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Comparison of Relaxation Responses of Cavernous and Trigonal Smooth Muscles from Rabbits by Alpha$_1$-Adrenoceptor Antagonists; Prazosin, Terazosin, Doxazosin, and Tamsulosin

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α_{1A}-adrenergic receptor (AR) primarily mediates the contraction of the prostatic and cavernous smooth muscles. Among clinically available α_1-AR antagonists for the medical management of benign prostatic hyperplasia (BPH), tamsulosin has a modest selectivity for α_{1A}- and α_{1D}- over α_{1B}-ARs. To compare the effects of various α_1-AR antagonists on relaxation responses of cavernous and trigonal smooth muscles, isometric tension studies with relatively selective (tamsulosin) and non-selective (prazosin, doxazosin, and terazosin) α_{1A}-AR antagonists, were conducted in the cavernous and trigonal muscle strips of rabbits (n=10 each). Tamsulosin had the strongest inhibitory effect on contraction of trigonal smooth muscle among the various α_1-AR antagonists, and the inhibitory activities of prazosin, doxazosin, and terazosin were not statistically different. All α_1-AR antagonists caused concentration-dependent relaxation of the cavernous muscle strips. Tamsulosin was shown to have greater potency than prazosin (more than 100-fold), doxazosin (more than 1000-fold), and terazosin (more than 1000-fold), in relaxation of cavernous smooth muscle. In conclusion, tamsulosin might be the most effective drug among the four commonly used α_1-AR antagonists for the medical management of BPH. Tamsulosin might be a potential substitute for phentolamine in combination with vasoactive agents as an intracavernous injection therapy for patients with erectile dysfunction.

Key Words : *α_1-Adrenergic alpha-eceptor antagonist; Muscle smooth cavernous; Bladder, trigone; Relaxation*

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INTRODUCTION

The rationale for blocking of α_1-adrenergic receptors (ARs) in benign prostatic hyperplasia (BPH) is based on functional and pharmacologic studies which provide evidence that contractile response of human prostatic smooth muscle is mediated via α_1- rather than α_2-ARs (1). An autoradiography has localized these receptors to the muscular stroma rather than the glandular epithelium (1, 2). Recently, both pharmacologic and molecular cloning techniques in human prostatic tissues have revealed the existence of at least three subtypes (α_{1A}, α_{1B}, and α_{1D}) of α_1-ARs (1, 4-6). Moreover, RNase protection studies have shown that the α_{1A}-subtype represented more than 70% of the total mRNA in the human prostate (7). Among the selective α_1-AR antagonists (doxazosin, terazosin, prazosin, and tamsulosin), commonly used for the medical management of BPH, doxazosin, terazosin, and prazosin have identical activities at all subtypes of α_1-AR but tamsulosin has a modest selectivity for α_{1A}- over α_{1B}- but not α_{1D}-AR (1, 8, 9). It has been reported that tamsulosin was more than 10 times more potent than doxazosin, terazosin, and alfuzosin in blocking phenylephrine-induced prostatic contraction in dogs (9). Clinical studies have demonstrated that tamsulosin provides equivalent clinical efficacy compared to other selective α_1-AR antagonists, such as terazosin, doxazosin, and alfuzosin, with fewer side effects (10, 11, 12).

It is known that contractions of cavernous smooth muscle in response to norepinephrine seem to be mediated predominantly by the activation of α_1-ARs, as in the prostate, although both α_1- and α_2-ARs in corpus cavernosum tissue have been identified (13). Traish et al. (14) demonstrated that α_{1A}- and α_{1D}-AR were more abundant than α_{1B}-AR in the cavernous smooth muscles. Therefore, it would be expected that the relaxation response of cavernous smooth muscle with tamsulosin would be better than with a non-

selective α_{1A}-AR antagonist, such as prazosin, doxazosin, and terazosin. However, there are no studies comparing the effect of these drugs on the relaxation of the cavernous smooth muscles.

The aim of this study was to compare the effects of various α_1-AR antagonists, which have been commonly used in the treatment of BPH, on relaxation responses of cavernous and trigonal smooth muscles from rabbits.

MATERIALS AND METHODS

Preparation of cavernous and trigonal strips

New Zealand White rabbits (3-3.5 kg, n=10) were killed with a blow to the head and exsanguinated. The entire penis and bladder were surgically removed. The corpus cavernosum was carefully dissected freeing it from the urethra, tunica albuginea, and surrounding connective tissue. The bladder was opened and the trigonal smooth muscles were stripped of mucosa and serosa. The trigonal strips were taken in an oblique direction from the internal urethral orifice toward one of the ureteral orifices. The excised cavernous and trigonal smooth muscles were immediately placed in 100% oxygen-saturated HEPES-buffered physiological salt solution and studied within 1 hour. Muscle strips were trimmed to a size of $0.2 \times 0.2 \times 1.0$ cm and mounted. To record isometric tension, the strips were attached by a silk tie to a fixed support on one end and to a wire-connected force transducer (52-9545, Harvard, U.K.) and polygraph (50-8630, Harvard, U.K.) at the other end. The tissue was placed in a 25 mL organ chamber containing HEPES-buffered physiological salt solution which was bubbled with 100% O_2 and maintained at 37℃, pH 7.4. The resting tension for each strip was adjusted to an optimal tension at which contraction by norepinephrine and potassium chloride (trigonal strips only), respectively, was maximal and the developed tension that developed was recorded.

Chemicals and solutions

Norepinephrine (NE), HEPES, potassium chloride (KCl), calcium chloride ($CaCl_2$), and magnesium chloride ($MgCl_2$) were purchased from Sigma Chemical Co. (U.S.A.). Terazosin and prazosin were obtained from Il Yang Pharmaceutical Co. (Korea). Doxazosin and tamsulosin were purchased from Pfizer (U.S.A.) and Yamanouchi Pharmaceutical Co. (Japan), respectively.

The composition of the HEPES-buffered physiological salt solution was as follows: 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, 11 mM glucose, pH titrated to 7.4 with 1 N NaOH.

Electrical field stimulation of trigonal smooth muscles

The trigonal strips were placed under an initial tension of 0.5 g and left to equilibrate for 30 min. The basal tone of the strips was then raised using KCl (20 mM above basal, substituted for equivalent NaCl in the HEPES-buffered physiological salt solution). The strips were equilibrated for 1 hr with several changes of solution in the chamber. After equilibration, an electrical field stimulation was applied using an electronic stimulator (Harvard, U.K.). Square wave pulses of 2 ms duration and voltage of 15 V at differing frequencies (2, 4, 8, 16, and 32 Hz) were delivered for 10 s at intervals of 10 min. After electrical stimulation, strips were washed with fresh physiologic salt solution more than 2 times over 30 min and tension was allowed to relax to the baseline level. After incubation with prazosin, doxazosin, terazosin, and tamsulosin (all 10^{-4} M) for 20 min, electrical stimulation was repeated as described. Frequency-response curves (2-32 Hz) were then constructed. The drugs were added according to a randomized administration order.

Contractile tension was expressed in grams. Study results were expressed as a percent inhibition of the contraction induced by electrical field stimulation in the absence of α_1-AR antagonists.

Pharmacological responsiveness of cavernous smooth muscles

To determine effective concentration $(EC)_{50}$ values for NE, concentration-response curves to NE (10^{-9}-10^{-4}M) were obtained from cavernous smooth muscle. Relaxation responses induced by various α_1-AR antagonists (prazosin, doxazosin, terazosin, and tamsulosin) were then studied in the cavernous strips in which tone had been elicited with EC_{50} of NE. Concentration-response curves were determined by adding successive logarithmic increments of the agents from 10^{-9} to 10^{-4} M to the chamber.

After a concentration-response curve to each agent was constructed, the strip was washed with fresh physiologic salt solution more than 2 times over 1 hr and tension was allowed to relax to the baseline level. Contractile tension was expressed in grams. Relaxation study results with α_1-AR antagonists were expressed as percent relaxation of the contraction induced by NE. Individual EC_{50} values for each agent were determined.

Statistical analysis

Data were expressed as the mean ± standard error (SEM) with n representing the number of specimens. The concentration-response curves or frequency-response curves to various α_1-AR antagonists in the cavernous or trigonal smooth muscles were analyzed comparatively using the one way

analysis of variance (ANOVA) test. $P<0.05$ was considered to be statistically significant for all tests.

RESULTS

Effect of various α_1-AR antagonists on inhibition of contraction induced by electrical field stimulation in trigonal smooth muscle

All trigonal muscle strips which were not incubated with α_1-AR antagonists, were shown to contract in response to electrical field stimulation. The higher frequencies of stimulation produced substantially greater contractions in all the strips. A voltage of 50 V and a frequency of 8-32 Hz were found to give the largest magnitude of contraction (data not shown).

When the strips were stimulated at 50 V and 8 Hz, tamsulosin, prazosin, doxazosin, and terazosin inhibited the contractions by 81%, 19%, 10%, and 5%, respectively (Fig. 1). Tamsulosin had the strongest inhibitory effect ($p<0.01$, $n=10$) on contraction among the various α_1-AR antagonists tested, and the inhibitory activities of prazosin, doxazosin, and terazosin were not statistically different (Fig. 1).

Effect of various α_1-AR antagonists on relaxation of cavernous smooth muscle

All α_1-AR antagonists caused concentration-dependent relaxation of the cavernous muscle strips which pre-contracted with norepinephrine (Fig. 2). The concentration-response curve to tamsulosin showed a leftward shift when compared to prazosin while the curve for doxazosin and terazosin demonstrated a rightward shift (Fig. 2).

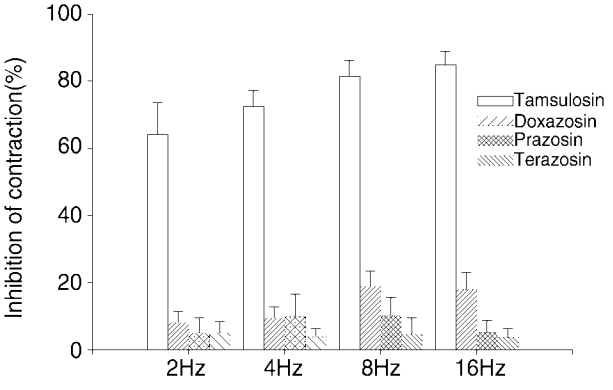

Fig. 1. Comparison of the inhibitory effects of tamsulosin, doxazosin, prazosin, and terazosin on contraction of rabbit trigonal smooth muscles (n=10). Tamsulosin is the strongest in inhibiting contraction among the 4 α_1-AR antagonists ($p<0.01$ by ANOVA test).

Fig. 3 shows mean EC_{50} (-log M) values for relaxation response to tamsulosin, prazosin, doxazosin, and terazosin. The EC_{50} for tamsulosin (8.89 ± 0.35) was significantly ($p<0.05$ by ANOVA, $n=10$) lower than for prazosin (6.58 ± 0.15). The EC_{50} for prazosin was significantly ($p<0.05$) lower than for doxazosin (5.70 ± 0.35) and terazosin (5.48

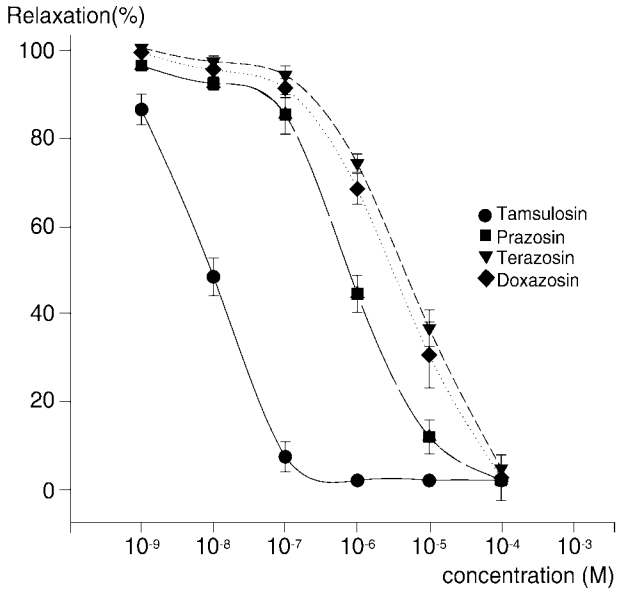

Fig. 2. Comparison of the relaxation responses of rabbit cavernous smooth muscles (n=10) induced by various α_1-AR antagonists (●; tamsulosin, ■; prazosin, ▼; terazosin, ◆; doxazosin). The concentration-response curve to tamsulosin shows leftward shift compared with that to prazosin, while the curve to doxazosin and terazosin demonstrats rightward shift ($p<0.01$ by ANOVA test).

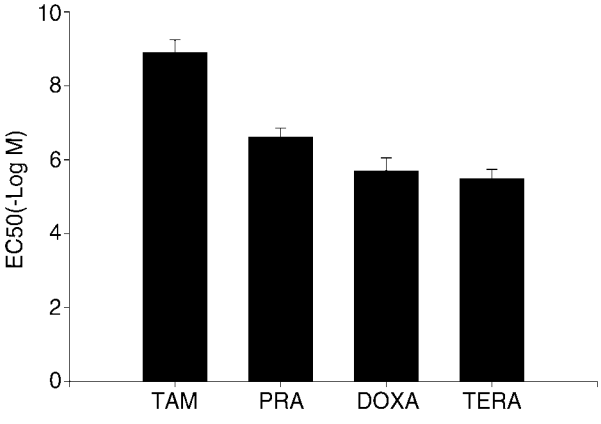

Fig. 3. Comparison of EC_{50} values (-log M) for relaxation responses to various α_1-AR antagonists in rabbit cavernous smooth muscles (n=10). TAM; tamsulosin, PRA; prazosin, DOXA; doxazosin, TERA; terazosin. The most potent drug is tamsulosin, followed in order by prazosin, doxazosin, and terazosin ($p<0.01$ by ANOVA test).

±0.26) while no difference was noted in EC_{50} between doxazosin and terazosin. Tamsulosin was shown to have greater potency than prazosin (more than 100-fold), doxazosin (more than 1000-fold), and terazosin (more than 1000-fold).

DISCUSSION

It has been repeatedly demonstrated that α_{1A}-AR is the predominant receptor in the human prostate, bladder neck, and urethra, and is the primary mediator of prostatic smooth muscle contraction (1, 5, 15-17). Furthermore, hyperplastic prostatic tissues contain more binding sites for α_1-ARs and are more responsive than normal tissues (18, 19). Extensive efforts have been conducted to identify α_1-AR antagonists with a selective affinity to α_{1A}-ARs, assuming that such drugs will have the potential to maintain their beneficial effect on BPH without blocking α_{1B}- and α_{1D}-ARs, possibly causing cardiovascular side effects (1, 5, 10, 15-17). Among long-acting α_1-AR antagonists, selective pharmacological properties have been demonstrated for tamsulosin (20). Reported estimates of the selectivity of tamsulosin for α_{1A}-ARs compared to α_{1B}-ARs range from 3.9 to 38 fold, and for α_{1A}- over α_{1D}-ARs range from 3 to 20 fold (1, 9, 20, 21). The fact that this functional selectivity may have clinical relevance is supported by the observation that tamsulosin provides clinical efficacy equivalent to other selective α_1-AR antagonists (terazosin, doxazosin, and alfuzosin) with significantly fewer adverse effects (10, 11). Lee and Lee (12) also reported that tamsulosin in fixed doses was as effective as terazosin in treating symptomatic BPH with a markedly better safety profile.

Our experiment comparing the effect of relatively selective (tamsulosin) and non-selective (prazosin, terazosin, and doxazosin) α_{1A}-AR antagonists on the tone of rabbit trigonal smooth muscle, demonstrated that tamsulosin was the most potent drug in blocking electrical stimulation-induced contraction. Therefore, tamsulosin might be the most effective drug among the various α_1-AR antagonists which have commonly been used to date for the medical treatment of symptomatic BPH.

The presence of adrenergic nerves has been demonstrated in cavernous and helicine arteries as well as in cavernous smooth muscles of humans and several animal species (13). In the flaccid state of the penis, these structures are kept contracted mainly by release of norepinephrine acting on postjunctional α-ARs (13, 22). The presence of both α_1- and α_2-ARs in corpus cavernosum has been reported (23, 24). However, a selective α_2-AR agonist (clonidine) was less potent and had less intrinsic activity than a selective α_1-AR agonist (phenylephrine) and norepinephrine, suggesting the functional predominance of α_1-AR in cavernous smooth muscle (25-27). In the cavernous artery, on the other hand, clonidine was a more contractile agent but had a lower efficacy than phenylephrine and norepinephrine (25). Thus, α_1-ARs predominate functionally in the human cavernous tissue while both α_1- and α_2-ARs seem to be important in the human cavernosal artery. However, Gupta et al. (24) reported that cavernous smooth muscles in the rabbit expressed post-synaptic α_2-ARs which play an important role in regulating smooth muscle tone. Expression of these receptors in human cavernous smooth muscle has also been demonstrated (28).

Because tone of the prostatic and cavernous smooth muscles is mediated primarily by α_1-ARs, improvement in erectile potency can be an additional benefit of α_1-AR antagonists in BPH patients with concomitant erectile dysfunction. It has been reported that prazosin caused prolonged erection (29). In contrast, Lepor et al. (30) reported that erectile dysfunction occurred in 7% of patients with symptomatic BPH who were treated with terazosin. To determine effect of α_1-AR antagonists on bladder outlet and corpus cavernosum, we performed isometric tension studies in both strips of trigonal and cavernous smooth muscles.

Since three subtypes of α_1-AR mRNA have been identified in human cavernous smooth muscles as well as in the prostate, with the α_{1A}- and α_{1D}-ARs predominating (14, 22, 23, 28), it is likely to expect that tamsulosin would be the most potent drug for inducing penile erection among the clinically-available selective α_1-AR antagonists. The present study demonstrates that all selective α_1-AR antagonists investigated caused concentration-related relaxation of rabbit cavernous smooth muscle, with tamsulosin the most potent among them.

We found that tamsulosin, which has a high affinity antagonist at functional α_1-ARs with a selectivity of $\alpha_{1D} \geq \alpha_{1A} > \alpha_{1B}$, was more than 100 times as potent as non-selective α_1-AR antagonists (prazosin, doxazosin, and terazosin) in the relaxation of cavernous smooth muscle. These findings suggest that contraction of rabbit cavernous smooth muscle might be mediated predominantly via α_{1A}- and α_{1D}-ARs rather than α_{1B}-ARs. Traish et al. (14) reported that α_{1A}- and α_{1D}- ARs were more abundant than α_{1B}-ARs in human cavernous smooth muscles.

It is well known that prazosin interacts with α_2- as well as α_1-ARs. It binds with high affinity to α_{2B}- and α_{2C}-ARs, and with low affinity to α_{2A}-ARs (6, 31). Gupta et al. (24) demonstrated that activation of post-synaptic α_2-ARs caused contraction of the rabbit cavernous smooth muscle. Better relaxation response (about 10 times more potent) of cavernous smooth muscle to prazosin compared to doxazosin or terazosin was noted in the present study, despite the lack of evidence for α_1- AR subtype selectivity. This suggests that contraction of rabbit cavernous smooth muscle could be mediated by activation of α_2- as well as α_1-

ARs.

In summary, among the various α_1- AR antagonists which have been commonly used for the medical management of BPH, tamsulosin (a relatively selective α_{1A}- AR antagonists) was the most potent drug in blocking electrical stimulation-induced contraction of rabbit trigonal smooth muscle and in relaxation of cavernous smooth muscle. Further in vitro and in vivo investigations are needed to study the potential use of tamsulosin, instead of phentolamine in combination with vasoactive agents, as an intracavernous injection therapy for patients with erectile dysfunction. Tamsulosin might be the most effective drug among the four commonly used α_1-AR antagonists for the management of BPH.

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