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The expression of functional postsynaptic α_2-adrenoceptors in the corpus cavernosum smooth muscle

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1 The purpose of this study was to determine if corpus cavernosum smooth muscle expresses functional postsynaptic α_2-adrenoceptors (AR).

2 The α_2-adrenoceptor agonist UK 14,304 elicited concentration-dependent contractions in rabbit corpus cavernosum smooth muscle (CCSM). The half-maximal response occurred at 0.32 ± 0.03 μM and the maximum contraction at 10 μM UK 14,304.

3 Pretreatment of CCSM strips with selective α_2-adrenoceptor antagonists, rauwolscine and RS-15385, produced rightward shifts in the dose-response curves to UK 14,304 (pA_2 values 7.1 and 8.5, respectively). In contrast, these antagonists did not alter contraction induced by the α_1-adrenoceptor agonist phenylephrine (PE) or oxymetazoline. UK 14,304-induced contractions were also inhibited by prazosin ($pA_2 = 9.08$).

4 UK 14,304-induced contractions, unlike those to PE, were highly dependent on the presence of extracellular Ca^{2+}.

5 [^{3}H]-rauwolscine bound to CCSM membranes with high affinity ($K_d = 1.5$ nM). [^{3}H]-rauwolscine binding was displaced by unlabelled rauwolscine, RS-15385, UK 14,304 and prazosin, but not by PE.

6 UK 14,304 inhibited forskolin and prostaglandin E_1 (PGE_1)-induced increases in intracellular cyclic AMP concentration in primary cultures of rabbit CCSM cells.

7 These results demonstrate that CCSM expresses G_i-coupled postsynaptic α_2-adrenoceptors, and activation of these receptors causes contraction of trabecular smooth muscle.

Keywords: α_2-Adrenoceptors; corpus cavernosum smooth muscle; penile erection, UK14,304; rauwolscine; RS-15385-197; prazosin; oxymetazoline; cyclic AMP

Introduction

During erection the penis accumulates blood under pressure. This function depends on the dilatation of the resistance arterial bed of the penis and the relaxation of the trabecular smooth muscle of the corpora cavernosa (Lue & Tanagho, 1988). Noradrenaline (NA), released from adrenal glands and sympathetic nerve terminals, is thought to contract trabecular smooth muscle by interacting predominantly with postsynaptic α_1-adrenoceptors (Benson *et al*., 1980; Adaikan & Karim, 1981; Steers *et al*., 1984; Hedlund & Andersson, 1985a; Saenz de Tejada *et al*., 1989; Christ *et al*., 1990, 1991; Steers, 1990). This contraction leads to detumescence (flaccidity). However, in recent years the presence of postsynaptic α_2-adrenoceptors has also been demonstrated in a number of tissues including blood vessels. At least three α_2 subtypes (α_{2A}, α_{2B} and α_{2C}) have been identified to date based on antagonist affinity, radioligand binding and molecular cloning studies (see reviews by Bylund, 1992; Hieble *et al*., 1995). Activation of these guanine nucleotide binding protein (G_i)-coupled receptors results in a decrease in intracellular adenosine 3′:5′-cyclic monophosphate (cyclic AMP) concentration (Jakobs *et al*., 1986), and potent contraction in blood vessels (Van Meel *et al*., 1981; Aburto *et al*., 1995; Medgett & Ruffolo, 1988).

The effects of α_2-adrenoceptor agonists and antagonists on corpus cavernosum smooth muscle contractility have been investigated (for a review see Andersson & Wagner, 1995). Clonidine, a partial α_2-adrenoceptor agonist, which also interacts with imidazoline receptors, causes contraction of corpus cavernosum (Hedlund & Andersson, 1985a). Furthermore, NA-induced contractions in corpus cavernosum are inhibited by the α_2-adrenoceptor antagonist rauwolscine (Hedlund & Andersson, 1985a; Saenz de Tejada *et al*., 1989). However, the presence of postsynaptic α_2-adrenoceptors in corpus cavernosum smooth muscle and their physiological relevance to erectile function has been questioned mainly due to the lack of selectivity of the agents used. For instance, prazosin was more effective in blocking NA-induced contractions than rauwolscine in most studies (Hedlund & Andersson, 1985a; Saenz de Tejada *et al*., 1989, for a review see Andersson & Wagner, 1995). Furthermore, high concentrations of yohimbine inhibited phenylephrine-induced contractions (Hedlund & Andersson, 1985a; Saenz de Tejada *et al*., 1989). In the absence of any radioligand binding or molecular studies, most investigators have concluded that NA causes contraction in corpus cavernosum smooth muscle by interacting predominantly with α_1-adrenoceptors (Hedlund & Andersson, 1985a; Saenz de Tejada *et al*., 1989). In the present study using three different approaches i.e. isometric tension measurements, radioligand binding studies and second messenger (cyclic AMP) formation, we demonstrate that corpus cavernosum smooth muscle expresses functional postsynaptic α_2-adrenoceptors.

Methods

Tissue procurement

Penile corpus cavernosum tissue was obtained from male New Zealand White rabbits (3.0–3.5 kg) as previously described

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(Saenz de Tejada *et al*., 1989). The protocol was approved by the Animal Care Committee at the Boston University Medical Center. Freshly dissected corporal strips (measuring $\sim 2 \times 2 \times 10$ mm) were placed in physiological salt solution (PSS, pH 7.4) of the following composition (in mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and CaEDTA 0.026, for organ chamber studies. A maximum of five strips was obtained from each animal for organ chamber studies. Some tissue strips were frozen immediately in liquid nitrogen for membrane preparation.

Organ chamber studies

Corpus cavernosum strips were mounted to force transducers (Grass instruments FT03, Quincy, MA) in 25 ml organ baths (37°C) containing PSS and aerated with 5% CO_2/95% air to maintain a pH of 7.4. Optimal isometric tension for contraction was attained as described previously (Saenz de Tejada *et al*., 1989). The maximum contraction induced by 80 mM KCl was determined for each strip before the experimental protocols were started. Dose-responses to various adrenoceptor agonists were obtained by cumulative additions of drugs to the chambers in half log increments starting at 1 nM and continuing until the maximal contraction was achieved with each treatment. All inhibitors were added to organ chambers 30 min before initiation of dose-responses. At the end of the experiment, 100 μM papaverine was administered to determine baseline tension.

Data analysis

The results are expressed as % of 80 mM KCl contraction (means $\pm$ s.e.) for each agonist concentration. Statistical analysis of the data was performed by ANOVA followed by Student-Newman-Keuls test, unless indicated otherwise; $P < 0.05$ denotes significant differences. The affinities (pA_2 values) of antagonists for α-adrenoceptors were calculated as described by Arunlakshana and Schild (1959).

Preparation of crude corpus cavernosum tissue membranes

Briefly, frozen tissue was pulverized, the powder was weighed and homogenized (1 g 6 ml^{-1} buffer) with a Polytron in 20 mM PIPES buffer (pH 7.4) containing 0.25 M sucrose, 1 mM EDTA, 1 mM EGTA, 0.5 mM PMSF, aprotinin (20 iu ml^{-1}), pepstatin (0.1 μg ml^{-1}), bacitracin (100 mg ml^{-1}) and leupeptin (10 μg ml^{-1}). The homogenate was centrifuged at $1000 \times g$ for 15 min at 2°C and the supernatant was transferred to a clean tube. The $1000 \times g$ pellet was re-homogenized in the same buffer and centrifuged at $1000 \times g$ for 15 min at 2°C. The two $1000 \times g$ supernatants were combined and recentrifuged at $100{,}000 \times g$ for 45 min at 2°C. The supernatant was discarded and the crude membrane pellet was resuspended in buffer, washed twice by resuspension and centrifugation. The final pellet was resuspended in 20 mM PIPES buffer containing 0.15 M NaCl and used for receptor binding assay.

[^{3}H]-rauwolscine binding to α_2-adrenoceptors

Saturation binding studies Binding properties of specific α_2-adrenoceptor antagonist rauwolscine, a structural analogue of yohimbine, to rabbit CC membranes were examined as described for α_1-adrenoceptors (Traish *et al*., 1995). Briefly, aliquots (200 μl, in triplicate) of the crude membranes were dispensed into siliconized tubes and incubated at 25°C for 1 h with 0.2 ml of buffer containing increasing concentrations of [^{3}H]-rauwolscine in the absence (total binding) or presence (nonspecific binding) of a 100 fold molar excess of unlabelled rauwolscine. At the end of the incubation, the protein bound radioactivity was separated from free radioactivity by vacuum filtration on GF/B Whatman glass microfibre filters pretreated with 0.3% polyethylenimine. Filters were then washed four times with 5 ml of phosphate buffer at 2°C. The bound radioactivity was extracted by placing the filters in scintillation vials containing 10 ml of scintillation fluid at room temperature for 16 h before being counted. Specific binding was determined by subtracting nonspecific binding from binding. The specific binding data were analysed according to Scatchard (1949) as described earlier (Traish *et al*., 1995). The non-specific binding in the experiments did not exceed 5% of the total binding and was linear as a function of unlabelled rauwolscine concentration.

Displacement binding studies Aliquots of membrane suspensions were incubated at 25°C for 1 h with [^{3}H]-rauwolscine or [^{3}H]-prazosin in the absence or presence of increasing concentrations of unlabelled competitors ($10^{-9} - 10^{-4}$ M) as described by Traish *et al*. (1995). At the end of the incubation, samples were rapidly filtered under vacuum by use of Whatman GF/B filters presoaked in 0.3% polyethylenimine to reduce non-specific binding. The binding in the control incubation was taken as 100%. The binding in the incubations with unlabelled competitors was expressed as % of control and plotted as a function of log molar concentration.

Cyclic AMP measurements

Rabbit corpus cavernosum smooth muscle cells (RCCSMC) were cultured and characterized as described for human corpus cavernosum (Moreland *et al*., 1995). RCCSMC were incubated in DMEM (37°C, pH 7.4) for 15 min in the presence of phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX, 10 μM). Cells were then treated with the appropriate concentrations of various agents for 5 min. At the end of the incubations the media were aspirated and the reaction stopped by addition of ice-cold trichloroacetic acid (6%). The cells were then scraped, and homogenized in a glass-glass homogenizer. Total extract was centrifuged at $2500 \times g$ and supernatant was extracted with water-saturated diethyl ether. The upper ether layer was discarded and the lower aqueous was lyophilized. [^{3}H]-cyclic AMP standard (15,000 d.p.m. 20 μl^{-1}) was added to each sample before homogenization and extraction to determine recovery at the end of the experiment. Dried extracts were reconstituted in 0.5 M sodium phosphate buffer, acetylated and cyclic AMP determined by radioimmunoassay. The recovery of cyclic AMP in control assays was $\sim$90%.

Materials

UK 14,304 (5-bromo-6-[2-imidazoline-2-ylamino]-quinoxaline), rauwolscine hydrochloride and prazosin hydrochloride were purchased from RBI (Natick, MA). RS-15385-197 (delequamine HCl) was a gift from Roche Bioscience (Palo Alto, CA) and prostaglandin (PGE_1) from Upjohn-Pharmacia (Kalamazoo, MI). All other drugs and chemicals were from Sigma (St. Louis, MO). [^{3}H]-rauwolscine was from NEN-Dupont (Boston, MA) and cyclic AMP radioimmunoassay kits from BTI (Stoughton, MA). Six well clusters for cell culture were obtained from Costar (Cambridge, MA) and foetal bovine serum from Summit Biotechnology (Greeley,

CO). Other cell culture supplies were from GIBCO (Grand Island, NY). UK 14,304 and isobutyl methylxanthine (IBMX) were dissolved in dimethylsulphoxide (DMSO) and PGE_1 in 95% ethanol. The rest of the drugs were dissolved in double distilled water.

Results

[^{3}H]-rauwolscine binding in corpus cavernosum

[^{3}H]-rauwolscine bound corpus cavernosum membranes with high affinity ($K_d = 1.5$ nM) (Figure 1). The maximum number of binding sites (B_{max}) was 110 fmol mg^{-1} protein. [^{3}H]-rauwolscine binding was inhibited in a concentration-dependent manner by unlabelled rauwolscine, RS-15385 and the selective α_2-adrenoceptor agonist UK 14,304. Furthermore, the non-selective α-adrenoceptor antagonists prazosin and phentolamine, but not α_1-adrenoceptor-selective agonist phenylephrine (PE), inhibited [^{3}H]-rauwolscine binding (Figure 2a and b). Concentrations of rauwolscine, RS-15385, phentolamine and prazosin required for half-maximal displacement of [^{3}H]-rauwolscine (IC_{50} values) were approximately 2 nM, 0.9 nM, 30 nM and 300 nM, respectively. In separate experiments, binding of [^{3}H]-prazosin was effectively inhibited by unlabelled prazosin (IC_{50} ~0.7 nM) and phentolamine (IC_{50} ~40 nM), as well as high (μM) concentrations of α_2-adrenoceptor selective ligands UK 14,304, rauwolscine and RS-15385 (Figure 2c).

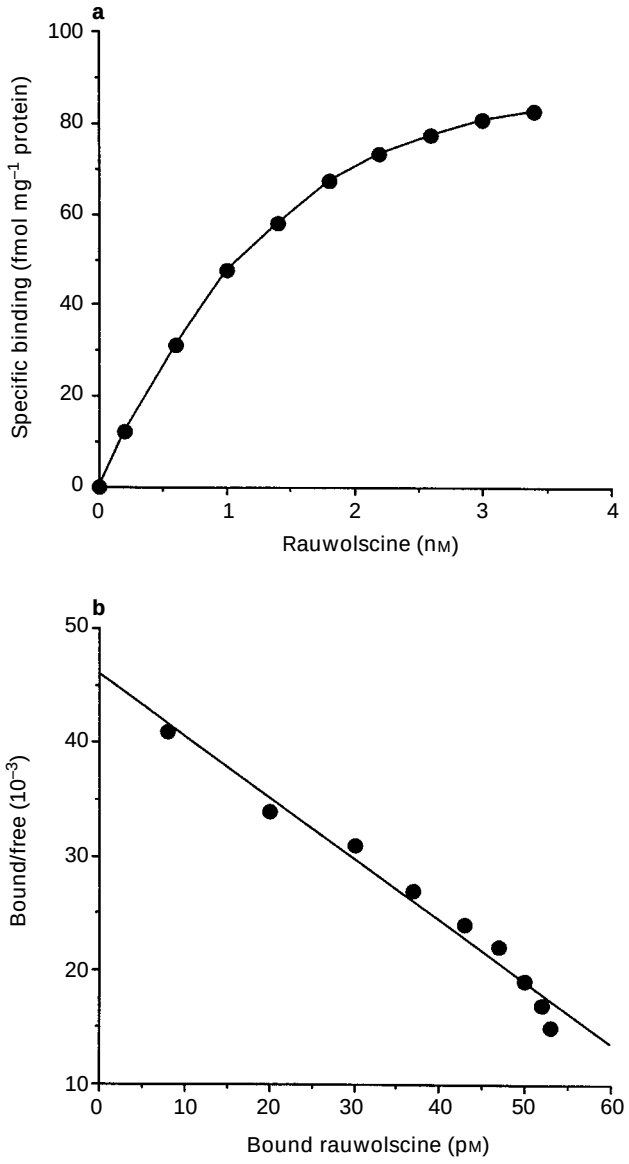

Figure 1 [^{3}H]-rauwolscine binding in corpus cavernosum. Aliquots of rabbit corpus cavernosum membranes were incubated with increasing concentrations of [^{3}H]-rauwolscine (0.05–3.5 nM) in the absence (total binding) or presence (nonspecific binding) of unlabelled rauwolscine. The specific binding is represented in (a). The affinity (K_d) and maximum number of binding sites (B_{max}) were determined by Scatchard analysis (b). Results are representative of 3 experiments.

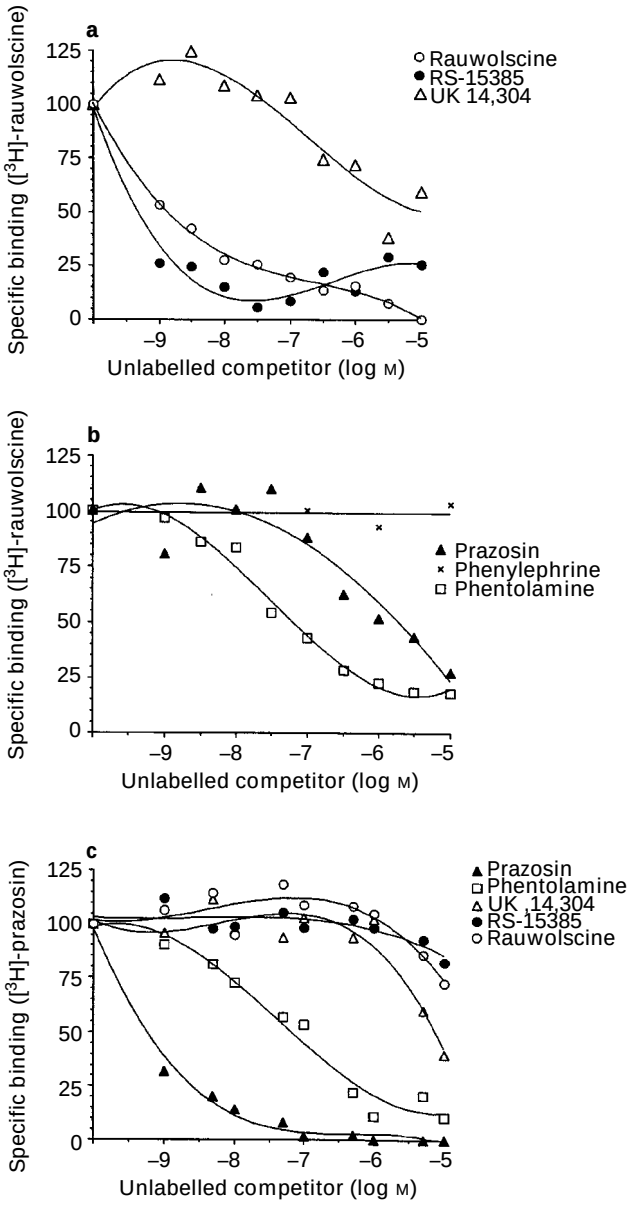

Figure 2 Displacement of [^{3}H]-rauwolscine and [^{3}H]-prazosin binding in corpus cavernosum. (a) Aliquots of rabbit corpus cavernosum membranes were incubated [^{3}H]-rauwolscine (2 nM) for 1 h in the absence and presence of α_2-adrenoceptor selective ligands. (b) Similar studies were carried out in the absence and presence of prazosin, phenylephrine and phentolamine. (c) In some experiments, membranes were incubated with [^{3}H]-prazosin (1 nM, 1 h) in the absence and presence of indicated concentrations of prazosin, phentolamine, UK 14,304, RS-15385 and rauwolscine. Results are representative of 3 experiments.

Effects of α_2-adrenoceptor specific ligands on corpus cavernosum smooth muscle tone

UK 14,304 caused concentration-dependent contractions in corpus cavernosum smooth muscle. Significant increases in tension were first observed with 0.03 μM UK 14,304, and the concentration required for half-maximal response (EC_{50}) was 0.32 ± 0.03 μM. The maximum contraction, which was approximately 80% of that induced by 80 mM KCl, occurred at 10 μM UK 14,304. The EC_{50} for UK 14,304 was in the proximity of the values obtained in tissues with established α_2-adrenoceptor mediated contractions (Daly *et al*., 1988; Aburto *et al*., 1995).

Rauwolscine inhibited UK 14,304-induced contraction of CCSM. Significant increases in EC_{50} values of UK 14,304 were observed in the presence of 0.1 μM rauwolscine. An insurmountable inhibition of UK 14,304-induced contractions was observed in the presence of 10 μM rauwolscine (Figure 3a). Similarly, RS-15385-197 (3 – 300 nM), a highly selective inhibitor of α_2-adrenoceptors (MacKinnon *et al*., 1992), significantly shifted the UK 14,304 dose response curve to the right (Figure 3b). The pA_2 values for rauwolscine and RS 15385-197 against the UK 14,304 dose-responses, as determined by Schild analysis, were 7.10 and 8.5, respectively (Figure 3 insets and Table 1). These values are similar to those found in blood vessels, human platelet, rat neonatal lung and other tissues (Fowler *et al*., 1984; Daly *et al*., 1988; MacKinnon *et al*., 1992; Hieble *et al*., 1995).

Effects of rauwolscine on phenylephrine-induced contractions

The α_1-adrenoceptor agonist, phenylephrine (PE), caused concentration-dependent contractions in corpus cavernosum. The threshold for initiation of contraction with PE was approximately 10 nM, the EC_{50} was approximately 1 μM. The maximum increase in tension was observed with 30 μM PE, which was approximately 150% of that observed with 80 mM KCl. PE-induced contractions were inhibited in the presence of 10 nM prazosin ($pA_2 = 8.3$, Figure 4a), but not 0.1 μM rauwolscine (Figure 4b). However, 1 μM rauwolscine produced a rightward shift in the PE concentration-response curve suggesting that rauwolscine at high concentrations interacts with α_1-adrenoceptors.

Effect of prazosin on UK 14,304-induced contractions

Prazosin, which originally was considered a specific α_1-antagonist, also binds to α_{2B}-adrenoceptors with high affinity and inhibits their activity (reviews by Bylund, 1992; Hieble *et al*., 1995). In corpus cavernosum, pretreatment with 3 – 30 nM prazosin inhibited UK 14,304-induced contractions. A further insurmountable inhibition of UK 14,304-induced contractions was seen in the presence of 100 nM prazosin (Figure 5). The pA_2 value of prazosin against UK 14,304 was 9.08 (Figure 5 and Table 1).

Effects of oxymetazoline in the corpus cavernosum

Oxymetazoline, an imidazoline derivative, has been suggested to be a mixed α_1/α_2-agonist. It has also been shown to have high affinity for α_{2A}-adrenoceptors (Hieble *et al*., 1995). In corpus cavernosum, oxymetazoline caused a concentration-dependent increase in tension (Figure 6a). The threshold for initiation of contraction was approximately 1 nM and EC_{50} was $\sim$20 nM. The maximum increase in tension, which was

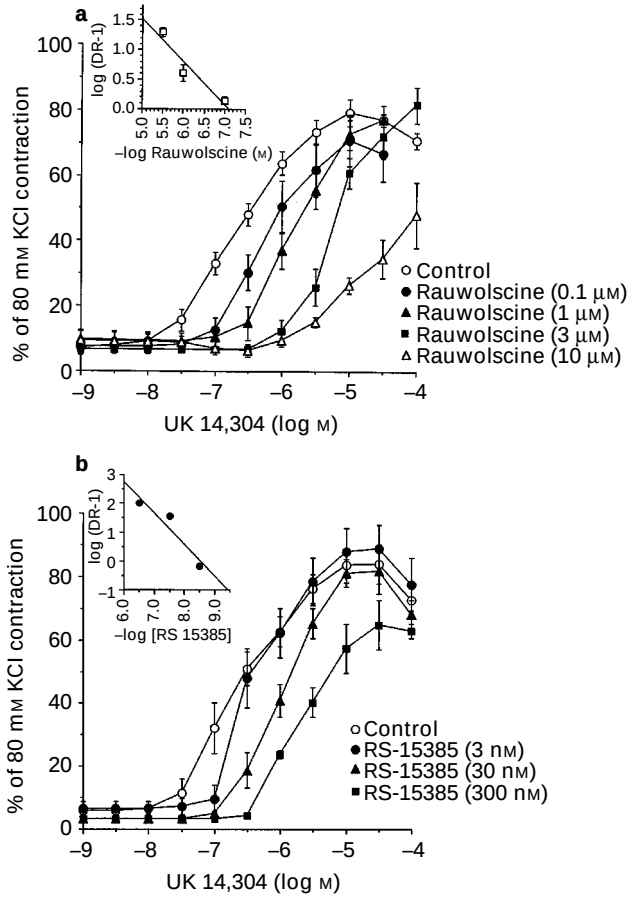

Figure 3 Effects of α_2-adrenoceptor-selective agents on rabbit corpus cavernosum smooth muscle tone. Rabbits corpus cavernosum strips were optimally stretched in the organ chambers as described in Methods. (a) Concentration-responses to the selective α_2-adrenoceptor agonist UK14,304 were obtained in the absence and presence of the indicated concentrations of rauwolscine. Inset shows Schild plot of rauwolscine antagonism of UK 14,304 contractile response. Results are mean of 6 tissue strips from different animals. Significant inhibition of contractile responses to 100 nM – 1 μM UK 14,304 were observed in the presence of all concentrations of rauwolscine used ($P < 0.05$ vs control). In the presence of 1 – 10 μM rauwolscine, contractions were also significantly different from control for 30 nM – 3 μM UK 14,304. (b) Similar studies were carried out in the absence and presence of indicated concentrations of RS-15385-197. Results are mean of 6 tissue strips from different animals. Inset shows Schild plot of RS-15385-197 antagonism of UK 14,304 contractile response. RS-15385, at 3 nM, caused significant inhibition of contraction by 100 nM UK 14,304. In the presence of 30 and 300 nM RS-15385, contractions were also significantly different from control for 30 nM – 3 μM UK 14,304 ($P < 0.05$ vs control). In (a) and (b), vertical lines show s.e.mean.

Table 1 Affinities for α_2-adrenoceptor antagonists in rabbit corpus cavernosum strips contracted with UK 14,304 or oxymetazoline

Antagonist	*Agonist*	*pA_2*	*Slope*	r
Raulwolscine	UK 14,304	7.10	−0.725	0.95
RS-15385-197	UK 14,304	9.40	−1.087	0.99
Prazosin	UK 14,304	9.08	−0.640	0.95
Prazosin	Oxymetazoline	8.20	−1.082	0.99

EC_{50} and dose ratios were derived from the data in Figures 2, 4 and 5. Antagonist affinities (pA_2) were calculated as described by Arunlakshana and Schild (1959). *r* is the coefficient of correlation.

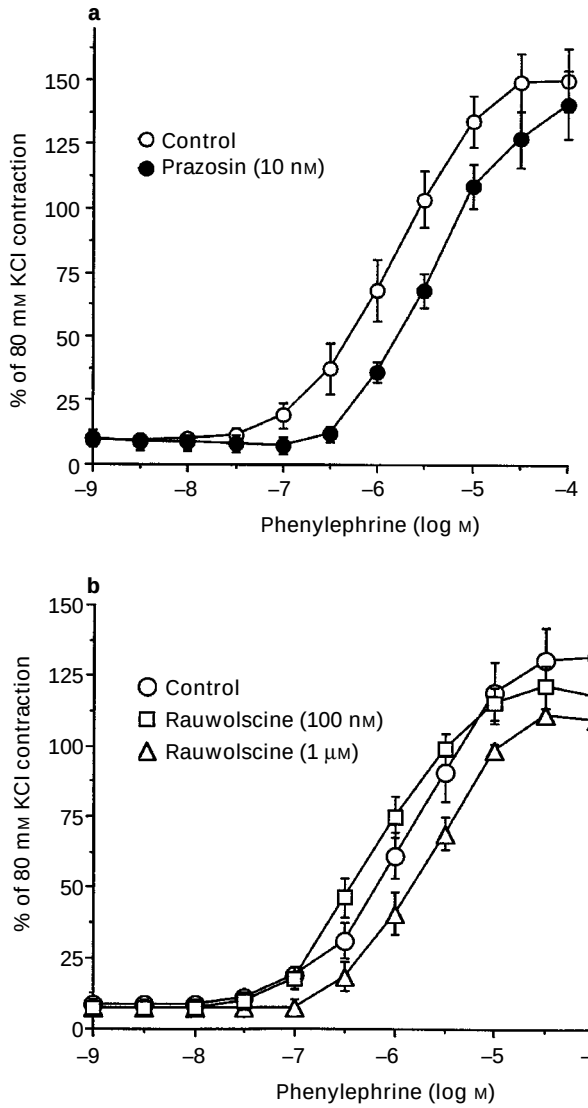

Figure 4 Effects of the α_1-adrenoceptor agonist phenylephrine in rabbit corpus cavernosum smooth muscle tone. Concentration-responses to phenylephrine were constructed in the absence and presence of indicated concentrations of (a) prazosin (30 min pretreatment), and (b) rauwolscine. Results are mean ± s.e. of 6 tissue strips from different animals. Significant inhibition of contractions induced by 100 nM – 10 μM phenylephrine was observed in the presence of 10 nM prazosin (a) and 1 μM rauwolscine (b) ($P<0.05$ vs control).

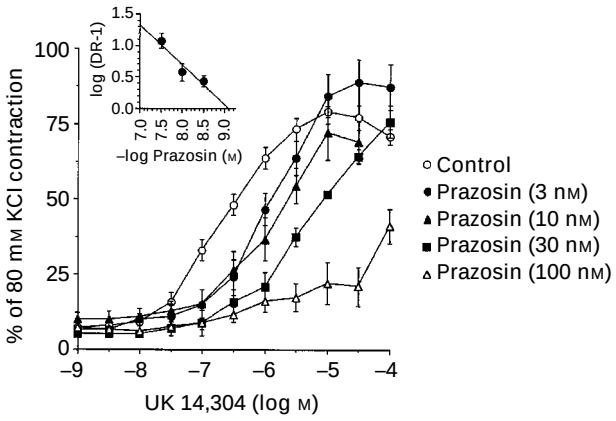

Figure 5 Inhibition of UK 14,304-induced contractions by prazosin. Concentration-responses to UK14,304 were constructed in the absence and presence of various prazosin concentrations. Inset shows Schild plot of prazosin antagonism of UK 14,304 contractile response. Results are mean of 6 tissue strips from different animals and vertical lines show s.e.mean. Significant inhibition of contractions induced by 100 nM – 1 μM UK 14,304 were observed in the presence of all concentrations (30 min pretreatment) of prazosin used ($P<0.05$ vs control). Higher concentrations of prazosin (10 – 100 nM) caused a significantly greater shift in the UK 14,304 dose-response curve.

approximately 110% of that caused by 80 mM KCl, was observed with 50 nM oxymetazoline. Pretreatment of strips with either rauwolscine (100 nM) or RS-15385-197 (10 nM – 1 μM) did not affect oxymetazoline-induced contractions (Figure 6a and Figure 6b). In contrast, prazosin shifted oxymetazoline-induced contraction dose-response curves to the right ($pA_2=8.2$), without affecting the maximum contraction (Figure 6c).

Oxymetazoline has also been suggested to be a partial agonist for α_2-adrenoceptors (see review by Hieble *et al*., 1995). Pretreatment with a partial agonist inhibits/shifts agonist responses to the right (Limbird, 1986). In corpus cavernosum, pretreatment with 1 and 10 nM oxymetazoline did not inhibit UK 14,304-induced contractions. In fact, the UK 14,304 dose-response curve was shifted to the left in the presence of 1 nM oxymetazoline (Figure 7a). On the other hand, pretreatment with 10 nM oxymetazoline inhibited in PE-induced contractions (EC_{50} for PE ~1.15 vs 1.5 μM; maximum contraction 152 vs 130%) (Figure 7b).

Effect of extracellular Ca^{2+}-removal on UK 14,304 and phenylephrine-induced contractions

Corpus cavernosum strips in organ chambers were incubated in a Ca^{2+}-free PSS containing 0.5 mM EGTA for 5 min. Following this, strips were thoroughly washed with Ca^{2+}-free medium (without EGTA), and incubated in the same medium for another 10 min before the agonist dose-responses were constructed. Removal of Ca^{2+} from the incubation medium resulted in greater than 80% inhibition of the UK-induced contraction (Figure 8a). In contrast, PE-induced contractions were only partially inhibited in the absence of extracellular Ca^{2+} (Figure 8b).

Effect of UK 14,304 on intracellular cyclic AMP concentration

The effects of UK 14,304 on cyclic AMP concentrations increased by forskolin, a direct activator of adenylyl cyclase, or the prostaglandin E receptor agonist PGE_1, were studied in rabbit cultured CCSM cells. As shown in Figure 9, forskolin and PGE_1 alone increased intracellular cyclic AMP levels by 200 and 20 fold, respectively. Pretreatment with UK 14,304 significantly inhibited forskolin and PGE_1-induced cyclic AMP formation.

Discussion

The presence of postsynaptic α_1-adrenoceptors and their role in the regulation of corpus cavernosum smooth muscle contraction in response to noradrenaline is well documented (reviewed by Andersson & Wagner, 1995). The results of the present study indicate that functional postsynaptic α_2-adrenoceptors are also present in corpus cavernosum smooth muscle. This conclusion is supported by the following observations: (i) the

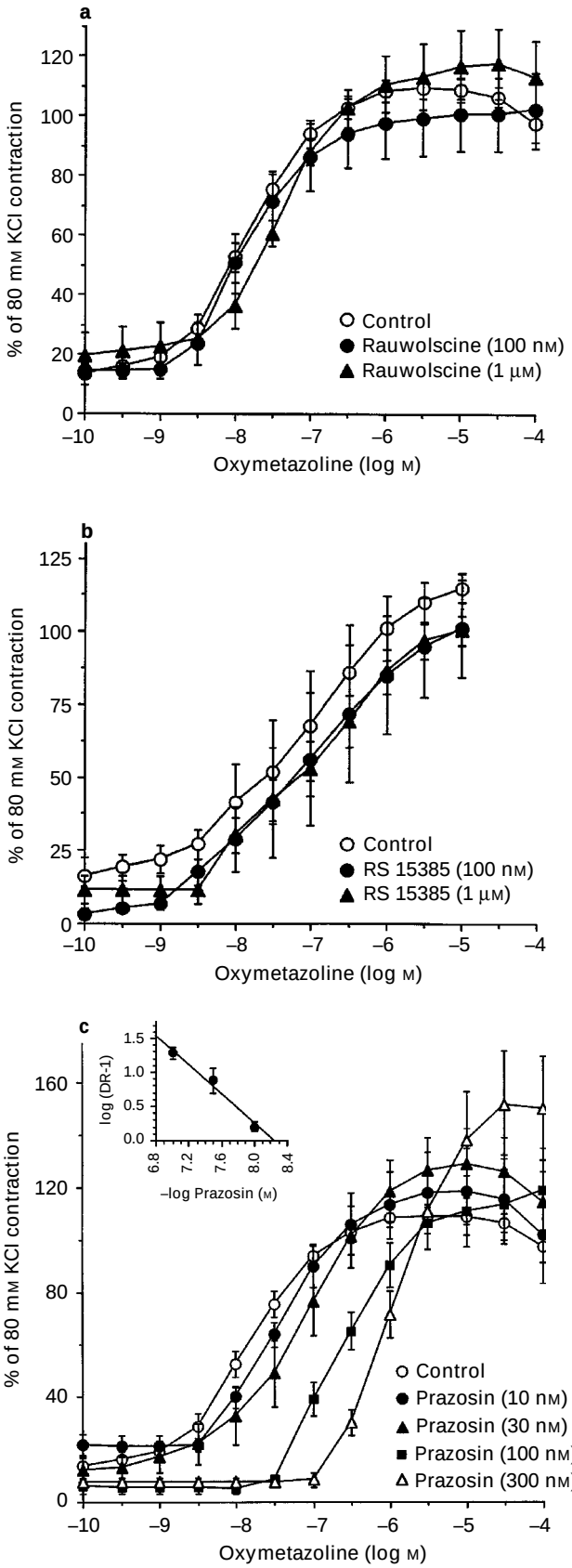

Figure 6 Effects of adrenoceptor antagonists on oxymetazoline-induced contractions in rabbit corpus cavernosum. Concentration-responses to oxymetazoline were constructed in the absence and presence of various concentrations of (a) rauwolscine, (b) RS-15385-197 and (c) prazosin. Inset shows Schild plot of prazosin antagonism of oxymetazoline contractile response. Results are mean of 6 tissue strips from different animals; vertical lines show s.e.mean. Significant inhibition of oxymetazoline (3 nM – 300 nM)-induced contractions was observed in the presence of 100 and 300 nM prazosin ($P<0.05$ vs control).

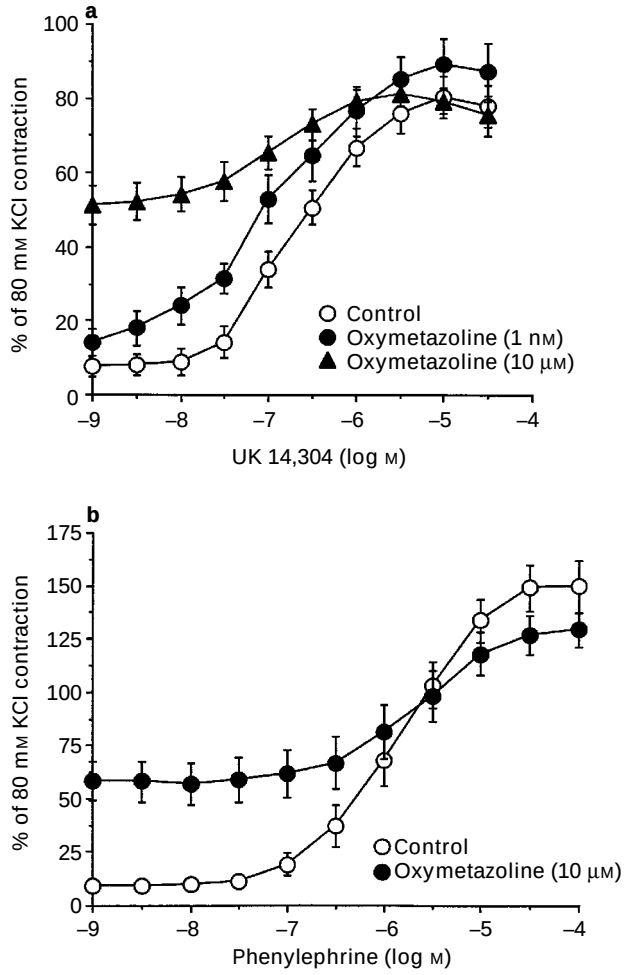

Figure 7 Effects of oxymetazoline pretreatment on UK 14,304 and phenylephrine-induced contractions. (a) Rabbit corpus cavernosum strips were pretreated with the indicated concentrations of oxymetazoline for 30 min. Following this, UK 14,304 concentration-responses were constructed. A significant increase in the contractile response to UK 14,304 (3 nM – 1 μM) was observed in the presence of 1 nM oxymetazoline ($P<0.05$ vs control). (b) Phenylephrine dose-responses were constructed in the absence and presence of 10 nM oxymetazoline. Results are mean of 4 tissue strips from different animals; vertical lines show s.e.mean. Oxymetazoline (10 nM) significantly inhibited contractions induced by 30 μM phenylephrine ($P<0.05$ vs control).

α_2-adrenoceptor-specific agonist UK 14,304 caused concentration-dependent contractions which were inhibited by selective α_2-adrenoceptor antagonists rauwolscine and RS-15385-197, (ii) rabbit corpus cavernosum exhibited saturable, high affinity [^{3}H]-rauwolscine binding which was displaced by ligands selective for α_2-adrenoceptor, but not by the α_1-adrenoceptor-specific agonist PE; and (iii) UK 14,304 lowered forskolin and PGE_1-induced increases in intracellular cyclic AMP concentration in cultured corpus cavernosum smooth muscle cells. In further support of this, inhibition of NA-induced contractions of corpus cavernosum smooth muscle by rauwolscine (100 nM; selective for α_2-adrenoceptors in corpus cavernosum) has been demonstrated (Hedlund & Andersson, 1985a; Saenz de Tejada *et al.*, 1989). This effect of rauwolscine on NA response was similar to that observed on α_2-selective agonist UK 14,304-mediated contractions in the present study.

It is now well documented that prazosin interacts with α_1- as well as α_2-adrenoceptors. It binds with high affinity to α_{2B} and α_{2C}-adrenoceptors, and with low affinity to α_{2A}-adrenoceptors

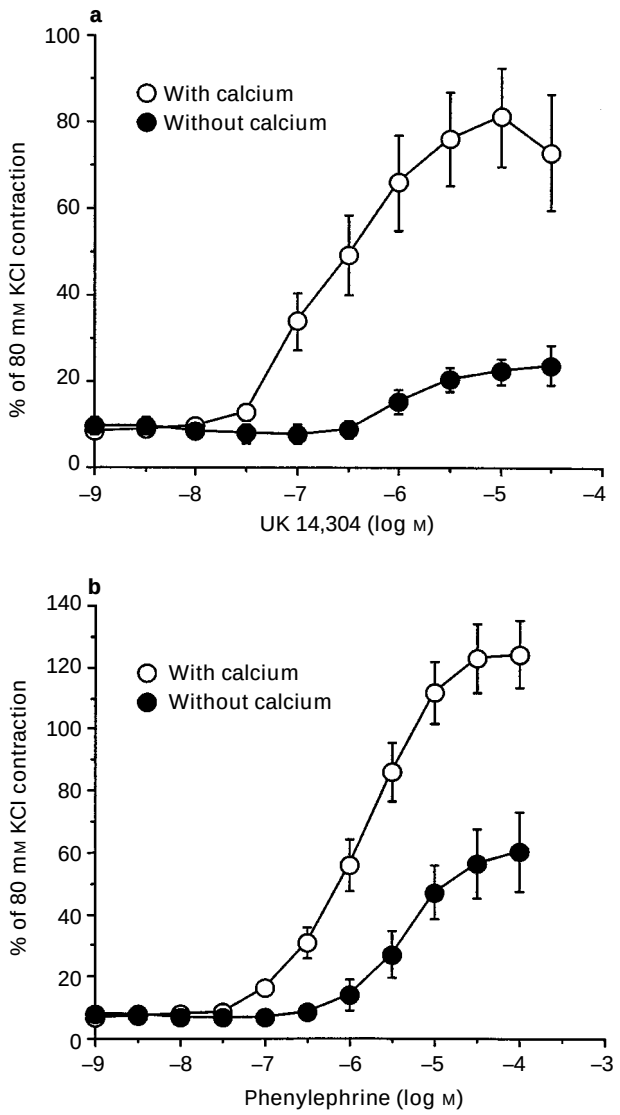

Figure 8 Effects of removal of extracellular Ca^{2+} on the contractions induced by UK 14,304 and phenylephrine. Rabbit corpus cavernosum strips were incubated in normal PSS or Ca^{2+}-free PSS containing 0.5 mM EGTA for 5 min. Following this, strips were washed with normal or Ca^{2+} free PSS (without EGTA), respectively, and incubated in the same media for another 10 min before construction of agonist dose-response curves for (a) UK14,304 and (b) phenylephrine. Results are mean of 4 tissue strips from different animals; vertical lines show s.e.mean.

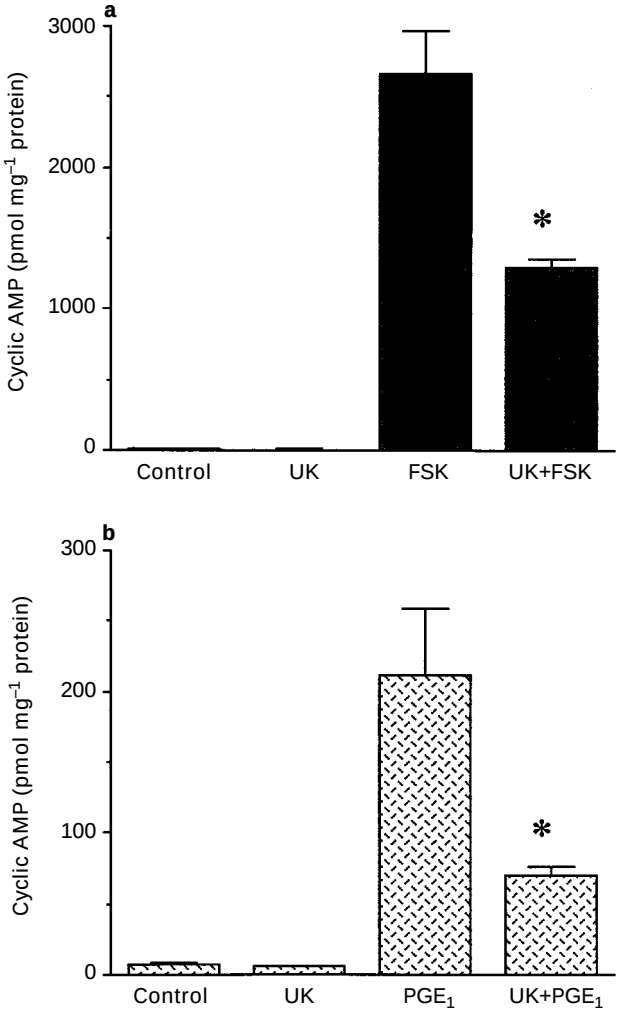

Figure 9 Effect of UK 14,304 on intracellular cyclic AMP concentration. Rabbit corpus cavernosum smooth muscle cells were incubated for 5 min with (a) forskolin (10 μM) or (b) PGE_1 (2 μM) in the absence and presence of 10 μM UK 14,304 (UK). Cyclic AMP levels were determined by radioimmunoassay as described in Methods. Results are mean$\pm$s.e. of 3 experiments. *Indicates $P<0.05$ vs forskolin or PGE_1 alone by paired t test.

(reviews by Bylund 1992; Hieble *et al.*, 1995). In lower urinary tract tissues, the pA_2 values of prazosin for α_1-receptors range between 7.77 to 8.3 (Honda *et al.*, 1985; Testa *et al.*, 1993; Deplanne & Draznin, 1996). In agreement with this, prazosin inhibited contractions of CCSM induced by the α_1-agonist phenylephrine with a pA_2 value of 8.3 in the present study. Moreover, prazosin exhibited high affinity for α_2-adrenoceptors, as it shifted UK 14,304-induced contraction dose-response curves to the right ($pA_2=9.08$) and inhibited [^{3}H]-rauwolscine binding. Furthermore, [^{3}H]-prazosin binding was inhibited by UK 14,304 as well as high concentrations of rauwolscine and RS-15385. These results indicate that in corpus cavernosum smooth muscle, prazosin inhibits contractions mediated by α_{2B} and/or α_{2C} subtypes in addition to those mediated by α_1 subtypes. The pA_2 value of prazosin against UK 14,304 in our study was lower than those obtained for α_{2B}- and α_{2C}-adrenoceptors in other species and tissues (reviews by Bylund 1992; Hieble *et al.*, 1995). This suggests that prazosin is a more potent antagonist for α_2- compared to α_1-subtypes in CCSM. However, due to the fact that the slope of the Schild plot was significantly different from unity in our study, this conclusion will have to be validated by additional means.

In contrast to prazosin, oxymetazoline exhibits high affinity for α_{2A}-adrenoceptors. It has also been demonstrated to be an α_{1A}-agonist (reviews by Bylund, 1992; Hieble *et al.*, 1995). In rabbit CCSM, oxymetazoline caused concentration-dependent contractions, which were inhibited by high concentrations of prazosin, but not by selective α_2-antagonists rauwolscine and RS-15385. These results suggest that oxymetazoline may cause contraction of corpus cavernosum smooth muscle by interacting predominantly with α_1-adrenoceptors. In support of this notion, oxymetazoline has been shown to displace specifically α_1-adrenoceptor ligand binding in CCSM (Traish *et al.*, 1995). However, in the present study, the affinity of prazosin ($pA_2=8.2$) against oxymetazoline was at least 10 fold higher than that obtained for α_{1A}-adrenoceptors in other systems. Recently, the presence of another α_1-subtype exhibiting low

affinity for prazosin (α_{1L}) has also been demonstrated (Oshita *et al*., 1993). Thus, oxymetazoline may cause contraction by interacting with α_{1L}-adrenoceptors in corpus cavernosum, as also found in rabbit urethra (Deplanne & Galzin, 1996). The maximum contraction induced by oxymetazoline, in our study, was also significantly less than that caused by the full α_1-agonist PE. This could be due to the fact that oxymetazoline interacts only with α_{1A} and/or α_{1L} subtypes, whereas PE is an agonist for all α_1-subtypes. Alternatively, it may only be a partial agonist for α_1-receptors in the corpus cavernosum. In further support of this, PE-induced contractions were slightly inhibited when corpus cavernosum strips were pretreated with oxymetazoline (Figure 7).

Rauwolscine, in contrast to prazosin, exhibited much lower affinity against UK 14,304-induced contractions ($pA_2 = 7.1$) in our study. This value was higher than that obtained in rabbit saphenous vein (7.78, Alabaster *et al*., 1985). However, one must exercise caution when comparing data from different tissues or species as prazosin did not block UK 14,304-induced contractions in saphenous vein (Alabaster *et al*., 1985). The slope of the Schild plot for rauwolscine against UK 14,304 contraction response was also significantly different from unity, suggesting that it may interact with more than one α-subtype in CCSM. In fact, higher concentrations of rauwolscine inhibited PE-induced contraction of CCSM in the present study. Alternatively, the low affinity of rauwolscine for α_2-adrenoceptors may be related to the absence of the α_{2A}-subtype in CCSM as also suggested by a lack of effect of α_2-adrenoceptor antagonists on oxymetazoline-induced contraction of the corpora. On the other hand, RS-15387 was a selective, high affinity antagonist for α_2-adrenoceptors in CCSM as indicated by the low pA_2 and IC_{50} values against UK 14,304 and rauwolscine, respectively (Figures 2 and 3).

UK 14,304 significantly reduced intracellular cyclic AMP concentrations in rabbit corpus cavernosum smooth muscle cells stimulated with forskolin and prostaglandin E_1 (Figure 9). These agents are known to cause relaxation of corpus cavernosum smooth muscle by elevating intracellular cyclic AMP concentration. Forskolin activates adenylyl cyclase directly, whereas PGE_1 interacts with specific G-protein-coupled receptors (Hedlund & Andersson, 1985b; Palmer *et al*., 1994; reviewed by Seamon & Daly, 1981; Porst, 1996). α_2-Adrenoceptors are known to couple to inhibitory guanine nucleotide binding protein (G_i) and their activation results in the inhibition of adenylyl cyclase activity and attenuation of cyclic AMP production in a number of cells and tissues (Jakobs *et al*., 1986). This pathway is thought to be responsible for a number of physiological effects elicited by α_2-adrenoceptor activation in other tissues. The results indicate that α_2-adrenoceptors in corpus cavernosum, like other mammalian tissues, are coupled to G_i.

The maximal contractile response upon activation of α_2-adrenoceptors by UK 14,304 was approximately half of that for α_1-adrenoceptor activation by PE. This may be related to differences in the G-protein coupling and signalling pathways underlying α_1 and α_2-adrenoceptor activation. Our data in cultured cells clearly indicate that α_2-adrenoceptors in CC are coupled to G_i and may elicit contractile response by lowering intracellular cyclic AMP. Furthermore, the responses mediated by α_2-adrenoceptor stimulation, particularly contraction of smooth muscle, have been shown to require influx of extracellular Ca^{2+} (Van Meel *et al*., 1981; Scarborough & Carrier, 1984). α_1-Adrenoceptors, on the other hand, are coupled to G_q/G_{11} and activate phospholipase C, which hydrolyzes phosphatidylinositol-4,5-bisphosphate, leading to formation of two second messengers. First is inositol 1,4,5-trisphosphate, which causes release of Ca^{2+} from intracellular stores. The other product of this hydrolysis, diacylglycerol, activates protein kinase C which is thought to play a key role in the contraction of smooth muscle (Danthuluri & Deth, 1984). The combination of increased intracellular Ca^{2+} and protein kinase C activity is thought to contribute to a greater/full response after α_1-adrenoceptor stimulation (Campbell *et al*., 1985; reviewed by Minneman, 1988). In the present study, removal of Ca^{2+} from the incubation medium caused greater than 80% inhibition of UK 14,304-induced contraction (Figure 8). Furthermore, nifedipine inhibited UK 14,304-induced contractions in the presence of normal extracellular Ca^{2+} (data not shown). In contrast, removal of extracellular Ca^{2+} resulted in only $\sim$50% inhibition of PE-induced contractions. These results suggest that α_2-adrenoceptor mediated contraction of corpus cavernosum smooth muscle is facilitated mainly by influx of extracellular Ca^{2+} presumably via voltage-operated Ca^{2+} channels, similar to that shown for α_2-adrenoceptors in vascular smooth muscle (Van Meel *et al*., 1981; Scarborough & Carrier, 1984) and G_i-coupled muscarinic acetylcholine receptors in airway smooth muscle (Fleischmann *et al*., 1997).

In summary, corpus cavernosum smooth muscle expresses postsynaptic α_2-adrenoceptors which play an important role in the regulation of smooth muscle tone. Activation of α_2-, in addition to α_1-adrenoceptors, may contribute to the noradrenaline-induced contractile response in corpus cavernosum *in vivo*. Further studies in corpus cavernosum are warranted to enable development of α_2-selective agents which may enhance penile erection in males with erectile dysfunction.

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