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Short communication

KMD-3213, a novel α_{1A}-adrenoceptor antagonist, potently inhibits the functional α_1-adrenoceptor in human prostate

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Abstract

KMD-3213, (−)-(*R*)-1-(3-hydroxypropyl)-5-[2-[[2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl]amino]propyl]indoline-7-carboxamide, is a novel and selective α_{1A}-adrenoceptor antagonist. The potency of this drug to antagonize functional α_1-adrenoceptor-mediated contraction in human prostatic smooth muscle was evaluated and compared with that of other α_1-adrenoceptor antagonists. KMD-3213 inhibited noradrenaline-induced contractions with an apparent pK_B value of 9.45 ± 0.039, indicating a potency similar to that of tamsulosin. The affinity of prazosin for prostatic α_1-adrenoceptors is given as potency for the α_{1L}-adrenoceptor with an estimated pA_2 value of 8.84 ± 0.044. The data obtained in this study suggest that KMD-3213, an α_{1A}-adrenoceptor-selective antagonist, has strong affinity for the α_{1L}-adrenoceptor in the human prostate. © 1997 Elsevier Science B.V.

Keywords: α_1-Adrenoceptor antagonist; KMD-3213; Prostate; (Human); Benign prostatic hypertrophy

1. Introduction

In benign prostatic hypertrophy patients, a significant increase in the number of α_1-adrenoceptors in the hypertrophied prostate has been reported (Yamada et al., 1987). The prostatic muscle tone mediated by the α_1-adrenoceptor is, therefore, thought to be one of the important components of bladder outlet obstruction. Although the therapeutic use of several α_1-adrenoceptor antagonists has been successful to relieve such obstruction (Kawabe et al., 1990), these antagonists cause hypotension or orthostatic hypotension in some cases.

Recently, native and cloned α_1-adrenoceptor subtypes (α_{1A}, α_{1B} and α_{1D}) have been characterized, fully based on operational (pharmacological), structural, and transductional criteria (reviewed by Hieble et al., 1995). Each of these subtypes exhibits a different expression pattern in various tissues. In particular, the α_{1A}-adrenoceptor is preferentially expressed in human prostate (Price et al., 1993), especially in hypertrophied prostate (Moriyama et al., 1996; Nasu et al., 1996). Moreover, the contractile response to noradrenaline is mediated by the α_{1A}-adrenoceptor (Marshall et al., 1995). These findings suggest a possibility for the development of an α_{1A}-adrenoceptor antagonist specific for the prostate and useful in the treatment of benign prostatic hypertrophy.

Shibata et al. (1995) demonstrated that KMD-3213, a novel α_1-adrenoceptor antagonist, has a significantly higher affinity for the human cloned α_{1A}-adrenoceptor, with a K_i value of 0.036 nM, than for α_{1B}- and α_{1D}-adrenoceptors (583- and 56-fold, respectively). Furthermore, KMD-3213 has a higher tissue selectivity for rabbit prostate than for rabbit and rat aortae (Yamagishi et al., 1996).

In the present study, we compared the effect of KMD-3213 on the functional α_1-adrenoceptor in human prostate with that of other α_1-adrenoceptor antagonists, i.e., prazosin and tamsulosin. In this way we hoped to determine the relative merit of KMD-3213 in the treatment of urinary outlet obstruction associated with benign prostatic hypertrophy.

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2. Materials and methods

Fresh human prostatic tissue was obtained from 18 males with an average age of 69.7 ± 1.72 who had had no treatment with α_1-adrenoceptor antagonist related to benign prostatic hypertrophy or hypertension for at least 1 week. The subjects were undergoing either open prostatectomy or transurethral resection of the prostate for benign prostatic hypertrophy according to ethical approval and with fully informed consent given. The burned surface of the prostatic tissue was carefully removed from the samples obtained from transurethral resection of the prostate. Strips of prostatic tissue, approximately 10–20 mm × 2–3 mm × 2 mm, were suspended under 1 g of resting tension in an organ bath at 37°C containing 10 ml of Krebs solution of the following composition (mM): NaCl, 111; KCl, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.5. Each bath was continuously bubbled with a gas mixture consisting of 95% O_2 and 5% CO_2. After 1 h for equilibration, cumulative concentrations of noradrenaline as an agonist were added to produce concentration-response curves twice. The second concentration-response curve was regarded as the control. Reproducibility of the noradrenaline-induced concentration-response between the second and third curves was confirmed (Fig. 1a). Antagonists in various concentrations were added to the bath 30 min before the third noradrenaline-induced concentration-response curve was made. In all cases, the Krebs solution also contained 1 μM propranolol to block β-adrenoceptors, 0.1 μM yohimbine to block α_2-adrenoceptors, and 0.1 μM desipramine and 10 μM corticosterone to inhibit intra- and extra-neuronal uptake, respectively. Responses of developed tension to noradrenaline were plotted as percentages of the maximum increase for each concentration-response curve. Then, individual EC_{50} (50% effective concentration) values were determined. The dissociation constant (K_B) of each antagonist was determined from the following equation: K_B = antagonist [M]/(CR − 1), where CR is the concentration ratio between EC_{50} values of control curves and those of curves for antagonist treatment. In the case of prazosin, assuming competitive antagonism, pA_2 values, which are expressed as the negative logarithm of K_B, were estimated from Schild plots made by plotting the log of (CR − 1) against the log of the molar concentration of antagonist (Arunlakshana and Schild, 1959).

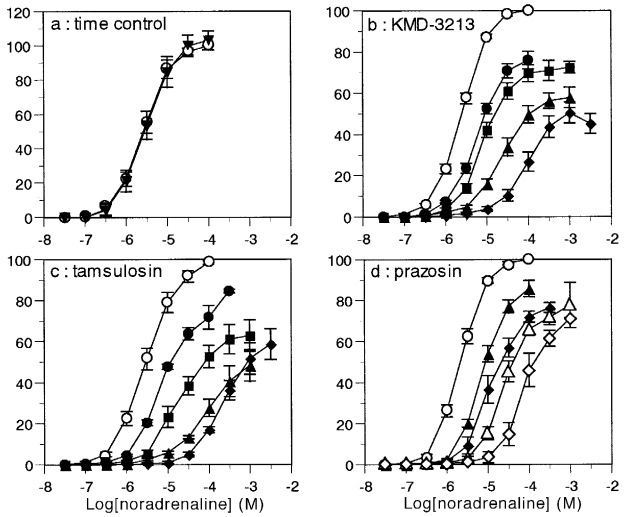

Fig. 1. Effect of KMD-3213, tamsulosin and prazosin on noradrenaline-induced contractions (%, values on ordinate in all panels) in human hypertrophied prostate. (a) Response to cumulative dosing with noradrenaline, with a second curve (○) as a control and a third curve (▼) as a time control. (b) Control (○) and the effect of KMD-3213 at 0.3 nM (●), 1 nM (■), 3 nM (▲) and 10 nM (♦). (c) Control (○) and the effect of tamsulosin at 0.3 nM (●), 1 nM (■), 3 nM (▲) and 10 nM (♦). (d) Control (○) and the effect of prazosin at 3 nM (▲), 10 nM (♦), 30 nM (▽) and 100 nM (◇).

2.1. Drugs

(−)-(*R*)-1-(3-hydroxypropyl)-5-[2-[[2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl]amino]propyl]indoline-7-carboxamide (KMD-3213), and tamsulosin HCl were synthesized in the Central Research Laboratories, Kissei Pharmaceutical (Matsumoto, Japan). Prazosin HCl, (−)-noradrenaline bitartrate, desipramine HCl and corticosterone were purchased from Sigma (St. Louis, MO, USA). Propranolol HCl was purchased from Wako (Osaka, Japan) and yohimbine HCl was purchased from Funakoshi (Tokyo, Japan). All other chemicals were purchased from Nacalai Tesque (Kyoto, Japan). KMD-3213, tamsulosin HCl and prazosin HCl were dissolved in dimethyl sulfoxide. KMD-3213 was diluted to appropriate concentrations with Hartmann's solution of the following composition (w/v%): NaCl, 0.60; KCl, 0.03; $CaCl_2$, 0.02; sodium lactate, 0.31. The other antagonists were prepared with physiological saline (0.9% NaCl solution) to the appropriate concentrations.

3. Results

Noradrenaline produced concentration-dependent contractions of human prostatic preparations with a pD_2 value of 5.55 ± 0.039. KMD-3213, tamsulosin and prazosin had no influence on the resting tension of the preparations (data not shown). Fig. 1 shows the concentration-response curves for noradrenaline in the absence or in the presence of various concentrations of the three antagonists. There was no time-dependent depression of noradrenaline-induced contraction in the human prostate (Fig. 1a). Each drug shifted the curve to the right in a concentration-dependent manner. However, all three drugs reduced the maximum response to noradrenaline (Fig. 1b–d). Apparent pK_B or pA_2 values for KMD-3213 and other antagonists are summarized in Table 1. KMD-3213 potently inhibited noradrenaline-induced contraction in human isolated

Table 1
Affinities of KMD-3213, tamsulosin and prazosin for α_1-adrenoceptor in human hypertrophied prostate

Drugs	pK_B (pA_2) ± S.E.	Slope	(95% CL)
KMD-3213	9.45 ± 0.039	–	–
Tamsulosin	9.68 ± 0.108	–	–
Prazosin	8.84 ± 0.044[a]	0.95	(0.854–1.044)

Data are presented as the means ± S.E.(pA_2 or pK_B) or the mean with 95% confidence limits (slope).
[a] pA_2 value obtained from Schild analysis. Slope was not different from unity.

prostate tissue with a pK_B value of 9.45 ± 0.039, $n = 23$, which was close to that of tamsulosin ($pK_B = 9.68 \pm 0.108$, $n = 19$). The rank order of potency of the antagonistic effect on human prostatic α_1-adrenoceptor(s) was tamsulosin ≥ KMD-3213 > prazosin.

4. Discussion

This is the first study to have evaluated the affinity of KMD-3213, a novel α_1-adrenoceptor antagonist (Shibata et al., 1995; Yamagishi et al., 1996), for the functional α_1-adrenoceptor(s) in human prostatic smooth muscle. In these experiments, KMD-3213 was nearly as potent as tamsulosin, inhibiting the noradrenaline-induced contractions of human prostatic tissue with an apparent pK_B value of 9.45 ± 0.039. Such a pK_B value is close to that obtained with rabbit prostatic smooth muscle, but not to the values for rat aortal smooth muscles (Yamagishi et al., 1996). KMD-3213 also reduced the maximum contractile response to noradrenaline (see Fig. 1b). This effect may have been due to the insurmountability of KMD-3213, and was not unique to KMD-3213. Tamsulosin and other antagonists with high affinity and slow dissociation for the receptors also showed this effect. The same phenomena with KMD-3213 and tamsulosin, however, were also observed in rabbit isolated prostate; those antagonists competitively antagonized the α_1-adrenoceptor in rat aorta (Yamagishi et al., 1996).

In previous studies using Chinese hamster ovary cells stably expressing the three cloned human α_1-adrenoceptors (α_{1A}, α_{1B} and α_{1D}) as well as native α_1-adrenoceptor(s) in human prostate tissue, Shibata et al. (1995) characterized the selective affinity of KMD-3213 toward these α_1-adrenoceptor subtypes. KMD-3213 potently inhibited [^{125}I]HEAT binding to the cloned human α_{1A}-adrenoceptor, with a K_i value of 0.036 nM (similar to that of tamsulosin), but had 583- and 56-fold lower potency for the α_{1B}- and α_{1D}-adrenoceptors, respectively. Furthermore, KMD-3213 could identify high- and low-affinity sites on native α_1-adrenoceptors in human prostate, the K_i values of which corresponded well to those for the cloned human α_{1A}- and α_{1B}-adrenoceptors, respectively. Recently, Moriyama et al. (1996), Nasu et al. (1996) and Price et al. (1993) demonstrated that the α_{1A}-adrenoceptor is the predominant subtype in the human prostate, and that noradrenaline-induced contraction of human prostate is mediated by this adrenoceptor subtype (Marshall et al., 1995). Those observations are in good agreement with our present results.

α_1-Adrenoceptors have also been separated into α_{1H}, α_{1L} and α_{1N} subtypes (Muramatsu et al., 1995). Prazosin has a higher affinity for the α_{1H}-adrenoceptor than for the α_{1L}- and α_{1N}-adrenoceptors. The α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors may be included in the α_{1H} group of the latter subclassification because of their high affinity for prazosin ($pK_i > 9$). Moreover, Muramatsu et al. (1994) demonstrated that the contractile response to noradrenaline in human prostatic tissue is mediated through the α_{1L} subtype, owing to its lower affinity for prazosin (pA_2 value 8.3). The estimated pA_2 value for prazosin obtained from the present study is also given as potency for the α_{1L}-adrenoceptor (pA_2 value 8.84). Indeed, RS-17053, a selective α_{1A}-adrenoceptor antagonist, had a lower pA_2 value (7.3) for human lower urinary tract smooth muscle in spite of having a high affinity for the native α_{1A}-adrenoceptor or the cloned α_{1A}-adrenoceptor (e.g., perfused rat kidney: pA_2 value of 9.8, human cloned: pK_i value of 9.2) (Ford et al., 1996). These results suggest that, even though the antagonist has a high affinity for the α_{1A}-adrenoceptor, it may not be able to inhibit noradrenaline-induced or neurogenic contraction in human prostate without having high affinity for the α_{1L}-adrenoceptor.

Furthermore, Yamagishi et al. (1996) in a functional study on KMD-3213 antagonism of *l*-phenylephrine-induced contractions in isolated rabbit prostate found a pK_B value of 10.0, suggesting that KMD-3213 has a higher affinity for rabbit prostate as well as for human prostate. Hiraoka et al. (1995) also indicated that the contractile response to noradrenaline is mediated through the α_{1L}-adrenoceptor in rabbit prostate. These findings suggest that KMD-3213 has a strong affinity for the α_{1L}-adrenoceptor as well as the α_{1A}-adrenoceptor subtype.

In conclusion, we confirmed that KMD-3213 inhibits noradrenaline-induced contractions in human isolated hypertrophied prostatic tissues as potently as tamsulosin. It has been reported that tamsulosin has higher affinity for the α_1-adrenoceptor in human prostate (Yamada et al., 1994) and has been used successfully for the treatment of benign prostatic hypertrophy (Kawabe et al., 1990). KMD-3213, therefore, may reduce the urethral resistance, and could be a useful drug for the treatment of bladder outlet obstruction associated with benign prostatic hypertrophy.

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