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Role of intracellular Ca^{2+} in the K channel opener action of CGRP in the guinea-pig ureter

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1 The aim of this study was to assess the role of sarcoplasmic reticulum (SR) calcium (Ca^{2+}) in the smooth muscle relaxant and hyperpolarizing actions of calcitonin gene-related peptide (CGRP) in the guinea-pig ureter.

2 CGRP (0.1 μM) rapidly and transiently reduced myogenic phasic contractions (twitches) produced by electrical field stimulation (EFS). Approximately 70% of the response to CGRP was antagonized by glibenclamide (1 μM).

3 Cyclopiazonic acid (CPA, 10 μM), ryanodine (100 μM) and thapsigargin (1 μM) reduced only the glibenclamide-sensitive component of the response to CGRP (0.1 μM) but did not modify the mechano-inhibitory effect of cromakalim (3 μM). A low concentration of CPA (1 μM), assumed to produce a limited impairment of Ca^{2+} uptake from the stores, prolonged the duration of the inhibitory response to CGRP. Pre-exposure to caffeine (5 mM) inhibited the suppression of twitches by CGRP or cromakalim.

4 When the frequency of EFS was increased, the suppression of twitches by CGRP was reduced. Under these conditions, CPA (1 μM) again prolonged the duration of the inhibitory response to CGRP.

5 CGRP (0.1 μM) and cromakalim (3 μM) markedly depressed the phasic component of contractions to 80 mM KCl. CPA (10 μM) antagonized the inhibitory effect of CGRP but not that of cromakalim. Inhibition of the tonic contraction to 80 mM KCl by CGRP was insensitive to CPA.

6 In sucrose gap experiments, a 5 min exposure to CGRP (0.1 μM) or cromakalim (3 μM) produced a sustained membrane hyperpolarization. Caffeine (5 mM) produced a glibenclamide-sensitive transient hyperpolarization followed by a sustained depolarization. When tested in a Ca^{2+}-free medium the hyperpolarization produced by CGRP, cromakalim or caffeine was reduced. In normal Krebs, pre-exposure to CPA (10 μM, 60 min) only abolished the hyperpolarization induced by CGRP. In contrast, 5 min after a caffeine challenge (5 mM) the hyperpolarizations induced by CGRP or cromakalim were reduced. The CGRP-induced hyperpolarization was insensitive to apamin (0.1 μM) or charybdotoxin (0.1 μM).

7 We conclude that the K channel-opening action of CGRP in the guinea-pig ureter requires the mobilization of intracellular Ca^{2+} from a caffeine- and CPA-sensitive store, leading to transient activation of glibenclamide-sensitive K channels. The K channel-opening action of caffeine appears to involve Ca^{2+} mobilization from a store which is insensitive to depletion by CPA.

Keywords: Guinea-pig ureter; calcitonin gene-related peptide; K_{ATP} channels; glibenclamide; sarcoplasmic reticulum; cyclic AMP; protein kinase A

Introduction

The 37 amino acid residue neuropeptide, calcitonin gene-related peptide (CGRP) is a major transmitter for local regulation of motility in the mammalian ureter: when released from the peripheral endings of capsaicin-sensitive primary afferent neurones, CGRP produces a profound receptor-mediated inhibition of the evoked motility of the ureter (Maggi & Giuliani, 1991; 1994). Either exogenous or endogenous CGRP produces hyperpolarization of the ureter smooth muscle by activating glibenclamide-sensitive K channels (Maggi *et al.*, 1994a; Santicioli & Maggi, 1994): this effect largely accounts for the ability of CGRP to suppress evoked phasic contractions triggered by action potentials from latent pacemakers in the ureter smooth muscle. In addition, a fraction of the relaxant action of CGRP in the ureter is glibenclamide-resistant (Maggi *et al.*, 1994a; 1995). The intensity of the glibenclamide-resistant relaxant response to CGRP depends upon the nature of the applied stimulus: about 30% of the inhibitory effect of CGRP on phasic contractions of the ureter can be observed in the presence of glibenclamide whilst the large inhibition of the tonic component of the contraction produced by KCl is almost completely glibenclamide-resistant (Maggi *et al.*, 1994a).

In previous studies, we established that elevation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) is involved in mediating the glibenclamide-sensitive inhibitory action of CGRP in the guinea-pig ureter (Maggi *et al.*, 1995; Santicioli *et al.*, 1995). The main pieces of evidence favouring this causal link are as follows: (a) CGRP selectively elevates cyclic AMP levels in the ureter; (b) an antagonist of cyclic AMP at its binding site on cyclic AMP-dependent protein kinase (PKA), as well as PKA inhibitors, selectively block the glibenclamide-sensitive action of CGRP; (c) the same drugs also inhibit the glibenclamide-sensitive response to forskolin and 3-isobutyl-1-methylxanthine, which elevate cyclic AMP by stimulating adenylate cyclase and inhibiting phosphodiesterase, respectively. The involvement of cyclic AMP and PKA has likewise been proposed to account for K channel activation by CGRP in arterial and gallbladder smooth muscles (Quayle *et al.*, 1994; Zhang *et al.*, 1994a,b). On the other hand, some observations, and especially the transient nature of K channel activation as opposed to the sustained kinetics of cyclic AMP elevation produced by CGRP, argue against a simple parallel relationship between CGRP receptor occupancy, cyclic AMP/PKA activation and K channel opening in the guinea-pig ureter. Considering the inherently transient nature of K channel activation by CGRP in the ureter, we became interested in the

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possibility that mobilization/uptake of Ca^{2+} from the sarcoplasmic reticulum (SR) (Grover & Khan, 1992 for review) may be involved. The SR Ca^{2+} ATP-ase is a known target for cyclic AMP-dependent PKA. PKA can either increase Ca^{2+} mobilization by e.g. phosphorylating the ryanodine-receptor Ca^{2+}-induced Ca^{2+}-release channel (CICR) (Coronado *et al.*, 1994 for review) or, by phosphorylating the SR protein, phospholamban, PKA can increase the efficiency of the pump in removing free intracellular Ca^{2+} (Suematsu *et al.*, 1984; Komori & Bolton, 1989; Murray, 1990 for review). Furthermore, studies using ryanodine and cyclopiazonic acid (CPA), two drugs known to affect SR Ca^{2+} handling (Goeger & Riley, 1989; Deng & Kwan, 1991; Coronado *et al.*, 1994 for review) have indicated that the SR may be involved in regulating the resting membrane potential of the guinea-pig ureter (Maggi *et al.*, 1994b,c).

In the first part of this study we used ryanodine, CPA and thapsigargin (Thastrup *et al.*, 1990; Inesi & Sagara, 1992) as probes to assess the possible importance of Ca^{2+} handling by the SR in the effect of CGRP: evidence was obtained that these three agents inhibit the effect of CGRP in the ureter, and that their action is restricted to the glibenclamide-sensitive component of the response to CGRP. Following these results, the hypothesis was advanced that CGRP might induce Ca^{2+} mobilization from the SR leading to activation of K channels. To probe this hypothesis, we tested (a) the effect of low concentrations of CPA, assumed to produce a moderate impairment of Ca uptake but not a depletion of SR Ca^{2+} stores on the response to CGRP and, (b) the effect of caffeine, as a tool to produce Ca^{2+} release and depletion from the internal store(s).

Methods

General

Male albino guinea-pigs weighing 250–300 g were stunned and bled. The ureters (from the inferior renal pole to their entry into the bladder) were excised, cleaned of adhering fat and connective tissue and placed in oxygenated and warmed Krebs solution (95% O_2 and 5% CO_2, pH 7.4 at 37°C) which was used in all experiments. The Krebs solution had the following composition (mM): NaCl 119, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.5, KCl 4.7, $CaCl_2$ 2.5 and glucose 11. For some experiments, a low-K (1.2 mM) Krebs solution was prepared, in which KCl was replaced by an equimolar amount of NaCl. The Krebs solution routinely contained Bay K 8644 (1 μM) to enable recording of regular action potentials and contraction in response to direct electrical stimulation of ureter smooth muscle (cf. Maggi *et al.*, 1994b,d). Because of the existence of a capsaicin-sensitive inhibitory innervation, all experiments were performed in capsaicin-pretreated ureters (10 μM for 15 min) to prevent the release of sensory neuropeptides by applied depolarizing stimuli (Maggi & Giuliani, 1991).

Organ bath experiments

A segment about 2 cm long was excised from the middle portion of the ureter and was mounted in a 5 ml organ bath for isotonic recording (load 2 mN) of mechanical activity along its longitudinal axis, as described previously (Maggi & Giuliani, 1991). Twitch contractions were evoked by direct electrical field stimulation (EFS) of ureter smooth muscle: trains of pulses (10 Hz for 1 s, 60 V) of long width (5 ms) were automatically delivered through platinum wire electrodes placed at the top and bottom of the organ bath (electrical field stimulation, EFS) by means of a GRASS S88 stimulator. The electrical stimuli were delivered at a frequency of 1 every 60 or 15 s.

In other experiments, we studied the effect of drugs on the phasic and tonic contraction of the ureter induced by the hypertonic addition of 80 mM KCl. These experiments were performed in capsaicin-pretreated ureters but Bay K 8644 was not present.

Sucrose gap experiments

A single sucrose-gap, modified as described in detail by Artemenko *et al.* (1982) and Hoyle (1987) was used to investigate changes in membrane potential and mechanical activity in response to electrical stimulation. The ureters were superfused with oxygenated Krebs solution at a rate of 1 ml min^{-1}. The temperature of the solution was kept constant at 35±0.5°C. In some experiments action potentials and phasic contractions of the guinea-pig ureter were evoked for 30 min at 15 s intervals by EFS (1–2 ms pulse width, 20–40 V) and the response to CGRP was determined before and after the 'exercise' period.

Some experiments were performed in a nominally Ca^{2+}-free medium containing 1 mM ethylediaminetetraacetic acid (EDTA). The effect of CGRP, cromakalim or caffeine on membrane potential was determined after 30 min superfusion with the Ca^{2+}-free medium. In other experiments, the effect of cromakalim, caffeine and CGRP was studied in a low-K^+ medium, prepared as described above for organ bath experiments.

Statistical analysis

Each value is mean±s.e.mean. Statistical analysis was performed by Student's *t* test for paired or unpaired data or by means of analysis of variance, as appropriate. A P level <0.05 was considered statistically significant.

Drugs

Drugs used were: human αCGRP, charybdotoxin and apamin (Peninsula), cromakalim (SmithKlineBeecham), cyclopiazonic acid (CPA), glibenclamide, caffeine and capsaicin (Sigma), ryanodine and 1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)-phenyl]-3-pyridine carboxylic acid methyl ester (Bay K 8644) (Calbiochem), thapsigargin and forskolin (RBI). Caffeine (0.25 M) was dissolved in distilled water. Stock solutions of CGRP (0.1 mM), charybdotoxin (0.1 mM), apamin (0.1 mM) and ryanodine (10 mM) were prepared in distilled water. Stock solutions of cromakalim, CPA, glibenclamide, thapsigargin and forskolin (all at 10 mM) were prepared in 100% dimethylsulphoxide. Stock solutions of capsaicin and Bay K 8644 (10 mM for both drugs) were prepared in 100% ethanol.

Results

General

Direct stimulation by EFS (10 Hz, 5 ms pulse width, 60 V, trains of 1 s duration every 60 s) produced phasic contractions (twitches) of the guinea-pig ureter smooth muscle. In capsaicin-pretreated ureters and in the presence of 1 μM Bay K 8644, twitch amplitude was stable for several hours, ranging from 45–60% of the contraction to 80 mM KCl.

Thapsigargin (10 nM–3 μM), ryanodine (3–100 μM) and CPA (1–30 μM) all produced a concentration- and time-dependent enhancement of twitches (Figure 1). The time course of the effect produced by 1 μM thapsigargin, 100 μM ryanodine and 10 μM CPA (the concentrations selected for further experiments, see below) is shown in Figure 1. The effect of CPA and ryanodine was rapid in onset, peaked at 10–30 min and re-equilibrated at a new steady state 40–60 min after drug administration. In contrast, the effect of thapsigargin showed a latency of approximately 5 min, was slower in development and reached a plateau at 30–50 min from administration (Figure 1). After 60 min all these drugs had produced a comparable and steady effect on the amplitude of twitches. This exposure time was used in subsequent experiments.

Effect of CPA, ryanodine and thapsigargin on the inhibitory action of CGRP

A maximally-effective concentration of CGRP (0.1 μM) produced a transient large inhibition or even complete suppression (in 14 out of 24 cases) of twitches (range 80–100% inhibition at the peak of CGRP action; Figure 2a). After 15 min exposure to CGRP, the amplitude of twitches had recovered to about 75–90% of baseline. In the presence of 1 μM glibenclamide, CGRP failed to suppress the twitches totally: the effect of CGRP was converted to a slowly developing partial inhibition of the evoked contractions (peak at 5–10 min from administration) (Table 1).

Thapsigargin (1 μM), ryanodine (100 μM) and CPA (10 μM) all inhibited the response to CGRP in the absence of glibenclamide (Table 1): as shown in Figure 2a, the early CGRP-induced transient suppression of twitches was never observed in the presence of thapsigargin, ryanodine or CPA. As compared to the effect observed in the respective control ureters, CPA appeared to be the most effective inhibitor of the response to CGRP, since only 15% of the inhibitory effect remained in the presence of CPA; indeed, in 3 out of 9 cases

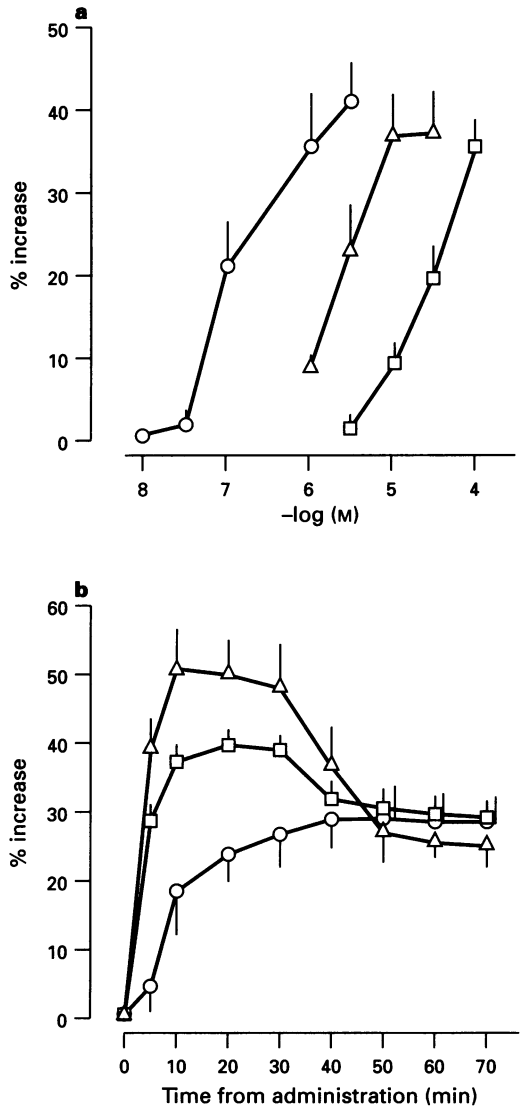

Figure 1 (a) Concentration-dependent potentiation of the response to EFS by thapsigargin (○), cyclopiazonic acid (CPA) (△) and ryanodine (□). (b) Time course of the potentiation of the EFS-induced contractions of the guinea-pig isolated ureter by 1 μM thapsigargin (○), 10 μM CPA (△) or 100 μM ryanodine (□). Each value is mean ± s.e.mean of 6–15 experiments.

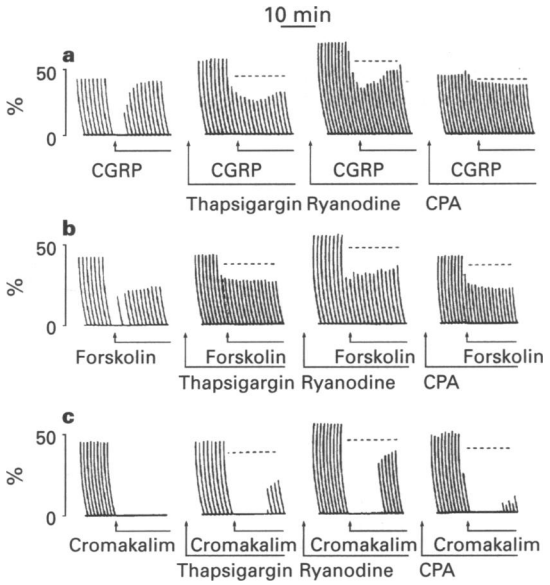

Figure 2 Effect of 60 min exposure to 1 μM thapsigargin, 100 μM ryanodine or 10 μM cyclopiazonic acid (CPA) on the inhibition of electrical stimulation-induced twitch contractions of guinea-pig ureter induced by 0.1 μM CGRP (a), 10 μM forskolin (b) or 3 μM cromakalim (c). Tracings represent experiments in ureters from different animals. Vertical lines are % of the maximal contraction to 80 mM KCl, and horizontal dotted lines indicate the amplitude of the control response to EFS before drug administration. Note that CGRP and forskolin produced a transient suppression of the response to electrical stimulation: the suppressant effect, which is glibenclamide-sensitive, was prevented by thapsigargin, ryanodine or CPA, while the suppressant effect of cromakalim was unchanged.

Table 1 Effect of 60 min exposure to cyclopiazonic acid (CPA), ryanodine and thapsigargin on the inhibitory effect of CGRP on EFS-induced twitch contractions of the guinea-pig isolated ureter

	Effect of CGRP (0.1 μM)			
	Peak inhibition in absence of glibenclamide (%)	*Time to peak (min)*	*Inhibition at 15 min (%)*	*Peak inhibition in presence of 1 μM glibenclamide (%)*
Control	91 ± 4	2 ± 0.1	16 ± 4	32 ± 3*
CPA 10 μM	14 ± 5*	9.5 ± 2*	18 ± 3	24 ± 3
Control	96 ± 4	2.0 ± 0.2	10 ± 2	31 ± 5**
Ryanodine 100 μM	58 ± 4*	2.8 ± 0.3	21 ± 3	37 ± 4
Control	84 ± 6	2.0 ± 0.3	20 ± 3	30 ± 3**
Thapsigargin 1 μM	44 ± 4*	7.5 ± 0.9*	40 ± 6	36 ± 4

Each value is mean ± s.e. of the mean of 5–9 experiments. *Significantly different from control, $P < 0.05$. **Significantly different from peak inhibition obtained in the absence of glibenclamide, $P < 0.05$.

tested, the response to CGRP was totally abolished in the presence of CPA. Thapsigargin and CPA also delayed the time to peak of the inhibitory effect of CGRP (Table 1).

The slowly developing partial inhibitory effect produced by CGRP in the presence of 1 μM glibenclamide was not reduced by thapsigargin or ryanodine; CPA produced a slight, non significant reduction of this effect (Table 1).

Effect of CPA, ryanodine and thapsigargin on the inhibitory action of forskolin

Forskolin (10 μM) produced a prompt inhibition (range 54–100%) of twitches, peaking at 1–2 min from administration and, in 9 out of 24 cases tested totally suppressed twitches (Figure 2b). As shown previously (Maggi *et al.*, 1996), the early peak of inhibition produced by forskolin is glibenclamide-sensitive, although to a lesser extent than the peak response to CGRP. The suppressant effect of forskolin was never observed in ureters pretreated with CPA, ryanodine or thapsigargin (Figure 2b). Data in Table 2 indicate that the three drugs significantly reduced the peak inhibitory effect of forskolin and that thapsigargin and CPA both delayed the time to peak of the inhibitory response to forskolin. The delayed inhibitory effect of forskolin, measured at 15 min from its administration to the bath was unchanged by thapsigargin, ryanodine or CPA (Table 2).

Effect of CPA, ryanodine and thapsigargin on the inhibitory action of cromakalim

Cromakalim (3 μM) invariably and totally suppressed twitches ($n=18$; Figure 2c). In 12 out of 18 cases tested, the total suppression was maintained throughout the 15 min observation period.

Neither thapsigargin, ryanodine nor CPA prevented the suppressant action of cromakalim or affected the time to peak of its action (Figure 2c). With each one of the three drugs, however, a somewhat faster recovery of evoked contractions from suppression induced by cromakalim was observed: thus the time of suppression of twitches averaged 12±1, 7.8±2, 8.6±2 and 7±2 min in controls and in the presence of thapsigargin, ryanodine and CPA, respectively ($n=5-18$).

Effect of a low concentration of CPA on the response to CGRP

The above experiments indicate that Ca^{2+} mobilization from the SR is in some way involved in the K channel opening by CGRP in guinea-pig ureter. In the previous experiments we used a high concentration and a prolonged contact time to study the effect of CPA, thapsigargin and ryanodine. Under those conditions, the Ca^{2+} store is likely to have been depleted. In the following experiments we studied the effect of a short contact time with a low concentration (1 μM for 10 min) of CPA on the response to CGRP. We speculated that the K channel activated by CGRP in the guinea-pig ureter may be Ca^{2+}-sensitive and that CGRP could act by producing a transient Ca^{+} release from the store. Should this hypothesis be correct, a moderate degree of impairment of the SR Ca^{2+} pump may actually increase or prolong the effect of CGRP, i.e. produce an effect opposite to that observed after exhaustion of the store. Furthermore, since 'exercise' selectively decreases the K channel opener action of CGRP in the ureter (Maggi *et al.*, 1996) these experiments were conducted at two different EFS driving frequencies, i.e. by delivering 1 stimulus every 60 or 15 s.

At a driving frequency of 1 stimulus every 60 s, CGRP suppressed twitches for 5.5±0.7 min ($n=8$); the duration of this suppressant effect was almost doubled by 1 μM CPA (Figure 3). The inhibitory effect of CGRP was significantly reduced and shortened when tested at a driving frequency of 1 stimulus every 15 s (cf. Maggi *et al.*, 1996). CPA (1 μM for 10 min) potentiated and prolonged the response to CGRP at this driving frequency (Figure 3).

Effect of caffeine and interactions with the inhibitory action of CGRP

In a previous study no contractile response to caffeine was detected in the guinea-pig ureter (Burdyga & Magura, 1986). We speculated that caffeine, by mobilizing Ca^{2+} from the SR, might produce an inhibitory effect in the guinea-pig ureter, possibly through K channel activation.

In a first series of experiments, a cumulative concentration-response curve to caffeine (1–10 mM) was produced in 4 ureters at a driving frequency of 1 stimulus every 60 or every 15 s: caffeine produced a concentration-dependent inhibition of twitches which was similar at the two driving frequencies

Table 2 Effect of 60 min exposure to cyclopiazonic acid (CPA), ryanodine and thapsigargin on the inhibitory effect of forskolin on EFS-induced twitch contractions of the guinea-pig isolated ureter

	Effect of forskolin (10 μM) *Peak inhibition* (%)	*Time to peak* (min)	*Inhibition at 15 min* (%)
Control	74±8	1.7±0.2	49±4
CPA 10 μM	39±6*	4.1±1.1*	38±6
Control	75±6	1.5±0.2	53±9
Ryanodine 100 μM	53±3*	1.9±0.2	45±4
Control	96±2	1.7±2	50±9
Thapsigargin 1 μM	63±2*	4.0±1.0*	59±4

Each value is mean±s.e. of the mean of 7, 8 and 6 experiments for CPA, ryanodine and thapsigargin, respectively. *Significantly different from controls, $P<0.05$.

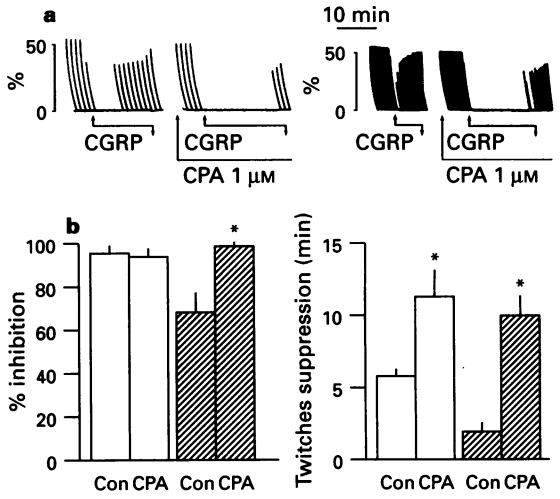

Figure 3 Effect of a low concentration of cyclopiazonic acid (CPA, 1 μM) on the inhibitory effect produced by CGRP (0.1 μM) on twitches produced by direct electrical stimulation of ureter smooth muscle. (a) Electrical stimuli were delivered at a driving frequency of 1 stimulus every 60 s (left) or 15 s (right): CPA markedly prolonged the suppressant effect of CGRP at both driving frequencies. Note that when ureters were driven at a higher frequency the duration of the suppressant effect of CGRP is shorter than at a lower driving frequency. (b) Effect of a low concentration of CPA (1 μM for 10 min) on peak inhibition (left) and duration of the suppressant effect (right) produced by CGRP (0.1 μM) on twitches produced by direct electrical stimulation at a driving frequency of 1 stimulus every 60 s (open columns) or every 15 s (hatched columns). Each value is mean± s.e.mean of 9–11 experiments. *Significantly different from control (Con), $P<0.05$.

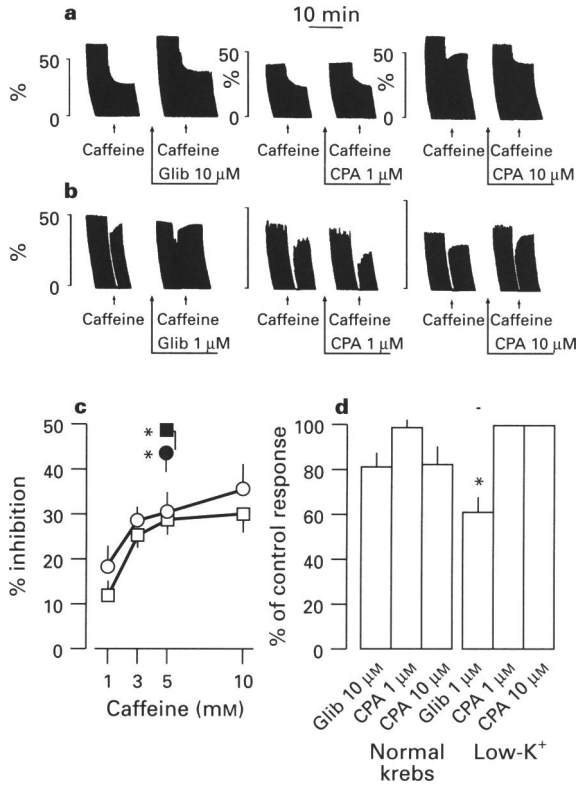

Figure 4 (a,b) Inhibitory effect of caffeine (5 mM) on twitch contractions produced by direct electrical stimulation of ureter smooth muscle in (a) normal Krebs solution or (b) low K^+ Krebs solution in the absence and presence of glibenclamide (Glib, 10 μM, 15 min before) and cyclopiazonic acid (CPA; 1 μM for 10 min or 10 μM for 60 min). (c) Concentration-dependent inhibition by caffeine of the amplitude of twitch contractions produced by direct EFS of ureter smooth muscle at a driving frequency of 1 stimulus every 15 s (○) or every 60 s (□). The solid symbols indicate the inhibitory effect produced by 5 mM caffeine administered as a single concentration to the bath at a driving frequency of 1 stimulus every 15 s (●) or every 60 s (■). Each value is mean ± s.e.mean of 4 experiments. (d) Effect of glibenclamide (Glib, 10 μM 15 min before) and cyclopiazonic acid (CPA, 1 μM 10 min before or 10 μM 60 min before) on the peak inhibition by caffeine of twitch contractions produced by direct EFS of ureter smooth muscle in normal or low K^+ Krebs solution. *Significantly different from the control response to CGRP, $P<0.05$.

(Figure 4c). The effect of caffeine peaked within 5 min from its application and was either maintained or showed little recovery during the 5 min observation time.

At 10 mM, caffeine produced the appearance of spontaneous activity having an amplitude similar to that of the EFS-evoked contractions while simultaneously depressing twitches (not shown). When 5 mM caffeine was applied as a single concentration to the bath it produced a significantly larger inhibition of twitches than that observed during its cumulative addition to the bath at both driving frequencies (Figure 4c).

From the above, a concentration of 5 mM caffeine was selected for further experiments, in which twitches were evoked at a driving frequency of 1 stimulus every 15 s (Figure 4a and d). Caffeine (5 mM) produced a prompt and partial inhibition of twitches evoked by EFS; the peak inhibitory effect was quite variable from one preparation to another (44 ± 3% inhibition, $n=42$). Neither glibenclamide (10 μM for 15 min) nor CPA (1 μM for 10 min or 10 μM for 60 min) affected twitch inhibition induced by caffeine (Figure 4a and d).

Since caffeine produces a small and very transient hyperpolarization in the sucrose gap (see below), we tested the effect of caffeine after lowering the K^+ content of the Krebs solution from 5.9 to 1.2 mM (Figure 4b and d).

We showed previously (Santicioli & Maggi, 1994; Maggi *et al.*, 1994a) that lowering extracellular K^+ concentration markedly enhances the glibenclamide-sensitive relaxation produced by forskolin and CGRP. Fifteen to twenty min from the application of the low K^+ medium the twitch amplitude had decreased to 71 ± 3% of control ($n=9$) and, in some preparations, the amplitude of the twitches became irregular (e.g. Figure 4b middle panel).

When tested in a low-K^+ medium, caffeine (5 mM) invariably produced a prompt, complete and transient (1.44 ± 0.2 min, $n=9$) suppression of twitches (Figure 4b and d). This complete suppression was abolished in the presence of 1 μM glibenclamide (15 min before). CPA (1 μM for 10 min) did not significantly affect the peak or the duration of the transient twitch suppression produced by 5 mM caffeine in low-K^+ medium (suppression time was 1.54 ± 0.3 and 2.04 ± 0.5 min before and after application of CPA, $n=6$, NS).

Following application of 10 μM CPA for 60 min the suppressant effect of caffeine in low-K^+ medium was preserved, albeit slightly reduced in duration (from 1.7 ± 0.2 to 1.2 ± 0.25 min, $n=5$, $P<0.05$).

Effect of caffeine on twitch inhibition by CGRP and cromakalim in normal Krebs and in a low-K^+ medium

In these experiments the ureters were driven by EFS at a frequency of 1 stimulus every 15 s and the inhibitory effect of CGRP (0.1 μM) or cromakalim (3 μM) was studied before and 5 min after application of caffeine (5 mM). The suppression produced by CGRP lasted for 2.4 ± 0.8 min ($n=5$) in 5 out of 11 ureters; caffeine produced 38 ± 5% inhibition of twitches and, in the presence of caffeine, the inhibitory effect of CGRP was greatly reduced (33 ± 7% inhibition of residual twitches amplitude, $n=11$). In the presence of caffeine, in no instance did CGRP completely suppress the twitches. Caffeine also reduced the glibenclamide- (1 μM) resistant response to CGRP which averaged 31 ± 3% inhibition of twitches before and 22 ± 3% 5 min after the challenge with caffeine ($n=6$, $P<0.05$).

Cromakalim (3 μM, $n=8$) promptly and invariably blocked twitches in the absence of caffeine: the suppressant effect lasted for 11 ± 1.2 min, after which a spontaneous recovery was observed. A previous challenge with 5 mM caffeine significantly reduced the suppressant effect of cromakalim: the peak inhibitory effect averaged 59 ± 12% ($n=8$); cromakalim transiently blocked twitches in only 2 out of 8 cases for 2 and 3 min. A transient suppression of twitches was always observed upon washout suggesting that removal of caffeine enabled twitch suppression by cromakalim.

Comparable experiments were then performed in low-K^+ medium: in these conditions CGRP blocked twitches in all cases tested for 4 ± 0.5 min ($n=6$) and cromakalim invariably blocked twitches for > 15 min ($n=5$). Caffeine (5 mM) likewise transiently blocked twitches in all cases tested ($n=11$): at 5 min from its application, twitches had recovered 30–70% of their original amplitude. At this stage, the application of CGRP produced a transient inhibition of twitches (61 ± 11% inhibition, $P<0.05$) but failed to block them; in sharp contrast, the suppressant effect of cromakalim was totally unaffected by previous application of caffeine.

Effect of CPA on the inhibitory action of CGRP on KCl-induced contractions of the ureter

Application of 80 mM KCl produces distinct phasic and tonic contraction in the guinea-pig isolated ureter: CGRP exerts a profound inhibitory effect on both components of the response to KCl while cromakalim selectively inhibits the phasic response (Maggi *et al.*, 1994a). Furthermore, the inhibitory effect of both CGRP and cromakalim on phasic contractions is glibenclamide-sensitive, while the effect of CGRP on the tonic contractions is glibenclamide-resistant. Since CPA produced a large inhibition of phasic contractions to EFS and also apparently reduced the effect of CGRP in the presence of glib-

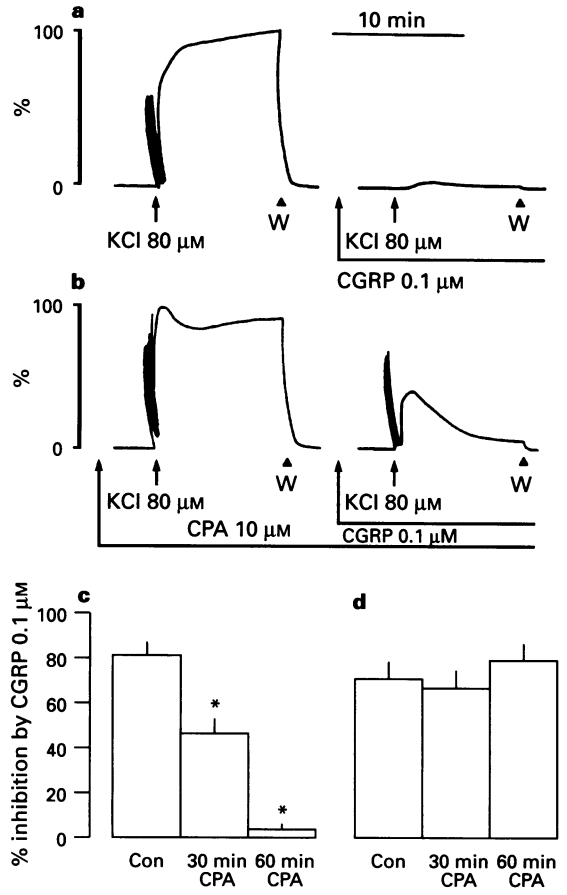

Figure 5 Effect of CGRP (0.1 μM) on the phasic and tonic components of the contractile response of the guinea-pig ureter to 80 mM KCl, in the absence (a) and presence (b) of 10 μM CPA (60 min). W = washout. Experiments were performed on paired ureters from the same animal, one of which served as a control and the other received CPA. After having established a control response to KCl, a second challenge was performed 60 min later; CGRP was administered 5 min before the second application of KCl. Note that CGRP blocked both the phasic and the tonic components of the response to KCl. In the presence of CPA, the inhibitory action of CGRP on the phasic response to KCl was largely prevented while the inhibitory effect on the tonic response (measured at 10 min from application of KCl) was preserved. Effect of 30 or 60 min pre-exposure to 10 μM CPA on the inhibitory effect of CGRP on the phasic (c) and tonic (d) components of the response to 80 mM KCl. Each value is mean ± s.e.mean of 9–12 experiments. *Significantly different from control, $P<0.05$.

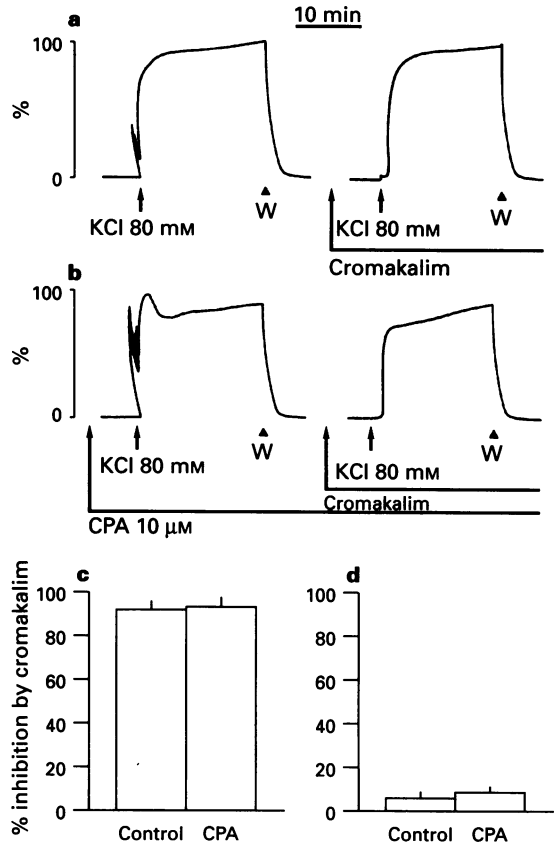

Figure 6 Effect of cromakalim (3 μM) on the phasic and tonic components of the contractile response of the guinea-pig ureter to 80 mM KCl, in the absence (a) and presence (b) of 10 μM cyclopiazonic acid (CPA) (60 min). W = washout. Experiments were performed on paired ureters from the same animal, one of which served as a control and the other received CPA. After having established a control response to KCl, a second challenge was performed 60 min later; cromakalim was administered 5 min before the second application of KCl. Note that cromakalim selectively blocked the phasic component of the response to KCl. In the presence of CPA, the inhibitory action of cromakalim on the phasic response to KCl was unaffected. (c and d) Effect of 60 min pre-exposure to 10 μM CPA on the inhibitory effect of cromakalim on the phasic (c) and tonic (d) components of the response to 80 mM KCl. Each value is mean ± s.e.mean of 4 experiments. *Significantly different from control, $P<0.05$.

enclamide, it appeared worthwhile to examine its effect on CGRP-induced inhibition of phasic and tonic contractions to KCl. The effect of CPA (10 μM, 30 and 60 min before) on the response to KCl has been detailed in a previous study (Maggi *et al.*, 1994c): briefly, CPA increased the amplitude of the phasic response to KCl without affecting the tonic response; a hump in the contraction to KCl was interposed between the phasic and the tonic responses in the presence of CPA (Figure 5b). As shown in Figure 5, CPA largely prevented the inhibitory action of CGRP on the phasic response to KCl, its effect being greater after 60 than 30 min contact time. In contrast, the CGRP-induced inhibition of the tonic response still occurred in the presence of CPA (Figure 5). Interestingly, the hump seen in CPA, peaking at 1–2 min from KCl administration remained well evident in the presence of CGRP (Figure 5).

Figure 6 shows the results of corresponding experiments performed with 3 μM cromakalim, which largely inhibited or suppressed the phasic response to KCl without affecting the tonic contraction. CPA (10 μM, 60 min before) did not prevent the action of cromakalim, but the hump produced by CPA was not evident in the presence of cromakalim.

Sucrose gap

Figure 7 shows a comparison of the changes in membrane potential observed during a 5 min superfusion of the guinea-pig ureter with caffeine (5 mM), CGRP (0.1 μM), cromakalim (3 μM) and forskolin (10 μM).

CGRP and cromakalim produced a sustained hyperpolarization of the membrane while the hyperpolarization induced by caffeine and forskolin was transient and, in the case of caffeine it was replaced by a depolarization within 3 min from its application (Figure 7). The depolarization produced by caffeine and forskolin recovered rapidly upon changing the superfusion medium to a drug-free Krebs solution.

Superfusion with 1 μM glibenclamide for 15 min totally abolished ($n=4$) the caffeine-induced hyperpolarization (0.64 ± 0.05 mV before glibenclamide, $n=4$; Figure 8a) while the subsequent depolarization was unchanged (0.61 ± 0.15 and 0.64 ± 0.14 mV in the absence and presence of glibenclamide,

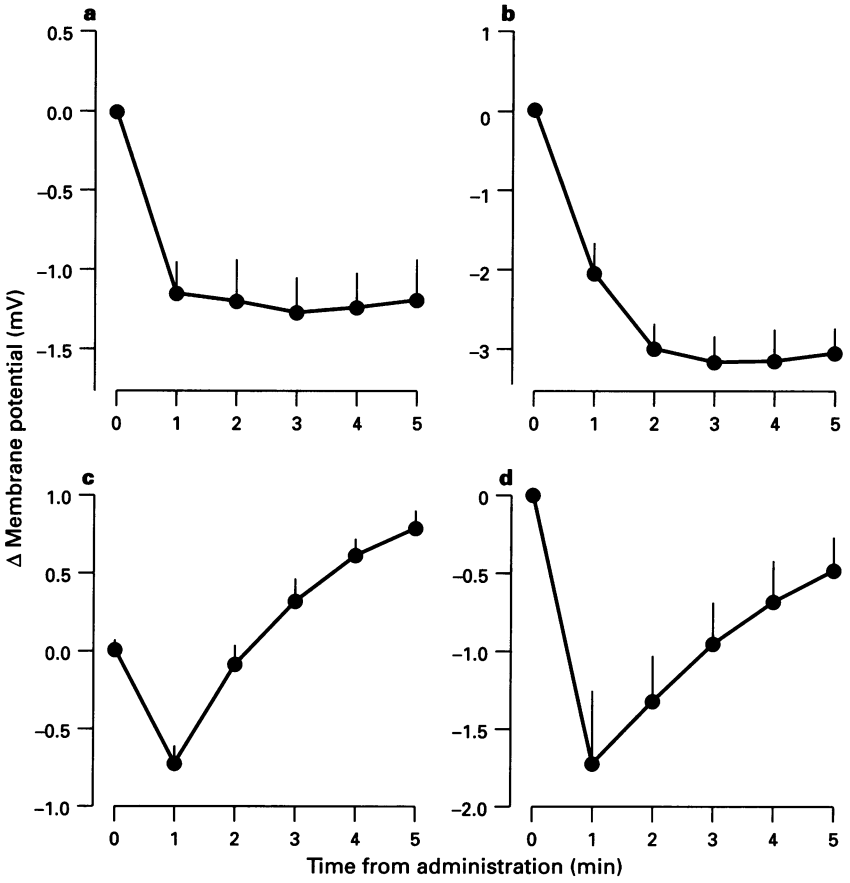

Figure 7 Changes in membrane potential produced during a 5 min superfusion with (a) CGRP (0.1 μM), (b) cromakalim (3 μM), (c) caffeine (5 mM) or (d) forskolin (10 μM). Note that CGRP and cromakalim produced a sustained hyperpolarization while the hyperpolarization induced by caffeine or forskolin was transient. The caffeine-induced hyperpolarization was followed by a sustained depolarization within 3 min of drug application. Each value is mean ± s.e.mean of 5–7 experiments.

respectively). We showed previously that the hyperpolarization produced by CGRP, cromakalim or forskolin is likewise abolished by 1 or 10 μM glibenclamide (Santicioli & Maggi, 1994; Santicioli *et al.*, 1995).

The involvement of K channels in the response to caffeine was further suggested by the observation that, when elicited in a low-K^+ medium, the hyperpolarization was significantly enhanced (by 66%, Figures 8b and 9a). The hyperpolarization produced by CGRP or cromakalim was likewise enhanced in low-K^+ medium, by 186 and 82%, respectively (Figure 9a). The depolarization produced by caffeine was unchanged when evoked in a low-K^+ medium (0.74 ± 0.08 and 82 ± 0.11 mV, $n=3$, NS).

In further experiments we compared the effect of prolonged (30 min) superfusion with Ca^{2+}-free EDTA (1 mM)-containing Krebs solution or of a prolonged (60 min) application of 10 μM CPA on the changes in membrane potential produced by CGRP (0.1 μM for 15 s), cromakalim (3 μM for 15 s) or caffeine (5 mM for 5 min).

As shown in Figure 9b, superfusion with Ca^{2+} -free medium reduced the hyperpolarization produced by caffeine, cromakalim and CGRP by about 50, 50 and 75%, respectively ($P<0.05$ for each drug). The depolarization produced by caffeine was slightly increased by this procedure (from 0.74 ± 0.1 to 1.08 ± 0.12 mV, $n=5$, $P<0.05$). In three experiments, the period of superfusion with Ca^{2+}-free EDTA-containing medium was extended to 60 min before the caffeine challenge: in these experiments the control hyperpolarization to caffeine was 0.66 ± 0.07 mV which was reduced to 0.29 ± 0.13 mV after 60 min superfusion with the Ca^{2+} -free medium. Therefore doubling the period of superfusion with Ca^{2+} -free medium did not produce a further inhibition of the hyperpolarizing effect of caffeine.

Figures 8 and 9c show that after 60 min superfusion with 10 μM CPA the hyperpolarization produced by CGRP was almost abolished, while that induced by caffeine or cromakalim was not significantly affected. In particular the hyperpolarization induced by caffeine was slightly reduced as compared to control response (from 0.70 ± 0.08 to 0.54 ± 0.07 mV, $n=5$, NS). The depolarization induced by caffeine was unchanged by CPA (0.52 ± 0.12 and 0.66 ± 0.11 mV, $n=5$, NS).

A previous challenge with 5 mM caffeine, 5 min before, significantly reduced the hyperpolarization induced by CGRP (0.1 μM for 15 s) or cromakalim (3 μM for 15 s) by 46 and 55%, respectively. The hyperpolarization induced by cromakalim was reduced from 1.58 ± 0.11 to 0.86 ± 0.15 mV ($n=5$, $P<0.05$) while that induced by CGRP was reduced from 1.13 ± 0.07 to 0.51 ± 0.06 mV ($n=4$, $P<0.05$).

Neither charybdotoxin (0.1 μM for 5 min) nor apamin (0.1 μM for 5 min) affected the CGRP-induced hyperpolarization: the effect of CGRP averaged 1.16 ± 0.20 and 0.92 ± 0.10 mV in the absence and presence of charybdotoxin, respectively ($n=5$) and 1.27 ± 0.13 and 1.16 ± 0.07 mV in the absence and presence of apamin, respectively ($n=3$).

Discussion

Role of sarcoplasmic reticulum in electromechanical coupling of the guinea-pig ureter

In previous studies (Maggi *et al.*, 1994b,c) we showed that CPA and ryanodine produce a sustained depolarization of the membrane, enhance the amplitude of twitches and prolong the duration of the contractile cycle of the guinea-pig ureter. As

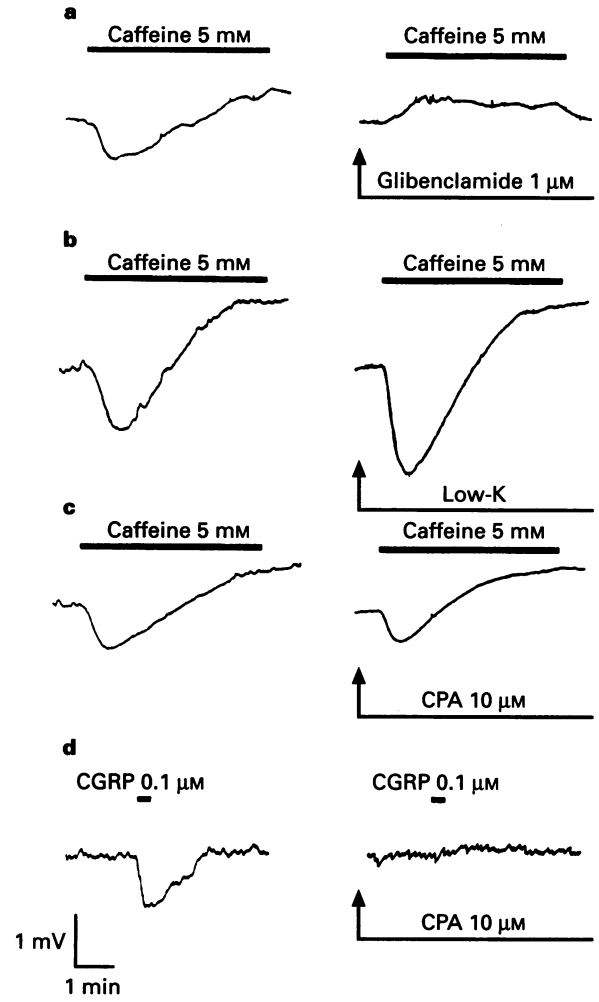

Figure 8 Biphasic changes in membrane potential of the smooth muscle of guinea-pig ureter observed during a 5 min superfusion with 5 mM caffeine; note that caffeine induced a transient hyperpolarization followed by a more sustained depolarization. The hyperpolarization was blocked by 1 μM glibenclamide (a) and enhanced when evoked in a low K medium (b). Effect of cyclopiazonic acid (10 μM for 60 min) on membrane hyperpolarization produced by caffeine (c) or CGRP (d).

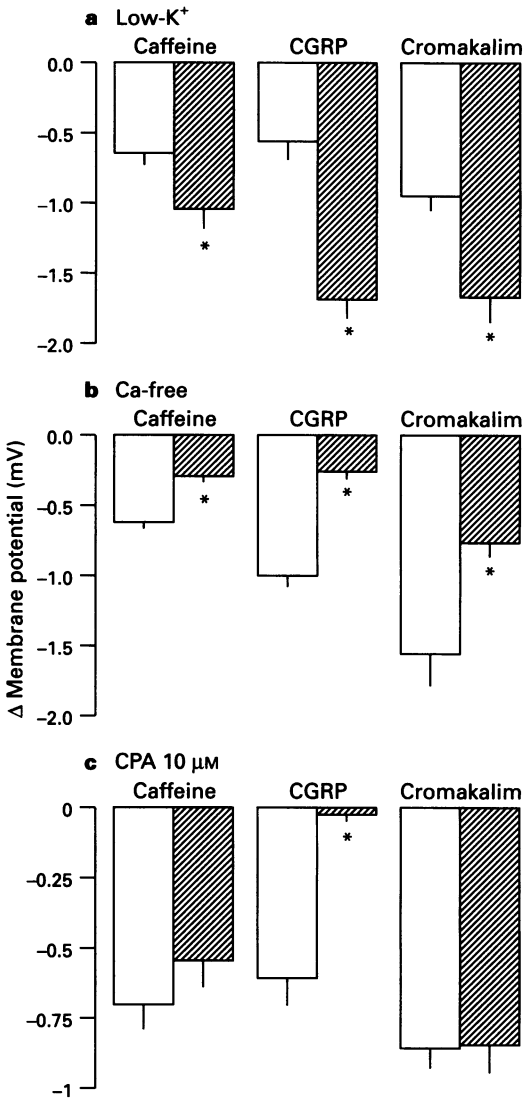

Figure 9 (a) Effect of superfusion with a low-K medium on the hyperpolarization produced by caffeine (5 mM), CGRP (0.1 μM) or cromakalim (3 μM). Open columns show the response to each agent in normal Krebs solution. Hatched columns represent the response obtained after 30 min superfusion with a low-K medium. (b) Effect of superfusion with a Ca-free medium (plus EDTA 1 mM) on the hyperpolarization produced by caffeine (5 mM), CGRP (0.1 μM) or cromakalim (3 μM). Open columns show the response to each agent in normal Krebs solution. Hatched columns represent the response obtained after 30 min superfusion with a Ca-free medium. (c) Effect of cyclopiazonic acid (CPA, 10 μM for 60 min) on the hyperpolarization produced by caffeine (5 mM), CGRP (0.1 μM) or cromakalim (3 μM). Open columns show the response to each agent in normal Krebs solution. Hatched columns represent the response obtained in the presence of CPA. All values are mean ± s.e.mean of 3–4 experiments. *Significantly different from the control response, $P<0.05$.

shown here, another blocker of SR Ca^{2+} ATPase, thapsigargin also enhances the EFS-evoked twitches. The comparable effect produced by three chemically-unrelated drugs which affect Ca^{2+} uptake/release from SR is strongly indicative that the observed changes are indeed secondary to their effect on SR function.

Thapsigargin (Thastrup *et al.*, 1990; Sagara *et al.*, 1992a,b) and CPA (Goeger & Riley, 1989; Deng & Kwan, 1991) have been characterized as selective inhibitors of SR Ca^{2+} ATPase. CPA interferes with the binding of Ca^{2+} to the high affinity sites of the ATPase (Goeger & Riley, 1989; Seidler *et al.*, 1989). Thapsigargin irreversibly inhibits the high-affinity binding of Ca^{2+} to the enzyme, thereby forming a catalytically-inactive, dead-end complex (Sagara *et al.*, 1992a,b). On the other hand, ryanodine interacts with the Ca^{2+}-induced Ca^{2+}-release (CICR) channel of the SR (Coronado *et al.*, 1994 for review). In spite of their different molecular targets/modes of action, the prolonged exposure to high concentrations of these three agents is expected to produce a common final effect, i.e. depletion of the SR Ca^{2+} store. The failure of these three agents to inhibit twitches produced by repetitive cycles of stimulation indicates that the SR does not provide activator Ca^{2+} for excitation-contraction coupling in guinea-pig ureter; rather, the re-uptake function of the SR appears important for terminating the contractile event (Maggi *et al.*, 1994b,c). Imaizumi *et al.* (1989) presented evidence suggesting that Ca^{2+} release from the store could be relevant for activating K channels and regulating the membrane potential in guinea-pig ureter smooth muscle. The sustained depolarization and increased excitability of the ureter produced by store depletion by CPA or ryanodine suggests that spontaneous discharge of Ca^{2+} from the SR in the vicinity of the plasma membrane normally participates in the setting of the resting membrane potential of the guinea-pig ureter (Maggi *et al.*, 1994b,c) possibly by activating a class of Ca^{2+}-dependent K channels. As will be discussed below, the present data indicate that the main physiological neuro-

transmitter of the guinea-pig ureter, CGRP activates a parallel mechanism in producing K channel activation, hyperpolarization and suppression of latent pacemakers.

Sarcoplasmic reticulum and activation of glibenclamide-sensitive K channels by CGRP

In previous studies we established that the hyperpolarizing and twitch-suppressant action of CGRP is blocked by low concentrations of glibenclamide (IC_{50} 0.13 μM; Maggi *et al.*, 1994a; 1995). CGRP also induces a glibenclamide-resistant selective elevation of cyclic AMP in guinea-pig ureter (Santicioli *et al.*, 1995) and functional evidence has been obtained linking cyclic AMP elevation and protein kinase A (PKA) activation to the glibenclamide-sensitive component of CGRP action (Maggi *et al.*, 1995). This, and the notion that CGRP activates ATP-sensitive K channels (K_{ATP}) via a cyclic AMP/PKA-dependent pathway in other guinea-pig smooth muscles (Zhang *et al.*, 1994a,b; Quayle *et al.*, 1994), provides the basis for speculating that CGRP likewise activates K_{ATP} in the guinea-pig ureter (Santicioli *et al.*, 1994; 1995; Maggi *et al.*, 1994a; 1995). The present results provide evidence linking a mobilization of intracellular Ca^{2+} to K channel activation by CGRP: in fact, CPA, thapsigargin and ryanodine prevent the activation of a class of glibenclamide-sensitive K channels by CGRP or forskolin while having little or no effect on the response to cromakalim. These results indicate that the chain of events initiated by adenylate cyclase activation (direct or receptor-mediated by forskolin and CGRP, respectively) leading, via cyclic AMP accumulation (Santicioli *et al.*, 1995), to the opening of K channels, involves a step related to Ca^{2+} handling from SR. The most straightforward hypothesis to account for these findings, an involvement of Ca^{2+}-dependent K channels, can be excluded in view of the negative results obtained with charybdotoxin and apamin, which failed to affect the CGRP-induced hyperpolarization.

Following the results of experiments obtained with maximally-effective concentrations of CPA, ryanodine and thapsigargin, we speculated that CGRP, by releasing Ca^{2+} from the SR store, produces a transient elevation of intracellular Ca^{2+} in the vicinity of the plasma membrane to activate a class of glibenclamide-sensitive K channels. This was supported by the observation that the hyperpolarization induced by caffeine is blocked by glibenclamide and CPA: thus we conclude that CGRP and caffeine similarly activate a class of glibenclamide-sensitive K channels through a step involving mobilization of intracellular Ca. Taken together, these results raise the question as to whether ATP-sensitive K channels in guinea-pig ureter might be Ca^{2+}-dependent. Although ATP-sensitive K channels are usually considered to be Ca^{2+}-independent, their remarkable heterogeneity (Ashcroft & Ashcroft, 1990 for review) leaves open the possibility that certain classes are modulated by elevation of intracellular Ca^{2+}. Indeed certain ATP-sensitive K channels are blocked by an elevation of cytoplasmic Ca^{2+} (e.g. Kakei & Noma, 1984; Findlay, 1987; Hussain & Wareham, 1994; Hehl *et al.*, 1994): thus it may be speculated that Ca^{2+} released by CPA, ryanodine or thapsigargin had inactivated ATP-sensitive K channels, thus blocking the glibenclamide-sensitive action of CGRP. However, this interpretation does not account for the glibenclamide-sensitive hyperpolarization produced by caffeine. On the other hand, ATP-sensitive K channels have been shown to be activated by an elevation of intracellular Ca^{2+} in the rat portal vein (Kajioka *et al.*, 1990); moreover, a reduced hyperpolarization to cromakalim in Ca^{2+}-free medium has been noted in previous studies (e.g. Nakao *et al.*, 1988; Gelband *et al.*, 1989). That the K channel activated by CGRP- and cromakalim in the guinea-pig ureter may be Ca^{2+}-sensitive is indirectly supported by the observation that superfusion with a Ca^{2+}-free medium reduced the hyperpolarization induced by either agent, although the use of EDTA in our experiments may have influenced the results by lowering the Mg concentration (e.g. Kozlowski *et al.*, 1989).

In a previous study (Santicioli *et al.*, 1995) we noted a dissociation between the ability of CGRP to produce a sustained elevation of cyclic AMP and the transient nature of the glibenclamide-sensitive suppression of evoked contractions of the guinea-pig ureter. 'Exercise', produced by increasing the frequency of EFS driving, reduced and shortened the CGRP-induced hyperpolarization without affecting the hyperpolarization induced by cromakalim (Maggi *et al.*, 1996): this may suggest a relationship between the ability of CGRP to activate K channels and cell metabolism. An ability of CGRP to activate K channels through Ca^{2+} mobilization from the SR is compatible with these observations. On the one hand, the CGRP-sensitive Ca^{2+} store may be transiently exhausted following CGRP challenge, accounting for the transient nature of hyperpolarization despite the progressively increasing elevation of cyclic AMP; on the other hand, 'exercise' could increase the activity of the SR Ca^{2+} pump thus reducing/shortening the intensity/duration of the Ca^{2+} transient induced by CGRP and of the resulting hyperpolarization. The results of experiments using a low concentration of CPA and caffeine are both in agreement with the second explanation.

In fact, a low concentration of CPA markedly prolonged the glibenclamide-sensitive component of CGRP action, and counteracted the adverse effect of 'exercise', while a high concentration of CPA blocked the hyperpolarization produced by CGRP. Thus CPA exerts a dual effect of K channel activation by CGRP. In the presence of 10 μM CPA, the glibenclamide-resistant relaxant action of CGRP was also slightly affected, an effect not evident with thapsigargin or ryanodine. Since the inhibition by CGRP of the response to KCl was unchanged by CPA we can exclude a direct interaction of CPA with CGRP receptors. Moreover, CPA did not prevent the selective suppressant effect of cromakalim on the phasic response to KCl, further demonstrating the selectivity of its CGRP-blocking action.

Caffeine-induced activation of glibenclamide-sensitive K channels

Caffeine is a tool widely used to produce Ca^{2+} mobilization from the SR. In many smooth muscles, the caffeine-induced Ca^{2+} release sustains a transient contraction and depletion of the store is used to infer whether a given agent produces contraction by mobilizing Ca^{2+} from the SR (e.g. Iino, 1989; Ganitkevish & Isenberg, 1992; Missiaen *et al.*, 1992 for review). The results of previous studies have consistently failed to detect contraction or elevation of intracellular Ca^{2+} following caffeine application to the guinea-pig ureter (Burdyga & Magura, 1986; Aaronson & Benham, 1989). However, Imaizumi *et al.* (1989) showed that application of 5 mM caffeine induces contraction of single ureter cells and transiently enhances spontaneous transient outward currents. Since there is little evidence that the SR contributes to activator Ca^{2+} for electromechanical coupling in guinea-pig ureter, while SR Ca^{2+} cycling may be important for setting of membrane potential and regulation of excitability (see Maggi *et al.*, 1994b,c), we speculated that Ca^{2+} release by caffeine may be too small to produce a consistent and measurable elevation of intracellular Ca^{2+} and contraction in the ureter, but, if occurring in the vicinity of the membrane, a small elevation of free Ca^{2+} could transiently activate K channels. This hypothesis is supported by the observation that caffeine induces a transient hyperpolarization in the guinea-pig ureter, which is enhanced in a low-K^+ medium. The observation that the hyperpolarization induced by caffeine is also glibenclamide-sensitive basically agrees with the idea that the K channels activated by cromakalim and CGRP in the guinea-pig ureter are Ca^{2+}-sensitive.

The functional and electrophysiological experiments presented here indicate that caffeine produces at least three distinct effects: (a) a transient, glibenclamide-sensitive membrane hyperpolarization which is sufficiently intense and long-lasting to produce a transient, glibenclamide-sensitive relaxation when magnified by lowering extracellular K^+; (b) a more sustained

depolarization, unaffected by glibenclamide, which is probably responsible for the appearance of 'spontaneous' contractions in organ bath experiments upon application of 5–10 mM caffeine and (c) a glibenclamide-resistant relaxant effect of unspecified origin. Overall, the profile of changes in membrane potential produced by caffeine more closely resembles that produced by forskolin than that produced by CGRP, although the hyperpolarization by caffeine is more sensitive to blockade by glibenclamide than that produced by forskolin (cf. Santicioli *et al.*, 1995). In spite of its complex profile of action, the hyperpolarization produced by caffeine is consistent with the proposal that Ca^{2+} mobilization from the SR activates glibenclamide-sensitive K channels in the guinea-pig ureter. The observation that a previous application of caffeine significantly reduced the hyperpolarization and relaxation of CGRP mirrors a number of experimental situations in which a previous challenge with caffeine has been shown to prevent or reduce smooth muscle contraction produced by agonists which mobilize Ca^{2+} from the SR store (Iino, 1989; Ganitkevich & Isenberg, 1992; Missiaen *et al.*, 1992). Interestingly, a fraction of the hyperpolarization produced by CGRP was caffeine-resistant, suggesting that the Ca^{2+} stores mobilized by these two agents may not exactly overlap. This is further indicated by the observation that a prolonged application of 10 μM CPA did not affect the hyperpolarization produced by caffeine, but suppressed the response to CGRP. A prolonged superfusion with Ca^{2+}-free medium plus EDTA reduced but did not abolish the hyperpolarization to CGRP, suggesting that a fraction of the Ca^{2+} mobilized by this neuropeptide is tightly bound to the store. The present findings suggest the existence of two SR Ca^{2+} stores accessible to differential mobilization by CGRP and caffeine, both of which lead to activation of glibenclamide-sensitive K channels. This idea is consistent with recent studies indicating the existence of spatially and functionally distinct SR Ca^{2+} pools amenable of differential modulation by thapsigargin and caffeine (e.g. Tribe *et al.*, 1994) or operationally defined as CPA-sensitive and CPA-insensitive, respectively (Low *et al.*, 1992). Interestingly, the refilling of the CPA-insensitive store described by Low *et al.* (1992) in dog mesenteric artery appears to be enhanced by Bay K 8644, which was also present in our experiments.

In conclusion, the present findings provide pharmacological evidence that Ca^{2+} mobilization from a partially caffeine-sensitive store may be involved in the K channel activation by CGRP in the guinea-pig ureter. According to our previous results on the signal transduction pathway initiated by occupancy of CGRP receptors in the guinea-pig ureter, the Ca^{2+} mobilization by CGRP may involve an elevation of cyclic AMP and PKA activation (Santicioli *et al.*, 1995). The present results imply that glibenclamide-sensitive K channels in the guinea-pig ureter are also sensitive to elevation of intracellular Ca^{2+} and that the Ca^{2+} stores mobilized by CGRP and caffeine possess differential sensitivity to SR Ca^{2+} pump blockade by CPA.

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