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Short communication

Inhibition by tamsulosin of tension responses of human hyperplastic prostate to electrical field stimulation

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Abstract

Tamsulosin (10^{-10}–10^{-9} M) or prazosin (10^{-9}–10^{-8} M) concentration dependently blocked the tension responses to electrical field stimulation (0.3 ms duration, 80 V and 20 Hz) in human hyperplastic prostate with IC_{50} values of $(1.93 \pm 0.26) \times 10^{-10}$ M and $(2.11 \pm 0.21) \times 10^{-9}$ M, respectively. The relative potency of tamsulosin with reference to prazosin was 10.96. The pA_2 values for tamsulosin and prazosin against phenylephrine-induced contractions were 10.05 ± 0.16 and 9.25 ± 0.07, respectively. The relative potency of tamsulosin with reference to prazosin was 6.31. In the presence of prazosin to block α_1-adrenoceptor-mediated responses, nifedipine (10^{-5} M), but not tamsulosin (10^{-9} M), significantly blocked the tension responses in human hyperplastic prostate induced by increasing $[Ca^{2+}]_o$ concentrations (10^{-4} to 3×10^{-3} M) in a Ca^{2+}-free environment pre-depolarized with 60 mM K^+. Additionally, the effects of prazosin and tamsulosin on electrical field stimulation-evoked [^{3}H]noradrenaline release were studied on the S_3/S_2 ratios. It appeared that both drugs had little effect on this release reaction, with S_3/S_2 ratios of 0.96 ± 0.02 and 0.90 ± 0.02, respectively. These results indicate that tamsulosin is a potent antagonist against endogenous sympathetic stimulation in human hyperplastic prostate.

Keywords: Tamsulosin; Electrical field stimulation; α_1-Adrenoceptor stimulation; Prostate, human

1. Introduction

Benign prostatic hyperplasia refers to the progressive enlargement of the prostate and its increased dynamic α-adrenoceptor-mediated tone leading to urinary outflow obstruction seen uniquely in aging men. That the predominance of α_1-adrenoceptors in human prostate mediates its contraction is well recognized (Hedlund et al., 1985). Additionally, functional and binding studies have shown at least two subtypes of α_1-adrenoceptors in human prostate, i.e., α_{1A}- and α_{1B}-adrenoceptor subtypes (Lepor et al., 1993; Teng et al., 1994). With the model of electrical field stimulation, the contractile responses of human prostate were compared with those of rat vas deferens and rat spleen. It is suggested that the α_{1A}-adrenoceptor subtypes in human prostate are functionally confined to the synaptic region (Guh et al., 1995).

Tamsulosin is a selective and potent α_1-adrenoceptor antagonist which in vitro tension studies and binding assays have shown to function in prostatic tissues (Yamada et al., 1987; Chapple et al., 1994). The present study sought to evaluate the effect of tamsulosin on muscle contraction of human hyperplastic prostate in response to electrical field stimulation in order to define its potency against endogenous adrenergic stimulation of this tissue.

2. Materials and methods

2.1. Human prostatic tissues

Human hyperplastic prostates were obtained at operation from symptomatic benign prostatic hyperplasia patients (23 males), aged 53–77 years, by transurethral resection of prostate or open prostatectomy. All these patients were diagnosed to have benign prostatic hyperplasia by the combination of prostatism symptoms, digital rectal examination, transrectal ultrasonography of the prostate, and

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urodynamic studies (including uroflowmetry, cystometry, and urethral pressure profile). The specimens were used for in vitro isometric experiments.

2.2. In vitro isometric experiments

Immediately after removal, the specimens were cut into strips (3 × 15 mm), and mounted in a thermostatically controlled organ bath (37°C) containing Krebs solution (5 ml). The tissue bath solution was bubbled with a mixture of CO_2 (5%) and O_2 (95%). The tissues were equilibrated for 90 min with four changes of solution and maintained under an optimal tension of 1 g before specific experimental protocols were initiated. Contractions were recorded isometrically via a force-displacement transducer (Grass, model 7DAG) connected to a Grass polygraph. Cumulative concentration-response curves (1:3) for phenylephrine-induced contractions were determined in the absence or presence of the indicated antagonists. The tissues were allowed to equilibrate with each antagonist for 15 min before each cumulative concentration response was obtained. For electrical field stimulation, the tissues were mounted vertically into two parallel platinum ring electrodes in organ baths. Intramural nerve stimulation was performed by means of an electronic stimulator (Grass model S88) delivering square pulses of 0.3 ms duration at supramaximum voltage (80 V over the electrodes) and 20 Hz for 5 s. The almost complete inhibition of the response by tetrodotoxin (0.1 μM) confirmed that the contractions induced by transmural stimulation were nerve-mediated ($n = 8$).

The contractile effect of calcium was evaluated in a high-K^+ (60 mM) solution without Ca^{2+}. The high-K^+ solution was prepared by substituting an equimolar amount of KCl for NaCl. The tissues were incubated in KCl (60 mM)/Ca^{2+}-free medium in the presence of prazosin (10^{-7} M) to block α_1-adrenoceptor-mediated responses, and then incubated in the absence or presence of tamsulosin (10^{-9} M) or nifedipine (10^{-5} M) at 37°C for 15 min. Cumulative concentrations of Ca^{2+} (10^{-4} to 3×10^{-3}M) were then used to evoke contraction.

2.3. Release of [^{3}H]noradrenaline from prostatic strips

The tissues were loaded with 1-(7,8)-[^{3}H]noradrenaline (3 μCi/ml) for 60 min at 37°C in Krebs solution, aerated with a mixture of CO_2 (5%) and O_2 (95%). After incubation with [^{3}H]noradrenaline, the tissues were washed with Ca^{2+}-free Krebs solution containing 0.04 mM EDTA for 90 min (with changes of the bathing solution every 10 min); after 90 min of washout, the tissues were incubated in Krebs solution for a further 20 min. After the above experimental procedure, the tissues were incubated in Krebs solution, and the solution was changed every 3 min and collected. From the amount of tritium in the tissue and in the efflux sample, fractional rates of loss (FRL, per 3 min) of tritium were calculated, i.e., the amount of tritium which appeared in the solution during any given 3 min collection period was expressed as a fraction of the amount of tritium present in the tissue at the beginning of the respective collection period. At the end of the experiments, the tissues were blotted, homogenized in 0.3 ml of 37% perchloric acid and extracted overnight. After centrifugation of the extract (at 3000 × g for 10 min), the tritium content of the supernatant and of the collection samples was determined.

During the experiments, the tissues were stimulated electrically three times: at the beginning of the 3rd, 13th and 23th collections (6, 36 and 66 min, respectively). Stimulation parameters were 0.3 ms duration at 80 V and 20 Hz for 5 s. The second stimulation (S_2) was used as a control; the third stimulation (S_3) was performed in the absence or presence of the drug applied. The drug used was added to the solution 15 min before the third stimulation (S_3). Effects of drugs were expressed as the ratio S_3/S_2 of the overflow of tritium evoked by the two stimulation periods.

2.4. Drugs and solutions

The composition of the Krebs solution (pH 7.4) used was (mM): NaCl 118.4, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 1.9 and glucose 11.7. Additionally, desmethylimipramine (100 nM), corticosterone (40 μM) and propranolol (1 μM), known to block the neuronal and extraneuronal uptake of noradrenaline and β-adrenoceptors, respectively, were present.

The following drugs were used: prazosin HCl, nifedipine, propranolol, desmethylimipramine HCl and corticosterone (all purchased from Sigma Chemical Co., St. Louis, MO, USA); phenylephrine HCl (Denmarks Apotekerforening, Copenhagen); 1-(7,8)-[^{3}H]noradrenaline (specific activity: 12.0 Ci/mmol, Amersham) and tamsulosin (gift from Yamanouchi Pharmaceutical Co., Tokyo, Japan). Drugs were dissolved in dimethyl sulfoxide and the final concentration of dimethyl sulfoxide in the bathing solution did not exceed 0.1% and had no effect on muscle contraction ($n = 6$).

2.5. Data analysis

In each experiment, agonist-elicited concentration-response curves in the presence of the indicated concentrations of each antagonist were related to the control concentration-response curve, the maximum response of which was taken as 100%. The concentration of phenylephrine necessary to give a half-maximum response in the presence of each concentration of antagonist was divided by the concentration giving a half-maximum response in the absence of antagonist to determine the dose ratio. The data were plotted by the method of Arunlakshana and Schild (1959) as the −log (antagonist concentration M) vs. the

log (dose ratio − 1); when the dose ratio was 2, the −log (antagonist concentration) was taken as pA_2 value from the Schild plot. Additionally, the concentration of antagonist needed to produce half-maximal inhibition (IC_{50}) in the absence of antagonist was determined, and the −log (antagonist concentration) was taken as pIC_{50} value. The experimental results are expressed as means ± S.E.M. and are accompanied by the number of observations. Statistical significance was assessed by means of Student's t test and P values less than 0.05 were considered significant.

3. Results

3.1. Phenylephrine-induced responses

Prazosin and tamsulosin caused concentration-dependent parallel rightward shifts of the concentration-response curve of phenylephrine in human hyperplastic prostate tissue. Schild plots were constructed for the effects of prazosin and tamsulosin at various concentrations. The slopes of these regressions did not differ significantly from negative unity (for prazosin, -1.14 ± 0.06; for tamsulosin, -1.19 ± 0.02). The pA_2 values were calculated to be 9.25 ± 0.07 and 10.05 ± 0.16 for prazosin and tamsulosin, respectively; the relative potency of tamsulosin with reference to prazosin was 6.31 (Table 1).

3.2. Electrical field stimulation-induced responses

The contractile responses to electrical field stimulation in human hyperplastic prostate were concentration dependently blocked by pretreatment with tamsulosin (10^{-10}–10^{-9} M) or prazosin (10^{-9}–10^{-8} M) (Fig. 1). The pIC_{50} values of prazosin and tamsulosin against field stimulation were 8.69 ± 0.04 and 9.73 ± 0.05 ($n = 4$), respectively. The relative potency of tamsulosin with reference to prazosin was 10.96 (Table 1).

Table 1
Effects of prazosin and tamsulosin on tension responses stimulated by phenylephrine and by electrical field stimulation of human hyperplastic prostates

Drugs	Phenylephrine			Electrical field stimulation		
	pA_2	Relative potency	n	pIC_{50}	Relative potency	n
Prazosin	9.25 ± 0.07	1	5	8.69 ± 0.04	1	4
Tamsulosin	10.05 ± 0.16	6.31	5	9.73 ± 0.05	10.96	4

Values are expressed as means ± S.E.M. n = number of individual experiments.

3.3. Effects of tamsulosin and nifedipine on high K^+-induced Ca^{2+}-dependent contraction

In the presence of prazosin (10^{-7} M) to block α_1-adrenoceptor responses, the cumulative addition of Ca^{2+} (10^{-4} to 3×10^{-3} M) caused a stepwise increase of muscle tension in isolated human hyperplastic prostate, which was pre-depolarized with 60 mM K^+ in a Ca^{2+}-free medium. The maximum tension attained at 3×10^{-3} M Ca^{2+} was 0.54 ± 0.04 g and was taken as 100%. Tamsulosin (10^{-9} M) had little effect on this Ca^{2+}-induced muscle contraction under the above conditions. The EC_{50} value of calcium in the presence of tamsulosin was $(8.2 \pm 0.4) \times 10^{-4}$ M compared to a control value of $(7.4 \pm 0.3) \times 10^{-4}$ M which is not significantly different. However, nifedipine (10^{-5} M) almost completely abolished this Ca^{2+}-induced contraction in human hyperplastic prostate.

3.4. Effects of prazosin and tamsulosin on electrical field stimulation-evoked [^{3}H]noradrenaline release

Electrical field stimulation significantly evoked the release of tritium, which amounted to $5.3 \pm 1.0\%$, $4.8 \pm 0.9\%$ and $4.5 \pm 1.1\%$, respectively for the first (S_1), second (S_2)

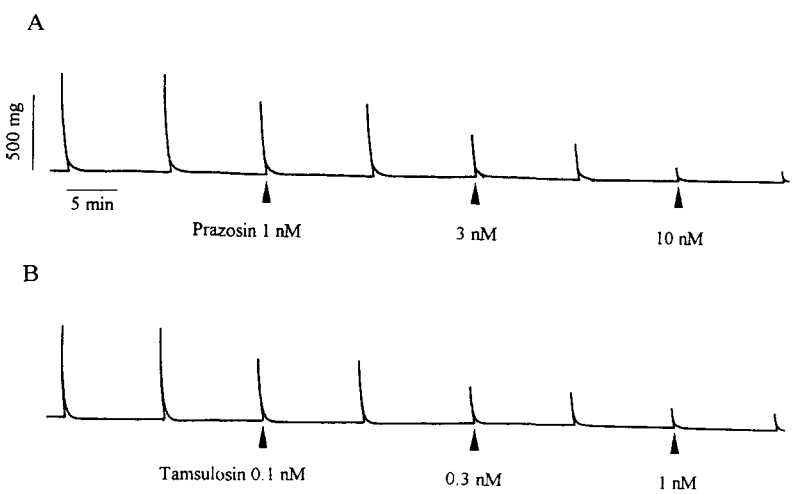

Fig. 1. Representative traces of the inhibitory effect of prazosin (A) and tamsulosin (B) on the contraction induced by electrical field stimulation in strips of human hyperplastic prostates. Electrical field stimulation was given at intervals of 10 min. The traces were obtained from one of the four experiments.

and third (S_3) stimulation. The S_3/S_2 ratio was calculated and was used to examine the effect of drugs on [^{3}H]noradrenaline release. The S_3/S_2 ratios were 0.94 ± 0.01, 0.96 ± 0.02 and 0.90 ± 0.02 ($n = 4$) for dimethyl sulfoxide (0.1%, control), prazosin (10 nM) and tamsulosin (1 nM), respectively, indicating that both prazosin and tamsulosin had little effect on the [^{3}H]noradrenaline release evoked by electrical field stimulation in human hyperplastic prostate.

4. Discussion

This study examined the effect of tamsulosin on contractile responses to phenylephrine and electrical field stimulation in human hyperplastic prostate. Tamsulosin has potent antagonistic effect on human prostate in an in vitro functional study with externally applied noradrenaline (Chapple et al., 1994). However, its effect on noradrenaline released on nerve stimulation, the real pathophysiology of benign prostatic hyperplasia, has not been documented before. In the present study, the effect of tamsulosin on contractions in response to phenylephrine and electrical field stimulation were examined in human hyperplastic prostate; the relative potencies of tamsulosin with reference to prazosin were calculated and compared, since prazosin exhibited no selectivity for α_1-adrenoceptor subtypes (Hanft and Gross, 1989). The results in this study showed that tamsulosin exhibited greater potency against field stimulation-induced contraction compared to that against phenylephrine. Guh et al. (1995) has suggested that the major subtype mediating contractions to neuronally released noradrenaline in human prostate is the α_{1A}-adrenoceptor subtype; additionally, the contraction in response to exogenously applied noradrenaline is mediated by both α_{1A}- and α_{1B}-adrenoceptor subtypes (Teng et al., 1994). Michel and Insel (1994) demonstrated that the affinity of tamsulosin for the α_{1A}-adrenoceptor subtype was ten times that for the α_{1B}-adrenoceptor subtype. It is suggested that the greater potency of tamsulosin against field stimulation-induced contractions in human hyperplastic prostate is due to the high affinity of tamsulosin for the α_{1A}-adrenoceptor subtype.

The α_{1A}-adrenoceptor subtype requires the influx of extracellular Ca^{2+} through dihydropyridine-sensitive channels to cause smooth muscle contraction (Minneman, 1988). In the present study, tamsulosin (10^{-9} M) had no effect on high K^+ (60 mM)-depolarized Ca^{2+}-induced contractions in human hyperplastic prostate; in contrast, nifedipine (10^{-5} M) almost completely abolished this Ca^{2+}-induced contraction. These results suggest that tamsulosin had no effect on the voltage-operated calcium channels in human hyperplastic prostate. Additionally, the effect on presynaptic noradrenaline release could also influence the field stimulation-mediated contractions in this tissue. In this study, the data showed that both prazosin (10 nM) and tamsulosin (1 nM) had little effect on the noradrenaline release evoked by electrical field stimulation.

In summary, we have demonstrated that tamsulosin is a potent antagonist against field stimulation-induced contractions in human hyperplastic prostate and this antagonistic effect is due mainly to its high affinity for the α_{1A}-adrenoceptor subtype.

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