Major difference between rat and guinea-pig ureter in the ability of agonists and caffeine to release Ca^{2+} and influence force

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1. We have investigated the internal Ca^{2+} store and its ability to affect contraction by simultaneously measuring force and Ca^{2+} in the ureter from guinea-pig and rat. Both species responded in a similar manner to electrical stimulation and depolarization with high-K^+, generating plateau-type action potentials and increasing intracellular calcium ($[Ca^{2+}]_i$) and force.

2. In the guinea-pig, carbachol had no effect on $[Ca^{2+}]_i$ and force in the resting ureter. In contrast, resting rat ureter always responded with a large $[Ca^{2+}]_i$ rise and maintained force to carbachol in Ca^{2+}-containing solution, and in Ca^{2+}-free solution it showed a transient increase in $[Ca^{2+}]_i$ and force. This Ca^{2+} release and force development was also present in both polarized and high-K^+-depolarized preparations and was insensitive to nifedipine, suggesting the presence of a receptor-coupled pathway of Ca^{2+} release in rat ureter.

3. Caffeine was able to produce a release of Ca^{2+} from the internal store of guinea-pig ureter and elicit contraction. However, rat ureter failed to respond to caffeine. In the presence of La^{3+}, the caffeine response in the guinea-pig ureter and carbachol response in the rat ureter, elicited in Ca^{2+}-free solutions, were always increased and prolonged and could be repeatedly evoked, suggesting similarity in Ca^{2+} uptake behaviour of the store in both species.

4. Ryanodine blocked the caffeine responses of the guinea-pig ureter elicited both in Ca^{2+}-containing and Ca^{2+}-free solutions, both in the absence and presence of La^{3+}. However, ryanodine failed to prevent the rat ureter responding to carbachol, suggesting that carbachol was releasing Ca^{2+} from a ryanodine-insensitive channel in the sarcoplasmic reticulum (SR).

5. Cyclopiazonic acid, which inhibits the SR Ca^{2+}-ATPase, abolished the effects of both caffeine and carbachol in Ca^{2+}-free solutions in guinea-pig and rat, respectively.

6. We conclude that there is a major difference in the mechanisms of Ca^{2+} release in the internal Ca^{2+} store of smooth muscle from guinea-pig and rat ureter. The data suggest that the guinea-pig store is purely a calcium-induced calcium release (CICR)-type store and that the rat store is a pure receptor-operated Ca^{2+} store.

In smooth muscle the internal Ca^{2+} store plays an important role in agonist-induced contraction (van Breemen & Saida, 1989; Somlyo & Somlyo, 1990). It has also become apparent that the Ca^{2+} store in smooth muscle (and other tissues) is not homogeneous – it is often referred to as comprising an IP_3-sensitive part (IP_3-induced-calcium release, IICR) and a Ca^{2+}-sensitive part (calcium-induced-calcium release, CICR; e.g. Iino, 1990). Agonists may differentially affect the Ca^{2+} release from these stores, thus partially explaining their potencies in modulating contraction. It has long been known that smooth muscles vary in the size of their sarcoplasmic reticulum (SR; Devine & Somlyo, 1972), but recently it has been suggested that the relative size of the components of the Ca^{2+} store differs in smooth muscles. Thus pulmonary smooth muscle has IICR and CICR stores which appear to be of equal size, whereas in portal vein the

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CICR store is only about a quarter of the size of the IICR store (Iino, 1990). The myometrium has little or no CICR and caffeine does not produce a contraction (Kanmura, Missiaen & Casteels, 1988; Savineau & Mironneau, 1990).

Thus the functional importance of the Ca^{2+} stores is variable in different types of smooth muscle. Little, however, is known about any species variation in the same types of smooth muscle. In cardiac muscle substantial species variation in the functional importance of the SR has been reported (Fabiato, 1982). Studies on the Ca^{2+} store of smooth muscles have often been carried out on different species. Thus if there are species variations, the differences attributed to say phasic *vs.* tonic smooth muscles or different size blood vessels may, in some studies, be due to the species. There have been few systematic studies of the effect of agonists on intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) and force in the same smooth muscle from different species and thus species effects have generally been overlooked. Much previous work has also been performed in isolated smooth muscle cells and thus the functional relevance of any results has remained unknown.

We report here the results of a study which investigates the internal Ca^{2+} store of the smooth muscle of ureter, using two inhibitors of SR function: ryanodine, which acts on CICR channels (Meissner, 1986), and cyclopiazonic acid, a mycotoxin which directly inhibits the SR ATP-dependent Ca^{2+}-ATPase (Goeger, Riley, Dorner & Cole, 1988). We have compared the effects of two Ca^{2+}-mobilizing agents, carbachol and caffeine, on the smooth muscle of ureter from rat and guinea-pig, and have found dramatic species differences. Rat ureter has a store from which Ca^{2+}can be released, in the absence of external Ca^{2+}, and produce force, when stimulated by agonists but not caffeine, i.e. an IICR store. In contrast, the guinea-pig ureter appears to have a Ca^{2+} store which responds to caffeine but not agonists, i.e. a CICR store.

METHODS

Male rats (~200 g) or guinea-pigs (~300 g) were anaesthetized with chloroform and then killed by cervical dislocation. The ureters were dissected, cleared of any fat, and cut into strips around 3 mm in length. To measure intracellular Ca^{2+} the ureters were incubated in a solution of the membrane-permeant form of the Ca^{2+}-sensitive fluorophore indo-1 (20 μM; Cambridge BioScience, Cambridge, UK) for 2–3 h at room temperature (20 °C). Tissues were rinsed and then placed in a 200 μl bath on the stage of an inverted Nikon microscope. One end of the tissue was fixed and the other end attached to a force transducer. The tissue was stimulated by silver electrodes, 3–5 V, every 20 s (duration 50–100 ms) when required. Simultaneous electrical and mechanical recordings were obtained using a double sucrose-gap method (Bülbring & Tomita, 1969). Action potentials were evoked by just-suprathreshold depolarizing current pulses (in the order of 10^{-7}A) of short duration (20–50 ms) to avoid the possible influence of prolonged depolarization on the shape and amplitude of the action potentials.

The tissue was superfused with oxygenated, buffered Krebs solution (pH 7·4), of the following composition (mM): NaCl, 154; KCl, 5·9; $CaCl_2$, 3; $MgSO_4$, 1·2; glucose, 11·5; Hepes, 11. When the tissue was depolarized by elevating K^+ to 140 mM, K^+ was isosmotically substituted for Na^+. The effects of agonists and caffeine were examined in 0 Ca^{2+} solution (see below) after Ca^{2+} loading the stores in high-K^+- and Ca^{2+}-containing solution. In Ca^{2+}-free solutions the $CaCl_2$ was omitted and 3 mM EGTA added. Carbachol was used at a final concentration of 10^{-5}–10^{-4} M, caffeine at 5–20 mM, and oxytocin at $\leqslant 5$ μM. La^{3+} (5 mM), ryanodine (50–100 μM; Calbiochem) and cyclopiazonic acid (25–50 μM) were used to investigate store function in some experiments. All chemicals unless stated otherwise were obtained from Sigma. Experiments were carried out at both 20 and 37 °C. In preliminary studies it was found that at 20 °C the caffeine responses of the resting guinea-pig ureter were larger and more regular than at 37 °C, in agreement with previous studies (Burdyga & Magura, 1986, 1988), and so the experiments were carried out at room temperature. However, before starting these experiments all responses were examined at 37 °C, and it was found that qualitatively the results are the same, irrespective of temperature. To load the store and produce Ca^{2+} release, the following procedure was followed; firstly the tissue was stimulated with high-K^+ solution for 40 s to load the store and then either Ca^{2+} was removed from the high-K^+ solution, or, in most experiments, the high-K^+ solution was replaced with Ca^{2+}-free Krebs solution. To produce Ca^{2+} release 1–5 min after Ca^{2+} removal, carbachol or caffeine were applied for 5–10 s. EGTA (3 mM) was added to all Ca^{2+}-free solutions without La^{3+}. In experiments in which La^{3+} was used, no EGTA was added and hence trace quantities of Ca^{2+} will have been present.

For $[Ca^{2+}]_i$ measurements the tissue was loaded with the membrane form of indo-1 (20 μM) for 2–3 h at 20 °C. After washing in control solution, the tissue was excited at 340 nm and the indo-1 fluorescence emission signals at 400 and 500 nm were recorded. The ratio of these signals (F_{400}: F_{500}) provides a measure of $[Ca^{2+}]_i$. Although we did not calibrate our signals, at the end of some experiments ($n = 3$) we used ionomycin to set $[Ca^{2+}]_i$ and confirmed that our Ca^{2+} records were below R_{max} (maximum signal) and that the indo-1 signals were not saturated (Himpens & Somlyo, 1988). Values are given as means ± S.E.M., and n is the number of animals.

RESULTS

Response to electrical stimulation and high-K^+ depolarization

Ureter muscle strips placed in an organ bath normally remained quiescent but readily responded with excitation to high K^+ or electrical stimulation (Fig. 1). When 140 mM K^+ is applied both tissues depolarize and generate one or several action potentials which appear on the rising phase of the depolarization and then undergo a sustained depolarization. The contractile response to 140 mM K^+ consists of fast phasic contraction associated with the action potentials and then a complex pattern of contracture associated with the sustained depolarization normally referred to as tonic component. When stimulated by suprathreshold depolarizing current pulses the ureter of

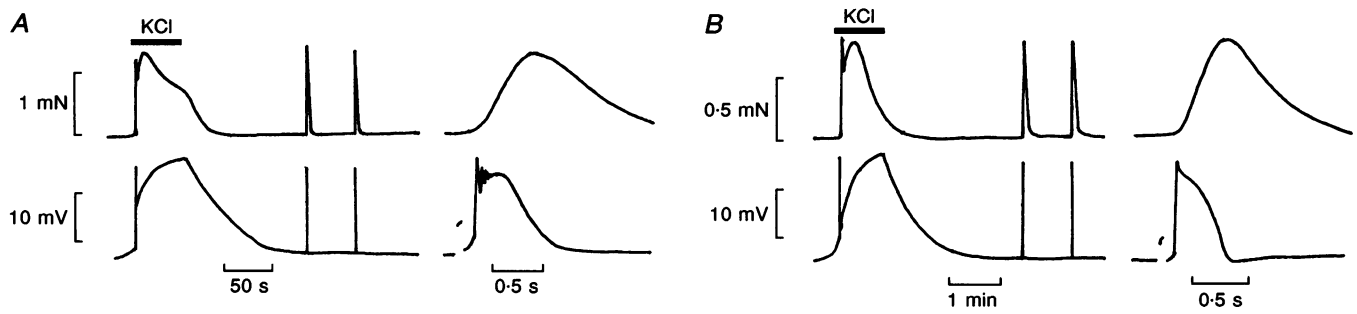

Figure 1. Simultaneous recordings of force and electrical activity in guinea-pig and rat ureteric smooth muscle

A, guinea-pig; *B*, rat. Top traces, force; bottom traces, electrical activity. External $[K^+]$ was raised to 140 mM for the period indicated by the bar. The preparations were then stimulated by suprathreshold depolarizing current pulses, which evoked plateau-type action potentials and twitches in both species. The electrical records were obtained by the double sucrose-gap method, at two different recording speeds.

both species generates action potentials accompanied by the phasic contractions. The action potential in both species consists of the initial fast spike component (trains in guinea-pig and a single spike in rat) and subsequent slow plateau component (Fig. 1). The simultaneous $[Ca^{2+}]_i$ traces show that the contractile responses to both high-K^+ depolarization and electrical stimulation were closely correlated to changes of $[Ca^{2+}]_i$ in both tissues (Fig. 2). Thus guinea-pig and rat ureters respond in a similar manner, in terms of $[Ca^{2+}]_i$ and contraction, to electrical stimulation and high-K^+ depolarization.

Response to carbachol and oxytocin

In guinea-pig ureter, application of carbachol normally potentiated the phasic contractions evoked by electrical stimulation (Fig. 3, inset), but in no case did it or oxytocin produce any changes in $[Ca^{2+}]_i$ or force in resting muscle ($n = 8$); Fig. 3*A* shows a typical example, and a 'test' response to high-K^+ depolarization.

In marked contrast, application of carbachol and oxytocin to resting rat ureter produced a clear increase in $[Ca^{2+}]_i$ and consequently contraction in this muscle ($n = 8$). A typical example is shown in Fig. 3*B*. Carbachol was also able to produce an increase in $[Ca^{2+}]_i$ and contraction in the absence of external Ca^{2+}, in either polarized or depolarized preparations, confirming its action on the intracellular Ca^{2+}store (Fig. 3*B* and subsequent figures). In the presence of the Ca^{2+} channel blocker nifedipine (10^{-5} M), fully depolarized tissue still responded with a transient single contracture after complete loss of any responsiveness to both electrical stimulation and high K^+ (Fig. 4). The response to carbachol in the absence of Ca^{2+} was transient and not as large as in the presence of external Ca^{2+}, suggesting a role for Ca^{2+} entry in maintaining contraction.

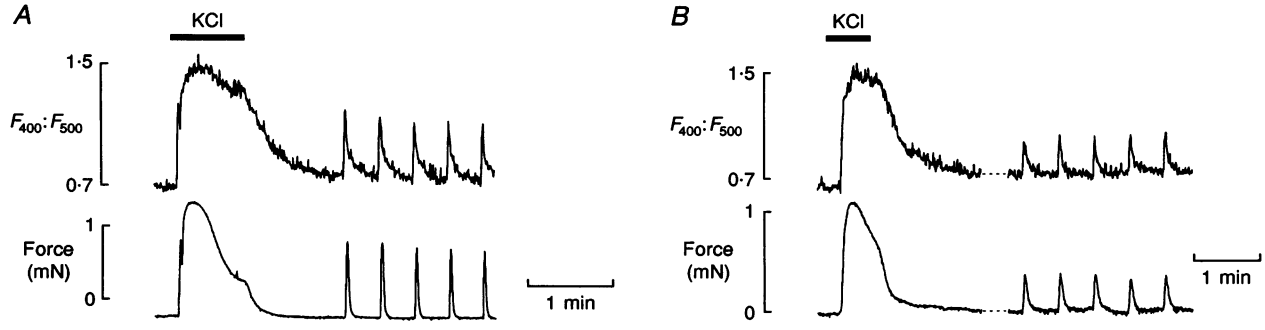

Figure 2. Simultaneous recording of $[Ca^{2+}]$ and force in guinea-pig and rat ureteric smooth muscle loaded with indo-1

A, guinea-pig; *B*, rat. Top traces, $[Ca^{2+}]$; bottom traces, force. External $[K^+]$ was raised to 140 mM for the period indicated by the bar. The preparations were then stimulated electrically, which produced brief Ca^{2+} transients and twitches. In this and all subsequent figures the Ca^{2+} records are the ratio of the fluorescence signal from the tissue at 400 and 500 nm. The dotted line in this and subsequent figures indicates a break in the recording.

Responses to caffeine and agonists in the absence and presence of La^{3+}

To investigate the nature of the internal Ca^{2+} store in the ureter, we applied caffeine, which can elicit Ca^{2+} release by activating CICR channels in the SR. We also investigated the effects of La^{3+} on caffeine and carbachol responses, as La^{3+} blocks surface membrane Ca^{2+} efflux and will therefore potentiate and prolong the effects of any Ca^{2+} released from internal stores by slowing its removal from the cytoplasm. In agreement with previously reported results (Burdyga & Magura, 1986), caffeine produced dose-dependent transient contractures of resting guinea-pig ureter, both in 0 Ca^{2+} and Ca^{2+}-containing solutions, which were significantly potentiated by La^{3+}. Caffeine (20 mM) produced a rise in $[Ca^{2+}]_i$ which was accompanied by contraction (Fig. 5*A*, typical of 5 other experiments). When La^{3+} was present, it potentiated the Ca^{2+} transients and mechanical responses produced by caffeine (Fig. 5*A*). Also, in the presence of La^{3+} the tissue was able to respond to several caffeine applications with $[Ca^{2+}]_i$ release and contraction (Fig. 5*A*), whereas in the absence of La^{3+} one caffeine application emptied the store (Burdyga & Magura, 1986). There was also a gradual increase in the indo-1 fluorescence ratio (Fig. 5). However, even in the presence of La^{3+} neither carbachol nor histamine, a strong stimulant in guinea-pig ureter (Shuba, 1981), elicited Ca^{2+} transients or tension in guinea-pig ureter (not shown). Thus in guinea-pig ureteric smooth muscle there is a caffeine-sensitive Ca^{2+} store but it is not accessed by carbachol or histamine.

Rat ureter under identical experimental conditions produced no response to caffeine ($n = 7$; not shown). However, rat ureter produced Ca^{2+} transients and mechanical responses to carbachol applications (in 0 Ca^{2+}) in the presence of La^{3+}. Both the Ca^{2+} transients and force produced by carbachol in the presence of La^{3+} were potentiated compared with those in the absence of La^{3+} and could be evoked by several carbachol applications (Fig. 5*B*). Thus in rat ureter the Ca^{2+} store is released by carbachol, but little or no release is seen with caffeine.

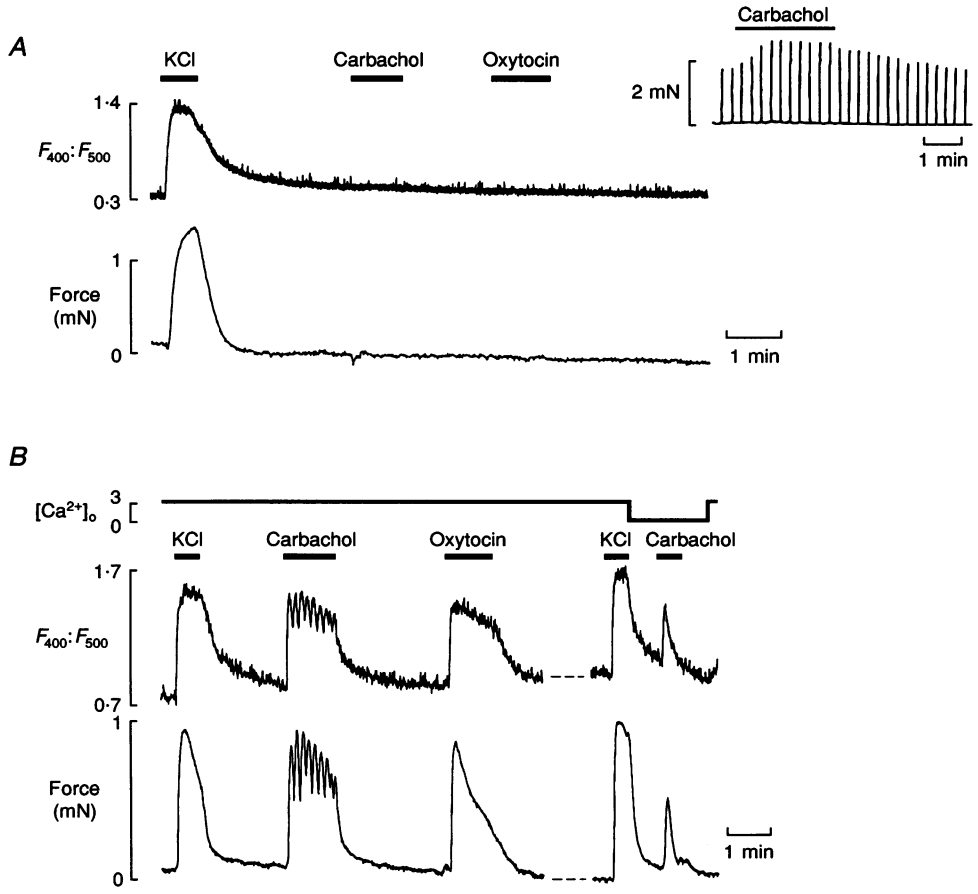

Figure 3. The response of ureteric smooth muscle to agonist

Simultaneous $[Ca^{2+}]$ (top trace) and force (bottom trace) records from guinea-pig (*A*) and rat (*B*). Following depolarization with high K^+ (140 mM), carbachol (10^{-4} M) and oxytocin (1 μM) were applied for the period indicated by the bars. No change in Ca^{2+} or force occurred in response to either agonist in guinea-pig ureter, unlike rat ureter. Removal of external Ca^{2+} did not prevent the response to agonist in the rat, as shown at the right-hand end of panel *B*. The inset shows the effect of carbachol (10^{-4} M) on the evoked phasic contractions of guinea-pig ureter.

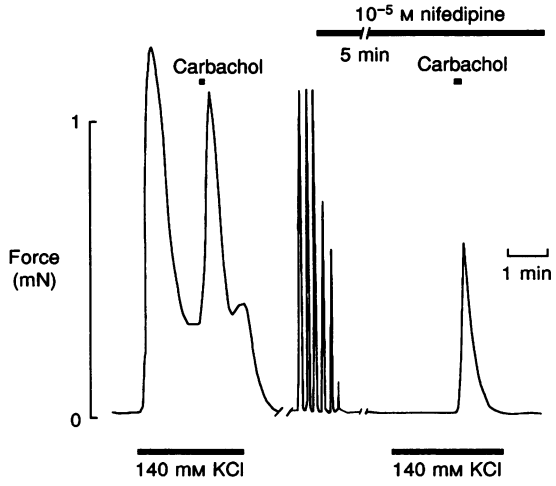

Figure 4. Effect of nifedipine on response of rat ureteric smooth muscle to carbachol
Carbachol responses of rat ureter taken in high-K^+ (140 mM)-depolarized tissue before and after application of nifedipine (10^{-5} M).

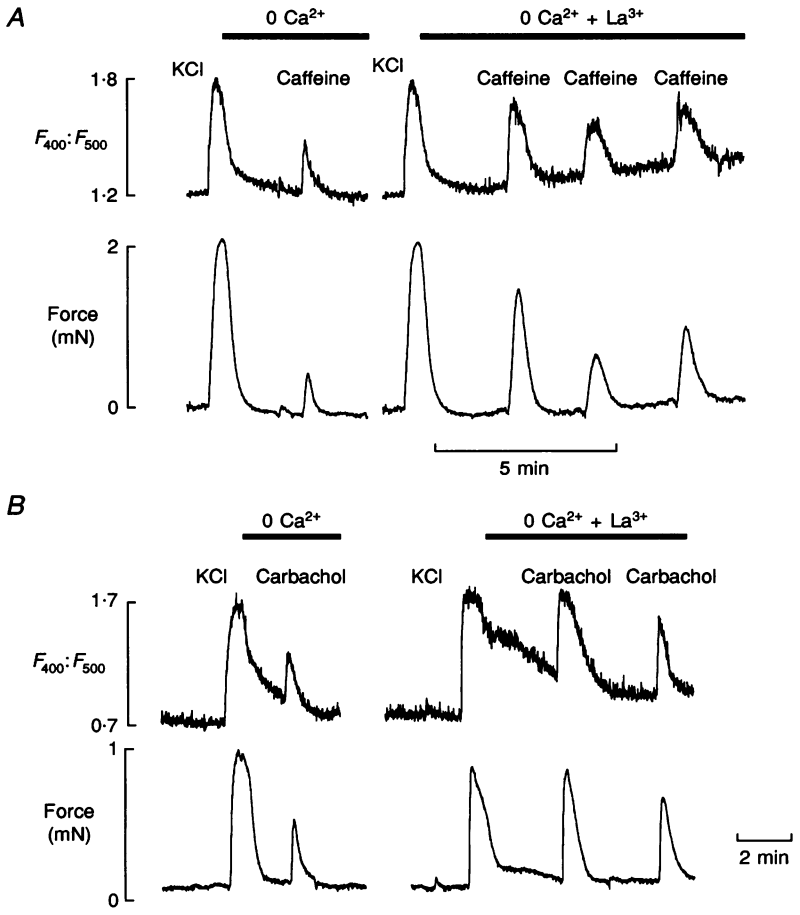

Figure 5. Effect of La^{3+} on response of ureteric smooth muscle to Ca^{2+} releasing agents
A, the response of guinea-pig ureter to caffeine (20 mM) in the absence and presence of La^{3+} (5 mM). *B*, the response of rat ureter to carbachol (10^{-4} M) in the absence and then presence of La^{3+}. Simultaneous $[Ca^{2+}]$ (top traces) and force data (bottom traces) were obtained.

Effects of ryanodine and cyclopiazonic acid on responses to caffeine and carbachol

To investigate further the nature of the Ca^{2+} release from the ureter internal stores we used two pharmacological probes: ryanodine and cyclopiazonic acid. Ryanodine is used to identify CICR channels and blocks SR function by converting the Ca^{2+}-sensitive channel to a low conducting, open state, hence preventing the internal store from holding Ca^{2+} (Meissner, 1986; Lai & Meissner, 1989). Ryanodine (100 μM) irreversibly blocked the caffeine response of guinea-pig ureter both in normal Krebs and Ca^{2+}-free solutions. As in other types of smooth muscle the effects of ryanodine were strongly use dependent, i.e. the effects were most apparent after the store had been stimulated by caffeine in the presence of ryanodine. This is well illustrated in Fig. 6 in which the effects of ryanodine on caffeine responses, in the presence of La^{3+}, which preserves the functional activity of the store (see Fig. 5), were examined. Figure 6 shows that the guinea-pig ureter still responded with a large Ca^{2+} transient and tension to the first challenge with caffeine in the presence of La^{3+} and ryanodine, but a second application of caffeine produced substantially smaller Ca^{2+} transients and no force. Also, in the presence of La^{3+}, but not in its absence, ryanodine slowed relaxation of the Ca^{2+} transient and mechanical response elicited by the first application of caffeine (Fig. 6). This suggests that ryanodine at this concentration is acting as an agonist rather than an antagonist of CICR channels, thus making the SR leaky to the Ca^{2+} and thereby preventing Ca^{2+} uptake. In the rat ureter the Ca^{2+} transient and force elicited by carbachol in Ca^{2+}-free solution, both in the absence and presence of La^{3+}, were unaffected by ryanodine (50–100 μM, $n = 6$; not shown).

Unlike ryanodine, cyclopiazonic acid inhibits SR function by blocking Ca^{2+}-ATPase (Seidler, Jona, Vegh & Martonishi, 1989). In preliminary studies it was found that cyclopiazonic acid (5–20 μM) blocks ATP-dependent Ca^{2+} accumulation into the SR microsomal fraction of pig ureter, as tested by oxalate sensitivity (Th. V. Burdyga, L. Prishchepa & S. A. Kosterin, unpublished data). Figure 7 clearly shows that cyclopiazonic acid completely inhibited the caffeine responses in guinea-pig ureter in Ca^{2+}-free solution ($n = 3$; Fig. 7*A*) and in Ca^{2+}-containing solution (not shown), and also inhibited the carbachol response in rat ureter in Ca^{2+}-free solution (Fig. 7*B*).

DISCUSSION

These data clearly show that there is a marked species difference in the mechanism of Ca^{2+} release from the intracellular Ca^{2+} store of the smooth muscle of the ureter, which produced functional effects. Irrespective of the method used to alter intracellular Ca^{2+} (depolarization, agonist, electrical stimulation or caffeine), $[Ca^{2+}]_i$ rose before force and there was a good correspondence between the rise of $[Ca^{2+}]_i$ and the amount of force. As shown throughout this

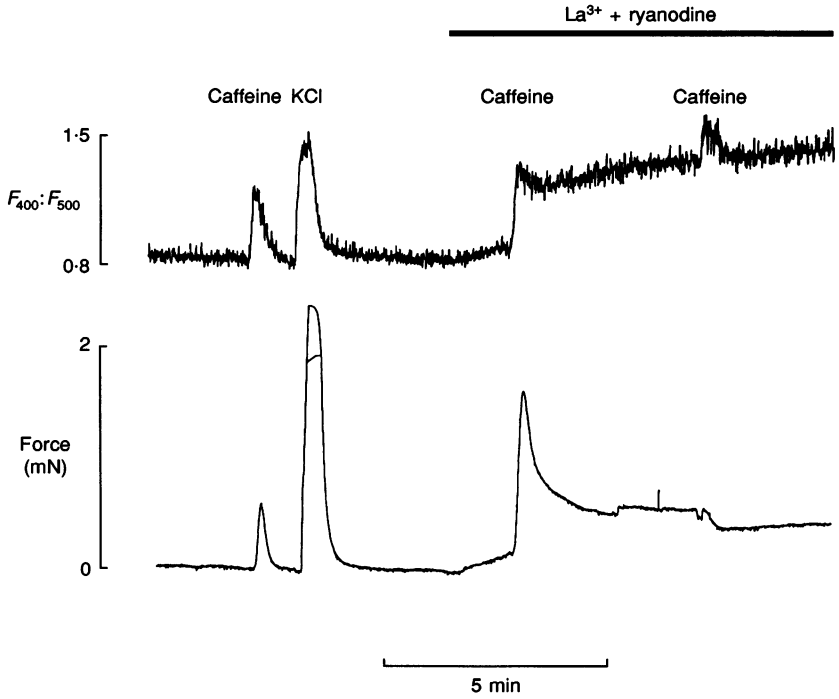

Figure 6. The effect of ryanodine on caffeine reponses in the presence of La^{3+} in guinea-pig ureter

The effect of ryanodine (100 μM) on the caffeine (20 mM) responsiveness of guinea-pig ureter bathed in Krebs solution containing La^{3+} (5 mM).

study, significant quantities of Ca^{2+} can be released from the SR by Ca^{2+}-releasing agents in the absence of external Ca^{2+}. This is in agreement with previous reports in other smooth muscles (e.g. Karaki, Kubota & Urakawa, 1979; Bond, Kitazawa, Somlyo & Somlyo, 1984). The contractions were transient and smaller than those occurring in the presence of external Ca^{2+}, as shown in Figs 3 and 4. This was as expected from previous studies, as extracellular Ca^{2+} entry is responsible for maintaining contraction in many smooth muscles and Ca^{2+} released from stores is responsible for the rapid initial activation of contractions (Ratz & Murphy, 1987; Himpens & Somlyo, 1988; van Breemen & Saida, 1989).

Carbachol and histamine were unable to produce a rise in $[Ca^{2+}]_i$ in the guinea-pig ureter. Oxytocin was able to stimulate both Ca^{2+} and force in the rat but not in the guinea-pig, either at rest or during evoked activity. Previous workers have already noted the species difference in contractile response to oxytocin (e.g. Barrigon & Tamargo, 1986). Although the agonists were used at both maximal and submaximal concentrations, we cannot directly rule out the possibility that insufficient IP_3 was produced in the guinea-pig. However, even when La^{3+} was added to prevent surface membrane Ca^{2+} efflux, no elevation of Ca^{2+} or tension occurred. In addition, in the rat the concentrations used produced very large effects on tension and $[Ca^{2+}]_i$. Finally in longitudinal intestinal muscle, which also appears to lack an IICR store, introduction of IP_3 into permeabilized cells failed to produce an elevation of $[Ca^{2+}]_i$ (Kuemmerle, Murthy & Makhlouf, 1994). Thus in resting preparations, agonists in the concentrations used do not appear to be able to produce a release of Ca^{2+} from the intracellular store in the smooth muscle of the ureter from guinea-pigs, and potentiation of the evoked phasic contractions is likely to be controlled by modulation of the ionic channels responsible for the action potential generation and its configuration (Shuba, 1981).

In rat ureter clear effects of carbachol on $[Ca^{2+}]_i$ and force were seen in all preparations, and as mentioned above the rises in $[Ca^{2+}]_i$ and force were seen both in polarized and

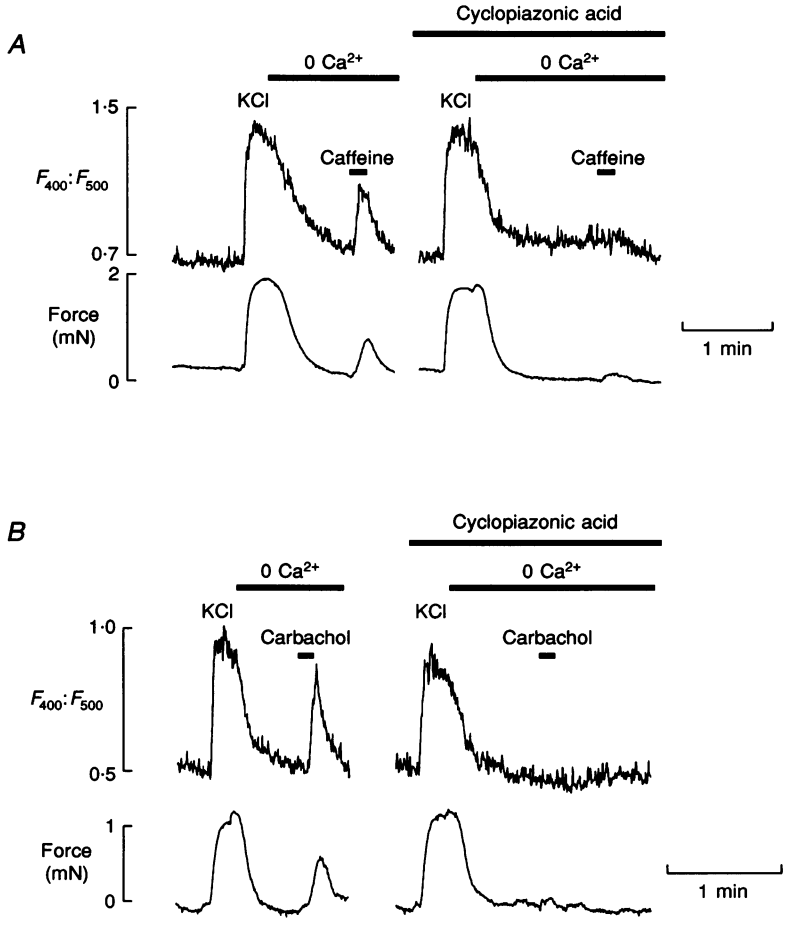

Figure 7. Effect of cyclopiazonic acid on response of ureteric smooth muscle to Ca^{2+} releasing agents

A, the response of guinea-pig ureter to caffeine (10 mM) in the absence and presence of cyclopiazonic acid (50 μM). *B*, the response of rat ureter to carbachol (10^{-4} M) in the absence and presence of cyclopiazonic acid. Simultaneous $[Ca^{2+}]$ (top traces) and force (bottom traces) data were obtained. The responses to caffeine and carbachol were obtained in 0 Ca^{2+} solution containing 3 mM EGTA.

depolarized cells, and in both the presence and absence of external Ca^{2+}, suggesting an effect on the internal Ca^{2+} store. To test this further we added cyclopiazonic acid, which is reported to be a selective inhibitor of the SR Ca^{2+}-ATPase in muscles, including smooth muscles (Goeger *et al.* 1988; Seidler *et al.* 1989; Uyama, Imaizumi & Watanabe, 1992; Shima & Blaustein, 1992; Watanabe *et al.* 1993; Munro & Wendt, 1994). In the absence of external Ca^{2+}, little or no change in resting $[Ca^{2+}]_i$ occurred in the presence of cyclopiazonic acid, in agreement with earlier findings on urinary bladder (Munro & Wendt, 1994). There was little or no effect of cyclopiazonic acid on the high-K^+ response, which is also in agreement with previous work and the fact that the high-K^+ contraction is due to Ca^{2+} entry, not Ca^{2+} release (Himpens & Somlyo, 1988; Munro & Wendt, 1994). Consistent, however, with its action on the SR Ca^{2+}-ATPase, cyclopiazonic acid inhibited responses to carbachol in Ca^{2+}-free solution. Thus it appears that carbachol increases $[Ca^{2+}]_i$ by release of Ca^{2+} from internal stores.

Although caffeine has several possible actions (Butcher & Sutherland, 1962; Sato, Ozaki & Karaki, 1988) in many tissues, including a wide variety of smooth muscle, it is capable of releasing Ca^{2+} from internal stores, and hence it has become a generally used probe for Ca^{2+}-induced Ca^{2+} store release (Chapman & Miller, 1974; Yamamoto, Iino, Yamazawa & Endo, 1991; Hirose, Iino & Endo, 1993). Thus the absence of tension and the small or absent Ca^{2+} response to caffeine in the rat ureter was surprising, especially as carbachol was readily able to release Ca^{2+}. The rat ureter thus resembles the rat myometrium, which also does not contract or release Ca^{2+} in response to caffeine (Kanmura *et al.* 1988; Savineau & Mironneau, 1990). These two tissues may represent 'pure' IICR-sensitive stores.

The lack of a caffeine-sensitive store in rat ureter contrasts markedly with the guinea-pig ureter where caffeine was the only agent tested which was able to stimulate an increase in $[Ca^{2+}]_i$ and contraction, in agreement with earlier force observations (Burdyga & Magura, 1986). The caffeine responses were inhibited by both ryanodine and cyclopiazonic acid, which agrees with observations in other smooth muscles (Shima & Blaustein, 1992). As found by other workers, the sensitivity to ryanodine in smooth muscle of the ureter was low and its effects were time and use dependent (Kanmura *et al.* 1988; Aoki & Ito, 1988; Wagner-Mann, Hu & Sturek, 1992). Normally the first application of caffeine applied shortly (5 min) after introduction of ryanodine under control conditions produces similar $[Ca^{2+}]_i$ and force changes, but the effect of subsequent applications of caffeine, acting at the internal Ca^{2+} store, is greatly inhibited. This can be clearly seen for guinea-pig ureter in Fig. 6.

Although cardiac and skeletal muscle possesses only a CICR store, apart from our results on guinea-pig ureter, we are aware of only one previous demonstration, in rabbit intestinal longitudinal muscle (Kuemmerle *et al.* 1994), of smooth muscle lacking an IICR store. Permeabilized preparations of the intestinal longitudinal muscle cells did not release Ca^{2+} in response to added IP_3 and intact cells produced very little IP_3 and possessed few if any IP_3 receptors (Kuemmerle *et al.* 1994).

Thus guinea-pig ureter and rat ureter may sit at the extremes of the scale for dual mechanisms of Ca^{2+} release from the internal Ca^{2+} store: in guinea-pig ureter the release involves CICR and caffeine- and ryanodine-sensitive channels and in rat ureter the release involves IICR channels which are ryanodine and caffeine insensitive.

Previous work has shown that when La^{3+} is used to block surface membrane Ca^{2+} transport guinea-pig and rat ureter can generate several contractions in response to caffeine and carbachol, respectively, in 0 Ca^{2+} solutions (Burdyga & Magura, 1988; Burdyga & Kosterin, 1993). This is also consistent with these agents acting on the SR, and, as the data in Fig. 5 show, rises in $[Ca^{2+}]_i$ accompany the effects on contraction seen in the presence of La^{3+}. The rise in the background indo-1 fluorescence ratio seen in the presence of La^{3+} (Fig. 5) was not always associated with an increase in resting force in either rat or guinea-pig ureter, so it may be that La^{3+} is interacting with indo-1 to give slow artifactual rises in signal (Grynkiewicz, Poenie & Tsien, 1985). It is also possible that La^{3+} was elevating $[Ca^{2+}]_i$ but that this Ca^{2+} did not affect the contractile machinery, perhaps because it was 'compartmentalized' (e.g. Chen & van Breemen, 1992).

In conclusion, we have demonstrated that there are great differences in the mechanisms of Ca^{2+} release from the intracellular Ca^{2+} stores of ureteric smooth muscle from guinea-pigs and rats. Care must therefore be taken when comparisons are made about the stores in different smooth muscles, e.g. comparing sizes and mechanisms of Ca^{2+} release, that they are from the same species. It appears that guinea-pig ureter may possess only a CICR release mechanism whereas rat ureter has just a receptor-operated, possibly IICR, mechanism. Thus these tissues will provide useful experimental systems for testing drugs and models for stored Ca^{2+} release. That such differences should exist within the same smooth muscle is fascinating. The data also show that contractile responses occur as a result of Ca^{2+} mobilization by the different agents, although it is not clear yet how these differences are functionally translated, *in vivo*.

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