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ORIGINAL PAPER

Localizations and expressions of α-1A, α-1B and α-1D adrenoceptors in human ureter

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Abstract This study was designed to determine the expression and localization of the α-adrenoceptor (AR) subtypes in human ureteral tissue. The expression of the α-AR subtypes was examined by immunohistochemistry using subtype selective antibodies in proximal, mid and distal ureter. Ureter samples were obtained from pathological specimens of nephroureterectomy. A single pathologist scored the expression of receptor (grade 0, no staining; grade 1, 0–25% cells positive; grade 2, 26–50% cells positive; and grade 3, greater than 50%). We compared the mean grades of α-AR 1A, 1B and 1D and the percentage of receptor expression with grade 2 or more between receptor subtypes in each ureter level. The expression levels of all three subtypes are altered according to the level of ureter. In the proximal ureter, the mean grades of the α-1A, -1B and -1D receptors were 1.0 ± 0.5, 1.1 ± 0.6, and 2.1 ± 0.7. Corresponding grades in the mid and distal ureter were 1.1 ± 0.6, 0.8 ± 0.6 and 2.0 ± 0.8, and 2.0 ± 0.7, 1.8 ± 0.6 and 2.5 ± 0.5, respectively. When compared with percentage of high expression, in the proximal and mid ureter, α-1D high expression percentage was significantly higher than α-1A and -1B subtype (80, 10, 20, and 90, 20, 10%, respectively). In the distal ureter, α-1D expression was higher than α-1A and -1B subtype but there was no statistical significance (100, 80, 70%). The distal ureter had higher density of α-AR receptors than proximal and mid ureter. The expression of α-1A and α-1B AR in distal ureter was significantly higher than proximal and mid ureter. α-1D expression in distal ureter was also higher than proximal and mid ureter but was not statistically significant ($p = 0.28$, 0.19, respectively). Our results show that α-1A, -1B and -1D AR subtypes are localized in human ureter irrespective of location. The expression levels of subtypes are altered according to level of ureter and subtype.

Keywords Alpha adrenoceptor subtype · Ureter · Smooth muscle

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Introduction

Smooth muscle tone in the ureter is controlled in part by the sympathetic nervous system via adrenoceptors [1], and α- and β-adrenoceptor expressions have been demonstrated in the ureter [2]. Nerve-mediated contractions of the ureter are associated with α-adrenoceptor activation [3, 4]. Alpha 1 adrenoceptors (AR) have also been reported to mediate contractile responses in the ureter, and there is evidence that α1 receptors predominate in ureteral smooth muscle [5–7].

However, studies on α1 adrenoceptors and ureteral contraction have been limited, although the efficacies of α1 blockers on stone passage, especially of tamsulosin, have been well explored. Tamsulosin is the most commonly studied α1-blocker for the treatment of ureteral stones [8–13].

Gene cloning studies initially identified three subtypes of α-AR, i.e., α-1A, α-1B, and α-1D [14]. Tamsulosin has affinity for special subtypes of α1 AR, i.e., α-1A and α-1D receptors [15]. Thus, it is important that the localizations and distributions of α-1 AR subtypes in human ureter be determined, as this would represent supporting information concerning the mechanism underlying the action of tamsulosin during treatment of ureteral calculi.

Only two experimental studies have been conducted on the presence of α receptor subtypes in the human ureter [16, 17]. One study has shown that α-1D AR (at the mRNA and protein levels) is the most abundantly expressed subtype by RT PCR and receptor autoradiography using subtype-selective ligands, and that these expressions are predominantly localized in the distal ureter [16]. However, due to differences in protein stability and/or translation, mRNA expression patterns may not provide an accurate picture of protein expression. Furthermore, receptor autoradiography does not offer the necessary degree of resolution to determine receptor expression at the cellular or subcellular levels. Thus, some authors have used immunohistochemistry-based approaches to determine the expressions and localizations of α-AR subtype in various tissues, including prostate, urethra [18, 19].

Recently, Itoh et al. [17] demonstrated the presence of α-adrenergic receptor subtypes in the human ureter using an immunohistochemical approach.

We conducted this study to characterize α-AR subtype expressions in human ureter and to compare the expressions of α-AR subtypes at different ureteral levels using an immunohistochemistry approach.

Materials and methods

Materials

Rabbit polyclonal antibodies raised to synthetic peptide fragments corresponding to the carboxy-termini of human α-1A, -1B and -1D ARs were obtained from Affinity BioReagents, Inc (Golden, CO). Immunohistochemistry supplies were obtained from Dako (Carpentaria, CA).

Subjects

A total of ten ureter specimens were utilized in the study. Proximal, mid, and distal ureter pathology specimens of patients that underwent nephroureterectomy were used to prepare paraffin blocks. Mean patient age was 65.2 years (range 60–71). No patient received preoperative local radiotherapy and/or chemotherapy. No sample showed microscopic tumor invasion, and samples were cut into three pieces at the proximal, mid and distal ureter levels with the depth of 5 μm.

Immunohistochemistry

All steps were performed at room temperature, unless otherwise indicated. Immunohistochemistry was performed using the avidin–biotinperoxidase method. Paraffin-embedded tissues were sectioned at 4 μm and incubated overnight at 37°C, after being placed on silane-coated slides. Slides were then deparaffinized for 5 min in a histoclear bath and rehydrated by sequential washing using graded ethanol and phosphate-buffered saline (0.137 M NaCl, 0.047 M NaHPO4, pH 7.4). Slides were treated with hydrogen peroxide (3%)/methanol for 30 min to quench endogenous peroxidase activity. They were then immersed in 10 mmol/L sodium citrate buffer (pH 6.0) and heated for 6 min in a microwave oven at 600 W. After being allowed to cool for 15 min, slides were pretreated with 5% normal goat serum (DAKO, Carpentaria, CA) in phosphate-buffered saline, and then incubated with primary antibodies to α-AR 1A, 1B and 1D in a humid room for 60 min. This was followed by sequential incubation for 30 min with biotinylated rabbit secondary antibody (1:20 diluted) and avidin/biotinylated horseradish peroxidase solution (DAKO, Carpentaria). Staining was completed by exposing the tissue slides to diaminobenzidine for 5 min and they were then counterstained with hematoxylin. Slides were then mounted using glycerin.

Assessment of α-AR subtype expression

The expression rates of α-AR subtypes were assessed by inspecting at least five microscopic fields at 400×. A single pathologist scored expressions of the receptor from grade 0 (no stain) to grade 3 (profuse stain). Receptor immunoreactivities were graded from 0 to 3 as described by Tickoo et al. (grade 0, no staining; grade 1, <25% of cells stained; grade 2, 26–50% of cells stained; and grade 3, >50% of cells stained) [20].

To minimize the error of examination, the pathologist was blinded regarding level and type of receptor when he scored the expression of the subtype. He scored the expression of receptor in five areas of each slide and calculated the average score.

We compared the mean grades of α-AR 1A, 1B and 1D and the percentage of receptor expression with grade 2 or more between receptor subtypes in each ureter level.

Statistical analysis

Statistical analysis was done using SPSS 10.0. The Mann Whitney test was used to compare adrenergic receptor subtype expressions at each ureteral level. Differences were considered significant for p values of <0.05.

Results

Alpha 1A, 1B and 1 D AR subtypes were all localized in human ureter irrespective of ureteral level (Fig. 1). The expression levels of the three subtypes depended on ureteral level.

Comparison of mean grades of AR

In the proximal ureter, the grades of the α-1A, -1B and -1D receptors were 1.0 ± 0.5, 1.1 ± 0.6, and 2.1 ± 0.7. Corresponding grades in the mid and distal ureter were 1.1 ± 0.6, 0.8 ± 0.6, 2.0 ± 0.8, and 2.0 ± 0.7, 1.8 ± 0.6, 2.5 ± 0.5, respectively (Fig. 2). As compared to the α-1A and α-1B subtypes, the α-1D subtype was significantly more expressed in the proximal and mid ureter. In the distal ureter α-1D expression was higher than α-1A and -1B, but no significant difference was observed between the expressions of α-1A AR and α-1D AR ($p = 0.143$).

Comparison of high expression percentage of AR

In the proximal and mid ureter, the percentage of high α-1D expression was significantly higher than α-1A and -1B subtype. (80, 10, 20, and 90, 20, 10%, respectively). In the distal ureter, α-1D expression was higher than α-1A and -1B subtype but there was no statistical significance (100, 80, 70%) (Fig. 3). Distal ureter has a higher density of α-AR receptors than proximal or mid ureter. The expressions of α-1A AR and α-1B AR in distal ureter were significantly higher than in proximal and mid ureter ($p < 0.05$ respectively). α-1D expression in distal ureter was also higher than proximal and mid ureter. However, this was not statistically significant ($p = 0.28$, 0.19, respectively).

Discussion

In this study, we characterized the localizations of α-AR receptors in human ureteral tissue using an immunohistochemical approach. Several researchers have used this type of approach to examine the localizations of the expressions of α-AR subtypes in various tissues, e.g., in prostate, urethra [18, 19]. Recently, Itoh et al. demonstrated the presence of α-adrenergic receptor subtypes in the human ureter using an immunohistochemical approach [17].

Recent papers rekindled interest in the role of α1 ARs in the mediation of ureteral contractile activity, and found that

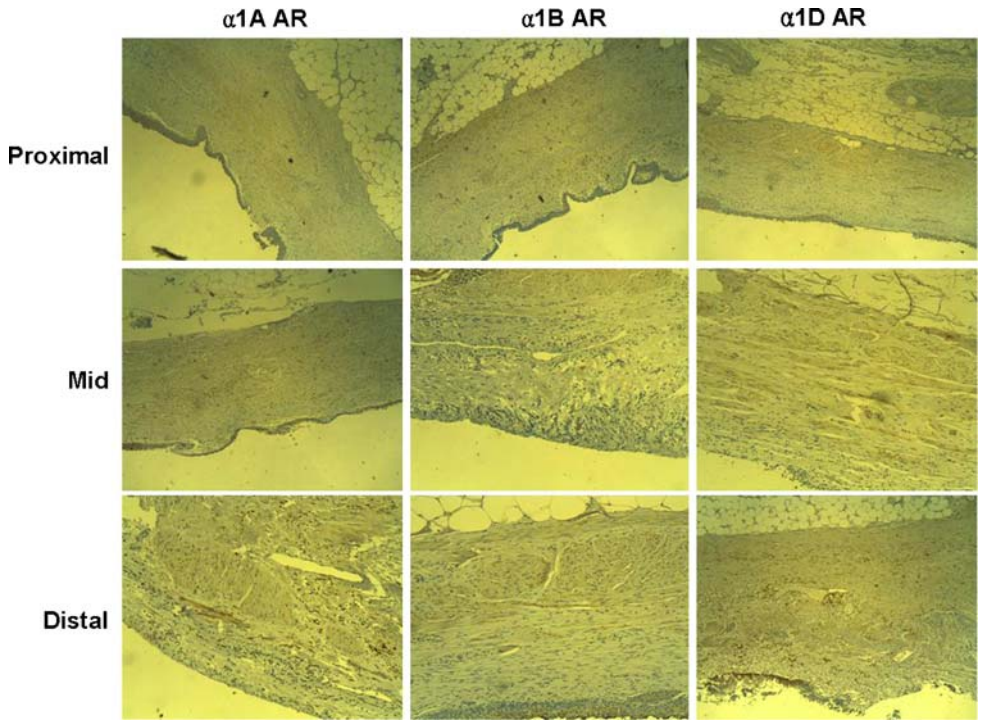

Fig. 1 Immunohistochemical localization of α-adrenoceptors in longitudinal sections of human ureter. All adrenoceptor subtypes are expressed in proximal, mid and distal ureter. Muscle layers exhibit brownish positive reaction to adrenoceptor antibody. Reduced from ×100

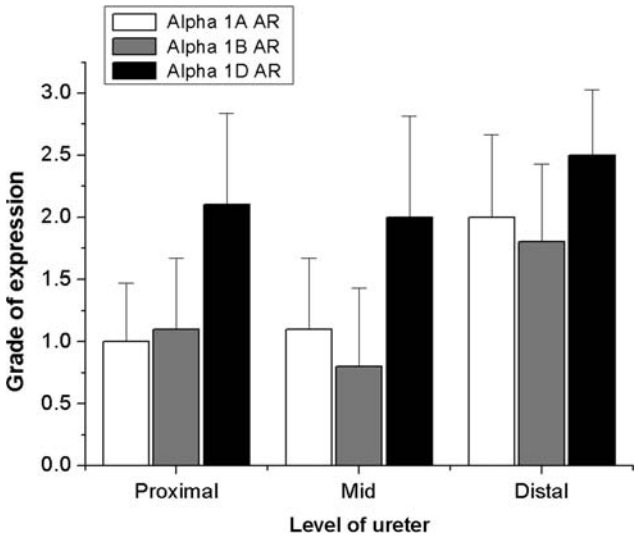

Fig. 2 Comparison of mean grades of adrenoceptor subtypes according to ureteral level

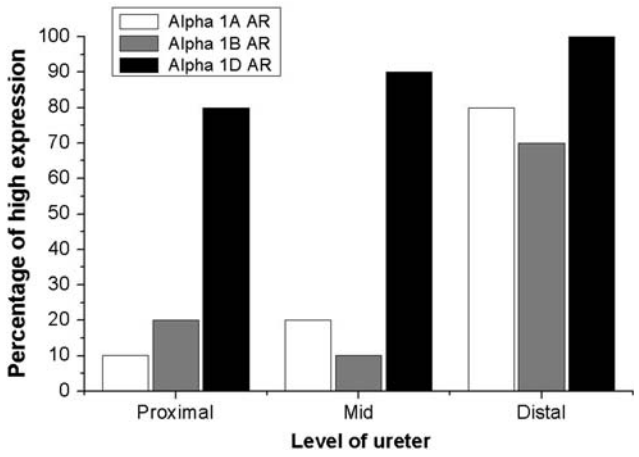

Fig. 3 Comparison of the percentage of receptor expression with grade 2 or more between receptor subtypes in each ureter level

treatment with mixed α-1A/D AR antagonist tamsulosin induces the rapid expulsion of ureteral stones [8–13]. These clinical findings may be explained by the results of the present study, which shows that α-1D ARs are the most commonly expressed subtype in the human ureter and distal ureter is rich with ARs.

Our results showed that α-1A and -1B AR are expressed as much as α-1D receptor in the distal ureter and no statistically significance was observed between the expressions of α-1A/1B and -1D AR. Our results are not consistent with previous reports [16, 17].

Yilmaz et al. reported that α1 antagonist, i.e., tamsulosin, doxazosin, and terazosin, are equally effective at inducing the expulsion of distal ureteral stones [11]. Our results show that all three α1 receptor subtypes are expressed in the distal ureter, which supports the finding that other α1 blockers have similar efficacies as tamsulosin in this context.

Consistent with the findings of a previous receptor-binding study [16, 17], we also found highest expressions of α-1D AR subtypes in distal ureter, which is consistent with a previous report of maximum α1 mRNA in distal ureter. In the distal ureter, the distribution of ARs was similar to another study [17]. However, α-1D distal ureter expression was not significantly higher than in the proximal or mid ureter according to our results. This may have been due to the rich expression of α-1D AR in other ureteral regions, which possibly reduced differentials versus the other subtypes.

A full understanding of the adrenergic physiology of the ureter is dependent upon precise knowledge of the localization of the individual α-ARs in the tissue. Until recently the only firm evidence available concerning the localizations of α1-AR subtypes in ureter was findings concerning the localizations of mRNA and of subtype selective ligands. Moreover, the expressions and localizations of receptor mRNAs may not accurately reflect receptor protein levels, due to variations in mRNA translation or protein stability. In addition, localization studies on receptor proteins in the ureter using selective ligands lack the necessary degree of sensitivity and resolution to determine expression at the cellular level. To overcome these issues, we used subtype selective antibodies to examine α-AR receptor expression. These studies enabled us to precisely locate and quantify the amounts of α-1A, α-1B and α-1D ARs in the human ureter.

However, the present study is limited because our evaluations of α-blocker receptor subtype expressions were based on subjective assessments. Nevertheless, our results confirm previous reports where the distal ureter has the highest density of α receptors, and α-1D AR is the most common receptor present in all portions of the ureter at the protein level.

Conclusions

Our results showed that the distal ureter has the highest density of α receptors, and α-1D AR is the most common receptor present in all portions of the ureter at the protein level. Our results also demonstrate that α-1A and -1B AR are expressed as much as α-1D AR, which supports the finding that α1 blockers other than tamsulosin are equally effective at inducing the expulsion of ureteral stones.

References

1. Malin JM, Deane RF, Boyarsky S (1970) Characterisation of adrenergic receptors in human ureter. Br J Urol 42:171

2. Weiss RM, Bassett AL, Hoffman BF (1978) Adrenergic innervation of the ureter. Invest Urol 16:123
3. Danuser H, Weiss R, Abel D, Walter B, Scholtysik G, Mettler D, Studer UE (2001) Systemic and topical drug administration in the pig ureter: effect of phosphodiesterase inhibitors alpha1, beta and beta2-adrenergic receptor agonists and antagonists on the frequency and amplitude of ureteral contractions. J Urol 166:714
4. Peters HJ, Parekh N, Popa G (1973) Effect of adrenergic and cholinergic agents on ureteral functions in dogs. Urol Int 34:137
5. Morita T, Ando M, Kihara K, Oshima H (1994) Function and distribution of autonomic receptors in canine ureteral smooth muscle. Neurourol Urodyn 13:315
6. Obara K, Takeda M, Shimura H, Kanai T, Tsutsui T, Komeyama T (1996) Alpha-1 adrenoreceptor subtypes in the human ureter: characterization by RT-PCR and in situ hybridization. J Urol 155(Suppl):472A
7. Hernandez M, Prieto D, Simonsen U, Rivera L, Barahona MV, Garcia-Sacristan A (1992) Noradrenaline modulates smooth muscle activity of the isolated intravesical ureter of the pig through different types of adrenoceptors. Br J Pharmacol 107:924
8. Dellabella M, Milanese G, Muzzonigro G (2003) Efficacy of tamsulosin in the medical management of juxtavesical ureteral stones. J Urol 170:2202
9. Resim S, Ekerbicer H, Ciftci A (2005) Effect of tamsulosin on the number and intensity of ureteral colic in patients with lower ureteral calculus. Int J Urol 12:615
10. De Sio M, Autorino R, Di Lorenzo G, Damiano R, Giordano D, Cosentino L, Pane U, Di Giacomo F, Mordente S, D'Armiento M (2006) Medical expulsive treatment of distal-ureteral stones using tamsulosin: a single-center experience. J Endourol 20:12
11. Yilmaz E, Batislam E, Basar MM, Tuglu D, Ferhat M, Basar H (2005) The comparison and efficacy of 3 different alpha1-adrenergic blockers for distal ureteral stones. J Urol 173:2010
12. Porpiglia F, Ghignone G, Fiori C, Fontana D, Scarpa RM (2004) Nifedipine versus tamsulosin for the management of lower ureteral stones. J Urol 172:568
13. Dellabella M, Milanese G, Muzzonigro G (2005) Randomized trial of the efficacy of tamsulosin, nifedipine and phloroglucinol in medical expulsive therapy for distal ureteral calculi. J Urol 174:167
14. Hieble JP, Bylund DB, Clarke DE, Eikenburg DC, Langer SZ, Lefkowitz RJ, Minneman KP, Ruffolo RR Jr (1995) International union of pharmacology. X. Recommendation for nomenclature of alpha 1-adrenoceptors: consensus update. Pharmacol Rev 47:267
15. Richardson CD, Donatucci CF, Page SO, Wilson KH, Schwinn DA (1997) Pharmacology of tamsulosin: saturation-binding isotherms and competition analysis using cloned alpha 1-adrenergic receptor subtypes. Prostate 33:55
16. Sigala S, Dellabella M, Milanese G, Fornari S, Faccoli S, Palazzolo F, Peroni A, Mirabella G, Cunico SC, Spano P, Muzzonigro G (2005) Evidence for the presence of alpha1 adrenoceptor subtypes in the human ureter. Neurourol Urodyn 24:142
17. Itoh Y, Kojima Y, Yasui T, Tozawa K, Sasaki S, Kohri K (2007) Examination of alpha 1 adrenoceptor subtypes in the human ureter. Int J Urol 14:749
18. Walden PD, Gerardi C, Lepor H (1999) Localization and expression of the alpha1A-1, alpha1B and alpha1D-adrenoceptors in hyperplastic and non-hyperplastic human prostate. J Urol 161:635
19. Nishi K, Latifpour J, Saito M, Foster HE Jr, Yoshida M, Weiss RM (1998) Characterization, localization and distribution of alpha1 adrenoceptor subtype in male rabbit urethra. J Urol 160:196
20. Tickoo SK, Alden D, Olgac S, Fine SW, Russo P, Kondagunta GV, Motzer RJ, Reuter VE (2007) Immunohistochemical expression of hypoxia inducible factor-1alpha and its downstream molecules in sarcomatoid renal cell carcinoma. J Urol 177:1258