Multiple sources of calcium for contraction of the human urinary bladder muscle

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1 KCl, carbachol, neurokinin A and endothelin produced concentration-dependent contractions of mucosa-free muscle strips from the dome of the human urinary bladder. The maximal response to carbachol or neurokinin A exceeded that to KCl, while the maximal response to endothelin approached that to KCl.

2 Nifedipine (1 μ M) abolished the response to KCl, reduced the response to carbachol or neurokinin A but had no effect on the response to endothelin. Bay K 8644 (1 μ M) markedly potentiated the response to KCl but had little or no effect on the response produced by the other stimulants.

3 Superfusion of the strips with a nominally calcium (Ca)-free medium containing EDTA (1 mM) for 30 min markedly reduced the response to carbachol, neurokinin A and endothelin, although a small response was still evident at high concentrations. Likewise, after a prolonged (60 min) superfusion of the strips with a high K (80 mM) Ca-free medium plus EDTA (1 mM) these three agonists still produced a small contractile response.

4 The nifedipine (1 μ M) resistant response to carbachol, neurokinin A or endothelin was markedly depressed by LaCl_3 (1 mM). In contrast, the nifedipine-(1 μ M) resistant response to carbachol was not modified by NiCl_2 (0.1 mM) or ω -conotoxin (0.1 μ M).

5 Caffeine produced divergent effects depending upon the temperature of incubation: a relaxation at 37°C and a concentration-dependent (2.5–20 mM) contraction at 25°C. The latter was markedly inhibited by procaine (3 mM) but unaffected by nifedipine (1 μ M).

6 After a prolonged (60 min) superfusion with a high K, Ca-free medium containing EDTA the response to carbachol (100 μ M) was abolished by previous exposure to procaine (3 mM). Conversely, the response to endothelin (1 μ M) was unaffected by procaine. The response to endothelin in these experimental conditions was also resistant to LaCl_3 (1 mM).

7 These findings indicate that multiple sources of Ca are mobilized for contraction of the human bladder muscle by different stimulants. Dihydropyridine- and voltage-sensitive Ca channels provide the major if not the sole source of Ca for the response to KCl, play some role in the response to muscarinic (carbachol) or NK-2 tachykinin receptor stimulation but are not involved in the response to endothelin. Carbachol, neurokinin A and endothelin all mobilize a Ca pool (either extracellular or located at membrane level) which is LaCl_3 -sensitive but nifedipine-resistant. Neither T- nor N-type channels appear to be involved in the response to carbachol. In addition, these agents mobilize a tightly bound Ca pool independently from membrane depolarization. This latter pool is probably a procaine-sensitive intracellular source of activator Ca mobilized by caffeine and carbachol. The failure of procaine to prevent the response to endothelin in high K, Ca-free medium raises the possibility that this peptide mobilizes an intracellular source of activator Ca, distinct from the caffeine- and carbachol-sensitive pool.

Introduction

Much evidence indicates that smooth muscles have multiple sources of calcium ions (Ca) which are uti-

lized for contraction (Somlyo & Somlyo, 1968; Bolton, 1979; Brading & Sneddon, 1980; Casteels & Droogmans, 1982; Van Breemen *et al.*, 1982; Bolton & Kitamura, 1983). Extracellular Ca enters the smooth muscle cells via two pathways, that is

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voltage-operated Ca channels (VOCs) and receptor-operated channels (see Bolton 1979, for review). In addition, release of intracellular Ca can be activated by several agonists either via receptor-mediated pathways or via a direct release from the storage site(s) such as in the case of caffeine (Bolton 1979; Brading & Sneddon, 1980; Itoh *et al.*, 1982; 1983; Leijten & Van Breemen, 1984).

Sources of activator Ca for contraction have been widely investigated for vascular and intestinal smooth muscle. Less information is available with regard to the detrusor muscle of the urinary bladder. Information in this field appears of particular interest because of the possible use of drugs with Ca entry blocking properties in the management of bladder hypermotility disorders such as detrusor instability or detrusor hyperreflexia.

Studies in animals have suggested that multiple sources of activator Ca are present in the bladder muscle. Ca entry via dihydropyridine-sensitive Ca channels has been proposed as a likely mechanism for bladder contraction in the detrusor muscle from rabbits, guinea-pigs or rats (Huddart & Butler, 1986; Batra *et al.*, 1987; Maggi *et al.*, 1988a). In the guinea-pig and rat isolated bladder muscle mobilization of intracellular Ca seems also a relevant process for agonist-induced contraction (Mostwin, 1985; Maggi *et al.*, 1988a). *In vivo* studies in anaesthetized rats suggested that Ca mobilization via nifedipine-resistant pathways might be a relevant mode of contraction during a physiological-like event, that is, volume-evoked bladder voiding (micturition reflex) (Maggi *et al.*, 1988a). Fovaeus *et al.* (1987) investigated the effect of nifedipine and Bay K 8644 on carbachol-induced contractions of human isolated bladder muscle and concluded that both voltage-dependent and receptor-operated Ca channels mediate this response.

Recently we studied the effect of endothelin, an endothelium-derived vasoconstrictor peptide proposed to act as an endogenous agonist at dihydropyridine-sensitive Ca channels (Yanagisawa *et al.*, 1988) in the human isolated bladder (Maggi *et al.*, 1989a) and found that the contractile response to this peptide was almost totally nifedipine-resistant while the KCl-induced contraction was abolished by nifedipine-pretreatment. These observations prompted us to re-address (Fovaeus *et al.*, 1987) the question of the sources of activator Ca for contraction of the human detrusor muscle. With this aim, four agonists were selected, that is KCl, carbachol, neurokinin A and endothelin, in order to assess whether different agonists might mobilize different Ca sources for contraction, and the effect of nifedipine, Bay K 8644, LaCl_3 and Ca-free media on the responses produced by these agents was investigated.

Methods

Specimens from the dome of human urinary bladder were obtained from 25 (18 males and 7 females) patients (age range 51–79 years) undergoing total cystectomy for carcinoma of the bladder base. Specimens were collected from patients showing no signs of urinary retention or detrusor instability. No patient received radio- or chemotherapy before intervention. Pre-anaesthetic medication was intramuscular atropine (1.0 mg) and diazepam (10 mg), 2 h before surgery. Anaesthesia was induced by thiopentone sodium (500 mg, i.v.) and maintained with NO_2/O_2 (1/2) and halothane (0.6–1%). During induction of anaesthesia, the patients received pancuronium bromide (6 mg, i.v.). All specimens appeared macroscopically normal, with no signs of tumour or inflammation. Tissues were placed in ice-cold physiological salt solution within 2–3 min from surgical removal. Most experiments were performed on strips which were stored overnight in ice-cold physiological salt solution and continuously gassed with 96% O_2 and 4% CO_2 as described previously (Maggi *et al.*, 1988b; 1989a). Some experiments were performed within 3 h of surgery ('fresh' strips).

From each sample, 4 to 6 small strips of mucosa-free muscular tissue were excised and mounted in 5 ml baths for isolated organs, maintained at 37°C, containing physiological salt solution continuously gassed with 95% O_2 and 5% CO_2 . In some experiments the bath temperature was kept at 25°C. Changes in isometric tension were recorded by means of a force-displacement transducer connected to a Basile 7050 pen recorder. Resting load was 10 mN.

After a 60–90 min equilibration period the strips were repeatedly exposed to KCl (80 mM, added to the bath) until responses were reproducible. This response served as an internal standard for all experiments.

Cumulative concentration-response curves to the various agonists were constructed as described by Van Rossum (1963), the next concentration being added when the effect of the preceding one had reached a steady state.

The composition (mM) of the physiological salt solution was as follows: NaCl 119, NaHCO_3 25, glucose 11, KCl 4.7, MgSO_4 1.5, KH_2PO_4 1.2 and CaCl_2 2.5.

In some experiments a nominally Ca-free solution was prepared by omission of CaCl_2 , replaced by NaCl. In other experiments a high K, Ca-free solution was prepared by adding 80 mM KCl to the Ca-free solution. In these 'Ca-free' solutions the contaminating Ca due to impurities present in the other salts was 6.7 μM . EDTA (1 mM) was added to both the Ca-free and the high K, Ca-free solutions. In

Figure 1 Typical tracings showing the contractile response of the human isolated bladder muscle strips to cumulative addition of KCl, carbachol or neurokinin A and inhibition of the response by 1 μM nifedipine (30 min beforehand, right section in each panel).

experiments involving the effect of LaCl_3 , the physiological salt solution had the following composition (mM): NaCl 144, glucose 11, Trizma 7.7 (Sigma) 5, KCl 4.7, MgSO_4 1.5 and CaCl_2 2.5 which was gassed with 100% oxygen. The high K, Ca-free solution used for experiments with LaCl_3 had the following composition (mM): NaCl 147.8, KCl 80, glucose 11, Trizma 5, EDTA 1, MgSO_4 1.5.

In experiments involving the Ca-free media, the strips were superfused with the modified solution for 30 or 60 min at a rate of 10 ml min^{-1} by means of a

peristaltic pump. Superfusion of the strips was stopped 5 min before application of the agonists which were added directly to the bath.

Statistical analysis

Each value given in Results section is mean $\pm$ s.e.mean. Statistical analysis of the data was made by Student's *t* test or analysis of variance, when applicable.

Figure 2 Effect of nifedipine ($1 \mu\text{M}$, 30 min beforehand, ●) on the concentration-response curve to carbachol, KCl, neurokinin A or endothelin in the human isolated bladder muscle strips; (○) control response. Each value is mean of at least 5 experiments; s.e. indicated by vertical bars. *Significantly different from controls, $P < 0.05$.

Drugs

Drugs used were: carbachol (Merck), atropine HCl (Serva), caffeine, nifedipine and NiCl_2 (Sigma), Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate, Bayer), ω -conotoxin fraction GVIA and porcine endothelin (Peninsula), LaCl_3 (Fluka) and procaine HCl (Gianni). Neurokinin A was synthesized by conventional solid-phase methods by Dr P. Rovero, Chemistry Department, Menarini Pharmaceuticals.

Results

General

The study was performed on 123 strips from 25 patients; 32 strips were studied within 3 h of surgery ('fresh' strips) while the remainders were stored over-

night. In some experiments, when reproducible contractile responses to KCl (80 mM, added to the bath) had been obtained, the strips were allowed to equilibrate for 60 min to determine whether spontaneous activity was present. This was observed in 8 out of 15 'fresh' strips (from 5 patients) and in 10 out of 50 'stored' strips (from 15 patients) ($P < 0.01$).

All strips responded to carbachol, neurokinin A, endothelin or KCl with concentration-related contractions. No major differences in sensitivity or maximal response to these agonists were observed in fresh vs. stored strips and therefore data obtained in these two different conditions were pooled.

The contractile response produced by carbachol, neurokinin A or KCl was readily reversed on washing. A second concentration-response curve to these agonists, made 45 min after the first one gave reproducible results ($n = 3$ each, data not shown). By contrast, the effect of endothelin was difficult to remove by washing (Maggi *et al.*, 1989a).

Figure 3 Effect of Bay K 8644 (1 μM, 30 min beforehand ●) on the concentration-response curve to carbachol, KCl, neurokinin A or endothelin in the human isolated bladder muscle strips; (○) control response. Each value is mean of at least 5 experiments; s.e. indicated by vertical lines. *Significantly different from controls, $P < 0.05$.

Effect of nifedipine

In accordance with our previous findings (Maggi *et al.*, 1989a), nifedipine (1 μM for 30 min) abolished the contractile response to KCl but had no significant effect on the response to endothelin (Figures 1 and 2). Carbachol- or neurokinin A- induced contractions were inhibited by nifedipine, the effect being more evident at low concentrations of the agonists. A response to carbachol or neurokinin A was always observed at the highest concentrations of the agonists (Figures 1 and 2).

Effect of Bay K 8644

Human bladder strips responded to Bay K 8644 (1 μM) with a contraction in 14 out of 18 cases. In responders, the magnitude of this contraction ranged between 8 and 37% of that to KCl (80 mM); it peaked in 5–10 min and then declined slowly to

baseline. At the time of administration of the various agonists (30 min after Bay K 8644), tension had fully recovered or was below 15% of the maximal response to KCl (80 mM). In the presence of Bay K 8644, the concentration-response curve to KCl was markedly enhanced as compared to controls and a higher maximal contraction was attained (Figure 3).

By contrast, the concentration-response curves to carbachol, neurokinin A or endothelin were barely influenced by Bay K 8644 (Figure 3) with the exception of low concentrations of the agonists (10 nM for carbachol, 1 and 3 nM for neurokinin A, 1 and 3 nM for endothelin) for which slightly higher contractions than controls were noted (Figure 3).

Effect of a Ca-free medium plus EDTA

The responses to carbachol, neurokinin A and endothelin were markedly reduced after superfusion (30 min) with a Ca-free medium containing 1 mM EDTA (Figure 4) although a response was still

Figure 4 Effect of 30 min superfusion with a Ca-free plus EDTA (1 mM, ●) solution on the concentration-response curve to carbachol, neurokinin A or endothelin in the human isolated bladder muscle strips; (○) control response. Each value is mean of at least 5 experiments; s.e. indicated by vertical lines. *Significantly different from controls, $P < 0.05$.

Figure 5 Effect of LaCl_3 (1 mM, 30 min beforehand, ●) on the concentration-response curve to carbachol, neurokinin A or endothelin in the human isolated bladder muscle strips; (○) control response. All experiments performed in presence of $1 \mu\text{M}$ nifedipine. Each value is mean of at least 5 experiments; s.e. indicated by vertical lines. *Significantly different from controls, $P < 0.05$.

Table 1 Contractile response to carbachol, neurokinin A or endothelin of detrusor strips from the human urinary bladder after prolonged (60 min) superfusion with a high K Ca-free medium plus EDTA

		A	B	C
	No. of experiments	Response to KCl (mN)	Response to high K, Ca-free medium (% of A)	Response to the agonist (% of A)
Carbachol	7	26 ± 4	85 ± 4	25 ± 7
Neurokinin A	3	21 ± 3	92 ± 3	5 ± 2*
Endothelin	6	20 ± 4	96 ± 4	18 ± 4

Each value is mean ± s.e.mean. The strips were first exposed to KCl (80 mM, added to the bath for 10 min) until reproducible responses were obtained (A). Then the strips were superfused with a high K (80 mM), Ca-free solution containing EDTA (1 mM). This produced at first a transient phasic contraction (B) followed by a slow return to baseline. At 60 min from the start of superfusion, carbachol (100 μ M) neurokinin A (1 μ M) or endothelin (1 μ M) was added to the bath (superfusion was stopped at 55 min) and its effect recorded (C).

*Significantly different from the response to carbachol, $P < 0.05$.

observed consistently at high concentrations (Figure 4).

Effects of LaCl_3 on the response to carbachol, neurokinin A or endothelin

To determine further the role of extracellular and/or membrane-bound Ca, the effect of LaCl_3 (1 mM for 30 min) was investigated. In these experiments, after having established reproducible responses to KCl, nifedipine (1 μ M) was added to the Krebs solution in order to block Ca influx via dihydropyridine-sensitive Ca channels. Figure 5 shows that LaCl_3 further depressed the nifedipine-resistant response to carbachol, neurokinin A and endothelin but failed to abolish completely the response produced by high concentrations of these agonists.

Effect of ω -conotoxin or NiCl_2 on the response to carbachol

These experiments were designed to assess whether the nifedipine-resistant but LaCl_3 -sensitive component of the response to an agonist such as carbachol might involve influx of extracellular Ca via T- or N-type channels. Therefore, concentration-response curves to carbachol were constructed in the presence of nifedipine (1 μ M, 30 min before), nifedipine plus NiCl_2 (0.1 mM, 30 min before) or nifedipine plus ω -conotoxin fraction GVIA (CTX, 0.1 μ M, 30 min before). As shown in Figure 6 neither NiCl_2 nor CTX, known blockers of T- and N-type channels, respectively, was capable of exceeding the inhibitory effect produced by nifedipine alone.

Effect of a high K, Ca-free medium plus EDTA

In these experiments, after having recorded reproducible responses to KCl (80 mM added to the bath), the strips were superfused with a high K (80 mM),

Ca-free medium plus EDTA (1 mM) at a rate of 10 ml min⁻¹ for 60 min. Exposure to this medium produced an immediate large contraction which peaked in 6–10 min and then declined slowly toward baseline. Full recovery of initial tension was achieved in 30–45 min. At 55 min from start of superfusion, the flow was stopped and the preparations allowed to equilibrate for 5 min. At this time carbachol (100 μ M, $n = 7$), neurokinin A (1 μ M, $n = 3$) or endothelin 1 μ M, $n = 6$) was added to the bath. As shown in Table 1, all the agonists produced a contraction,

Figure 6 Effect of NiCl_2 (0.1 mM, 30 min beforehand) or ω -conotoxin (0.1 μ M, 30 min beforehand) on the concentration-response curve to carbachol in the human isolated bladder muscle strips. All experiments performed in presence of 1 μ M nifedipine: (○) nifedipine alone; (□) nifedipine plus NiCl_2 ; (△) nifedipine plus ω -conotoxin. Each value is mean of 5 experiments; s.e. indicated by vertical lines.

Figure 7 Effect of a prolonged (60 min) superfusion with a high K, Ca-free medium, plus EDTA (1 mM) (●) on the concentration-response curve to carbachol in the human isolated bladder muscle strips; (○) control response. Each value is mean of 5 experiments; s.e. indicated by vertical lines. *Significantly different from controls, $P < 0.05$.

the response to neurokinin A being significantly smaller than that produced by carbachol or endothelin. The complete concentration-response curve to carbachol obtained under similar experimental conditions and compared to that obtained in controls is shown in Figure 7.

Effect of caffeine

At 37°C, caffeine (5–20 mM) had either no effect or produced a relaxation ($n = 4$ strips from 3 patients). In both cases, the contractile response to KCl (80 mM, added to the bath) was markedly reduced in the presence of caffeine (40–60% inhibition). In other experiments, the bath temperature was kept at 25°C. In these conditions caffeine (2.5–20 mM) produced a concentration-related contraction (Figure 8) which peaked 1–3 min after its addition and then faded to baseline. In two strips from different patients the effects of caffeine were investigated first at 37°C and then at 25°C. In both instances, caffeine produced a contraction only at the lower bath temperature. The caffeine- (5 mM) induced contractions were fully reproducible at 30 min intervals with changes of the bath solution every 5 min. Procaine (3 mM, 15 min beforehand) inhibited the caffeine-induced contractions of the human bladder while nifedipine (1 μM, 30 min beforehand) did not ($n = 4$ from 3 bladders). In fact the caffeine-(5 mM) induced contraction averaged 13 ± 3 and $16 \pm 4\%$ of the response to KCl before and after addition of nifedipine, respectively.

Figure 8 Non-cumulative concentration-dependent contractile response to caffeine (○) on the human isolated bladder muscle strips and its blockade by procaine (3 mM, □). Bath temperature was 25°C. Increasing concentrations of caffeine were added to the bath at 45 min intervals. Each value is mean of 4 experiments; s.e. indicated by vertical bars. *Significantly different from controls, $P < 0.05$.

Effect of procaine or LaCl_3 on the response to carbachol or endothelin in high K, Ca-free medium

In presence of procaine (3 mM, 15 min beforehand) the contractile response produced by 100 μM carbachol in a high K, Ca-free medium (plus EDTA) ($25 \pm 7\%$ of the response to 80 mM KCl in normal Krebs) was abolished ($n = 3$). By contrast, endothelin (1 μM) induced a response ($14 \pm 4\%$ of the response to KCl in normal Krebs) not significantly different from that ($18 \pm 4\%$, $n = 4$) observed in controls. The response to endothelin in high K, Ca-free medium was also unaffected by previous exposure to 1 mM LaCl_3 , being $15 \pm 3\%$ ($n = 4$) of the response to KCl in normal Krebs solution.

Discussion

In agreement with previous observations (Fovaeus *et al.*, 1987), we found evidence for the presence of dihydropyridine- and voltage-sensitive (L-type) Ca channels in the smooth muscle of the human urinary bladder. This pathway sustains entirely the contractile response to KCl and probably plays some role in the response to carbachol and neurokinin A, as indicated by the partial inhibitory effect of nifedipine. These latter agents exert their effect directly on

smooth muscle cells by activating muscarinic (Zappia *et al.*, 1986) and NK-2 tachykinin receptors (Maggi *et al.*, 1988b), respectively. It appears therefore conceivable that L-type Ca channels might be activated indirectly through membrane depolarization initiated by a receptor-mediated mechanism.

A large fraction of the response to carbachol or neurokinin A was insensitive to nifedipine, although largely dependent upon extracellular Ca as indicated by experiments using the Ca-free medium plus EDTA and antagonism by LaCl_3 .

We therefore interpret these findings as an indication that carbachol, neurokinin A or endothelin activate influx of extracellular Ca and/or mobilization of Ca pools associated with the plasma membrane, which can refill from the extracellular Ca via a nifedipine-resistant pathway (cf. Fovaeus *et al.*, 1987). This could occur through activation of receptor-operated mechanisms as proposed for many other smooth muscles (Somlyo & Somlyo, 1968; Bolton, 1979). The resulting contractile response could be operationally defined as nifedipine-resistant but LaCl_3 -sensitive. At least for carbachol, we can exclude T- or N-type voltage-sensitive channels as playing a role in the nifedipine-resistant component of the response, as it was unaffected by either NiCl_2 or ω -conotoxin (see Fox *et al.*, 1987; Miller, 1987).

In addition, since high concentrations of the three agonists were still able to induce a contraction in the absence of extracellular Ca, these findings suggest that they may mobilize Ca from an intracellular store (see below).

Caffeine induced a procaine-sensitive but nifedipine-resistant contraction of the human bladder. This occurred only at a low (25°C) bath temperature, while at 37°C a relaxant effect was observed. Caffeine has been shown to contract various smooth muscles by mobilizing an intracellular Ca pool from the sarcoplasmic reticulum (Weber & Hertz, 1968; Casteels & Raeymaekers, 1979; Haeusler *et al.*, 1981; Itoh *et al.*, 1982; 1983; Leijten & Van Breemen, 1984) but smooth muscle relaxation via inhibition of phosphodiesterases and other mechanisms has also been noted (Leijten & Van Breemen, 1984; Sato *et al.*, 1988). We could speculate that at 37°C the relaxant effect of caffeine prevails while at 25°C the relaxant action is somehow slowed or inhibited thus unmasking a contractile effect. However, a relaxant effect of caffeine was evident after waning of the contractile response at low temperature. These findings are in keeping with data of Sato *et al.* (1988) who showed that the transient elevation of intracellular Ca produced by caffeine on rat aorta is enhanced at low temperature (see also Karaki *et al.*, 1987).

In view of the above, we interpret the procaine-sensitive caffeine-induced contraction as evidence for

the existence of an intracellular Ca pool in the human bladder muscle. This pool is probably activated also by neurotransmitters via receptor-mediated stimulation. In fact, stimulation of either muscarinic or NK-2 tachykinin receptors produced a small but consistent contractile response after a prolonged incubation in a Ca-free medium plus EDTA and this effect was also observed in K-depolarized preparations.

The fact that procaine was able to block the response to carbachol or endothelin produced after a prolonged superfusion with a Ca-free depolarizing medium suggests that the contraction might involve Ca release from the sarcoplasmic reticulum (Endo, 1977; Itoh *et al.*, 1981). The ability of procaine to block Ca release from the internal store in the human urinary bladder muscle is further indicated by its action toward the caffeine response.

A final point deserving discussion is related to the nature of Ca pools mobilized by endothelin. After the original discovery (Yanagisawa *et al.*, 1988) the suggestion was made that endothelin may act as an endogenous direct activator of dihydropyridine (L-type) voltage-sensitive Ca channels in both vascular, tracheobronchial and cardiac muscle (Ishikawa *et al.*, 1988a,b; Uchida *et al.*, 1988; Tomobe *et al.*, 1988). However, in some smooth muscles, the contractile response to endothelin is largely or totally resistant to dihydropyridine type Ca channel blockers (Auguet *et al.*, 1988; Maggi *et al.*, 1989a,b). Here we confirm that the response to this peptide in the human bladder muscle is nifedipine-resistant and also substantially unaffected by Bay K 8644. Van Renterghem *et al.* (1988) showed that endothelin acts in a complex manner on the cell membrane: it stimulates metabolism of inositol phosphates (see also Sugiura *et al.*, 1989; Marsden *et al.*, 1989), inducing mobilization of Ca from intracellular stores (Miasiro *et al.*, 1988; Kai *et al.*, 1989), it opens both a Ca-sensitive K channel (transient hyperpolarization) and a non-specific cation channel (prolonged depolarization) which in turn may activate L-type voltage-dependent Ca channels (Van Renterghem *et al.*, 1988). Thus, the contribution of L-type Ca channels to tension generation by endothelin might vary from one smooth muscle to another depending upon the relative role of the various mechanisms involved.

In this study, we extended our observations (Maggi *et al.*, 1989a) about the Ca sources utilized by endothelin in producing a contraction of the human bladder muscle. Present data confirm the view that L-type Ca channels play a very minor if any role in the response to endothelin in this tissue. The response to endothelin can be divided into a large (about 80% of total tension generated), LaCl_3 -sensitive component (this fraction of the response conceivably overlaps with that prevented

by superfusion with Ca-free media) and a small (about 20% of total response) LaCl_3 -resistant response, probably reflecting Ca mobilization from internal store(s). In rat aortic smooth muscle cells in culture, Kai *et al.* (1989) showed that the intracellular Ca pool activated by endothelin overlaps the caffeine-sensitive one. This does not seem to occur in the human urinary bladder muscle, because the caffeine response was procaine-sensitive while that to endothelin (in high K, Ca-free medium) was procaine-resistant. These findings give rise to the hypothesis, which needs further experimental evaluation, that the Ca store mobilized by endothelin in the human bladder muscle might be distinct from that mobilized by caffeine (or carbachol).

In conclusion, the present findings indicate that multiple sources of activator Ca can be mobilized, in a differential manner, by several agonists which

induce contraction of the human urinary bladder muscle. Ca influx via L-type channels plays some role in the response to carbachol and neurokinin A but not in the response to endothelin. Receptor-operated and lanthanum ion-sensitive mechanisms promoting Ca influx could play a role in the response to carbachol, neurokinin A or endothelin. A caffeine- and procaine-sensitive intracellular Ca pool is mobilized by carbachol. Further, endothelin mobilizes a tightly bound (intracellular ?) Ca pool which is procaine-resistant. The relevance of these findings to the possible use of drugs interfering with Ca mobilization in the therapy of bladder motility disorders awaits elucidation of the nature of the Ca pools which are utilized *in vivo* for physiological (micturition contraction) and 'pathological' (detrusor instability) human bladder contractions.

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