Urol Res (1993) 21:319–323

Urological Research

Role of intracellular Ca^{2+} stores in smooth muscle contractions of the guinea pig vas deferens

P. Drescher, R. E. Eckert, P. O. Madsen

Urology Section, Veterans Hospital, and Department of Surgery University of Wisconsin, School of Medicine, Madison, WI 53705, USA

Received: 18 February 1993 / Accepted: 28 May 1993

Summary. Guinea pig vas deferens was used as an animal model for alpha-1 adrenoceptor (α_1-receptor) mediated contractions in human hyperplastic prostatic tissue. The selective α_1-receptor agonist, phenylephrine (PE), induced fully reversible, dose-dependent contractions antagonized by increasing concentrations of the α_1-receptor blockers prazosin (1–100 nM) and YM 617 (0.1–10 nM). Removal of extracellular Ca^{2+} reduced PE-evoked contractions in a time-dependent manner. Nifedipine (1–1000 nM), a blocker of voltage-dependent L-type Ca^{2+} channels (VDCC), inhibited the PE-induced response by up to 65%. Removal of extracellular Ca^{2+} abolished the α_1-agonist reactivity in a time-dependent fashion. To elucidate the participation of intracellular Ca^{2+} stores in α_1-receptor-mediated contractions, the tissue was pretreated with ryanodine (10 μM) or thapsigargin (0.1 μM), established inhibitors of Ca^{2+} release from intracellular pools. Both substances reduced the PE contractions by up to 80%. Nifedipine suppressed the remaining contractions completely. This provides evidence that Ca^{2+} influx through VDCC and Ca^{2+} release from intracellular stores contribute to α_1-receptor-mediated contractions in the guinea pig vas deferens and may be important in obstructive benign prostatic hyperplasia.

Key words: α_1-adrenoceptor – Benign prostatic hyperplasia – Ca^{2+} channels – Intracellular Ca^{2+} stores – Vas deferens

Increased smooth muscle tone in the human prostate plays a major role in bladder outlet obstruction caused by benign prostatic hyperplasia (BPH) [36]. The contractions of this tissue are mainly mediated by α_1-receptors, as demonstrated by receptor ligand binding and contraction studies [27]. Consequently, treatment of patients with α_1-receptor antagonists has improved obstructive symptoms in some patients with BPH [26].

This emphasizes the importance of focusing on α_1-receptor-induced contractions in hyperplastic prostatic tissue to develop new therapeutical approaches. Function and pharmacological properties of α_1-receptors in this tissue are well defined [8]. The guinea pig vas deferens with its abundance of α_1-receptors [11] seemed to be a suitable animal model for α-adrenergic-mediated increased smooth tone in BPH. Despite the known role of adrenergic neurotransmission in regulating these smooth muscle contractions, the cellular mechanism of this response is not fully understood. Smooth muscle contraction is induced by an increase in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$). Conflicting experimental results have been reported about the source of Ca^{2+} [24, 29]. The relative contribution of Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCC) and Ca^{2+} release from intracellular Ca^{2+} stores (sarcoplasmic reticulum) varies in different tissues [14] and is a crucial determinant of smooth muscle contraction. Ryanodine and thapsigargin have been shown to be useful tools for investigating the participation of intracellular Ca^{2+} stores in receptor-mediated contractions [31]. Ryanodine depletes Ca^{2+} from sarcoplasmic reticulum by holding Ca^{2+} release channels in a subconductance state, whereas thapsigargin inhibits Ca^{2+} transport into intracellular Ca^{2+} stores by blocking the Ca^{2+} adenosine triphosphatase (ATPase) selectively [33, 34].

The present study investigated the role of VDCC and intracellular Ca^{2+} stores in phenylephrine induced contractions of human hyperplastic prostatic tissue and guinea pig vas deferens.

Materials and methods

Prostatic tissue strips were obtained from patients undergoing transurethral resection of the prostate (TURP) because of BPH. The tissue was removed as a large chip from the bladder neck down to the verumontanum at the 6 o'clock position (central zone). It was placed

Correspondence to: P. O. Madsen, M. D., Ph.D.; FAX: 1(608)256-1901

immediately in Reznikoff solution (Ham's F12) nutrient mix with L-glutamine, $NaHCO_3$ 25 mM, penicillin/streptomycin 100 units/ml, 4 °C) and cut into strips measuring approximately 2×10 mm. Experiments were performed within 1–19 h after TURP. The same experiments were performed with vasa deferentia of guinea pigs (400–600 g) killed by cervical dislocation. After dissecting free from surrounding tissue, 10-mm sections were prepared. All tissue was transferred to a 10 ml perfusion bath containing Krebs' solution (in Mm): NaCl 118, KCl 4.6, $CaCl_2$ 1.2, $NaHCO_3$ 24, $MgSO_4$ 1.2, KH_2PO_4 1.6, glucose 11 (aerated with 5% CO_2, 95% O_2, 37 °C, pH 7.4).

Sections were mounted individually under a resting tension of 1 g for guinea pig vas and 0.5 g for human prostatic tissue. Equilibration was allowed for 60 min, with the resting tension being maintained. Isometric contractions of the tissue were registered on a Grass FTO3C force displacement transducer and recorded on a Grass 7D polygraph.

Agonist, dose–response curves were obtained noncumulatively, i.e. the agonist was washed out for 20 min immediately after the maximum contraction was achieved and before increasing the agonist dose. The maximum contraction was considered as 100%. To study α_1-receptor antagonists the tissue specimen was incubated with increasing concentrations of the respective antagonist for 15 min before adding 100 µM phenylephrine (PE). The tissue was incubated with increasing concentrations of nifedipine for 15 min [12] before adding PE (100 µM). The specimen was rinsed with Ca^{2+}-free solution (composition in mM: NaCl 104, KCl 10, $MgSO_4$ 5, KH_2PO_4 1, glucose 20, taurin 50, HEPES 5, aerated with 5% CO_2, 95% O_2, 37 °C, pH 7.4) for various time periods before PE was added in a concentration of 100 µM. Ryanodine (10 µM) or thapsigargin (0.1 µM) were applied with caffeine (1 mM) for 30 min before addition of 100 µM PE.

L-phenylephrine (Sigma), prazosin (Sigma), YM 617 (Yamanouchi Pharmaceutical), nifedipine (Sigma), caffeine (Sigma), ryanodine (Calbiochem), thapsigargin (Sigma), yohimbine (Sigma) and propranolol (Sigma) were prepared as stock solutions (1–1000 mM) and dissolved in the perfusion solution.

Data are reported as means±SEM. One-way or two-way analysis of variance and Student's *t*-test were used for statistical analysis, and correlations between different variables were calculated by Pearson's linear correlation coefficient. *P*-values < 0.05 were considered significant.

Results

PE-induced contractions

Figure 1 a shows the original recording of contractions of the guinea pig vas deferens during exposure to increasing concentrations of PE. These responses were comparable to those of human hyperplastic prostatic tissue (Fig. 1 b). The maximum response was calculated as the 100% value in the respective tissue. The PE-induced contractions consist of two parts: an initial phasic followed by a tonic component. Application of PE in the submicromolar range did not elicit any contractions ($n = 27$). Maximal responses were obtained with millimolar PE concentrations ($n = 24$). In the guinea pig vas deferens, the mean threshold concentration of PE was 3 µM ($n = 17$), the EC_{50} value was at 50 µM ($n = 21$) and with 1 mM PE a mean contraction of 96.4±1.5% ($n = 24$) was obtained. In the human prostatic tissue, a threshold concentration of 1 µM ($n = 4$), an EC_{50} of 30 µM ($n = 8$) was obtained whereby 0.5 mM PE induced a mean contraction of 94.5±3.4% ($n = 12$).

Blocking of PE-induced contractions by α_1-receptor antagonists

Prazosin and the investigational drug YM 617, two selective α_1-receptor antagonists, dose-dependently

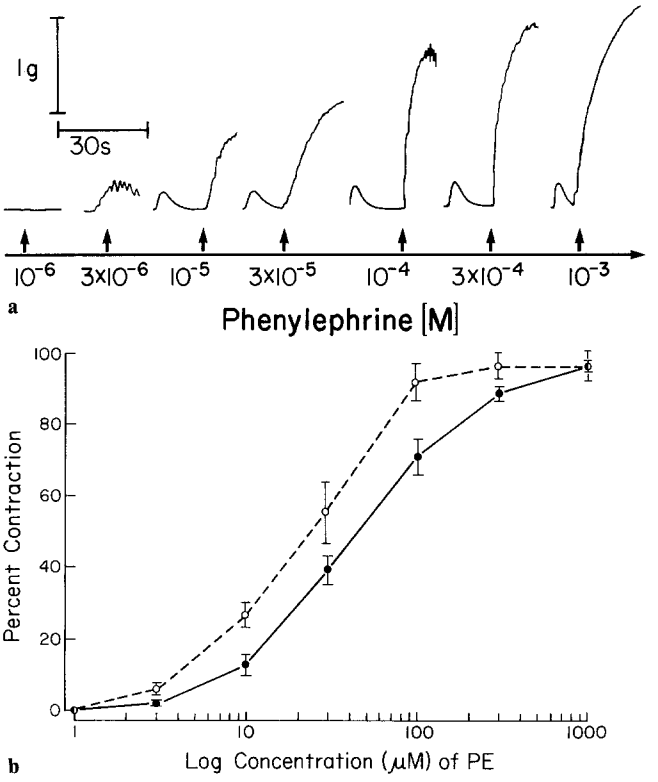

Fig. 1. a Original traces of characteristic contractions in guinea pig vas deferens induced by increasing concentrations of phenylephrine (PE). The *arrows* indicate administration of PE. **b** Comparison of dose-response curves of PE-induced contractions in human prostatic tissue (*continuous line*) and guinea pig vas deferens (*broken line*). Logarithm of PE micromolar concentration is plotted versus percent of maximum contraction. *Points* are means and *vertical bars* indicate±SEM ($n = 27$)

(1–100 nM) inhibited the PE-induced contractions (100 µM) in human prostatic tissue and guinea pig vas deferens. S-shaped dose response curves could be obtained for both tissues, with a left shift of the curve for human prostatic tissue, when the logarithmic concentration of antagonists was plotted against percentage of contractions evoked by 100 µM PE (Fig. 2). Prazosin, at a con-

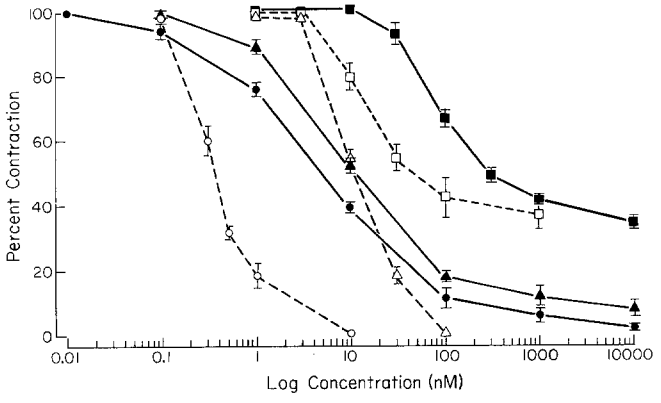

Fig. 2. Inhibitory effects of YM 617 (○ ●), prazosin (△ ▲) and nifedipine (□ ■) on contractions of human prostate strips (*broken line*) and guinea pig vas deferens (*continuous line*) evoked by 100 µM PE. Logarithm of drug nanomolar concentration is plotted versus percentage of control contraction. *Points* are means and *vertical bars* are±SEM ($n = 12$)

centration of 100 μM, abolished PE-elicited contractions in the vas deferens ($n = 7$). A 54.4±1.9% reduction was obtained with 10 nM in vas deferens ($n = 9$) and 57.1±2.1% reduction in human prostatic tissue. Application of 0.1 nM prazosin did not affect the ability of PE to induce smooth muscle contraction. Similar results were obtained with the potent α_1-antagonist YM 617 [18] which fully blocked α_1-receptor-mediated contraction at a concentration as low as 10 nM ($n = 7$, vas deferens) and 10 μM ($n = 6$, prostatic tissue). The EC_{50} value was in the range of 0.4 nM (51.7±2.5%, $n = 8$) for guinea pig vas deferens and 6 nM (48.8±1.7%, $n = 8$) for human prostatic tissue. Figure 2 summarizes the α_1-receptor blocking effects of prazosin and YM 617 in human prostatic tissue and guinea pig vas deferens. The α_2-receptor agonist clonidine (0.1 – 1000 μM, $n = 6$) did not induce contractions. The α_2-receptor antagonist yohimbine and the β-receptor antagonist propranolol (0.1 – 100 μM, $n = 6$) did not suppress the PE-induced contractions, indicating that these contractions in both tissues are exclusively mediated by α_1-receptors.

Nifedipine effect

The Ca^{2+} channel blocker nifedipine (1 – 1000 nM) was used to study the contribution of gated Ca^{2+} entry through VDCC to the contractions induced by PE (100 μM). Nifedipine at 1 μM reduced the PE-induced contraction by 65.2±3.2% ($n = 9$) in guinea pig vas deferens and 56.3±2.1% ($n = 6$) in prostatic tissue, whereas 300 nM inhibited the PE response by 48.6±4.3% ($n = 8$) in human prostatic tissue and 59.6±3.3% ($n = 9$) in guinea pig vas deferens. In Fig. 2 the inhibition of the PE-induced contraction by nifedipine is compared for prostatic tissue and guinea pig vas deferens. Removal of extracellular Ca^{2+} led to a time-dependent reduction of PE-induced contractions (not illustrated). After 30 min of constant bath perfusion with nominally Ca^{2+}-free solution (containing 1 mM EGTA) PE-induced contractions were abolished.

The human hyperplastic prostatic tissue and the guinea pig vas deferens showed a comparable reactivity at different concentrations of α_1-adrenergic agonists and antagonists as well as the L-type Ca^{2+} channel blocker nifedipine (Figs. 1, 2). Thus we conclude that the guinea pig vas deferens can be established as a reliable model for studying α_1-receptor mediated processes in human prostatic tissue.

Blocking of intracellular Ca^{2+} release

Vas deferens strips were incubated for 30 min with ryanodine (10 μM), a blocker of the sarcoplasmic Ca^{2+} release channel. To deplete intracellular Ca^{2+} stores the incubated strips were exposed to 1 mM caffeine [25] during incubation with ryanodine or thapsigargin. Caffeine itself elicited a small phasic contraction (not shown) of about 10% of the control contraction induced by 100 μM PE but did not inhibit contractions of PE. Figure 3 a shows original tracings of PE-induced vas deferens contractions before and after ryanodine exposure. After

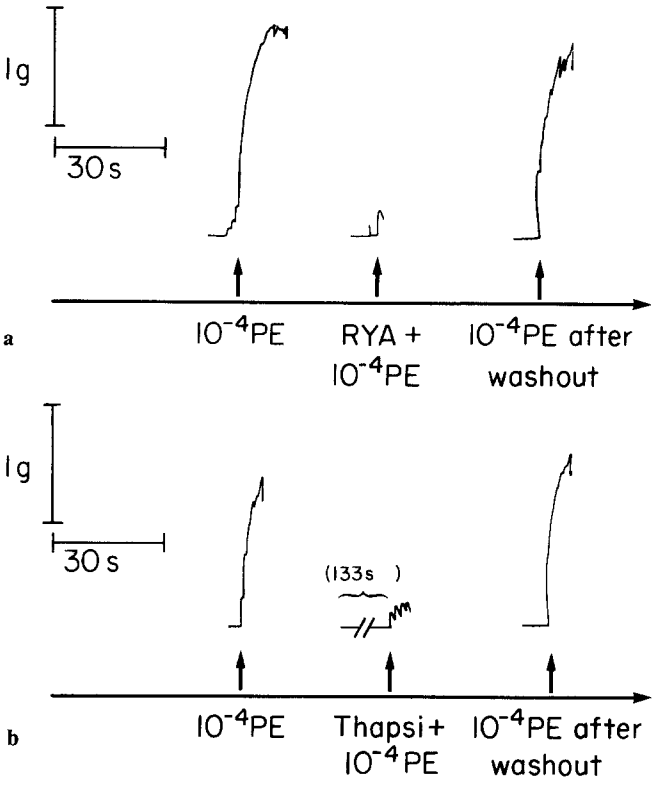

Fig. 3. a. Inhibition of PE-induced contraction by 10 μM ryanodine. The *first trace* shows the control, the *second trace* the contraction after incubation with 10 μM ryanodine (*RYA*) and 10 mM caffeine for 30 min. The *third trace* demonstrates the response to 100 μM PE after a washout period of 30 min. *Arrows* indicate the administration of 100 μM PE (10^{-4} PE). **b** Block of PE-induced contractions by 0.1 μM thapsigargin. The *first trace* shows the control, the *second trace* the contraction after preincubation with 0.1 μM thapsigargin (*Thapsi*) and 10 mM caffeine for 30 min with a delay of onset of 133 s. The *third trace* demonstrates the response to 100 μM PE after a washout period of 30 min. *Arrows* indicate administration of 100 μM PE (10^{-4} PE)

ryanodine treatment the PE-induced contraction was inhibited by 78.8±4.3% ($n = 15$).

To elucidate further the participation of intracellular Ca^{2+} release mechanisms thapsigargin (0.1 μM), which blocks sarcolemmal Ca^{2+} ATPases, was used in the same experimental protocol as ryanodine. Incubation with 0.1 μM thapsigargin exerted two effects: (1) a delay of the onset of contraction by 133±22 s ($n = 12$; control 8±4.5 s, $n = 27$) and (2) inhibition of the PE-evoked contractions by 73.3±3.5% ($n = 12$; Fig. 3 b). The contraction remaining following thapsigargin or ryanodine incubation could be totally blocked by 1 μM nifedipine ($n = 5$).

Discussion

The guinea pig vas deferens was successfully used as an animal model for α_1-adrenoceptor mediated contractions, which also play an important role in hyperplastic prostate. Guinea pig vas deferens [11] and hyperplastic prostatic tissue [8] have been proven to contain a vast supply of α_1-receptors, and our studies showed a striking similarity in the reaction of the two tissues to α_1 agonists

and antagonists (Fig. 1b). Incubation with Ca^{2+}-free solution and the VDCC blocker nifedipine also demonstrated a comparable pattern in the two tissues (Fig. 2). Our data indicate furthermore that besides Ca^{2+} influx through VDCC, intracellular Ca^{2+} release mechanisms play an important role in α_1-receptor-mediated contractions in guinea pig vas deferens.

To induce α_1-receptor-mediated contractions, PE was used as a selective α_1-receptor agonist with β-receptor stimulation only in high concentrations [17]. However, there was no β-adrenergic contribution to the contraction in either of the tissues investigated since propranolol incubation did not interfere with PE-elicited reactions. In accordance with other reports [6] the α_1-receptor was identified by use of the specific postsynaptic α_1-receptor antagonist prazosin [9], which abolished the PE-induced response in a dose-dependent manner. YM 617, a sulfonamide derivative of phenylethylamine [18], demonstrated even higher α_1-antagonistic activity in the nanomolar range (Fig. 2). This substance was recently tested in a multi-center study in patients with symptomatic BPH [23]. The inability of the α_2-receptor agonist clonidine to induce smooth muscle contractions supports the finding that smooth muscle contraction is an exclusively α_1-receptor-mediated response. Also the α_2-receptor antagonist yohimbine did not prevent PE-induced contractions. However, Bueltmann et al. [7] reported the existence of postjunctional α_2-adrenoceptors mediating contractions in mouse vas deferens.

Smooth muscle contractility is determined by $[Ca^{2+}]_i$ [35]. Two major systems are involved in the control of smooth muscle $[Ca^{2+}]_i$: (1) the plasmalemma, transmembrane VDCC under the control of the membrane potential, GTP-binding proteins and different intracellular second messenger systems, and (2) intracellular Ca^{2+} stores controlled by second messengers [22]. Agonists inducing smooth muscle contractions raise $[Ca^{2+}]_i$ either by opening VDCC [2], by releasing Ca^{2+} from intracellular stores [5] or by a combination of the two mechanisms. Application of the VDCC blocker nifedipine decreased the PE contraction by 65%. This is in accordance with other data which demonstrate that the contractile response to α_1-receptor agonists is only partially abolished by nifedipine in vas deferens [13, 15, 32, 37]. Removal of extracellular Ca^{2+} reduced α_1-receptor-mediated contractions in a time-dependent manner. Even after 15 min PE still elicited tissue contractions in a reduced fashion. The presence of this response shortly after incubation in Ca^{2+}-free solution indicates that PE mobilized intracellular Ca^{2+} stores. Prolonged incubation (30 min) in Ca^{2+}-free solution totally suppressed the PE response. Under physiological conditions, agonist-stimulated release of Ca^{2+} from intracellular stores is accompanied by repletion of these stores by Ca^{2+} influx from the extracellular space. However, if Ca^{2+} reloading of intracellular stores is prevented, subsequent stimuli do not evoke contractions over time [3, 5].

It has been reported that ryanodine, a neutral alkaloid of plant origin [21], inhibits the contractions of smooth muscle cells [33]. This inhibition is considered to be due to the depletion of sarcoplasmic reticulum by locking Ca^{2+}-induced Ca^{2+} release channels in a half-open state [16, 19] without affecting VDCC [16, 20]. This inhibitory effect is enhanced by caffeine, which specifically stimulates intracellular Ca^{2+} release from the sarcoplasmic reticulum [10]. A concentration of 10 μM ryanodine inhibited PE-induced contractions by nearly 80% (Fig. 3). Thapsigargin is useful for discriminating between different intracellular Ca^{2+} stores [1]. This sesquiterpene lactone [30] blocks the Ca^{2+} ATPase responsible for active Ca^{2+} uptake into intracellular pools [34] and has proven to be an effective tool in studying the mechanism of intracellular Ca^{2+} release in smooth muscle contraction. The delay of onset of contractions in vas deferens treated with thapsigargin varied and cannot be explained (Fig. 3). Poor tissue penetration cannot be excluded; however, other studies have shown reliable results with comparable concentrations and incubation times [28]. Thapsigargin and ryanodine inhibited PE-mediated responses in guinea pig vas deferens to a similar degree, which has also been shown in other smooth muscle tissues [31].

In conclusion, the guinea pig vas deferens provides a useful model for studying α_1-adrenoceptor induced contractions. Our data show that these contractions are mediated by Ca^{2+} influx through VDCC and Ca^{2+} release from intracellular Ca^{2+} pools. However, additional experiments with hyperplastic prostatic tissue need to be performed to evaluate the contribution of intracellular and extracellular Ca^{2+} stores to smooth muscle contractions in BPH.

Further studies are also required to identify the intracellular second messengers responsible for the opening of plasmalemma VDCC and intracellular Ca^{2+} release channels.

References

1. Bian J, Ghosh TK, Wang JC, Gill DL (1991) Identification of intracellular calcium pools: selective modification by thapsigargin. J Biol Chem 266:8801
2. Bolten TB (1979) Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev 59:606
3. Bond M, Kitazawa T, Somlyo AP, Somlyo AV (1984) Release and recycling of Ca^{2+} by the sarcoplasmic reticulum in guinea-pig portal vein smooth muscle. J Physiol (Lond) 355:677
4. Bourreau JP, Zhang ZD, Low AM, Kwan CY, Daniel EE (1991) Ryanodine and the adrenergic, purinergic stimulation in the rat vas deferens smooth muscle: functional and radioligand binding studies. J Pharmacol Exp Ther 256:1063
5. Breemen C van, Saida K (1989) Cellular mechanisms regulating $[Ca^{2+}]_i$ smooth muscle. Annu Rev Physiol 51:315
6. Breschi MC, Martinotti E, Ducci M, Fantoni B (1987) Postsynaptic effects of α agonists on adrenoceptors of the reserpinized rat vas deferens. Pharmacol Res Commun 19:415
7. Bueltmann R, Kuegelgen I von, Starke K (1991) Contraction-mediating α_2-adrenoceptor in the mouse vas deferens. Naunyn-Schmiedeberg's Arch Pharmacol 343:623
8. Caine M, Raz S, Zeigler M (1975) Adrenergic and cholinergic receptors in the human prostate, prostatic capsule and bladder neck. Br J Urol 47:193
9. Cambridge D, Davey MJ, Massingham R (1977) Prazosin, a selective antagonist of postsynaptic α-adrenoceptors. Br J Pharmacol 59:514

10. Endo M (1975) Mechanism of action of caffeine on the sarcoplasmic reticulum of skeletal muscle. Proc Jpn Acad 51:479
11. Fedan JS, Hogaboom GK, O'Donnel JP, Colby J, Westfall DP (1981) Contributions by purines to the neurogenic response of the vas deferens of the guinea pig. Eur J Pharmacol 69:41
12. Feddersen M, Vesperinas G, Lewin J, Huidobro-Toro JP (1988) Different calcium sources mobilized by noradrenaline and adenosine 5'-triphosphate (ATP): a basis of cotransmission in the rat vas deferens. Eur J Pharmacol 153:313
13. Fovaeus M, Andersson KE, Hedlund H (1987) Calcium channel blockade and contractile responses in the isolated human vas deferens. J Urol 138:654
14. Han CH, Abel PW, Minneman KP (1987) Alpha 1-adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. Nature 329:333
15. Hay DWP, Wadsworth RM (1983) The effects of calcium channel inhibitors on the twitches and noradrenaline contractions of the rat bisected vas deferens. Eur J Pharmacol 87:367
16. Hisayama T, Takayanagi I (1988) Ryanodine: its possible mechanism of action in the caffeine-sensitive calcium store of smooth muscle. Pflugers Arch 412:376
17. Hoffman BB, Lefkowitz RJ (1990) Catecholamines and sympathomimetic drugs. In: Goodman, Gilman (eds) The pharmacological basis of therapeutics. Pergamon Press, Oxford, p 187
18. Honda K, Nakagawa C, Terai M (1987) Further studies on ±-YM-617, a potent and selective α_1 adrenoceptor antagonist and its individual optical enantiomers. Naunyn-Schmiedeberg's Arch Pharmacol 336:295
19. Iino M, Kobayashi T, Endo M (1988) Use of ryanodine for functional removal of the calcium store in smooth muscle cells of the guinea pig. Biochem Biophys Res Commun 152:417
20. Ito K, Ikemoto S, Ota M (1989) Effects of ryanodine and 9,21-didehydroryanodine on caffeine induced contraction of rat and guinea pig aortae. Jpn J Physiol 21:691
21. Jenden DJ, Fairhurst AS (1969) The pharmacology of ryanodine. Pharmacol Rev 21:1
22. Kamm KE, Stull JT (1989) Regulation of smooth muscle contractile elements by second messengers. Annu Rev Physiol 51:299
23. Kawabe K, Ueno A, Takemoto Y (1990) Use of an alpha-1-blocker, YM 617, in the treatment of benign prostatic hypertrophy. J Urol 144:908
24. Khoyi MA, Westfall DP, Gerthoffer WT (1987) Effects of potassium and norepinephrine on calcium influx in guinea-pig vas deferens. Eur J Pharmacol 140:55
25. Leijten PAA, Breemen C van (1984) The effects of caffeine on the noradrenaline-sensitive calcium store in rabbit aorta. J Physiol 357:327
26. Lepor H (1991) The emerging role of alpha antagonists in the therapy of benign prostatic hyperplasia. J Androl 12:6
27. Lepor H, Gup DI, Bauman M, Shapiro E (1991) Comparison of alpha-1 adrenoceptors in the prostate capsule of men with symptomatic and asymptomatic benign prostatic hyperplasia. Br J Urol 67:493
28. Low AM, Gaspar V, Kwan CY, Darby JP, Bourreau JP, Daniel EE (1991) Thapsigargin inhibits repletion of phenylephrine-sensitive intracellular Ca^{++} pool in vascular smooth muscle. J Pharmacol Exp Ther 258:1105
29. Mimata T, Inomata H (1989) Effects of noradrenaline on current- and voltage-clamp muscle cells of the guinea-pig vas deferens in normal Krebs' solution. Eur J Pharmacol 163:181
30. Rasmussen U, Christensen SB, Sandberg F (1978) Thapsigargin and thapsigargicin, two new histamine liberators from *Thapsia garganica*. Acta Pharm Suecica 15:133
31. Shima H, Blaustein MP (1992) Modulation of evoked contractions in rat arteries by ryanodine, thapsigargin and cyclopiazonic acid. Circ Res 70:968
32. Smith CH, Bray M (1990) Direct and indirect contractile responses of the human vas deferens and actions of noradrenaline and of calcium antagonists. Exp Physiol 75:33
33. Sutko JL, Kenyon JL (1990) Actions of ryanodine. J Gen Physiol 96:439
34. Thastrup O (1990) Role of Ca-ATPases in regulation of cellular Ca^{2+} signalling, as studied with the selective microsomal Ca^{2+}-ATPase inhibitor, thapsigargin. Agents Actions 29:8
35. Walsh MP (1985) Calcium regulation of smooth muscle contraction. In: Marne D (ed) Calcium and cell physiology. Springer, Berlin Heidelberg New York, p 213
36. Walsh PC (1992) Benign prostatic hyperplasia. In: Walsh PC, Gittes RF, Perlmutter AD, Stamey TA (eds) Campbell's urology. Saunders, Philadelphia, p 1099
37. Yamada S, Suzuki M, Kato Y, Kimura R, Mori R, Matsumoto K, Maruyama M, Kawabe K (1992) Binding characteristics of naftopidil and alpha 1-adrenoceptor antagonists to prostatic alpha-adrenoceptors in benign prostatic hypertrophy. Life Sci 50:127