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Characterization of α_1-adrenoceptor subtypes in tension response of human prostate to electrical field stimulation

Jih-Hwa Guh, *Shih-Chieh Chueh, Feng-Nien Ko & [1]Che-Ming Teng

Pharmacological Institute and *Department of Urology, College of Medicine, National Taiwan University, Taipei, Taiwan

1 The effects of various α_1-adrenoceptor antagonists and nifedipine on tension responses of human prostate to electrical field stimulation were evaluated in this study.

2 Prazosin (3×10^{-10} to 10^{-8} M) and 5-methyl-urapidil (10^{-9} to 3×10^{-8} M) blocked concentration-dependently the tension responses to electrical field stimulation and completely abolished them in the maximal concentrations (10^{-8} M and 3×10^{-8} M, respectively); in contrast, chloroethylclonidine (CEC), in the maximal concentration of 100 μM, blocked these effects by only 50%.

3 The contractile responses of rat vas deferens and spleen to exogenously-applied α_1-adrenoceptor agonists were competitively inhibited by prazosin and 5-methyl-urapidil; in addition, the pA_2 values were calculated and the relative potencies with reference to prazosin were obtained. The relative potency of 5-methyl-urapidil in human prostate (0.105) was close to that in rat vas deferens (0.257), which contains primarily putative α_{1A}-adrenoceptors. However, it was much more than that in rat spleen (0.011), which contains primarily putative α_{1B}-adrenoceptors.

4 Nifedipine (10^{-8} to 10^{-6} M) inhibited concentration-dependently the contractile responses to electrical field stimulation in human prostate; in addition, the inhibition percentages were similar to those to exogenously-applied noradrenaline in rat vas deferens. In contrast, CEC (10 μM), which almost flattened the concentration-response curve of the rat spleen to phenylephrine, only partially inhibited (by 33.1%) the nerve-mediated contraction of human prostate.

5 The involvement of prejunctional α_2-adrenoceptors situated on the sympathetic nerve terminals of human prostate was also examined. Clonidine (3×10^{-9} to 3×10^{-7} M) blocked concentration-dependently the contractile response to electrical field stimulation of human prostate and this inhibitory effect was reversed by yohimbine (10^{-7} M). Additionally, the inhibitory effect of CEC (3×10^{-6} to 3×10^{-4} M) to the nerve-mediated contraction was also partially reversed by yohimbine (10^{-7} M).

6 It is suggested that the putative α_{1A}-adrenoceptors in human prostate may be functionally confined to the synaptic region whereas only minor populations of the putative α_{1B}- and/or α_{1C}-adrenoceptors exist in this region.

Keywords: Human prostate; electrical field stimulation; α_1-adrenoceptor subtypes; 5-methyl-urapidil; chloroethylclonidine

Introduction

A substantial amount of pharmacological research (Hedlund *et al.*, 1985; Kitada & Kumazawa, 1987; Yamada *et al.*, 1987) and clinical trials (Caine *et al.*, 1978; Shapiro *et al.*, 1981; Kirby *et al.*, 1987; Jardin *et al.*, 1991) on benign prostatic hypertrophy (BPH) has recently been undertaken. In these studies, it is well established that α-adrenoceptors are present in smooth muscle in BPH; in addition, α-adrenergic stimulation is an important factor in the development of urinary obstruction in BPH. Although both α_1- and α_2-adrenoceptors are identified within the human prostate, the contractile properties of the human prostate adenoma are mediated primarily by α_1-adrenoceptors (Hedlund *et al.*, 1985; Hieble *et al.*, 1985; Kitada & Kumazawa, 1987; James *et al.*, 1989).

More recently, at least three α_1-adrenoceptor subtypes have been demonstrated to exist by gene coding, i.e., $\alpha_{1A/D}$, α_{1B} and α_{1C} (Cotecchia *et al.*, 1988; Lomasney *et al.*, 1991; Schwinn *et al.*, 1991; Garcia-Sainz *et al.*, 1992; Schwinn & Lomasney, 1992). In human prostate, Chapple *et al.* (1991) suggested that the α_{1B} subtype forms the majority of the α_1-adrenoceptors, whereas Lepor *et al.* (1993) and Testa *et al.* (1993) using binding tests suggested that the dominant α_1-adrenoceptor subtype in the human prostate is the α_{1A} subtype. Price *et al.* (1993) investigated the mRNA expression of α_1-adrenoceptors in the human prostate with specific probes for the $\alpha_{1A/D}$, α_{1B} and α_{1C} subtypes, indicating that the predominant subtype is α_{1C}. In addition to exogenous adrenergic stimulation, endogenous adrenergic stimulation plays an important role in human prostate since the tone of prostatic smooth muscle regulated by the autonomic nervous system is thought to be the 'dynamic' component of bladder outlet obstruction by BPH (Caine, 1986). Furthermore, a rather dense network of adrenergic nerve fibres has been found within the smooth muscle layer of the prostatic glandular stroma (Vaalasti & Hervonen, 1980).

This study seeks to characterize the α_1-adrenoceptor subtypes involved in contraction after endogenous adrenergic stimulation in the smooth muscle of human prostate. We have employed 5-methyl-urapidil, selective for α_{1A}-adrenoceptors (Gross *et al.*, 1988; Hanft & Gross, 1989); chloroethylclonidine (CEC), which alkylates α_{1B}-adrenoceptors (Han *et al.*, 1978; Minneman, 1988); prazosin, which is a non-selective α_1-adrenoceptor antagonist (Hanft & Gross, 1989; Aboud *et al.*, 1993) and nifedipine, which inhibits $[Ca^{2+}]_0$ influx (Rampe *et al.*, 1985), to distinguish between the various α_1-adrenoceptor subtypes.

Methods

Human hyperplastic prostates were obtained at operation from 28 males, aged 53-78 years, by open prostatectomy or transurethral resection of the prostate. All these patients had

[1]Author for correspondence at: Pharmacological Institute, College of Medicine, National Taiwan University, No. 1, Jen-Ai Road, 1st Section, Taipei, 10018, Taiwan.

histories of prostatism, and were diagnosed to have BPH by a combination of rectal digital examinations, transrectal sonography of prostate and urodynamic studies (including uroflowmetry, urethral pressure profile and cystometry). The specimens were used for *in vitro* isometric tension experiments.

In vitro *isometric tension experiments*

Immediately after removal, the prostatic tissue was placed in Krebs solution. The specimens were cut into strips (1 × 3 × 15 mm), and mounted vertically between two parallel platinum ring electrodes in organ baths containing 5 ml of Krebs solution which was continuously bubbled with 95% O_2 plus 5% CO_2 at 37°C. Tissues were equilibrated for 60 min with three changes of solution and maintained under a resting tension of 1 g before specific experimental protocols were initiated. Intramural nerve stimulation was performed by means of an electronic stimulator (Grass model S88) delivering square pulses of 0.2 ms duration at supramaximum voltage (80 V over the electrodes) and 20 Hz for 5 s. Contractions were recorded isometrically via a force-displacement transducer (Grass, Model 7DAG) connected to a Grass polygraph. The almost complete inhibition of the response by tetrodotoxin (0.1 μM) confirmed that the contractions induced by transmural stimulation were nerve-mediated.

Rat vas deferens contraction

Whole rat vas deferens were mounted and equilibrated under the same conditions as human prostate for 60 min under a resting tension of 0.5 g. After the equilibration period, rat vas deferens were contracted twice to 10 μM noradrenaline and then washed and equilibrated for a further 30 min. Non-cumulative concentration-response curves for noradrenaline-induced contractions were determined in the absence or presence of the indicated antagonists and tissues were allowed to equilibrate with each antagonist for 30 min.

Rat spleen contraction

Rat spleens were hemisected and equilibrated under the same conditions as human prostate at a resting tension of 1 g and a concentration-response curve to phenylephrine was obtained in a cumulative manner in the absence or presence of the indicated antagonists.

Drugs and solutions

The composition of the Krebs solution (pH 7.4) used was (mM): NaCl 118.0, KCl 4.0, $MgSO_4$ 1.2, $CaCl_2$ 1.9, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.7. Additionally, desmethylimipramine (10 nM), corticosterone (40 μM) and propranolol (1 μM), known to block neuronal and extraneuronal uptake of noradrenaline and β-adrenoceptors, respectively, were present.

The following drugs were used: noradrenaline HCl, prazosin HCl, yohimbine HCl, clonidine HCl, nifedipine, propranolol HCl, desmethylimipramine HCl and corticosterone (all purchased from Sigma Chemical Co., St. Louis, U.S.A.); chloroethylclonidine dihydrochloride and 5-methyl-urapidil (Research Biochemical Inc. Natick, MA, U.S.A.); phenylephrine HCl (Denmarks Apotekerforening). Drugs were dissolved in distilled water, except for corticosterone (100% ethanol) and nifedipine (dimethylsulphoxide). The final concentration of dimethylsulphoxide in the bathing solution did not exceed 0.1% and had no effect on the muscle contraction.

Data analysis

Agonist elicited concentration-response curves in the presence of the indicated concentrations of each antagonist were related to the control concentration-response curves, of which the maximal response was taken as 100%. The concentration of antagonist necessary to give a half-maximal response in the presence of each concentration of antagonist was divided by the concentration giving a half-maximal response in the absence of antagonist to determine the dose ratio (DR). Data were plotted by the method of Arunlakshana & Schild (1959) as the −log (antagonist concentration) (M) vs the log (DR − 1) and when DR was 2, the −log (antagonist concentration) was taken as the pA_2 value from the Schild plot (Mackay, 1978).

The experimental results are expressed as means ± s.e. mean and accompanied by the number of observations. Statistical significance was assessed by Student's t test and P values less than 0.05 were considered significant.

Results

Effects of α_1-adrenoceptor antagonists on electrical field stimulation of human prostate

The contractile responses to transmural field stimulation were concentration-dependently blocked by pretreatment with prazosin (3×10^{-10} to 10^{-8} M), 5-methyl-urapidil (10^{-9} to 3×10^{-8} M) or CEC (3×10^{-6} to 10^{-4} M). At the maximal concentrations, both prazosin (10^{-8} M) and 5-methyl-urapidil (3×10^{-8} M) almost completely abolished these responses whilst CEC (10^{-4} M) inhibited these responses by only approximately 50% (Figure 1). Yohimbine (10^{-8} to 10^{-6} M) was also examined but had no effect on these responses (data not shown).

Effects of nifedipine on rat vas deferens and human prostate

Exogenously-applied noradrenaline stimulated concentration-dependently the contractions in rat vas deferens. Nifedipine (10^{-8} to 10^{-6} M) caused concentration-related reductions in these responses (Figure 2a, Table 1); in contrast, CEC (30 μM) was ineffective against these responses. In comparison with rat vas deferens, nifedipine (10^{-8} to 10^{-6} M) also inhibited concentration-dependently the contractions to electrical field stimulation in human prostate (Figure 2b). Furthermore, the inhibition percentages were similar to those for exogenously-applied noradrenaline in rat vas deferens (Table 1).

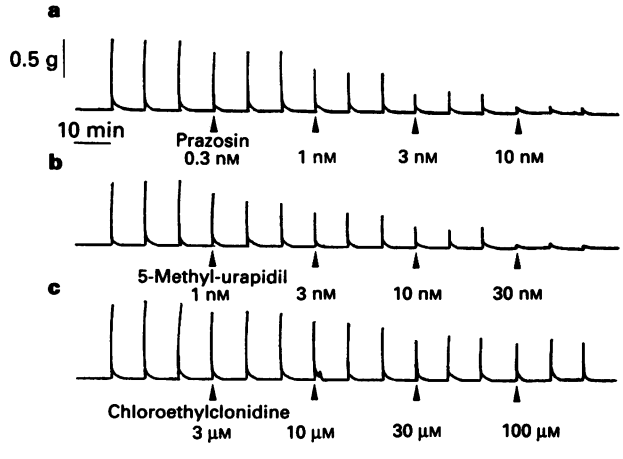

Figure 1 Representative traces of the inhibitory effect of prazosin (a), 5-methyl-urapidil (b) and chloroethylclonidine (c) on the contraction induced by transmural field stimulation in human hyperplastic prostates. Electrical stimulation was given at intervals of 10 min as described in Methods. The depicted traces were obtained from one of the five experiments.

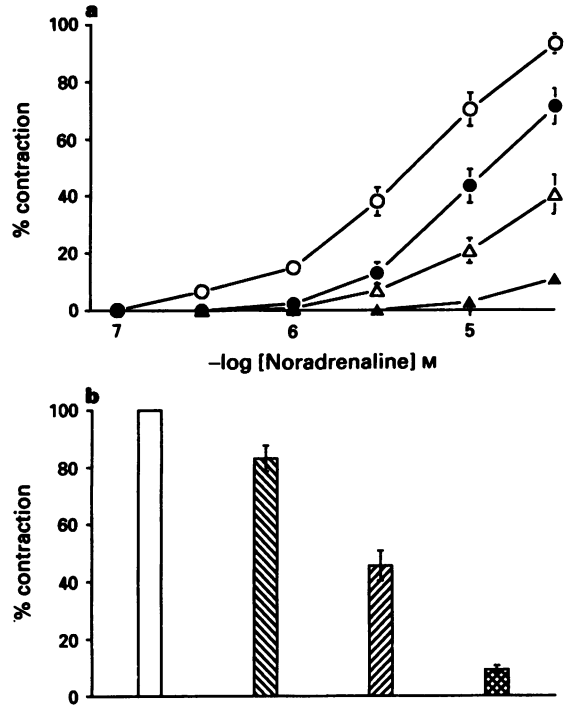

Figure 2 Effects of nifedipine on contractions to noradrenaline in rat vas deferens (a) and to transmural field stimulation in human hyperplastic prostates (b). Dimethylsulphoxide (0.05%, control) (○, □) or nifedipine, 10^{-8} M (●, ▨), 10^{-7} M (△, ▨) and 10^{-6} M (▲, ▨) was preincubated with tissues for 30 min. Values are the mean ± s.e.mean ($n = 5$).

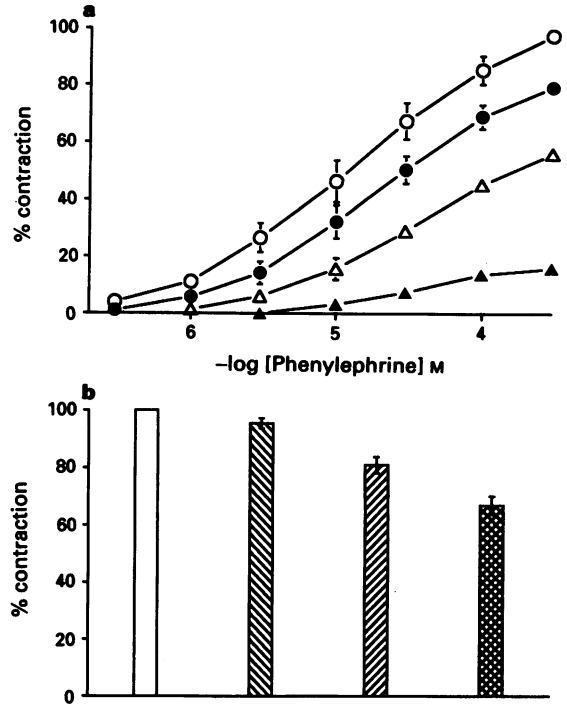

Figure 3 Effects of chloroethylclonidine on contractions to phenylephrine in rat spleens (a) and to transmural field stimulation in human hyperplastic prostates (b). Distilled water (control) (○, □) or chloroethylclonidine, 10^{-6} M (●, ▨), 3×10^{-6} M (△, ▨) and 10^{-5} M (▲, ▨) was preincubated with tissues for 30 min. Each values represents the mean ± s.e.mean ($n = 5$).

Table 1 The inhibitory effects of nifedipine and chloroethylclonidine on contractions to noradrenaline (30 μM) in rat vas deferens, to phenylephrine (300 μM) in rat spleens and to transmural field stimulation in human hyperplastic prostates

		Inhibition (%)		
Drugs (M)		*Vas deferens*	*Spleens*	*Prostate*
Nifedipine	10^{-8}	23.4 ± 6.7	-	16.8 ± 4.5
	10^{-7}	56.8 ± 7.3	-	54.4 ± 5.2
	10^{-6}	88.5 ± 1.6	-	90.8 ± 1.5
Chloroethylclonidine	10^{-6}	-	18.8 ± 0.7	4.6 ± 1.8
	3×10^{-6}	-	42.5 ± 1.8	19.0 ± 2.8
	10^{-5}	-	83.7 ± 1.6	33.1 ± 3.1

Values are expressed as means ± s.e.mean of 5 individual experiments.

Effects of CEC on rat spleen and human prostate

Phenylephrine stimulated concentration-dependently the contractions in rat spleen. CEC (1 to 10 μM) caused concentration-related reductions in these responses (Figure 3a, Table 1) and at a concentration of 10 μM, CEC almost flattened the concentration-response curve to phenylephrine; in contrast, nifedipine (1 μM) was ineffective on these responses. In comparison with rat spleen, CEC (1 to 10 μM) also concentration-dependently inhibited the contractions to electrical field stimulation in human prostate. At a concentration of 10 μM, however, CEC inhibited these responses by only 33.1% (Figure 3b, Table 1).

Effects of prazosin and 5-methyl-urapidil on rat vas deferens, rat spleen and human prostate

Prazosin and 5-methyl-urapidil produced parallel rightward shifts in the concentration-response curves of rat vas deferens to exogenously-applied noradrenaline and of rat spleens to phenylephrine without diminishing the maximal responses. The slopes of Schild plots were not significantly different from negative units (vas deferens: -1.10 ± 0.06 and -1.06 ± 0.04, respectively; spleen: -1.08 ± 0.07 and -1.04 ± 0.04, respectively); the pA_2 values were calculated (Table 2). Prazosin and 5-methyl-urapidil inhibited concentration-dependently the contractions to electrical field stimulation of human prostate and the half-maximal inhibition (IC_{50}) was determined (Table 2). In addition, the relative potencies of 5-methyl-urapidil with reference to prazosin in these tissues were obtained. Table 2 shows that the relative potency of 5-methyl-urapidil in human prostate (0.105) is close to that in rat vas deferens (0.257), but is about 10 fold that in rat spleen (0.011).

Effects of yohimbine on clonidine- and CEC-induced inhibitory responses in human prostate

Both clonidine (3×10^{-9} to 3×10^{-7} M) and CEC (3×10^{-6} to 10^{-4} M) inhibited concentration-dependently the contractions to electrical field stimulation in human prostate. The inhibitory effects were partially reversed by yohimbine (10^{-7} M); subsequently, the concentration-response curves were shifted to the right (Figure 4).

Discussion

This study has examined the effects of α_1-adrenoceptor antagonists on contractile responses to electrical field stimulation in human prostate. Prazosin, 5-methyl-urapidil and CEC all concentration-dependently blocked these responses. Both prazosin and 5-methyl-urapidil, at maximal concentrations, almost completely abolished these nerve-mediated responses whereas CEC only partially inhibited them. Also, further characterization of α_1-adrenoceptor subtypes mediat-

Table 2 Effects of α_1-adrenoceptor antagonists and nifedipine on tension responses stimulated by noradrenaline of rat vas deferens, by phenylephrine of rat spleens and by transmural field stimulation of human hyperplastic prostates

	Vas deferens (α_{1A})		*Spleen* (α_{1B})		*Prostate*	
Drugs	pA_2	*Relative potency*	pA_2	*Relative potency*	pIC_{50}	*Relative potency*
Prazosin	9.41 ± 0.41	1	9.11 ± 0.25	1	8.99 ± 0.13	1
5-MU	8.82 ± 0.43	0.257	7.15 ± 0.19	0.011	8.01 ± 0.17	0.105
CEC	No effect		Effective		Effective	
Nifedipine	Effective		No effect		Effective	

Values are expressed as means ± s.e.mean of 5 to 8 individual experiments. Abbreviations: 5-MU, 5-methyl-urapidil; CEC, chloroethylclonidine

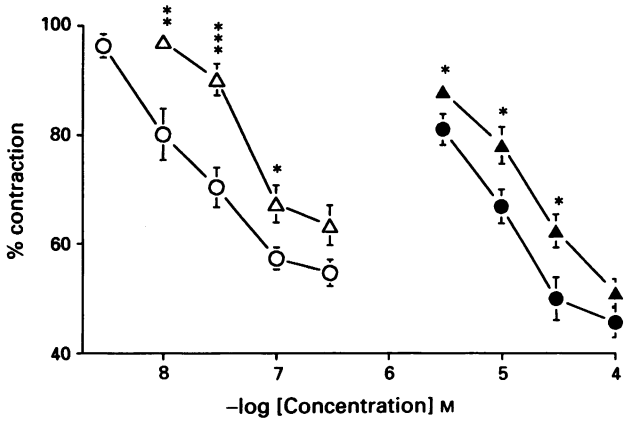

Figure 4 Effect of yohimbine on clonidine- and chloroethylclonidine-induced inhibition on contractions to transmural field stimulation in human hyperplastic prostates. Clonidine (○, △) or chloroethylclonidine (●, ▲) was preincubated in the absence (control) (○, ●) or presence (△, ▲) of yohimbine (10^{-7} M) with tissues for 30 min. Each point represents the mean ± s.e.mean ($n = 5$). $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ as compared with the respective control.

ing contractions elicited by neuronally-released noradrenaline was performed.

Based on affinities of a series of ligands for binding sites in rat vas deferens and spleen (Han *et al.*, 1987; Gross *et al.*, 1988), and on the ability of CEC to inactivate the α_{1B} but not the α_{1A}-adrenoceptor subtypes (Han *et al.*, 1987; Minneman *et al.*, 1988), it has been suggested that contractions to exogenously-applied noradrenaline are mediated predominantly by α_{1A}-adrenoceptors in rat vas deferens (Han *et al.*, 1987; Hanft & Gross, 1989), and by α_{1B}-adrenoceptors in rat spleen (Han *et al.*, 1987). In the present study, the contractions of rat vas deferens to noradrenaline have been used as a model for α_{1A}-adrenoceptors and contractions of rat spleen to phenylephrine as a model for α_{1B}-adrenoceptors. We found prazosin, a nonselective α_1-adrenoceptor antagonist (Hanft & Gross, 1989; Aboud *et al.*, 1993), and 5-methyl-urapidil, a selective α_{1A}-adrenoceptor antagonist (Gross *et al.*, 1988; Hanft & Gross, 1989), competitively inhibited the contractions to noradrenaline in rat vas deferens and those to phenylephrine in rat spleen. In rat spleen, phenylephrine but not noradrenaline was used because of the involvement of α_2- as well as α_1-adrenoceptors in the contractile responses (Kenakin & Novak, 1988). Table 2 shows that prazosin had similar pA_2 values in rat vas deferens and spleen; 5-methyl-urapidil exhibited greater potency (47 fold) in rat vas deferens than in rat spleen. However, it is difficult to obtain pA_2 values for prazosin and 5-methyl-urapidil to electrical field stimulation in human prostate. Therefore, the pIC_{50} values were calculated and the relative potencies with reference to prazosin were determined. The observed data revealed that the relative potency of 5-methyl-urapidil in human prostate was close to that in rat vas deferens whereas it was about 10 fold that in rat spleen.

A number of authors have reported that the smooth muscle contractions elicited by α_{1A}-adrenoceptor activation are dependent on Ca^{2+} influx through dihydropyridine-sensitive channels, while contractions elicited by the activations of other α_1-adrenoceptors are independent of extracellular Ca^{2+} influx (Han *et al.*, 1987; Minneman, 1988; Han & Minneman, 1990). In the present study, nifedipine (10^{-8} to 10^{-6} M) induced concentration-related reductions in noradrenaline-stimulated concentration-response curves in rat vas deferens but was ineffective against those in rat spleen. In addition, nifedipine also concentration-dependently inhibited the contractions to electrical field stimulation in human prostate; moreover, the respective inhibition percentage was consistent with that in rat vas deferens (Table 1). In rat spleen, the concentration-response curve to phenylephrine remained unaffected by nifedipine (1 μM) but was reduced in a concentration-related manner by CEC; moreover, it was almost flattened by 10 μM CEC. In contrast, CEC (10 μM) only partially inhibited (by 33.1%) the nerve-mediated contractions in human prostate. These data imply that the contractions elicited by neuronally-released noradrenaline in human prostate are mediated predominantly by the putative α_{1A}-adrenoceptors.

In a number of studies, 5-methyl-urapidil has been reported to have a high affinity for cloned α_{1C}-adrenoceptors (Goetz *et al.*, 1993; Kenny *et al.*, 1994; Michel & Insel, 1994). Furthermore, it has been suggested that α_{1C}-subtype forms the majority of the α_1-adrenoceptors in human prostate (Price *et al.*, 1993). However, α_{1C}-adrenoceptors are sensitive to alkylation by CEC (Garcia-Sainz *et al.*, 1992; Michel *et al.*, 1992). In the present study, the nerve-mediated contractions in human prostate was sensitive to blockade by 5-methyl-urapidil; but was only partially reduced by CEC. This response was unlikely to be mediated predominantly by α_{1C}-adrenoceptors. In addition, the concentrations of CEC used to block these responses were markedly higher than those required to inhibit the contractions to phenylephrine in rat spleen. Whether these inhibitions to neuronally-released noradrenaline by CEC in human prostate result from the alkylation of α_{1B} and/or α_{1C}-adrenoceptors remains doubtful since CEC also has an affinity for α_2-adrenoceptors (Michel *et al.*, 1993).

Clonidine, a selective prejunctional α_2-adrenoceptor agonist, was examined to assess the contribution of α_2-adrenoceptors in nerve-mediated contractions of human prostate. Clonidine (3×10^{-9} to 3×10^{-7} M) concentration-dependently inhibited the contractions to electrical field stimulation in human prostate; at the high concentration of 3×10^{-7} M, it produced maximal inhibition (by 42.7%) of these nerve-mediated responses. The clonidine-elicited inhibition was reversed by yohimbine (10^{-7} M) confirming the involvement of prejunctional α_2-adrenoceptors. In addition, yohimbine (10^{-7} M) also partially reversed the inhibitory effect of CEC on nerve-mediated contractions in human prostate. This implies that the observed CEC-sensitive effects in human pros-

tate are related at least partially to the activation of prejunctional α_2-adrenoceptors.

In summary, we have demonstrated in this paper that the major subtype mediating contractions to neuronally-released noradrenaline is the α_{1A}-adrenoceptor; in addition, activation of prejunctional α_2-adrenoceptors may partially inhibit these responses. Furthermore, the CEC-sensitive effects may be accounted for at least partially by the activation of prejunctional α_2-adrenoceptors.

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