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α_{1A}-Adrenoceptor mediated contraction of rat prostatic vas deferens and the involvement of ryanodine stores and Ca^{2+} influx stimulated by diacylglycerol and PKC

Richard P. Burt, *Christopher R. Chapple & [1]Ian Marshall

Department of Pharmacology, University College London, Gower Street, London WC1E 6BT and *Department of Urology, The Royal Hallamshire Hospital, Sheffield S10 2JF

1 The present study has investigated the α_1-adrenoceptor subtype mediating contraction of the rat isolated prostatic vas deferens and the possible effector mechanisms involved in this response by use of functional experiments.

2 Contractions to noradrenaline in the rat isolated prostatic vas deferens were antagonized by prazosin (9.4, 1.04 ± 0.19, pA_2 and Schild plot slope), 5-methyl urapidil (8.9, 1.10 ± 0.13), BMY 7378 (6.4, 1.53 ± 0.07) and RS 17053 (8.3, 1.13 ± 0.18). These affinities are consistent with the response being mediated by the α_{1A}-adrenoceptor subtype.

3 The contraction to noradrenaline at 37°C consisted of an initial phasic response, composed of many rhythmic contractile spikes and a more slowly developing tonic contraction. When the temperature was lowered to 25°C the phasic contraction became a smooth single response which was increased in magnitude.

4 In Ca^{2+}-free Krebs solution the tonic contraction to noradrenaline (10^{-4} M) was abolished, suggesting that this response was dependent on influx of extracellular Ca^{2+}. After 2 min in Ca^{2+}-free Krebs solution at 37°C and 25°C the phasic response to noradrenaline (10^{-4} M) was $38 \pm 2\%$ and $91 \pm 4\%$, respectively, compared with the phasic contraction to noradrenaline (10^{-4} M in normal Krebs solution) and after 30 min it was abolished at 37°C and was $7 \pm 1\%$ at 25°C. Ryanodine abolished the noradrenaline response in Ca^{2+}-free Krebs solution for 2 min at 25°C, while cyclopiazonic acid reduced it to $36 \pm 2\%$.

5 In normal Krebs solution at 25°C the protein kinase C inhibitor calphostin C reduced the tonic contraction to noradrenaline (10^{-5} M) from $36 \pm 8\%$ to $14 \pm 3\%$ compared with the phasic contraction to noradrenaline (10^{-4} M). The DAG kinase inhibitor R 59022 increased the contraction following the initial phasic response to a maximum of $107 \pm 17\%$ after 35 s, before dropping down to a well maintained contraction which was still greater in magnitude compared with the control. Nifedipine (3×10^{-7} M) reduced the tonic contraction from $49 \pm 6\%$ to $7 \pm 1\%$ but did not reduce the phasic response. Ryanodine (10^{-4} M) reduced the phasic contraction from $50 \pm 2\%$ to $7 \pm 1\%$ and the tonic response from $47 \pm 5\%$ to $27 \pm 5\%$.

6 The phorbol ester phorbol-12,13-dibutyrate at 25°C produced a transient contraction of the rat prostatic vas deferens, maximum response (10^{-5} M) $48 \pm 4\%$, compared with the maximum tonic response to noradrenaline. The contraction to PDBu (10^{-5} M) was reduced to $23 \pm 2\%$ by calphostin C (10^{-6} M) and to $15 \pm 1\%$ by nifedipine (3×10^{-7} M) and was abolished after 2 min in Ca^{2+}-free Krebs solution.

7 In conclusion, the α_{1A}-adrenoceptor mediated contraction to noradrenaline of the rat prostatic vas deferens appears to consist of an initial phasic component due to the release of intracellular Ca^{2+} from ryanodine-sensitive stores. These stores are depleted in the absence of extracellular Ca^{2+} and this depletion is slower at 25°C than at 37°C. The phasic contraction is followed by a tonic contraction involving activation of protein kinase C by diacylglycerol and influx of Ca^{2+} through nifedipine-sensitive channels.

Keywords: Rat prostatic vas deferens; α_1-adrenoceptors; noradrenaline; ryanodine; protein kinase C; R 59022; calphostin C; nifedipine

Introduction

α_1-Adrenoceptors have been characterized into three subtypes (α_{1A}-, α_{1B}-, α_{1D}-), in various tissues and their corresponding cDNAs have also been cloned (Ford *et al*., 1994; Heible *et al*., 1995). The cloned subtypes are defined by lower case subscripts (Bylund *et al*., 1994). The α_1-adrenoceptor mediating contraction of the rat epididymal vas deferens has been characterized in several studies as the α_{1A}-subtype (Aboud *et al*., 1993; Burt *et al*., 1995a) but the subtype in the prostatic vas deferns has not been fully characterized. Some early studies indicated that the α_1-adrenoceptor in the two halves of the rat vas may be different due to differing agonist potencies (McGrath, 1982). However, these differences could also be explained by differences in receptor reserve. Therefore the first aim of this investigation was to characterize the α_1-subtype mediating contraction of the rat prostatic vas deferens in functional experiments, by use of antagonists with known selectivity for the three cloned α_1-subtypes.

α_1-Adrenoceptors are G-protein-coupled receptors which are usually linked to activation of phospholipase C (PLC) (Minneman & Esbenshade, 1994) leading to production of inositol 1,4,5-trisphosphate (IP_3), which mobilizes Ca^{2+} from

[1] Author for correspondence.

intracellular stores (Berridge, 1993) and diacylglycerol (DAG), which stimulates protein kinase C (PKC) (Lee & Severson, 1994). Smooth muscle contraction nearly always involves a rise in intracellular [Ca^{2+}] due to release of Ca^{2+} from intracellular stores or influx of extracellular Ca^{2+}, or both. The α_{1A}-mediated contraction of the rat epididymal vas deferens seems to be dependent on activation of PKC by DAG and influx of extracellular Ca^{2+} through nifedipine-sensitive channels (Burt *et al*., 1996). However the α_{1B}-mediated contraction of the rat spleen appears to involve mobilization of intracellular Ca^{2+} and capacitative Ca^{2+} influx (Burt *et al*., 1995b). In some cells, including those from smooth muscle, there are intracellular Ca^{2+} stores which are insensitive to IP_3 but are sensitive to ryanodine and these stores have ryanodine receptors rather than IP_3 receptors on their surface (Sorrentino & Volpe, 1993; Ehrlich *et al*., 1994). Ryanodine inhibits responses involving release of Ca^{2+} from these stores. The mechanism by which receptor stimulation leads to release of Ca^{2+} from these ryanodine-sensitive stores is not at present clearly understood, but may involve Ca^{2+}-induced Ca^{2+} release or the putative second messenger cADP-ribose (Galione *et al*., 1991; Zucchi & Ronca-Testoni, 1997). The second aim of the present study was to investigate, by means of functional experiments, the possible mechanisms involved in the α_1-mediated contraction of the rat prostatic vas deferens.

Methods

Male Sprague-Dawley rats between 350–450 g were stunned and killed by cervical dislocation. The vasa deferentia were removed into Krebs solution (see below), associated blood vessels and mesentery were dissected away and were then bisected so that only the prostatic portion (about 20 mm in length) was used. The tissues were suspended in 5 ml tissue baths containing Krebs solution of the following composition (mM): Na^+143, K^+5.9, Ca^{2+}2.5, Mg^{2+}1.2, Cl^-128, HCO_3^- 25, HPO_4^{2-}1.2, SO_4^{2-}1.2 and glucose 11, at either 37°C or 25°C and bubbled with 95% O_2/5% CO_2. The vasa deferentia were placed under 0.5 g resting tension and equilibrated for 1 h. Changes in isometric tension were measured with Grass FT.03 transducers and recorded by Biopac Systems Inc. MP100WS for Windows.

To characterize the α_1-subtype in the rat prostatic vas deferens, following an initial contraction to noradrenaline (10^{-4} M) non-cumulative concentration-contraction curves to noradrenaline were constructed at 37°C with 15 min between additions. The curve was then repeated after 1 h or 2 h 15 min either in the absence or presence of an antagonist. The antagonists were equilibrated with the tissues for 45 min except for RS 17053, which was equilibrated for 2 h. This had been found necessary for RS 17053 in the rat epididymal vas deferens (Marshall *et al*., 1996). Cocaine and β-oestradiol (both 10^{-5} M) were included in the Krebs solution for these experiments to block neuronal and extraneuronal uptake, respectively.

To investigate the mechanism of contraction in the rat prostatic vas deferens experiments were performed on single reproducible contractions to noradrenaline at either 37°C or 25°C. Following an initial response to noradrenaline 10^{-4} M which produced a maximum contraction, reproducible responses were obtained either at 10^{-4} M or 10^{-5} M noradrenaline before a final addition of noradrenaline was made under relevant experimental conditions. Ca^{2+}-free Krebs solution always contained EGTA (10^{-3} M). Calphostin C was incubated with tissues for 1 h in bright light which is essential for its activity (Bruns *et al*., 1991). Nifedipine, ryanodine, cyclopiazonic acid, R 59022 and U-57,908 were all incubated for 30 min. Cocaine and β-oestradiol were not included in the Krebs solution. The effect of Ca^{2+}-free Krebs solution at 25°C on contractions to K^+ (50 mM) and the effect of temperature on a submaximal contraction to α-,β-methylene ATP (10^{-5} M) were also measured.

Data analysis

For the concentration-response curves, responses were calculated as a percentage of the maximum response in the initial curve. Phasic and tonic responses to individual additions of noradrenaline were calculated as a percentage of the maximum phasic contraction to noradrenaline (10^{-4} M) for each tissue at the appropriate temperature. Contractions to PDBu were calculated as a percentage of the maximum tonic contraction to noradrenaline (10^{-4} M) for each tissue. All responses were plotted as the mean of at least 4 separate experiments with vertical lines representing s.e.mean. Where error bars are not shown this is because they fall within the size of the symbol.

Statistical significance of differences between control and test means was tested for on raw data by means of a paired *t* test, except for comparison of means with phorbol-12,13-dibutyrate responses where an unpaired *t* test was used. A *P* value of less than 0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed with InStat (GraphPAD Software, San Diego, CA, U.S.A.).

For the antagonists prazosin, 5-methyl urapidil, BMY 7378 and RS 17053, Schild plots were constructed where the x axis intercept is equal to the pA_2 (Arunlakshana & Schild, 1959). A pK_B value was also calculated for BMY 7378 with concentration-ratios from the lowest concentration of BMY 7378 used, equal to log (concentration-ratio −1)−log [antagonist]. Curve fitting for the calculation of EC_{50} values by non-linear regression and linear regression for the calculation of pA_2 values was performed with Prism (GraphPAD Software, San Diego, CA, U.S.A.). When a maximum response in the presence of an antagonist was not reached this was estimated by curve fitting. Concentration-ratios were calculated with the second concentration-response curve in the absence and presence of antagonist.

Drugs and solutions

RS 17053 (*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α, α-dimethyl-1*H*-indole-3-ethanamine hydrochloride) was donated by Roche Bioscience. Prazosin hydrochloride was donated by Pfizer Central Research (Kent). Nifedipine, cocaine hydrochloride and β-oestradiol were obtained from Sigma and noradrenaline bitartrate, 5-methyl-urapidil, 8-[2-[4-(2-methoxy-phenyl)-1-piperazinyl]-8-azaspirol[4,5]decane-7,9-dione dihydrochloride (BMY 7378 dihydrochloride) and α-,β-methylene ATP were obtained from RBI. Calphostin C, R 59022 (6-(2-(4-((*p*-fluorophenyl)phenylmethylene)-1-piperidinyl)ethyl)-7-methyl-5H-thiazolo(3,2-a)pyrimidine-5-one), U-57,908 (1,6-bis-(cyclohexyloximinocarbonylamino)-hexane), phorbol-12,13-dibutyrate (PDBu), ryanodine and cyclopiazonic acid were obtained from Calbiochem. All stock solutions were made in distilled water and diluted to working concentrations in Krebs solution except for nifedipine, which was dissolved in ethanol and then diluted in distilled water, prazosin, RS 17053, β-oestradiol, R 59022 and U-57,908, which were dissolved in DMSO and then diluted in Krebs solution, and calphostin C and cyclopiazonic acid, which were dissolved in DMSO and further diluted in DMSO. Stock

solutions were stored frozen, except for noradrenaline which was prepared fresh each day. The final bath concentrations of DMSO when using calphostin C and cyclopiazonic acid were 1 in 500, which was found in preliminary experiments not to affect responses to noradrenaline.

Results

Contractions to noradrenaline were antagonized by prazosin (9.4, 1.04 ± 0.19, pA_2 and Schild plot slope, Figure 1a), 5-methyl urapidil (8.9, 1.10 ± 0.13, Figure 1b), BMY 7378 (6.4, 1.53 ± 0.07, Figure 1c) and RS 17053 (8.3, 1.13 ± 0.18, Figure 1d). The pK_B for BMY 7378 was 6.6 ± 0.1.

The contraction to noradrenaline (10^{-4} M, which produced a maximum response) in normal Krebs solution at 37°C consisted of an initial phasic contraction consisting of many rhythmic contractile spikes (0.56 ± 0.07 g), followed by a smaller tonic contraction (Figure 2a). At 25°C the phasic contraction to noradrenaline became a single smooth response and was larger in magnitude (2.64 ± 0.04 g, Figure 2b). The contraction to α-,β-methylene ATP (10^{-5} M) was 1.48 ± 0.10 g at 37°C and 1.56 ± 0.08 g at 25°C.

In Ca^{2+}-free Krebs solution the tonic response to noradrenaline (10^{-4} M) was abolished (Figure 2c). After 2 min in Ca^{2+}-free Krebs at 37°C and 25°C the phasic response to noradrenaline was $38 \pm 2\%$ and $91 \pm 4\%$, respectively, but after 30 min it was abolished at 37°C and was $7 \pm 1\%$ at 25°C

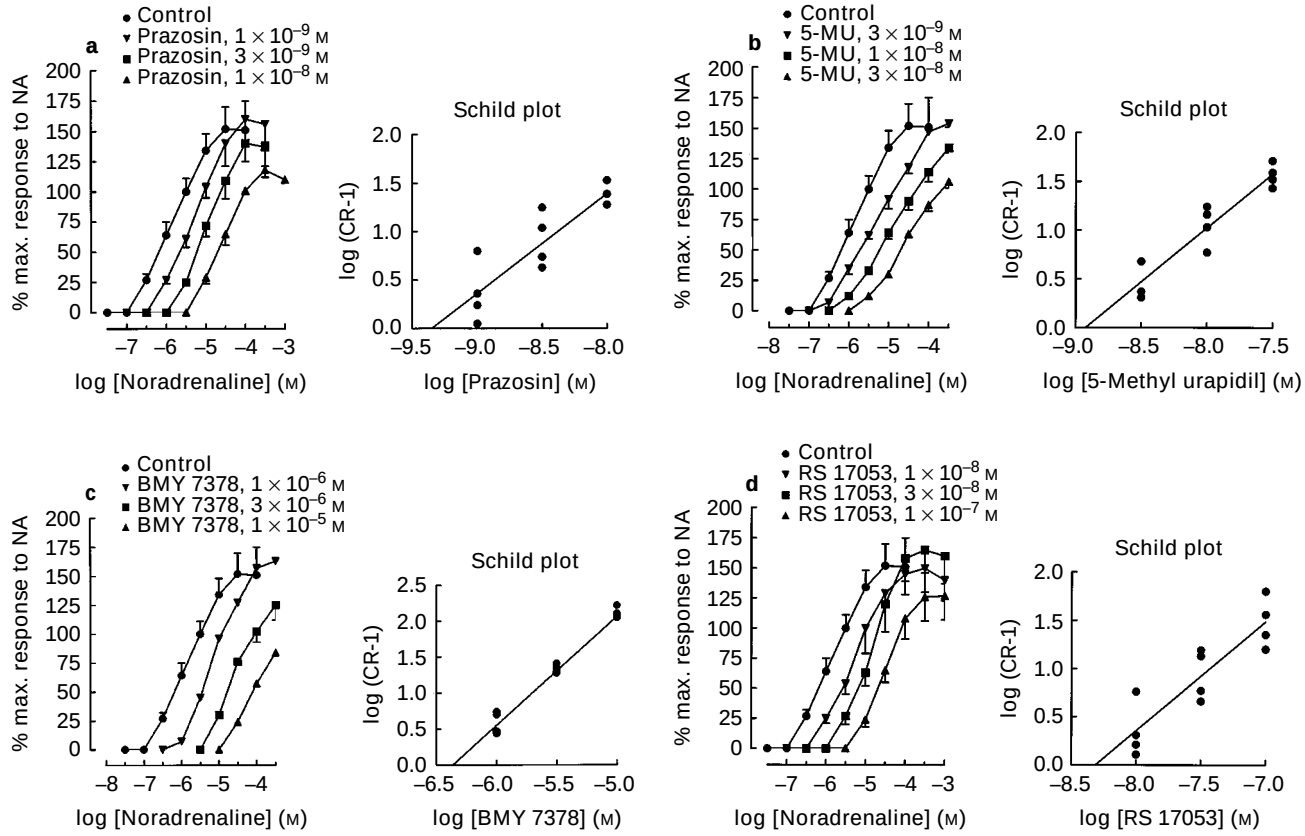

Figure 1 Antagonism of contractions to noradrenaline (NA) in the rat prostatic vas deferens by (a) prazosin, (b) 5-methyl urapidil (5-MU), (c) BMY 7378, and (d) RS 17053. Each plot represents the mean with s.e.mean of 4 separate experiments.

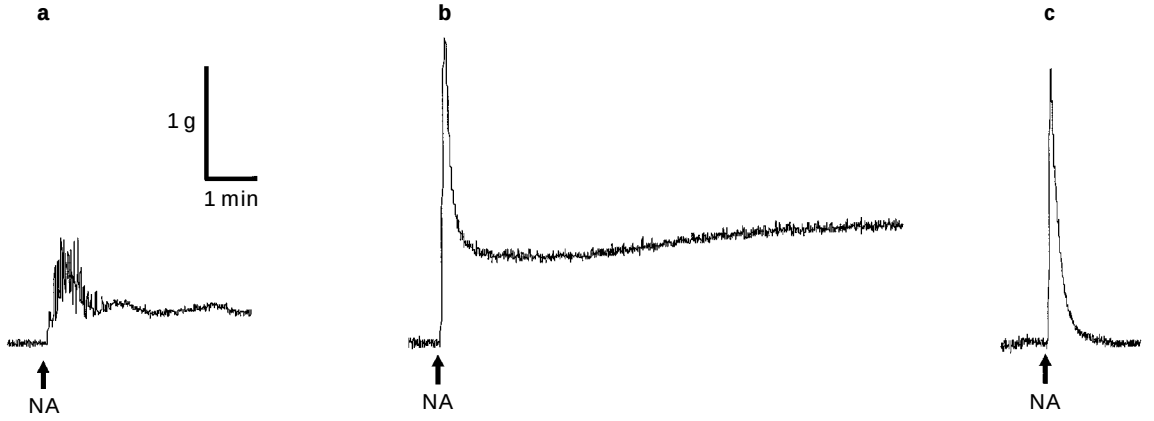

Figure 2 Typical recordings for a contraction to noradrenaline (NA, 10^{-4} M) in the rat isolated prostatic vas deferens at (a) 37°C (b) 25°C and (c) in Ca^{2+}-free Krebs solution at 25°C.

(Figure 3a). The contractile spikes of the phasic contraction at 37°C were not abolished in Ca^{2+}-free Krebs solution. Ryanodine (10^{-4} M, 30 min incubation) abolished the noradrenaline response in Ca^{2+}-free Krebs for 2 min at 25°C, cyclopiazonic acid (which depletes Ca^{2+} from intracellular stores) reduced it to $36\pm2\%$ ($P<0.05$) but with nifedipine (3×10^{-7} M) it was $92\pm3\%$ (Figure 3b). In normal Krebs at 25°C K^+ (50 mM) produced a $127\pm5\%$ contraction. However, after 2 min in Ca^{2+}-free Krebs this was reduced to $2\pm1\%$ ($P<0.05$), indicating extracellular Ca^{2+} had been removed under these conditions.

The preceding experiments showed that the phasic contraction was larger and reduced more slowly at 25°C compared with at 37°C, therefore all further studies were performed at 25°C. In normal Krebs solution at 25°C the protein kinase C inhibitor calphostin C reduced the tonic contraction to noradrenaline (10^{-5} M) from $36\pm8\%$ to $14\pm3\%$ ($P<0.05$) (Figure 4). The DAG kinase inhibitor R 59022 increased the contraction following the initial phasic response to a maximum of $107\pm17\%$ ($P<0.05$) after 35 s before dropping down to a well maintained contraction which was still significantly greater in magnitude compared with the control (Figure 5). Neither compound affected the initial phasic response. The noradrenaline contraction (10^{-5} M) was not potentiated by the DAG lipase inhibitor U-57,908 (10^{-5} M), control $55\pm4\%$ and in the presence of U-57,908, $47\pm4\%$. Nifedipine (3×10^{-7} M) reduced the tonic contraction from $49\pm6\%$ to $7\pm1\%$ ($P<0.05$) but not the phasic response (Figure 6). Ryanodine (10^{-4} M) reduced the phasic contraction from $50\pm2\%$ to $7\pm1\%$ ($P<0.05$) and also reduced the tonic response from $47\pm5\%$ to $27\pm5\%$ ($P<0.05$) (Figure 7).

In normal Krebs solution at 25°C the tonic contraction to noradrenaline in the presence of R 59022 (3×10^{-7} M) increased to $107\pm17\%$ after 35 s (as mentioned previously). This contraction in the presence of R 59022 at 25°C was reduced by calphostin C (10^{-6} M) to $49\pm6\%$ after 35 s ($P<0.05$) (Figure 8a). It was also reduced by nifedipine (3×10^{-7} M) to $12\pm2\%$ after 35 s ($P<0.05$) (Figure 8b). In the presence of ryanodine (10^{-4} M) the response was $126\pm14\%$ after 35 s (Figure 8c).

The phorbol ester phorbol-12,13-dibutyrate (PDBu) at 25°C produced a transient contraction of the rat prostatic vas deferens, with a maximum response of $48\pm4\%$ at 10^{-5} M (compared with the tonic contraction to noradrenaline, 10^{-4} M). The contraction to PDBu (10^{-5} M) was reduced to $23\pm2\%$ ($P<0.05$) by calphostin C (10^{-6} M) and to $15\pm1\%$

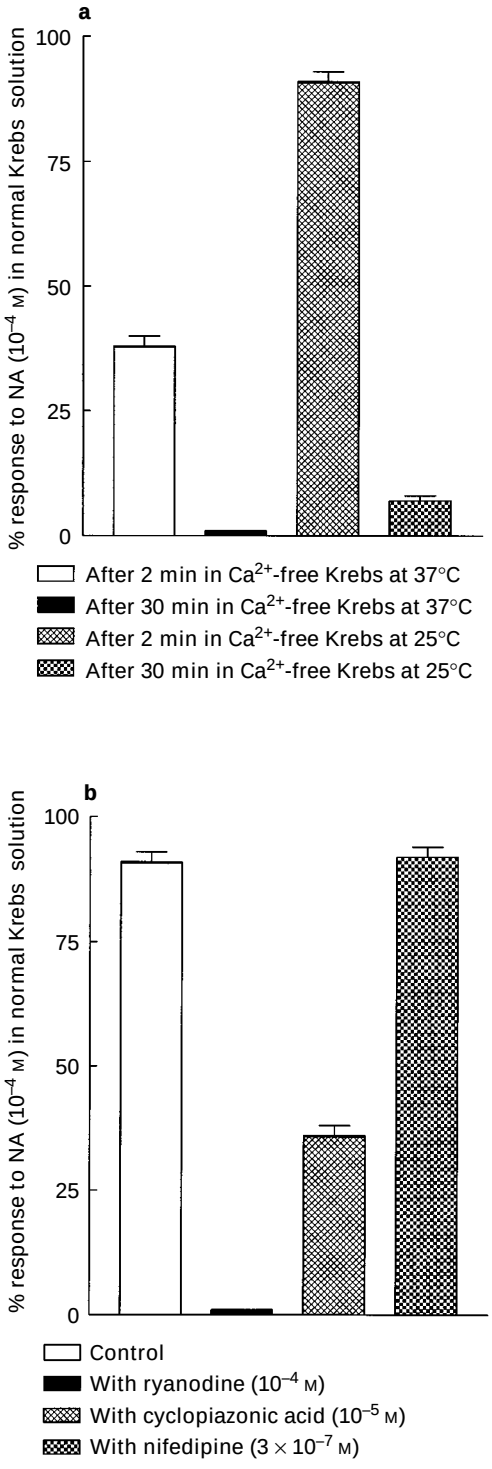

Figure 3 (a) The effect of removing extracellular Ca^{2+} on the phasic contraction to noradrenaline (NA, 10^{-4} M) in the rat prostatic vas deferens at either 37°C or 25°C. (b) The effect of nifedipine, cyclopiazonic acid and ryanodine on the contraction to NA (10^{-4} M) in rat prostatic vas deferens at 25°C after 2 min in Ca^{2+}-free Krebs solution (Control contraction). Each column represents the mean with s.e.mean of 4 separate experiments.

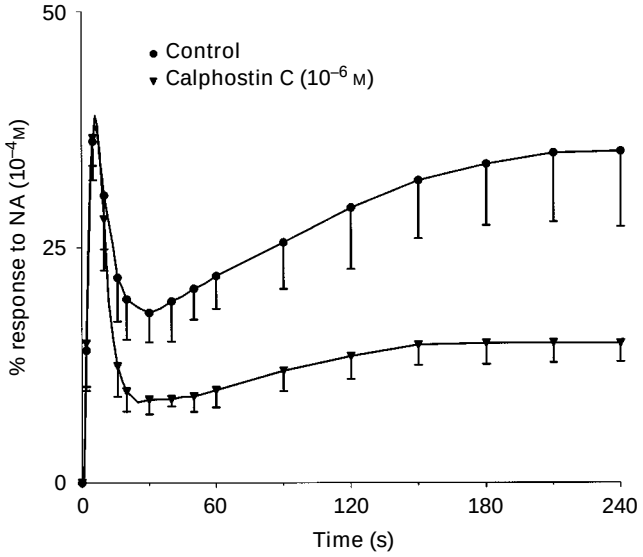

Figure 4 The effect of the selective PKC inhibitor calphostin C on the contraction to noradrenaline (NA, 10^{-5} M) at 25°C in normal Krebs solution. Each point represents the mean with s.e.mean of 4 separate experiments. For clarity only a proportion of the calculated points are illustrated on the figure.

($P<0.05$) by nifedipine (3×10^{-7} M) and was abolished after 2 min in Ca^{2+}-free Krebs solution (Figure 9).

Discussion

The first aim of this study was to characterize the α_1-adrenoceptor subtype mediating contraction to noradrenaline of the rat prostatic vas deferens. These contractions were competitively antagonized by the antagonists employed with the exception of BMY 7378 (Schild slope greater than unity). The affinity of prazosin was consistent with α_1-adrenoceptors. This is higher than would be found for an α_{1L}-subtype, as proposed under another classification system in which prazosin has a low affinity for α_1-adrenoceptors in some tissues (Muramatsu *et al*., 1990) and which had been suggested to mediate contraction of rat prostatic vas deferens (Ohmura *et al*., 1992). The relatively high affinity for the α_{1A}-adrenoceptor selective 5-methyl urapidil and the relatively low affinity for the α_{1D}-selective BMY 7378 suggests the response is mediated by α_{1A}-adrenoceptors.

The α_{1a}-selective antagonist RS 17053 may distinguish between subtypes of α_{1A}-adrenoceptors, as it has been shown to have a 100 fold lower affinity than expected for α_{1A}-adrenoceptors in some tissues (Ford *et al*., 1996; Marshall *et al*., 1996). However, there is no molecular evidence for this and it has also been suggested that the α_{1A}-subtype may exist in different affinity states which may be distinguished by RS 17053 (Ford *et al*., 1997). The affinity of RS 17053 in this study for the α_{1A}-adrenoceptors mediating contraction of the rat prostatic vas deferens (pA_2 8.3) was intermediate between its high and low affinities for the α_{1A}-adrenoceptors in the rat epididymal vas deferens and portal vein, respectively, (Marshall *et al*., 1996). This may possibly represent a third α_{1A}-subtype/affinity state for RS 17053; an intermediate affinity was also obtained for this antagonist on the α_{1A}-adrenoceptors mediating contraction of rat small mesenteric artery (Stam *et al*., 1996). Alternatively it may reflect a mixture of α_{1A}-adrenoceptors with high and low affinities for RS 17053.

The second aim of the study was to investigate the effector mechanisms involved in the α_{1A}-mediated contraction to noradrenaline of the rat prostatic vas deferens. This contraction consisted of two phases, an initial phasic response composed of many rhythmic contractile spikes and a more slowly developing tonic contraction. In contrast the α_{1A}-mediated contraction to noradrenaline in the epididymal vas deferens is greater in magnitude and consists of a single phasic response, with no rhythmic contractile spikes (Burt *et al*., 1996). In the prostatic vas deferens it has previously been shown that the phasic contraction to noradrenaline is sensitive to ryanodine and the tonic response is sensitive to nifedipine (Vesperinas *et al*., 1989). However, although this implied that the phasic response was dependent on the release of intracellular Ca^{2+}, the effect of removing extracellular Ca^{2+}

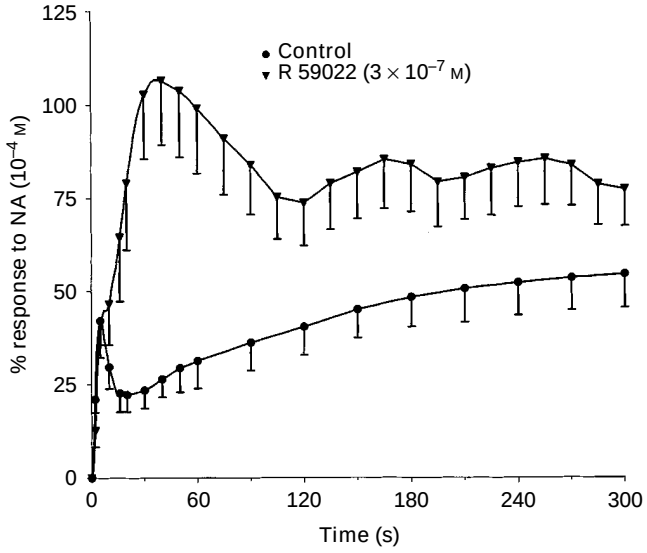

Figure 5 The effect of the DAG kinase inhibitor R 59022 on the contraction to noradrenaline (10^{-5} M) at 25°C in normal Krebs solution. Each point represents the mean with s.e.mean of 4 separate experiments. For clarity only a proportion of the calculated points are illustrated on the figure.

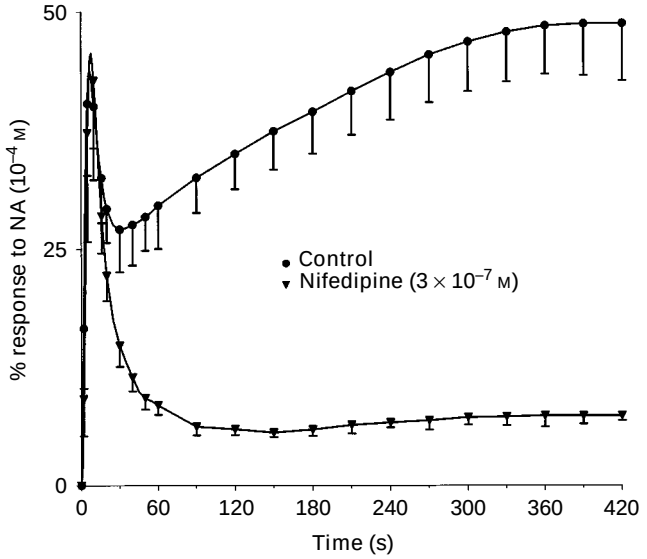

Figure 6 The effect of nifedipine on the contraction to noradrenaline (10^{-5} M) at 25°C in normal Krebs solution. Each point represents the mean with s.e.mean of 4 separate experiments. For clarity only a proportion of the calculated points are illustrated on the figure.

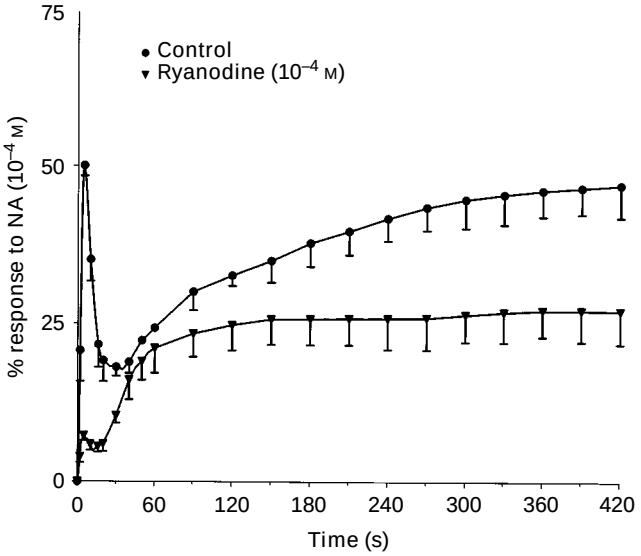

Figure 7 The effect of ryanodine on the contraction to noradrenaline (NA, 10^{-5} M) at 25°C in normal Krebs solution. Each point represents the mean with s.e.mean of 4 separate experiments. For clarity only a proportion of the calculated points are illustrated on the figure.

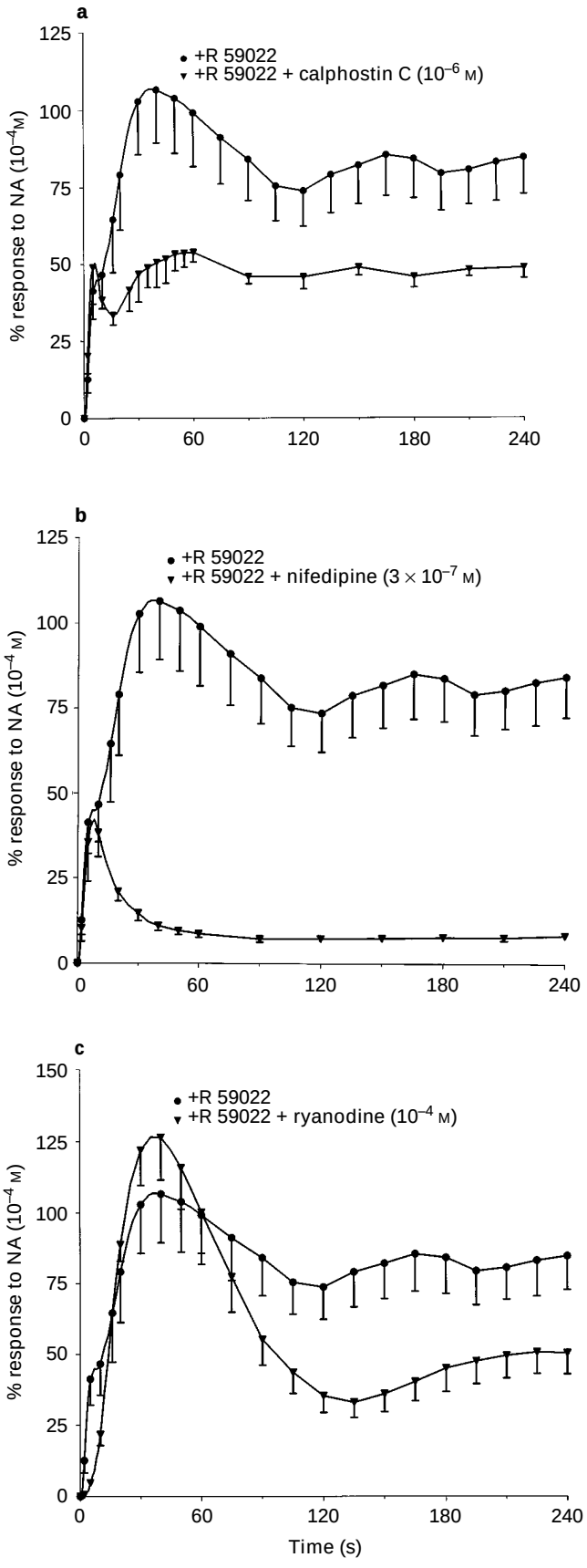

Figure 8 The effect of (a) calphostin C, (b) nifedipine and (c) ryanodine on the potentiated contraction to noradrenaline (10^{-5} M) in the presence of R 59022 (3×10^{-7} M) at 25°C in normal Krebs solution. Each point represents the mean with s.e.mean of 4 separate experiments. For clarity only a proportion of the calculated points are illustrated on the figure.

was not shown in the study. Preliminary experiments in this study suggested that the whole contraction to noradrenaline in the prostatic vas was dependent on extracellular Ca^{2+}, as there was no phasic or tonic response left after the tissues had been in Ca^{2+}-free Krebs solution for 30 min. Under these conditions contractions due to mobilization of intracellular Ca^{2+} can be shown in various smooth muscle preparations, such as contraction of rat spleen to phenylephrine (Burt *et al*., 1995b). However, some intracellular Ca^{2+} stores may be depleted by removal of extracellular Ca^{2+} over a period of time and this process may be slower at lower temperatures (Lalanne *et al*., 1984; Schwietert *et al*., 1993). Therefore responses to noradrenaline in the prostatic vas were studied after different periods of time in Ca^{2+}-free Krebs solution and the effect of lower temperature was also studied.

When the temperature was lowered to 25°C in normal Krebs solution the initial phasic contraction to noradrenaline became a single smooth response and was increased in magnitude. Why the rhythmic contractile spikes were abolished at the lower temperature is not clear. It is possible that Ca^{2+} is released from these intracellular stores in waves at 37°C producing the rhythmic contractile spikes, whereas at 25°C Ca^{2+} is released in one burst. Alternatively, the intracellular Ca^{2+} stores may be able to contain more Ca^{2+} at the lower temperature. The contraction to α-,β-methylene ATP was not increased at 25°C, suggesting that the effect of temperature on noradrenaline contractions is not a non-specific one on the tissue's contractile mechanism. Removal of extracellular Ca^{2+} at 37°C or 25°C always abolished the tonic contraction which developed in the prostatic vas deferens, suggesting that this part of the contraction was dependent on influx of extracellular Ca^{2+}. However, after 2 min in Ca^{2+}-free Krebs solution at 25°C nearly all the phasic response remained, but was reduced with increasing time in Ca^{2+}-free Krebs and more quickly at 37°C compared with 25°C. This suggested that the phasic contraction was due to release of intracellular Ca^{2+} and that the stores from which this Ca^{2+} is released are depleted in the absence of extracellular Ca^{2+} and

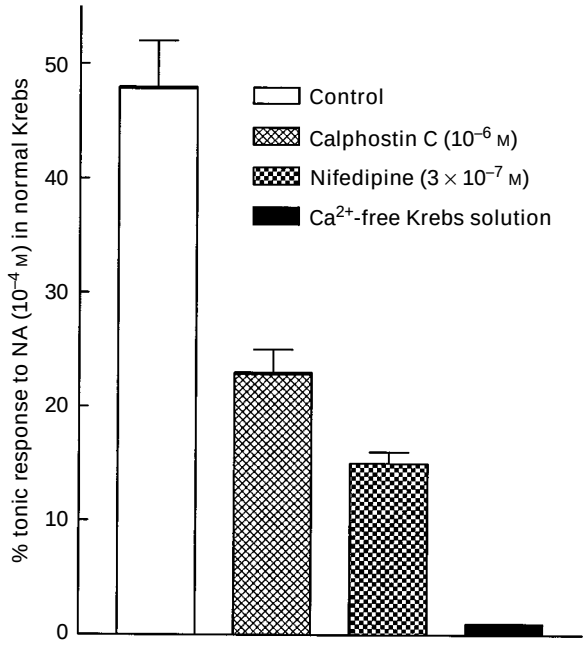

Figure 9 The effect of calphostin C, nifedipine and Ca^{2+}-free Krebs solution on the contraction to PDBu (10^{-5} M) in rat prostatic vas deferens at 25°C. Each column represents the mean with s.e.mean of 4 separate experiments.

that the depletion is slower at lower temperature; therefore all further experiments, discussed below, were performed at 25°C.

Cyclopiazonic acid is a Ca^{2+}-ATPase inhibitor which depletes Ca^{2+} from intracellular stores (Seidler *et al*., 1989; Deng & Kwan, 1991) and therefore its effect on the phasic contraction was investigated. This compound greatly reduced the phasic response to noradrenaline in both Ca^{2+}-free and normal Krebs solution, further indicating that this response was due to mobilization of Ca^{2+} from intracellular stores. Cyclopiazonic acid depletes Ca^{2+} from intracellular stores which are sensitive to IP_3 and ryanodine. However, ryanodine, which does not affect IP_3-sensitive Ca^{2+} stores (Palade *et al*., 1989), completely abolished the phasic contraction in Ca^{2+}-free Krebs and nearly abolished it in normal Krebs, showing that the phasic contraction was due to release of Ca^{2+} from a ryanodine-sensitive store in agreement with Vesperinas *et al*. (1989). This suggests that Ca^{2+} released by IP_3 is not directly responsible for any of this component of the response. However, IP_3 might cause enough Ca^{2+} release from the IP_3 sensitive stores to induce Ca^{2+}-activated Ca^{2+} release from the ryanodine-sensitive stores, but not enough to alone cause a contraction. Cyclic ADP-ribose has been shown to release Ca^{2+} from ryanodine-sensitive stores in some cells (Clapper *et al*., 1987; Galione *et al*., 1991) but it has not been shown, as yet, to act as a second messenger upon receptor stimulation. Nicotinic acid adenine dinucleotide phosphate (NAADP) has also been shown to mobilize intracellular Ca^{2+}, but from a pool which is insensitive to IP_3 cyclopiazonic acid and ryanodine, but can be blocked by nifedipine (Genazzani & Galione, 1997). However, the phasic contraction in the rat prostatic vas deferens was sensitive to ryanodine and cyclopiazonic acid and insensitive to nifedipine. The link between receptor stimulation and mobilization of Ca^{2+} from the ryanodine-sensitive stores is therefore unclear.

In some tissues influx of extracellular Ca^{2+} is stimulated by depletion of IP_3-sensitive intracellular Ca^{2+} stores, termed capacitative Ca^{2+} influx (Putney, 1986; 1990) and in these tissues the Ca^{2+} influx can also be stimulated by cyclopiazonic acid (Fasolato *et al*., 1994). However this mechanism does not appear to operate in the rat prostatic vas deferens, as cyclopiazonic acid did not produce a contraction in normal Krebs solution. This is unlike the α_{1B}-adrenoceptor mediated contraction in the rat spleen, which does involve capacitative Ca^{2+} influx (Burt *et al*., 1995b). It seems unlikely that release of intracellular Ca^{2+} is the stimulus for influx of extracellular Ca^{2+}, as ryanodine nearly abolished the phasic contraction in normal Krebs with a much smaller reduction of the tonic response.

In the rat prostatic vas deferens the noradrenaline tonic contraction was reduced by the highly selective PKC inhibitor calphostin C (Kobayashi *et al*., 1989). It was also greatly potentiated by the DAG kinase inhibitor R 59022 (de Chaffoy de Courcelles *et al*., 1985), which can inhibit the phosphorylation of DAG to phosphatidic acid by DAG kinase (Bishop & Bell, 1986; Kanoh *et al*., 1993). However, the contraction was not potentiated by the DAG lipase inhibitor U-57,908 (Yang *et al*., 1991), which inhibits the metabolism of DAG to arachidonic acid (Severson & Hee-Chong, 1986). The tonic contraction was also reduced by nifedipine, in agreement with Vesperinas *et al*. (1989). These results indicated that the tonic contraction to noradrenaline in the prostatic vas was dependent on activation of PKC by DAG and influx of Ca^{2+} through nifedipine-sensitive channels. They also indicate that DAG is metabolized primarily by DAG kinase in this tissue. This component of the response therefore is similar to the noradrenaline contraction in the rat epididymal vas deferens (Burt *et al*., 1996), which although it is a phasic response is dependent on extracellular Ca^{2+}. The potentiated response in the prostatic vas in the presence of R 59022 was also reduced by calphostin C and nifedipine, but the maximum potentiated response was not reduced by ryanodine (the tonic contraction proceeding this was reduced as was seen in the absence of R 59022). This suggests that the increased response in the presence of R 59022 was due to a potentiation of the contraction seen in the absence of R 59022 rather than activation of a different mechanism. The small tonic response to noradrenaline left in the presence of nifedipine (3×10^{-7} M) did not represent a nifedipine-insensitive component as this was abolished by nifedipine (10^{-6} M) (unpublished results). The larger tonic response to noradrenaline left in the presence of R 59022 and nifedipine (3×10^{-7} M) may be due to the potentiated tonic contraction competitively reducing the effects of nifedipine rather than representing a new nifedipine-insensitive response. Although nifedipine (3×10^{-7} M) was able to abolish a contraction to K^+ (50 mM) in this tissue, the greater sensitivity of this contraction to nifedipine may be due to different voltage-gated Ca^{2+} channels being involved compared with the contraction to noradrenaline.

The phasic contraction was not affected by calphostin C or R 59022 indicating that DAG and PKC are not involved in the release of Ca^{2+} from the intracellular stores. A direct coupling between L-type Ca^{2+} channels and the ryanodine receptor as in skeletal muscle (Sutko & Airey, 1996) is unlikely, because contractions to high K^+ were completely dependent on extracellular Ca^{2+}.

In the presence of R 59022 the potentiated response consisted of a large increase in the contraction following the initial ryanodine-sensitive phasic response, reaching a maximum after about 30 s which then declined again but became stable at a magnitude that was greater than the control tonic contraction. This made the contraction of the rat prostatic vas deferens to noradrenaline more similar to that in the epididymal vas deferens, suggesting that one reason the responses differ could be due to different rates of DAG formation/phosphorylation. It would seem likely that an increase in DAG levels is much less rate limiting for contraction in the epididymal vas deferens compared with the prostatic vas, as R 59022 produced a much smaller potentiation of the noradrenaline contraction in the epididymal portion (Burt *et al*., 1996).

Phorbol esters activate PKC by binding to the DAG binding site (Castagna *et al*., 1982) and the phorbol ester PDBu caused a contraction in the prostatic vas deferens which was reduced by calphostin C and nifedipine. This provides further evidence that activation of PKC in this tissue produces a contraction via influx of Ca^{2+} through nifedipine-sensitive channels. However, the PDBu contraction was smaller in magnitude and was not well maintained compared with the noradrenaline tonic contraction. This might reflect a relatively low efficacy of PDBu for the PKC isoform involved in the contraction (Ryves *et al*., 1991). It may also be that the release of intracellular Ca^{2+} by noradrenaline stimulates the translocation of a Ca^{2+}-dependent isoform of PKC to the cell membrane before activation by DAG (Haller *et al*., 1990). PDBu does not cause release of intracellular Ca^{2+} and this might also limit the response to the phorbol ester.

The potentiation of the tonic contraction by R 59022 suggests that DAG is formed by stimulation of the α_{1A}-adrenoceptors in the rat prostatic vas deferens. If DAG is produced via stimulation of phosphatidylinositol-specific PLC then IP_3 levels should also be increased. One possibility is that IP_3 releases Ca^{2+} from IP_3-sensitive Ca^{2+} stores which then

causes release of Ca^{2+} from ryanodine-sensitive stores by Ca^{2+}-activated Ca^{2+} release. The rise in intracellular [Ca^{2+}] due to release from intracellular stores might cause translocation of a Ca^{2+}-dependent PKC isoform to the cell membrane (Haller *et al*., 1990), where it would be activated by DAG leading to influx of extracellular Ca^{2+}. This mechanism would allow both phasic and tonic contractions to result from activation of phosphatidylinositol-specific PLC. The phasic noradrenaline contraction in Ca^{2+}-free Krebs solution was abolished by ryanodine, which might suggest that none of this response was due to release from IP_3-sensitive stores. However, it is possible that IP_3-mediated Ca^{2+} release may not be enough to cause a contraction but is enough to initiate Ca^{2+}-activated Ca^{2+} release from the ryanodine stores. It is also possible for DAG to be produced without an increase in IP_3 levels via stimulation of phosphatidylcholine-specific PLC or PLD.

In conclusion, the α_{1A}-adrenoceptor-mediated contraction to noradrenaline of the rat prostatic vas deferens appears to consist of an initial phasic component due to release of intracellular Ca^{2+} from ryanodine-sensitive stores. These stores are depleted in the absence of extracellular Ca^{2+} and this depletion is slower at 25°C than at 37°C. The phasic response is followed by a tonic contraction involving activation of PKC by DAG and influx of Ca^{2+} through nifedipine-sensitive channels. This is different from either the α_{1A}-mediated contraction of the rat epididymal vas deferens (Burt *et al*., 1996) or the α_{1B}-mediated contraction of the rat spleen (Burt *et al*., 1995b). The involvement of DAG in the tonic response suggests that the α_{1A}-adrenoceptors are linked to PLC or possibly PLD. However, the link between receptor stimulation and mobilization of Ca^{2+} from the ryanodine-sensitive stores in the rat prostatic vas deferens remains unclear. It is possible that the influx of extracellular Ca^{2+} is completely independent of the release of intracellular Ca^{2+}, which is unusual in smooth muscle cells.

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