this agonist was no longer considered. The wash-out period was 30 min for noradrenaline and 30 or 45 min for α,β-methylene-ATP and 60 min for ATP. After two identical responses had been elicited the effect of the test compound was investigated. A given concentration of compound(s) or vehicle was added to the organ bath and was left in contact with the tissue for 20 min before testing the agonist. The compounds tested were cromakalim, prazosin and suramin. Only one agonist and only one concentration of each compound under examination was tested on each preparation.

2.4. Drugs

The following drugs were used: (−)-noradrenaline bitartrate (Sigma), prazosin hydrochloride (Sigma), suramin sodium (a generous gift from Dr. A. Faggiotto, Bayer), cromakalim (Sigma), glibenclamide (Sigma), α,β-methylene-ATP lithium (Sigma), ATP disodium (Sigma). Noradrenaline (10^{-2} M) was dissolved in double-distilled water containing ascorbic acid (3 mg/l) and disodium edetate (1 mg/l) to prevent oxidation. Prazosin (10^{-3} M) was dissolved weekly in 0.5 ml of ethanol and 9.5 ml of double-distilled water. Dilutions were made daily in double-distilled water. Suramin (10^{-2} M) and α,β-methylene-ATP (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^{-1} M) was dissolved daily in double-distilled water. Cromakalim (10^{-2} M) was dis-

solved in ethanol 70% (v/v) and dilutions were made daily in double-distilled water. Glibenclamide (10^{-3} M) was dissolved in dimethyl sulfoxide (final concentration of solvent less than 0.5% and without effect).

2.5. Statistics

Results are expressed as mean values ± 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. The pD_2 values (expressed as $-\log EC_{50}$ for noradrenaline and as $-\log IC_{50}$ for cromakalim) were calculated graphically for noradrenaline and with GraphPadPrism (a data analysis package, San Diego, CA, USA) for cromakalim and expressed as means ± S.E.M.

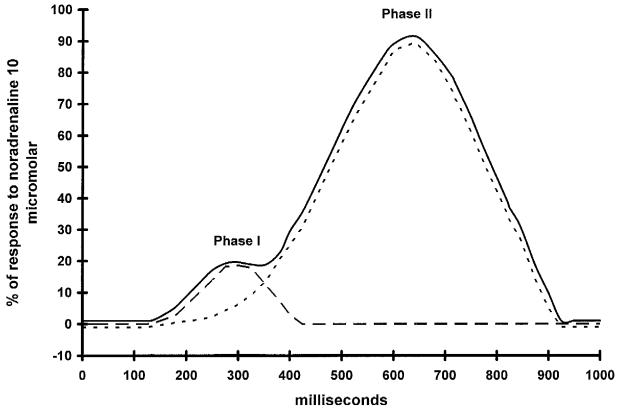

Fig. 1. Typical example of biphasic response induced by single-pulse field stimulation without treatment (solid line) and in the presence of suramin 3×10^{-4} M (dotted line), which abolished the first phase of the response, or of prazosin 1×10^{-8} M (dashed line), which abolished the second phase of the response.

3. Results

Prazosin, suramin and cromakalim were used in concentrations that did not alter the normally quescient tone of the preparations.

3.1. Transmural stimulation of epididymal and prostatic portions of rat vas deferens and action of test compounds

The mechanical response of the epididymal portion of rat vas deferens to single-pulse field stimulation was biphasic, as shown in Fig. 1. It consisted of an early phase ($phase_I$) peaking at 250–300 ms followed by a second slowly developing and larger phase ($phase_{II}$) peaking at 650 ms. The reproducibility of the biphasic response to single-pulse field stimulation was good for at least 180 min.

Suramin (3×10^{-4} M) completely abolished the first component of the contraction to single-pulse field stimulation, as shown in Fig. 1 and Table 1, leaving unaffected the second phase.

Prazosin at a concentration of 1×10^{-8} M almost completely abolished the second phase of the response to single-pulse field stimulation, leaving intact the first one (Fig. 1 and Table 1).

The combination of suramin and prazosin at the concentrations mentioned above completely abolished the first phase of the response to single-pulse field stimulation and deeply depressed the second one (Table 1).

Increasing concentrations of cromakalim ($0.01–1 \times 10^{-5}$ M) resulted in a concentration-dependent inhibition of the first twitch with a pD_2 ($-\log IC_{50}$) of 5.90 ± 0.11 and a maximum inhibition of 95.56 ± 4.44% (see Table 1 and Fig. 2). Even with the highest concentration of cromakalim used (1×10^{-5} M), the second phase was practically unaffected.

Then three concentrations of glibenclamide (3×10^{-7}–3×10^{-6} M) were tested: with all three concentrations both phases of the response to single-pulse field stimulation were unaffected (Table 2). Glibenclamide 3×10^{-7} M was not able to counteract in a significant manner the inhibitory effect of cromakalim while glibenclamide at

Table 1
Antagonism by prazosin, suramin and cromakalim of the response to single-pulse field stimulation of the epididymal portion of rat vas deferens

	Control		Treated	
	$Phase_I$	$Phase_{II}$	$Phase_I$	$Phase_{II}$
Vehicle ($n = 26$)	23.89 ± 4.11	86.66 ± 6.20	23.0 ± 2.28	87.01 ± 5.96
Prazosin 1×10^{-8} M ($n = 8$)	22.92 ± 4.11	92.74 ± 7.04	19.30 ± 3.44	4.63 ± 3.04 [a]
Suramin 3×10^{-4} M ($n = 6$)	25.92 ± 6.74	89.35 ± 21.36	0 [a]	88.07 ± 19.24
Prazosin 1×10^{-8} M + suramin 3×10^{-4} M ($n = 7$)	21.53 ± 4.03	72.17 ± 8.25	0 [a]	13.27 ± 2.15 [a]
Cromakalim 1×10^{-7} M ($n = 7$)	16.48 ± 1.81	97.97 ± 8.87	14.49 ± 1.73 [a]	96.85 ± 9.20
Cromakalim 3×10^{-7} M ($n = 6$)	24.69 ± 1.94	104.04 ± 11.02	16.65 ± 1.76 [a]	96.89 ± 13.91
Cromakalim 1×10^{-6} M ($n = 7$)	20.89 ± 2.85	92.50 ± 5.20	9.82 ± 0.22 [a]	87.74 ± 5.78
Cromakalim 3×10^{-6} M ($n = 7$)	26.13 ± 3.17	100.00 ± 8.32	6.63 ± 2.76 [a]	89.53 ± 8.02
Cromakalim 1×10^{-5} M ($n = 5$)	21.67 ± 4.00	95.81 ± 8.59	1.00 ± 0.99 [a]	86.08 ± 6.53

Results are expressed as the percentage (mean ± S.E.M.) of the force developed by the preparation in response to a test stimulation (noradrenaline 1×10^{-5} M). [a] Student's *t*-test for paired data, $P < 0.05$.

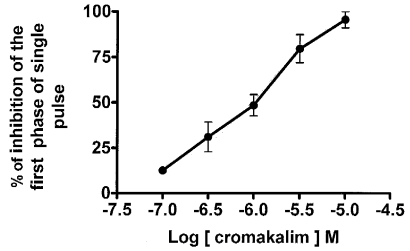

Fig. 2. Inhibitory effect of cromakalim on the first phase of the response (closed circle) to single-pulse field stimulation in the epididymal portion of rat vas deferens. Each point is the mean ± S.E.M. of 5–7 observations; where not visible the vertical bars lie within the sign. Percentage inhibition was obtained from data presented in Table 1.

concentrations of 1×10^{-6} M and 3×10^{-6} M attenuated the inhibitory effect of cromakalim by 57.75 ± 6.82% and 88.28 ± 4.67%, respectively. The slightly non-significant depression of the second phase induced by cromakalim was completely reversed with all concentrations of glibenclamide tested (Table 2). The mechanical response of the prostatic portion of rat vas deferens to single-pulse field stimulation was biphasic, consisting mainly of the first peak ($phase_I$) with a shoulder ($phase_{II}$) on the descending side of the peak. The first phase peaked at about 250 ms, amounting to 0.51 ± 0.04 g ($n = 16$), and the second one peaked at about 400 ms, amounting to 0.31 ± 0.03 g ($n = 16$) (data not shown). Suramin (3×10^{-4} M) strongly affected but did not completely abolish the first phase and moderately (31.83 ± 9.29%) but significantly inhibited the second phase (Table 3). Prazosin (1×10^{-8} M) completely abolished the second phase and slightly but significantly inhibited the first one (Table 3). The action of suramin 3×10^{-4} M in the presence of prazosin 1×10^{-8} M on $phase_I$ consisted of a marked but not complete inhibition (Table 3). Cromakalim (3×10^{-6} M, 1×10^{-5} M) inhibited the first phase by 39.49 ± 3.42% and 59.11 ± 4.48% respectively, leaving unaffected the second one. Higher concentrations caused no further inhibition (Table 3). In the presence of prazosin 1×10^{-8} M the inhibitory effect of cromakalim on the first phase was significantly ($P < 0.05$) increased (Table 3) when 1×10^{-5} M was used.

Table 3
Antagonism by prazosin, suramin and cromakalim alone and in combination of the response to single-pulse field stimulation of the prostatic portion of the rat vas deferens

	$Phase_I$	$Phase_{II}$
Cromakalim 3×10^{-6} M ($n = 6$)	60.51 ± 3.42 [a]	95.27 ± 3.45
Cromakalim 1×10^{-5} M ($n = 6$)	40.89 ± 4.48 [a]	80.07 ± 8.57
Cromakalim 3×10^{-5} M ($n = 6$)	55.94 ± 3.33 [a]	97.83 ± 2.17
Prazosin 1×10^{-8} M ($n = 5$)	73.85 ± 8.54 [a]	0 [a]
Prazosin 1×10^{-8} M + cromakalim 1×10^{-6} M ($n = 7$)	62.15 ± 8.98 [a]	4.51 ± 4.51 [a]
Prazosin 1×10^{-8} M + cromakalim 3×10^{-6} M ($n = 7$)	57.63 ± 4.70 [a]	4.46 ± 4.47 [a]
Prazosin 1×10^{-8} M + cromakalim 1×10^{-5} M ($n = 7$)	23.03 ± 3.56 [a]	0 [a]
Suramin 3×10^{-4} M ($n = 5$)	13.83 ± 3.43 [a]	68.17 ± 9.26 [a]
Suramin 3×10^{-4} M + prazosin 1×10^{-8} M ($n = 7$)	21.45 ± 5.47 [a]	4.12 ± 2.66 [a]

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

3.2. Action of exogenous spasmogens and of test compounds

Fig. 3 shows the concentration-response curves for noradrenaline and α,β-methylene-ATP obtained in a non-cumulative manner in the epididymal portion.

Noradrenaline (from 1×10^{-7} to 1×10^{-4} M) and α,β-methylene-ATP (from 1×10^{-7} to 3×10^{-4} M) induced a concentration-dependent contractile response in the epididymal portion of rat vas deferens with a pD_2 ($-\log EC_{50}$) of 5.52 ± 0.14 for noradrenaline ($n = 4$). In the case of α,β-methylene-ATP it was not possible to calculate the pD_2 value because even with the highest concentration used (3×10^{-4} M) the curve had no clear maximum. The concentration-response curve of α,β-methylene-ATP was shifted to the right by about 0.4 log unit compared with that of noradrenaline.

The effects of various concentrations of antagonists were studied on the response elicited by noradrenaline 1×10^{-5} M, a concentration of noradrenaline that produced a contraction similar to that induced by endogenous noradrenaline (i.e. the second phase of the response to

Table 2
Antagonism by glibenclamide of the inhibitory effect of cromakalim 1×10^{-5} M on the contractile response to single-pulse field stimulation of the epididymal portion of the rat vas deferens

	Control [a]		Treated [b]	
	$Phase_I$	$Phase_{II}$	$Phase_I$	$Phase_{II}$
Glibenclamide = 0 (from Table 1) ($n = 5$)	21.67 ± 3.98	95.81 ± 8.59	1.00 ± 0.99	86.08 ± 6.53
Glibenclamide 3×10^{-7} M ($n = 4$)	18.29 ± 4.47	101.81 ± 16.88	1.25 ± 1.25	103.4 ± 15.10
Glibenclamide 1×10^{-6} M ($n = 8$)	17.74 ± 1.74	96.05 ± 9.24	9.89 ± 1.02 [c]	108.1 ± 10.52
Glibenclamide 3×10^{-6} M ($n = 4$)	29.49 ± 4.09	97.23 ± 6.3	25.47 ± 2.23 [c]	101.73 ± 6.54

Results are expressed as the percentage (mean ± S.E.M.) of the response of the preparation to a test stimulation (noradrenaline 1×10^{-5} M). [a] Data obtained in the absence of cromakalim 1×10^{-5} M. [b] Data obtained in the presence of cromakalim 1×10^{-5} M. [c] Student's *t*-test for unpaired data, $P < 0.05$ between each set of data for glibenclamide-treated and untreated preparations.

Table 4
Antagonism by prazosin, suramin and cromakalim of the contractile response of the epididymal portion of the rat vas deferens to noradrenaline 1×10^{-5} M

Prazosin 1×10^{-8} M	Prazosin 1×10^{-7} M	Suramin 3×10^{-4} M	Cromakalim 1×10^{-5} M
48.50 ± 6.50 [a] ($n=6$)	5.54 ± 1.06 [a] ($n=7$)	102.72 ± 5.45 ($n=4$)	67.25 ± 2.78 [a] ($n=6$)

Results are expressed as the percentage (mean ± S.E.M.) of the force developed by the preparation in response to noradrenaline 1×10^{-5} M. [a] Denote responses significantly different from effects of vehicle (Student's *t*-test for paired data, $P<0.05$).

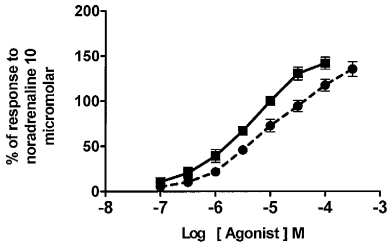

Fig. 3. Non-cumulative concentration-response curves for noradrenaline (closed square) and α,β-methylene-ATP (closed circle) on the epididymal portion of rat vas deferens. Each point is the mean ± S.E.M. of 4–6 observations; where not visible the vertical bars lie within the sign.

single-pulse field stimulation) (see Table 1). This concentration produced 61.77 ± 3.20% of the maximal contraction (see Fig. 3). The reproducibility of the contractile response to this concentration of noradrenaline was good (data not shown). The P_{2X} antagonist suramin at the concentration 3×10^{-4} M was devoid of an inhibitory effect (Table 4). The α_1-adrenoceptor antagonist prazosin (1×10^{-8} M, contact time 20 min) was able to inhibit by 51.46 ± 6.50% the response to exogenous noradrenaline.

A few experiments were carried out in order to detect if the maximum antagonistic effect of prazosin 1×10^{-8} M was reached with a 20-min contact time. Consequently the antagonism of prazosin was evaluated following 40-min contact time and the inhibition was 43.45 ± 3.17%. This value was not significantly different from that obtained with a 20-min contact time.

Experiments were carried out also to test prazosin at a concentration 10-fold higher (1×10^{-7} M). This concentration was able to almost completely abolish the response to exogenous noradrenaline: an inhibition of 94.45 ± 1.06% of the contraction induced by noradrenaline 1×10^{-5} M was reached.

Cromakalim at the highest concentration used in this study (1×10^{-5} M) was able to slightly (about 32.75 ± 2.78%) but significantly ($P<0.05$) depress the contraction induced by noradrenaline (Table 4).

In order to analyze the effects of cromakalim, prazosin and suramin on the responses due to P_{2X} purinoceptor activation, two concentrations of α,β-methylene-ATP were tested: the first one (1×10^{-6} M) produced the same level of contraction as that induced by endogenous ATP (i.e. the first phase of single-pulse field stimulation); the second one was 10-fold higher. α,β-Methylene-ATP used at these concentrations caused 16.04 ± 2.06% and 53.71 ± 5.11% of the maximum response obtained with this agonist, respectively (Fig. 3).

No significant difference was found between the contractile response to both concentrations of α,β-methylene-ATP tested using intervals of 30 min or 45 min between two following administrations. In both cases the reproducibility of the contractile response was good (data not shown). A 45-min interval was chosen as a rest standard period to test all the antagonists used.

Prazosin 1×10^{-8} M left unaffected the response to both the concentrations of α,β-methylene-ATP tested (Table 5). Suramin completely abolished the contractile response induced by both concentrations of α,β-methylene-ATP (Table 5). Cromakalim at the same concentration (1×10^{-5} M) that completely suppressed the response to endogenous ATP left unaltered the response induced by both concentrations of α,β-methylene-ATP (Table 5). The action of suramin and cromakalim on the response induced by ATP 1×10^{-3} M was studied in the prostatic portion. This response consisted of a phasic contraction on average equal to about the 70% of that obtained with electrical stimulation ($phase_I$). Good reproducibility of the response was obtained with a rest interval of 60 min between two following administrations. In the presence of cromakalim 1×10^{-5} M the response was practically unaffected ($n=5$); in the presence of suramin (3×10^{-4} M) the response was reduced by 59.13 ± 6.86% ($n=6$).

Table 5
Antagonism by prazosin, suramin and cromakalim of the response to α,β-methylene-ATP of the epididymal portion of rat vas deferens

	α,β-Methylene-ATP 1×10^{-6} M		α,β-Methylene-ATP 1×10^{-5} M	
	In absence	In presence	In absence	In presence
Vehicle ($n=8$)	25.78 ± 5.95	23.73 ± 5.95	66.65 ± 8.88	78.27 ± 9.70
Prazosin 1×10^{-8} M ($n=5$)	26.60 ± 2.90	22.79 ± 4.03	75.91 ± 5.07	79.67 ± 6.01
Suramin 3×10^{-4} M ($n=4$)	28.53 ± 1.36	0 [a]	65.80 ± 2.81	0 [a]
Cromakalim 1×10^{-5} M ($n=6$)	27.16 ± 1.97	25.27 ± 1.81	73.51 ± 3.99	77.52 ± 4.42

Results are expressed as the percentage (mean ± S.E.M.) of the force developed by the epididymal portion of the rat vas deferens in response to a test stimulation (noradrenaline 1×10^{-5} M). [a] Student's *t*-test for paired data, $P<0.05$.

4. Discussion

It is well known that the contractile response to single-pulse field stimulation in the epididymal portion of the rat vas deferens is biphasic. There is good evidence that the first rapid component is purinergic and that the second slower phase is adrenergic (McGrath, 1978; Brown et al., 1983; Sneddon and Westfall, 1984; Amobi and Smith, 1986; Bourreau et al., 1991).

First of all our data, confirming and extending previous observations in the epididymal portion (Amobi and Smith, 1986) and in the whole vas deferens (Mallard et al., 1992), evidenced that prazosin (1×10^{-8} M) abolished the adrenergic phase of the response to single-pulse field stimulation, leaving intact the purinergic component, and that suramin (3×10^{-4} M) selectively impaired the first (purinergic) component but was without effect on the second, noradrenergic component. The combination of prazosin and suramin almost completetely abolished both phases of the response to single-pulse field stimulation.

Secondly we demonstrated that the K^+ channel opener cromakalim was able to inhibit in a concentration-dependent fashion the first purinergic phase of the response to single-pulse field stimulation ($pD_2 = 5.90 \pm 0.11$).

The results obtained using three concentrations of glibenclamide (from 3×10^{-7} M to 1×10^{-6} M) demonstrated that this blocker of ATP-sensitive K^+ channels was able to reverse the inhibitory effect of cromakalim. These concentrations of glibenclamide are those commonly used (Cook and Quast, 1990; Grana et al., 1991b).

None of the concentrations of cromakalim tested significantly affected the adrenergic phase of the contraction induced by single-pulse field stimulation.

The contractile response to single-pulse field stimulation of the prostatic portion of rat vas deferens is variously described (McGrath, 1978; Brown et al., 1983; Aboud et al., 1993) but basically consists of a biphasic contraction with a main peak which has a shoulder on the descending side of the peak. Our data confirmed the observations reported above.

The inhibitory action of prazosin was different to that exerted by this α_1-adrenoceptor antagonist on the epididymal portion since not only was $phase_{II}$ completely inhibited but partially also $phase_I$. The effect of suramin was at least partially different from that exerted on the epididymal portion, because the inhibition of $phase_I$ was marked but not complete and a partial inhibition of the component commonly reported as adrenergic was also found. These data should be interpreted bearing in mind that the overlap of the purinergic component with the adrenergic one is greater in the prostatic portion than in the epididymal portion. The persistence of a modest but significant response in the presence of suramin and suramin plus prazosin could suggest the hypothesis of the involvement of other cotransmitters (Schlicker et al., 1989) or the presence of suramin-resistant receptor binding sites (Bultmann and Starke, 1994) that cannot be detected in the epididymal portion. The inhibitory effect of cromakalim on the purinergic component appears to be evident although it is less clear than that obtained in the epididymal portion. This inhibitory effect appears to be potentiated when prazosin is also present, possibly by an additive effect. The explanation for the incomplete inhibition is similar to the above-mentioned explanation for a residual effect in the presence of suramin. On the whole, it would seem that cromakalim and the antagonists suramin and prazosin have inhibitory actions that follow the same trend but are more easily recognized in the epididymal than in the prostatic portion. Therefore experiments were done in an attempt to analyze the mode of action of cromakalim, i.e., to determine whether a prejunctional and/or a postjunctional effect occurred. To this purpose we investigated the action of cromakalim on the epididymal portion versus α,β-methylene-ATP by using the concentration (1×10^{-5} M) that abolished the purinergic phase of the response to single-pulse field stimulation.

In the present study carried out with the epididymal portion of the rat vas deferens, the contractile effect of α,β-methylene-ATP was monophasic, rapid and transient. This effect seems to be due to the activation of P_{2X} purinoceptors (Bultmann et al., 1994). Under our experimental conditions, with two different concentrations of α,β-methylene-ATP (1×10^{-6} and 1×10^{-5} M) desensitization did not occur. Cromakalim, at the concentration that completely abolished the purinergic phase of the response to single shock, left unaffected the response to these two concentrations of α,β-methylene-ATP.

Therefore our results led to the conclusion that the inhibition exerted by cromakalim of the first phase of the response induced by single shock is a prejunctional effect and therefore that cromakalim inhibits the release of ATP. The ability of glibenclamide to antagonize the inhibitory effect of cromakalim strongly suggested that ATP-sensitive K^+ channels are involved. To our knowledge, this conclusion is new. Indeed, Docherty and Brady (1995) have demonstrated that apamin, which they regarded as an ATP-sensitive K^+ channel blocker, produced no significant effect on electrically evoked contractions of the prostatic portion of rat vas deferens, contractions which consist mainly of the first non-adrenergic phase.

Our results concerning noradrenergic transmission are supported by the finding that in guinea-pig isolated trachea (Burka et al., 1991), and in guinea-pig airways in vivo (Ichinose and Barnes, 1990) cromakalim modulates the release of neurotransmitter(s) from cholinergic and excitatory non-cholinergic and non-adrenergic nerve terminals.

In order to obtain a more general view of the problem, we tested in the epididymal portion also the antagonism by cromakalim and prazosin of the response to exogenous noradrenaline and the antagonism exerted by suramin of the contractile response produced by α,β-methylene-ATP and by noradrenaline. In the prostatic portion the action of

cromakalim and suramin against the effect of ATP was tested keeping in mind the variety of receptor binding sites suggested by Bultmann and Starke (1994) and our results obtained with electrical stimulation.

In the epididymal portion the response to noradrenaline (1×10^{-5} M) was unaffected by suramin, as reported by Mallard et al. (1992) in the whole rat vas deferens, but was modified by the other two compounds tested. Prazosin 1×10^{-8} M inhibited by about 50% the response to exogenous noradrenaline. The potential problem of antagonist equilibration time was ruled out by demonstrating that a 20- and a 40-min contact time produced a non-significantly different inhibition ($51.50 \pm 6.50\%$ and $43.45 \pm 3.17\%$, respectively). In contrast, prazosin 1×10^{-7} M practically completely abolished the response produced by exogenous noradrenaline.

The results presented in this study seem to suggest that the effectiveness of prazosin on the contractile response to endogenous noradrenaline (i.e. the second phase produced by single shock) was greater than that on the response induced by exogenous noradrenaline.

Cromakalim, at a concentration (1×10^{-5} M) that leaves practically unaffected the noradrenergic phase of the response to electrical stimulation, was able to inhibit, partially, the response to exogenous noradrenaline. The results obtained with the epididymal portion of rat vas deferens are in accordance with those obtained by Grana et al. (1991a) with the whole rat vas deferens.

Prazosin and cromakalim seem to have an opposite effectiveness on the response to exogenous and endogenous noradrenaline. It is well known that in the rat vas deferens, as in other preparations, the effectiveness of some antagonists may depend on the origin (i.e. neurally released or exogenously applied) of the agonist (Amobi and Smith, 1986; Bourreau et al., 1991).

In our experiments suramin was able to completely antagonize contractions elicited by α,β-methylene-ATP. These results are consistent with those obtained with other non-vascular preparations such as mouse vas deferens (Dunn and Blakeley, 1988; Von Kügelgen et al., 1989), whole rat vas deferens (Mallard et al., 1992) and pithed rat (Schlicker et al., 1989). Prazosin was found to be completely devoid of any antagonistic effect versus α,β-methylene-ATP.

In the prostatic portion the inhibitory action of suramin on the response to ATP was very marked but was not complete and it seems to confirm the presence of P_{2X} purinoceptors resistant to inhibition by suramin, as suggested by Bultmann and Starke (1994). This result is in accordance with what was seen with electrical stimulation. Cromakalim was completely inactive versus ATP: this result confirms that the action of cromakalim is prejunctional. The only partial inhibition of $phase_I$ is still an open question, but the hypothesis that in the prostatic portion the response to electrical stimulation is also mediated via other transmitters seems to be more likely.

In conclusion, the results presented in this study about the action of suramin and prazosin on the effects of endogenous and exogenous ATP confirm and extend previous observations, as do the results obtained for the action of prazosin and cromakalim on the effects of endogenous and exogenous noradrenaline.

The main outcome is that there is evidence that cromakalim can depress purinergic transmission by acting prejunctionally. This action was more evident when the epididymal portion was used, although the data obtained with the prostatic portion indicate a similar behaviour.

The mechanism by which cromakalim exerts this effect is not yet understood, as is true for the vasorelaxant mechanisms of K^+ channel openers (Quast, 1993). With regard to this question it might be of interest to emphasize that there are some similarities between the inhibitory effect of cromakalim and the inhibitory effect exerted by nifedipine on the first phase of the contractile response of the rat vas deferens (French and Scott, 1981; Brown et al., 1983; Bourreau et al., 1991; Aboud et al., 1993). However, further studies are needed to assess this.

Analysis of a possible difference between the prostatic and the epididymal portions as concerns the occurrence of different types of purinergic receptor or the involvement of other cotransmitters was not the purpose of the present work. A difference between prostatic and epididymal portions has been reported, for example, by Donoso et al. (1988) with respect to the effect of neuropeptide Y.

Acknowledgements

This work was supported by M.U.R.S.T. 60%. The authors wish to thank Mr. Andrea Losi for technical support.

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