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Evaluation of Tamsulosin and Alfuzosin Activity in the Rat Vas Deferens: Relevance to Ejaculation Delays

Simone Tambaro, Stefania Ruiu, Christian Dessi, Raymond Mongeau, Giorgio Marchese, and Luca Pani

Neuroscienze-PharmaNess S.c.a r.l., Cagliari, Italy

ABSTRACT

The effect of two α-adrenergic receptor antagonists widely employed in the therapy of benign prostatic hyperplasia, tamsulosin [(−)-(*R*)-5-[2-[[2-(0-ethoxyphenoxy) ethyl]amino]propyl]-2-methoxybenzenesulfonamide] and alfuzosin [(±)-*N*-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl) methylamino]propyl] tetrahydro-2-furancarboxamide], was investigated in the rat vas deferens. Because several clinical studies have shown that tamsulosin causes ejaculatory disorders, this study also evaluated the possible mechanisms implicated in these disorders by comparing the effect of tamsulosin with that of alfuzosin. Tamsulosin competitively antagonized the contractions induced by noradrenaline in vitro in the epididymal portion of the vas deferens with a potency pA_2 value of 9.2 ± 0.8. In the prostatic portion, tamsulosin increased the amplitude of intermittent spikes induced by exogenous noradrenaline (100–1000 μM). In both portions of the vas deferens, alfuzosin behaved as an α-adrenergic antagonist blocking the contractions induced by exogenous noradrenaline without altering spikes. The administration of tamsulosin (3 μg/kg i.v.) significantly reduced the contractions evoked by electrical pulses in the epididymal portion, whereas it increased those produced in the prostatic portion. Intravenous tamsulosin antagonized the contraction produced by exogenous noradrenaline, whereas alfuzosin administration (10 μg/kg i.v.) did not change the electrically induced contractions in both portions of the rat vas deferens and did not antagonize the contractions produced by exogenous noradrenaline. The fact that tamsulosin unusually enhances noradrenaline-induced intermittent spike contractions and nerve stimulation-induced twitches in the prostatic portions might be linked to its greater propensity to cause sexual dysfunctions.

Ejaculation disorders often occur following pharmacological treatment of benign prostatic hyperplasia (BPH) (Carbone and Hodges, 2003). BPH is a frequent disorder in elderly men characterized by the progressive enlargement of prostatic tissue resulting in an obstruction of the proximal urethra and disturbance of urinary flow. Symptomatic BPH consists of two components: a static component (modulated by androgens) related to the prostatic tissue mass and a dynamic component (modulated by α_1-adrenergic receptor) related to the prostatic smooth muscle tone. The ability of α_1-adrenoreceptor antagonists to improve the clinical symptoms of BPH was first demonstrated by Caine et al. (1976). It is believed that pharmacotherapy, which prevents the α-adrenoreceptor-mediated contraction of the prostatic smooth muscle, exerts its effect by reducing prostatic tissue size. The use of selective α_1-antagonists in BPH therapy is currently widely accepted (Lepor, 1993; Monda and Oesterling, 1993), but ejaculatory disorders might occur as a secondary effect of blocking α_1-adrenoceptors.

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Molecular and pharmacological studies have led to the classification of α_1-adrenoceptors into three subtypes: α_{1A}-, α_{1B}-, and α_{1D}-adrenoreceptors (Hieble et al., 1995; Michel et al., 1995). The α_{1A}-adrenoreceptor subtype is predominant in the human prostate and urethra (Price et al., 1993; Nasu et al., 1998) and is considered to play a predominant role in mediating the contractile response of the human prostate (Forray et al., 1994). Recently, tamsulosin has been introduced for the treatment of bladder outlet obstruction due to BPH and was shown to be the first clinically available antagonist which discriminates between α-adrenoceptor subtypes. In radioligand binding studies, this agent is selective for α_{1A}- and α_{1D}-adrenoceptors when compared with α_{1B}-adrenoceptors (Michel and Insel, 1994; Foglar et al., 1995). Another α_1-adrenergic antagonist used for BPH is alfuzosin, which is selective for the lower urinary tract in vivo but does not have selectivity for any of the three α_1-adrenoreceptor subtypes in vitro (Lefèvre-Borg et al., 1993; Leonardi et al., 1997).

Adrenergic blockers such as tamsulosin and alfuzosin have been reported to produce ejaculatory dysfunctions including anejaculation or retrograde ejaculation (Lepor, 1998; McK-

ABBREVIATIONS: BPH, benign prostatic hyperplasia; mN, millinewton; NA, noradrenaline; tamsulosin, (−)-(*R*)-5-[2-[[2-(0-ethoxyphenoxy) ethyl] amino]propyl]-2-methoxybenzenesulfonamide; alfuzosin, (±)-*N*-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl) methylamino]propyl] tetrahydro-2-furancarboxamide; ANOVA, analysis of variance.

eage and Plosker, 2002). However, a review of clinical literature shows that ejaculatory disorders are much more frequent for tamsulosin (4–18%) than for alfuzosin (<1%) (Giuliano et al., 2004). Normal ejaculation occurs through rhythmic contractions of adequate amplitude and frequency within secondary sex organs, including the vas deferens. To assess whether the different propensities of tamsulosin and alfuzosin to cause ejaculation problems is explained by differential pharmacological actions at the level of the vas deferens, we tested the effects of these drugs on the noradrenaline- and nerve stimulation-induced contractions of the epididymal and the prostatic portions of the rat vas deferens. These two models are complementary as exogenous noradrenaline produces contractions that are mediated predominantly by α_{1A}-adrenoceptors, whereas nerve stimulation evokes α_{1D}-adrenoceptor-mediated contractions in the rat vas deferens (Hanft and Gross, 1989; Aboud et al., 1993; Honner and Docherty, 1999).

Materials and Methods

Male Sprague-Dawley rats weighing 250 to 400 g (Charles River Italia, Calco, Italy) were housed at 22 ± 2°C on a 12-h light/dark cycle (lights on at 7:00 AM, off at 7:00 PM) with food and water available ad libitum. All experimental protocols were performed in strict accordance with the EC regulations for the care and use of experimental animals (86/609/EEC).

Rats were sacrificed by stunning and exsanguination. Vas deferens were excised and cleaned of adhering connective tissues. Each vas deferens was bisected transversely (terminal distance 1 cm from each end of the vas deferens), and prostatic and epididymal segments were set up in separate 10-ml organ baths containing Krebs bicarbonate solution with the following composition: 117.7 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.4 mM $NaHCO_3$, 10 mM glucose, and 2.5 mM $CaCl_2$. The solution was kept at 37°C and bubbled with 95% O_2 and 5% CO_2. The initial tension of the vas deferens was adjusted to 9.80 mN (1 g), and the preparation was allowed to equilibrate for 60 min to obtain a steady tension (approximately 3–5 mN) before the start of the experiment. During this period the bathing solution was replaced every 15 min.

In Vitro Studies. Tissues were exposed to cumulatively increasing concentrations of noradrenaline (NA) (0.01–1000 μM) to obtain concentration-response curves either in the absence (control) or in the presence of tamsulosin (1, 3, and 10 nM) or alfuzosin (3, 10, and 30 nM). Drugs were present in the bath 20 min before the first addition of NA. Increasing concentrations of NA were added to the organ bath after the response to the previous one had peaked. NA responses were calculated as a percentage of the maximal increase for each concentration-response curve, as well as in terms of the differences (in millinewton) between the induced contraction and the baseline tension. Moreover, it was possible to analyze the different components of the mechanical response to NA developed during the observation time. In general, the response evoked by NA consisted of a rapid phasic component followed by a second tonic component with spikes superimposed that assume the characteristic of rhythmic contractions. The phasic response was measured as the distance between the baseline and the highest point reached at the initial contraction. The tonic response was measured as the mean distance between the baseline and the lowest spike in the phase of stabilization 3 to 15 min after the administration of NA. The amplitude of the spikes was evaluated at this time. All three parameters were expressed in millinewtons.

In Vivo Studies. Rats were surgically implanted with a catheter into the right jugular vein under equithesin anesthesia (5 ml/kg i.p.). After one week, animals were treated with vehicle, tamsulosin (1–3 μg/kg i.v.), or alfuzosin (3–10 μg/kg i.v.) 10 min before sacrifice. Isometric contractions were evoked by stimulation with 1-ms pulses delivered at 0.05 Hz at supramaximal voltage (45–55 V) through a platinum electrode attached to the upper end of each bath and a stainless steel electrode attached to the lower end. Stimuli were generated by a Grass S88K stimulator, amplified (Multiplexing pulse booster 316S; Ugo Basile, Comerio, Italy), and then divided to yield separate outputs to four organ baths. Tetrodotoxin (1 μM) completely abolished the contractile response to electrical stimulation. Tissue preparations (epididymal and prostatic portions from rats treated with tamsulosin and alfuzosin) were exposed to cumulative concentrations of NA (0.01–1000 μM). Contractions were monitored by a computer using a date recording and analysis system (PowerLab 400) and linked via preamplifiers (Quadbridge) to an F10 transducer (2Biological Instruments, Besonzo, Va, Italy).

Materials. Tamsulosin and alfuzosin were synthesized at Sanofi-Synthelabo (Rueil-Malmaison, France). Compounds were dissolved in Krebs bicarbonate solution for in vitro studies and in saline for in vivo studies. NA bitartrate monohydrate was purchased from Sigma-Aldrich (St. Louis, MO) and was dissolved in 0.01 M HCl solution containing $NaHSO_3$ (0.1 mM) to prevent oxidation. Drugs were added to the baths in volumes of 10 μl.

Statistical Analysis. NA concentration-response curves were analyzed by nonlinear regression analysis using GraphPad (GraphPad Software Inc., San Diego, CA) and determination of pD_2 (-log EC_{50}) values were done with Prism software. The pA_2 values for competitive antagonists were calculated by Schild regression analysis (Arunlakshana and Schild, 1959). Data were plotted as log antagonist concentrations (molar) versus log (concentration ratios, −1). All values are shown as mean ± standard error of mean (mean ± S.E.M.) of n experiments. Statistical analyses were carried out using one-way or two-way ANOVA. When a significant ($P < 0.05$) effect was demonstrated, the Newman-Keuls post hoc test was applied to compare the effect of different drugs.

Results

Contraction Induced by Exogenous NA in Vitro. In both the epididymal and the prostatic portions of the rat vas deferens, NA produced a concentration-dependent contraction which in general consisted of an initial phasic component followed by a second slower tonic component with spikes superimposed (Fig. 1, A and B). NA produced contractions with pD_2 values of 5.00 ± 0.10 in the epididymal portion and 4.50 ± 0.06 in the prostatic portion (Fig. 2). The maximum contractions were 14.08 ± 0.83 and 10.15 ± 1.30 mN in the epididymal and prostatic portion, respectively (Fig. 3).

As shown in Fig. 2, A and C, tamsulosin produced a concentration-dependent rightward and a parallel shift of the concentration-response curve. This shows that it behaves as a competitive antagonist with pA_2 values of 9.20 ± 0.80 and 9.09 ± 0.90 in the epididymal and prostatic portion, respectively. Furthermore, the slope of the Schild plot was not significantly different from unity in either case (0.82 ± 0.14 and 0.98 ± 0.11, $P > 0.05$). On the other hand, as shown in Fig. 2, B and D, alfuzosin produced a concentration-dependent rightward parallel shift of the concentration response curve showing a competitive antagonist behavior with pA_2 values of 8.48 ± 0.60 and 8.00 ± 0.90 in the epididymal and prostatic portion, respectively. Again, the slope of the Schild plot was not significantly different from unity (1.12 ± 0.04 and 0.96 ± 0.05, $P > 0.05$). However, when the effects of tamsulosin and alfuzosin on NA-induced contractions were plotted in terms of differences between the contraction-induced and the baseline tension (millinewton; Fig. 3), instead of the percentage of the maximal response, an additional

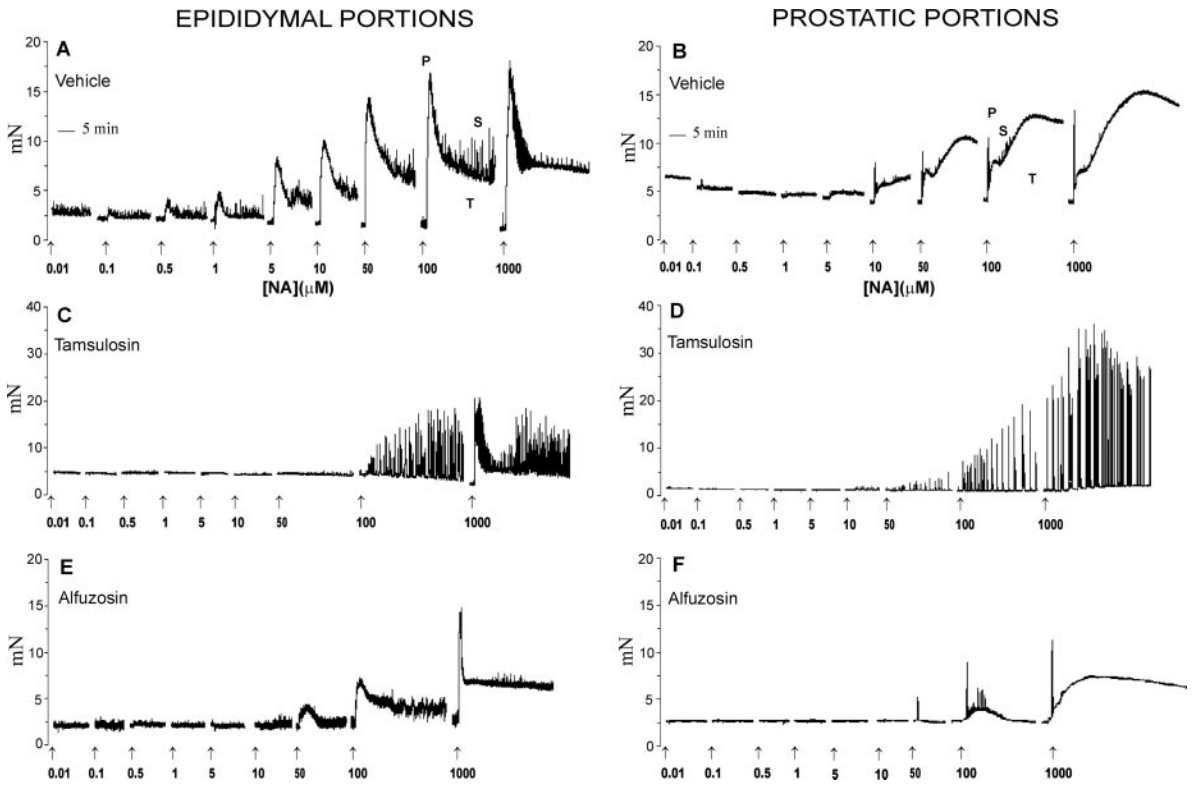

Fig. 1. Representative tracing showing features of the mechanical contractile response to various concentrations of NA (0.01–1000 μM) alone (A and B), in the presence of tamsulosin 10 nM (C and D), or alfuzosin 30 nM (E and F) in the epididymal portion and in the prostatic portion of the rat vas deferens. Panels A and B illustrate the phasic (P), tonic (T), and spike (S) components produced by 100 μM NA.

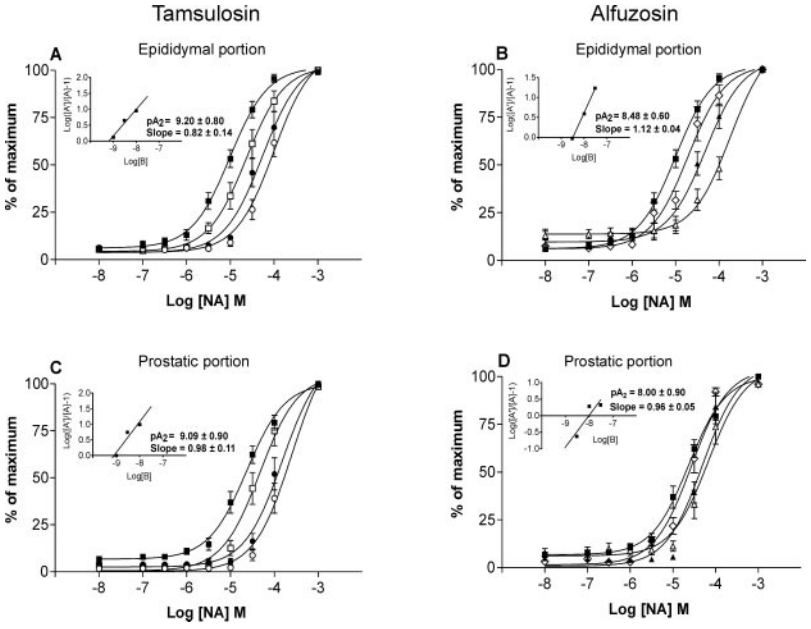

Fig. 2. Concentration-response curves of NA in the rat vas deferens in the absence (■) (control) and in the presence of tamsulosin 1 (□), 3 (●), and 10 nM (○) (A and C) or in the presence of alfuzosin 3 (◇), 10 (▲), and 30 nM (△) (B and D). Values are expressed as percentage of the maximum contractile response induced by NA for each experiment. Points represent the mean ± S.E.M. of at least 12 experiments.

effect of tamsulosin was observed (Fig. 3, A and C). In both portions, tamsulosin (1, 3, and 10 nM) increased the tension developed by exogenous NA. The phasic, tonic, and spike components of the mechanical response to noradrenaline (10, 100, and 1000 μM) in the presence of two compounds is shown in Table 1.

When NA-mediated contractions in the epididymal portion were analyzed in the presence of tamsulosin or alfuzosin, significant differences were observed among groups [phasic component, $F_{drug}(2,99) = 34.30$, $P < 0.01$, $F_{conc}(2,99) = 52.95$, $P < 0.01$, $F_{interact}(4,99) = 4.03$, $P < 0.05$; tonic component, $F_{drug}(2,99) = 16.63$, $P < 0.01$, $F_{conc}(2,99) = 27.61$, $P < 0.01$, $F_{interact}(4,99) = 0.611$, $P > 0.05$; spike amplitude, $F_{drug}(2,99) = 17.66$, $P < 0.01$, $F_{conc}(2,99) = 11.00$, $P < 0.01$, $F_{interact}(4,99) = 5.74$, $P < 0.01$].

In the epididymal portion (Fig. 1C, Table 1), tamsulosin (10 nM) reduced the phasic and the tonic contractions produced by 10 and 100 μM NA, but not those produced by 1000 μM

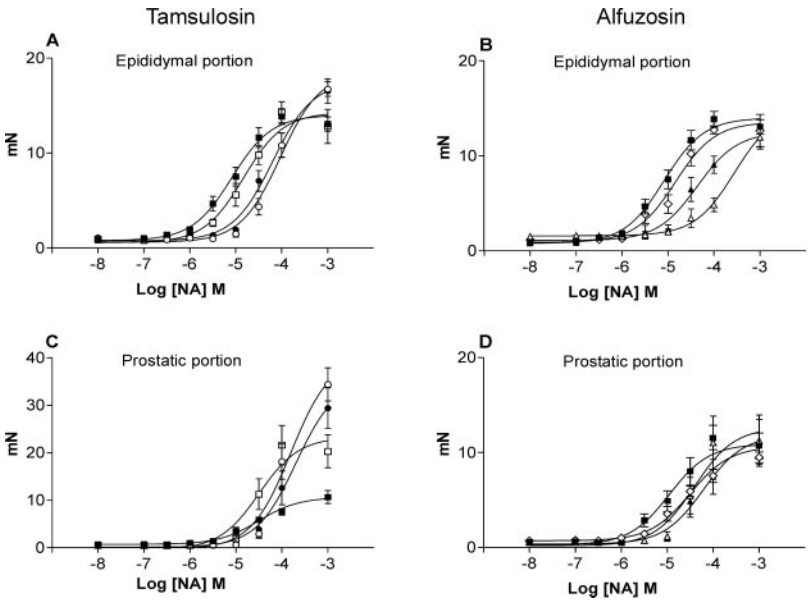

Fig. 3. Contractions produced by NA in the epididymal and in the prostatic portions of the rat vas deferens in the absence (■) (control) and in the presence of tamsulosin 1 (□), 3 (●), and 10 nM (○) (A and C) or in the presence of alfuzosin 3 (◇), 10 (▲), and 30 nM (△) (B and D). Values are expressed as differences between contraction induced and baseline tension (millinewton). Points represent the mean ± S.E.M. of at least 12 experiments.

NA, whereas it reduced only the amplitude of the spikes produced by low concentrations of NA (10 μM). At concentrations of 100 and 1000 μM NA, tamsulosin increased the amplitude of the intermittent spikes. Alfuzosin (30 nM) reduced all three components (phasic, tonic, and spikes) produced by concentrations of 10 to 100 μM NA and failed to alter the contractions induced by 1000 μM NA (Fig. 1E, Table 1).

When NA-mediated contractions in the prostatic portion were analyzed in the presence of tamsulosin or alfuzosin, significant differences were observed among groups [phasic component, $F_{drug}(2,99) = 50.70$, $P < 0.01$, $F_{conc}(2,99) = 6.46$, $P < 0.01$, $F_{interact}(4,99) = 2.21$, $P > 0.05$; tonic component, $F_{drug}(2,99) = 59.38$, $P < 0.01$, $F_{conc}(2,99) = 30.93$, $P < 0.01$, $F_{interact}(4,99) = 6.42$, $P < 0.01$; spike amplitude, $F_{drug}(2,99) = 11.99$, $P < 0.01$, $F_{conc}(2,99) = 6.42$, $P < 0.01$, $F_{interact}(4,99) = 3.42$, $P < 0.05$] (Table 1).

In the prostatic portion (Fig. 1D, Table 1), tamsulosin (10 nM) greatly reduced the phasic and the tonic contractions produced by NA (10, 100, and 1000 μM). Tamsulosin reduced the amplitude of the intermittent spikes of the contractions produced by 10 μM NA, but produced an unexpected 6- to 8-fold increase of the maximum amplitude of the intermittent spikes when the contractions were produced by 100 and 1000 μM NA. In contrast to tamsulosin, alfuzosin (30 nM) reduced all three contraction components produced by NA (10, 100, and 1000 μM; Fig. 1F, Table 1).

Effect of Treatment with Tamsulosin and Alfuzosin on Contractions Induced by Transmural Nerve Stimulation and Exogenous NA in Vivo. At an intravenous dose of 3 μg/kg (but not at 1 μg/kg), tamsulosin produced a significant inhibition of field stimulation-induced contractions in the epididymal portion of the rat vas deferens (Fig. 4A). On the other hand, in the prostatic portion, tamsulosin (3 μg/kg i.v.) produced a significant increase of contractions induced by field stimulation (Fig. 4C). In contrast to tamsulosin, alfuzosin (3–10 μg/kg i.v.) failed to affect the contractions induced by field stimulation in either the epididymal or in the prostatic portions (Fig. 4, B and D).

Tamsulosin (1–3 μg/kg i.v.) antagonized the response evoked by NA in the epididymal and prostatic portion of the rat vas deferens (Fig. 5, A and C). The efficacy of tamsulosin (3 μg/kg i.v.) in inhibiting each component (phasic, tonic, and spikes) of the NA-evoked response was analyzed as shown in Table 2. Tamsulosin action was less in vivo than in vitro, and in both portions of the rat vas deferens, it reduced only contraction components produced by low concentrations of NA (10 μM), but not those produced by high concentrations of NA (100–1000 μM). Finally, alfuzosin (3–10 μg/kg i.v.) did not antagonize the response evoked by NA in either the epididymal or the prostatic portions (Fig. 5, B and D; Table 2).

Discussion

Ejaculatory dysfunction associated with the therapeutic use of α_1-adrenoreceptor antagonists may result from an alteration of the contractility of the vas deferens smooth muscle. In the present study, we investigated the pharmacological effects of two α_1-adrenoreceptor antagonists, tamsulosin and alfuzosin, in the epididymal and in the prostatic portions of the rat vas deferens. Furthermore, we have tested the effects of these two drugs on the contractions induced by both exogenous NA (in vitro and in vivo) and nerve stimulation (in vivo).

The two α_1-adrenoceptor antagonists used in the present study behaved as competitive antagonists in the presence of NA. Interestingly, tamsulosin in the rat vas deferens had an antagonistic action toward NA (a pA_2 value) similar to that reported for the human prostate (Noble et al., 1997). Alfuzosin showed pA_2 values lower than those of tamsulosin, reflecting its lower affinity for adrenoceptors (Kenny et al., 1996; Michel et al., 1996; Richardson et al., 1997). It seems that similar adrenoceptor subtypes are involved in the contraction of both prostate and vas deferens smooth muscles. In fact, several studies have suggested that α_{1A}-adrenoceptors mediate the contractile response of exogenous NA in both portions of the rat vas deferens and prostate (Aboud et al., 1993; Burt et al., 1995, 1998; Lepor et al., 1995; Honner and Docherty, 1999).

We have also observed different effects of the two adren-

TABLE 1

In vitro effect of tamsulosin and alfuzosin on the phasic, tonic, and spikes components of the response to NA in the epididymal and in the prostatic portion of rat vas deferens

Tissue preparations were exposed to increasing concentrations of NA (10, 100, and 1000 μM) in the absence (vehicle) and in the presence of tamsulosin (10 nM) or alfuzosin (30 nM). The phasic response was measured as the distance between the baseline and the highest point reached at the initial contraction. The tonic response was measured as the mean distance between the baseline and the lowest spike in the phase of stabilization, 3 to 15 min after the administration of NA. The amplitude of the spikes was evaluated at this time. All three parameters were expressed in millinewtons. The values represent mean ± S.E.M of 12 experiments. Statistical analyses were carried out using a two-way ANOVA followed by a Newman-Keuls post hoc test.

Drugs Applied in Vitro	Phasic Component			Tonic Component			Spikes Amplitude		
	10 μM	100 μM	1000 μM	10 μM	100 μM	1000 μM	10 μM	100 μM	1000 μM
Epididymal portion									
Vehicle	7.7. ± 0.0.7	11.9 ± 1.1	13.0 ± 0.8	1.6 ± 0.3	3.6 ± 0.4	4.0 ± 0.4	1.9 ± 0.4	2.3 ± 0.3	2.5 ± 0.4
Tamsulosin	1.1 ± 0.3**	3.6 ± 0.5**	10.8 ± 0.7	0.0 ± 0.1**	1.1 ± 0.1**	3.6 ± 0.4	0.4 ± 0.1*	4.1 ± 0.5**	5.6 ± 0.9**
Alfuzosin	1.7 ± 0.3**	6.0 ± 1.0**	12.4 ± 1.3	0.1 ± 0.1**	1.5 ± 0.5**	3.8 ± 0.2	0.4 ± 0.1*	0.6 ± 0.1*##	2.2 ± 0.3##
Prostatic portion									
Vehicle	2.6 ± 0.5	4.5 ± 0.4	5.5 ± 0.5	1.3 ± 0.2	4.8 ± 0.6	6.1 ± 0.7	1.6 ± 0.2	2.9 ± 0.5	5.1 ± 1.0
Tamsulosin	0.4 ± 0.1**	0.5 ± 0.1**	0.5 ± 0.1**	0.1 ± 0.1**	0.1 ± 0.1**	0.7 ± 0.1**	0.6 ± 0.1*	16.6 ± 2.8**	28.5 ± 3.6**
Alfuzosin	0.7 ± 0.1**	1.7 ± 0.1**	2.4 ± 0.4**#	0.1 ± 0.1**	1.7 ± 0.4**##	3.7 ± 0.2**##	1.0 ± 0.1*	1.9 ± 0.5*##	2.6 ± 0.6*##

* $P < 0.05$ and ** $P < 0.001$ versus vehicle.
# $P < 0.05$ and ## $P < 0.01$ versus tamsulosin (10 nM).

ergic antagonists on each component of NA contractile response in the bisected rat vas deferens. Exogenous NA alone caused a contraction consisting of a rapid initial phasic response, followed by a slow tonic contraction with many rhythmic (intermittent) contraction spikes. Tamsulosin produced an unexpected increase in the intermittent spikes of the contractions in both portions of the vas deferens. The maximal increase of the intermittent spikes was recorded in the prostatic portion (about 550% versus control), whereas the phasic and tonic components were markedly reduced. The present results are interesting in view of similar data observed in the rat vas deferens pretreated with verapamil, an L-channel selective Ca^{2+} antagonist (Boselli et al., 1998). The three components of the contraction (tonic, phasic, and intermittent spikes) are all associated with a Ca^{2+} mobilization following activation of α-adrenoceptors (Han et al., 1987; Matsuki et al., 1996). The unexpected action of tamsulosin may be due to its interaction on the source of Ca^{2+} mobilized following α_1-adrenoreceptor activation. In fact, the interaction of exogenous NA with α-adrenoceptors results in the influx of extracellular Ca^{2+} through two distinct channels (Boselli et al., 1998). The first is verapamil- and nifedipine-sensitive, allows direct entry of Ca^{2+} into the muscle cells, is responsible for the phasic and tonic components of the contraction, and is predominant in the epididymal portion. The second channel, which is mainly responsible for the rhythmic spike contractions, is sensitive only to nifedipine and allows the Ca^{2+} entry that acts as a trigger and mobilizes intracellular Ca^{2+} prevailing in the prostatic portion. Boselli et al. (1998) suggested that this second channel is related to an ATP-sensitive K^+ channel. Furthermore, it is known that exogenous NA (upon α_1-adrenoreceptor stimulation) increases ATP outflow in a concentration-dependent manner in smooth muscle cells of the rat vas deferens (Vizi and Sperlágh, 1999). The unexpected increase of the rhythmic spikes in the presence of tamsulosin was observed only at high NA concentration when the latter has a preponderant agonistic effect on α_1-adrenoceptors. Therefore, it is possible that tamsulosin's potentiation effect on the intermittent spikes with its concomitant antagonistic action on the phasic and tonic contractions is not related to a simple action on adrenoceptors, but rather on the modulation of other processes such as those involving ATP or Ca^{2+} channels. For example, tamsulosin may have an antagonist action on the verapamil- and nifedipine-sensitive channels which are mainly responsible for the tonic and the phasic contractions. This additional putative action of tamsulosin, on the verapamil- and nifedipine-sensitive Ca^{2+} channels, may potentiate the activity of Ca^{2+}channels responsible for the spike contractions, predominant in the prostatic portion where we recorded the maximal increase of spikes. In any case, further studies will be necessary to unveil the receptors and second messenger systems involved in the whole spectrum of actions of tamsulosin on the contraction of the vas deferens. In contrast to tamsulosin, alfuzosin did not produce any significant increase in terms of maximum contraction of intermittent spikes. These data indicate that alfuzosin produces an adrenergic antagonist action with respect to NA contractile response in both portions of the rat vas deferens.

To further evaluate the pharmacological effect of these drugs in the rat vas deferens, comparative studies were carried out. On one hand, the assessment of contractions after

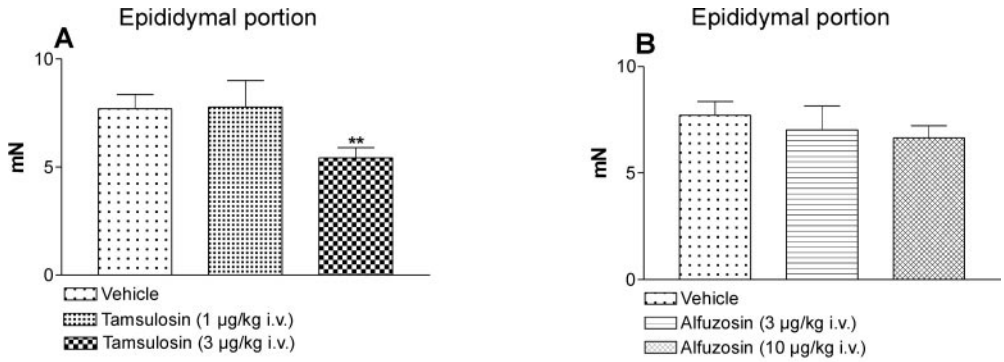

Fig. 4. Effects of intravenous vehicle or tamsulosin (1 and 3 μg/kg) on the contractile response to transmural nerve stimulation in the epididymal (A) and in the prostatic (C) portions, and effects of intravenous vehicle or alfuzosin (3 and 10 μg/kg) in the epididymal (B) and in the prostatic (D) portions. Responses are expressed as differences between the contraction induced and the baseline tension (millinewton). Each column represents the mean ± S.E.M. of 12 different experiments. Statistical differences were calculated using a one-way ANOVA followed by a Newman-Keuls test for multiple comparison (*, $P < 0.05$ and **, $P < 0.01$ versus vehicle).

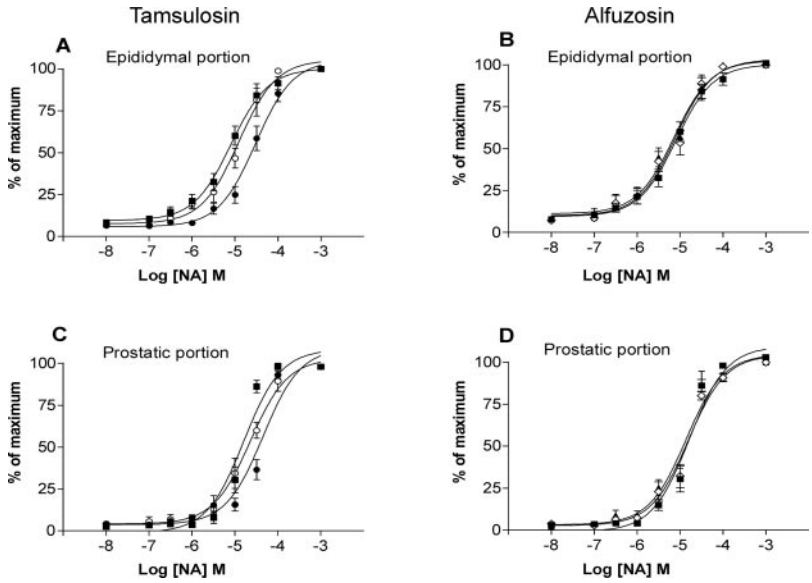

Fig. 5. The effect of intravenous vehicle (■), tamsulosin 1 μg/kg (○), and 3 μg/kg (●) on concentration-response curves evoked by NA in the epididymal (A) and in the prostatic (C) portions of the rat vas deferens, and the effect of intravenous vehicle (■), alfuzosin 3 μg/kg (◇), and 10 μg/kg (▲) in the epididymal (B) and in the prostatic (D) portions. Values are expressed as percentage of the maximum NA-mediated response for each experiment. Points represent the mean ± S.E.M. of at least 12 different experiments.

intravenous injection of tamsulosin and alfuzosin allowed determination of the efficacy of these drugs in the rat vas deferens after their normal diffusion within the tissue. On the other hand, electrical field stimulation of the sympathetically innervated vas deferens was performed, in addition to stimulation with exogenous NA, as this method allows observation of more physiological conditions of contraction. Tamsulosin induced a significant inhibition of the field stimulation-induced contractions (twitches) of the rat vas deferens in the epididymal portion, whereas in the prostatic portion it produced an increase of contractions. In contrast, alfuzosin had no effect in either portion.

The effects of intravenous tamsulosin in the prostatic and the epididymal portions have to be explained separately because they are likely to involve different mechanisms of action. The stimulation-evoked contractions with a single electrical pulse consists of a biphasic response; the earliest phase is linked to ATP and is known to predominate in the prostatic portion, whereas the second phase is mediated by α_1-adrenoceptors and predominates in the epididymal portion (Brown et al., 1983; Sneddon and Machaly, 1992). As far as the prostatic portion is concerned, the action of the drug seems linked to a regional variation of the endogenous neurotransmitters involved in the contractile response to nerve stimulation. The enhancement of the twitch response in the presence of tamsulosin reminds us of the similar action of this drug in vitro on intermittent spikes. This is interesting in view of Moritoki et al. (1987) who reported that Ca^{2+} antagonism augments the nonadrenergic response in the prostatic portion of the rat vas deferens. Here again, we believe that the profile of action of tamsulosin on contractions cannot sim-

TABLE 2

In vivo effect of i.v. tamsulosin and alfuzosin on the phasic, tonic, and spike components of the response to NA in the epididymal and in the prostatic portion of rat vas deferens

Tissue preparations were exposed to increasing concentrations of NA (10, 100, and 1000 μM). Animals were treated with vehicle or tamsulosin (3 mg/kg i.v.) or alfuzosin (10 mg/kg i.v.) 10 min before decapitation. The phasic response was measured as the distance between the baseline and the highest point reached at the initial contraction. The tonic response was measured as the mean distance between the baseline and the lowest spike in the phase of stabilization, 3 to 15 min after the administration of NA. The amplitude of the spikes was evaluated at this time. All three parameters were expressed in millinewtons. The values represent mean ± S.E.M of 12 experiments. The effect of the different drugs on the contractions evoked by NA (10 μM) were analyzed using a one-way ANOVA [one-way ANOVA: epidydimal portion phasic component, $F(2,33) = 5.56$, $P < 0.01$; tonic component, $F(2,33) = 6.04$, $P < 0.01$; spike amplitude, $F(2,33) = 4.42$, $P < 0.05$; prostatic portion phasic component, $F(2,33) = 6.00$, $P < 0.01$; tonic component, $F(2,33) = 7.10$, $P < 0.01$; spike amplitude, $F(2,33) = 9.89$, $P < 0.01$] followed by a Newman-Keuls post hoc test.

Drugs Injected Intravenously	Phasic Component			Tonic Component			Spikes Amplitude		
	10 μM	100 μM	1000 μM	10 μM	100 μM	1000 μM	10 μM	100 μM	1000 μM
Epididymal portion									
Vehicle	8.6 ± 1.7	11.4 ± 1.3	11.9 ± 2.0	1.3 ± 0.4	2.8 ± 0.4	2.8 ± 0.5	1.8 ± 0.4	2.4 ± 0.4	3.6 ± 0.6
Tamsulosin	3.7 ± 0.7**	12.2 ± 0.8	11.9 ± 1.3	0.2 ± 0.1**	2.5 ± 0.2	2.9 ± 0.4	0.6 ± 0.2*	2.6 ± 0.6	3.3 ± 0.7
Alfuzosin	8.4 ± 1.5#	12.1 ± 1.8	10.0 ± 0.9	0.9 ± 0.3#	2.4 ± 0.4	2.7 ± 0.3	1.4 ± 0.3	2.7 ± 0.4	3.2 ± 0.6
Prostatic portion									
Vehicle	2.0 ± 0.3	1.9 ± 0.2	2.7 ± 0.7	2.0 ± 0.5	2.8 ± 0.7	3.2 ± 0.6	2.8 ± 0.3	3.4 ± 0.6	3.9 ± 0.7
Tamsulosin	0.9 ± 0.1**	2.0 ± 0.3	2,7 ± 0.6	0.2 ± 0.1**	2.9 ± 0.3	3.1 ± 0.4	0.8 ± 0.1**	3.5 ± 0.5	3.5 ± 0.5
Alfuzosin	1,6 ± 0.3#	2.5 ± 0.4	2.6 ± 0.5	1.3 ± 0.3#	3.4 ± 0.5	3.6 ± 0.5	2.3 ± 0.5##	3.0 ± 0.7	4.0 ± 0.8

* $P < 0.05$ and ** $P < 0.001$ versus vehicle.
# $P < 0.05$ and ## $P < 0.01$ versus tamsulosin (10 nM).

ply be explained in terms of adrenoceptors activation and that Ca^{2+} channels or ATP release might be involved.

In the epididymal portion, the ability of tamsulosin to inhibit nerve-evoked contractions seems to involve α_{1D}-adrenoceptors, but not α_{1A}-adrenoceptors. Indeed, Honner and Docherty (1999) have shown that the α_{1D}-adrenoreceptor is the principal subtype implicated in the contractile response to nerve stimulation (but not in exogenous NA stimulation). Furthermore, in binding studies, tamsulosin showed higher affinity for α_{1D}-adrenoreceptors than did alfuzosin. In contrast, intravenous alfuzosin failed to affect the contractions induced by field stimulation and exogenous NA in both portions of the rat vas deferens despite the fact that the doses used were more than three times higher than those used for tamsulosin. Therefore, the lack of effect of in vivo alfuzosin might be explained in terms of lower receptor affinity. An alternative explanation for the failure of alfuzosin to antagonize the effect of nerve stimulation might have something to do with a less effective incorporation of this drug inside the vas deferens compared with that of tamsulosin following its intravenous administration. These explanations may account for the minor occurrence of ejaculatory disorders (retrograde ejaculation and/or anejaculation) reported in patients treated with alfuzosin compared with those treated with tamsulosin.

In conclusion, we propose that tamsulosin in the vas deferens exerts, in addition to its antagonist action on α_1-adrenoceptor-mediated contractions, a facilitation of the rhythmic spike contractions not mediated by adrenoceptors. This effect is best explained by an action of tamsulosin on verapamil- and nifedipine-sensitive Ca^{2+} channels. An abnormal increase of contractions in the prostatic portion of the vas deferens, such as that observed in the present study with tamsulosin, may cause ejaculatory dysfunctions by altering the progression and emission of sperm.

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Address correspondence to: Dr. Luca Pani, Institute of Neurogenetics and Neuropharmacology, Italian National Research Council (C.N.R.), Section of Neuropharmacology, President and CEO, Neuroscienze PharmaNess Scarl, Via Palabanda, 9. 09124, Calgliari, Italy. E-mail: luca.pani@ns.crs4.it