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Age-related changes in alpha$_1$-adrenoceptors in rat prostate

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Abstract The age-related changes in the density of alpha$_1$-adrenoceptors in the dorsolateral lobe of the rat prostate were evaluated in Wistar rats at 8, 52, and 104 weeks of age. [^{3}H]YM617, a newly synthesized alpha$_1$-adrenergic blocker, was used as the ligand. The mean maximum number of binding sites, or alpha$_1$-adrenoceptor density (B_{max}) ±SE of 104-week-old rats (11.0±1.2 fmol/mg protein) was significantly lower than that in the 8-week-old rats (37.0±4.3 fmol/mg protein) and 52-week-old rats (37.2±3.4 fmol/mg protein) (respectively, $P< 0.01$). In contrast, mean affinity (K_d) values ±SE of these groups showed no significant differences (8-week-old rats, 115.8±9.1; 52-week-old rats, 100.5±5.8; and 104-week-old rats, 116.4±9.8 pM). Mean volumes ±SE of muscle cells of the prostate were 3.7±1.1×10^3 μm^3 at 8 weeks, 30.0±6.2×10^3 μm^3 at 52 weeks, and 18.6±8.2×10^3 μm^3 at 104 weeks. Volumes for 8-week-old rats were significantly smaller than those for 52-week-old ($P< 0.01$) and 104-week-old rats ($P< 0.05$). However, the mean area density of the muscle cells showed no difference among the three groups: 20.1±2.2% at 8 weeks, 27.3±2.9% at 52 weeks and 20.3±3.4% at 104 weeks. In conclusion, the density of YM617-binding sites (alpha$_1$-adrenoceptors) in 104-week-old rats was lower than in 8- and 52-week-old rats. Muscle volume in the rat prostate was larger in rats aged 52 and 104 weeks than in 8-week-old rats, but no correlation was found between alpha$_1$-adrenoceptor density and the muscle volume or muscle density in aging.

Key words Alpha$_1$-adrenoceptor · Age-related change
Rat prostate · Morphometry

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Benign prostatic hypertrophy (BPH) is one of the most common problems encountered in elderly men. The obstructive symptoms accompanying BPH are now often treated with alpha$_1$-adrenergic blockers [6, 11]. Although age-related changes in alpha$_1$-adrenoceptor mechanisms have been examined in various organs in experimental animals [4, 14, 15, 20, 21], age-related changes in this receptor in human and animal prostates have received little attention. The purpose of this study was to examine the age-related changes in this receptor in the dorsolateral lobe of the prostate using membrane preparations from Wistar rats. [^{3}H]YM617 is a potent and selective alpha$_1$-adrenergic blocker. Because of its low nonspecific binding, it has been suggested that this compound would be a useful radioligand in analysis of the alpha$_1$-adrenoceptor [24], and it was therefore used in the present study. Furthermore, we also evaluated age-related muscular changes in the rat prostate using the morphometric method.

Materials and methods

Preparation of the dorsolateral lobe of rat prostates

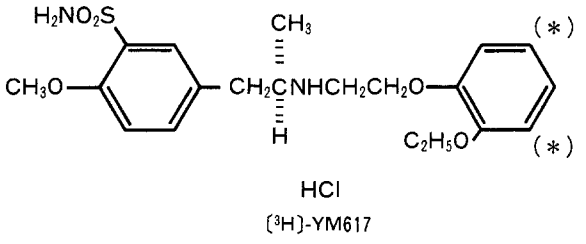
Under sufficient anesthesia with ethyl ether, the dorsolateral lobes of the prostate were removed from Wistar rats aged 8, 52, and 104 weeks (6 rats per group). The specimens were divided into two groups. For binding experiments the specimens were quickly frozen and stored at –80 °C. For morphometric examination they were embedded in OCT compound and stored at –80 °C until use.

Ligand

^{3}H-labeled YM617([^{3}H]YM617); (R)-(–)-5-[2-[[2[ethoxyring(n)-^{3}H](*o*-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide hydrochloride) was specially synthesized by Amersham Japan Co. Ltd. (Tokyo, Japan) for Yamanouchi Pharmaceutical Co. (Tokyo, Japan). The molecular weight of [^{3}H]YM617 was 412 and specific activity was 56.3 Ci/mmol (2.08 TBq/mmol) (Fig. 1).

Binding experiments using membrane preparations

The prostate fragments were weighed and minced into small pieces, then homogenized in 20 vol. of ice-cold 5 mM Tris-HCl buffer (pH



R-(−)-5-[2-[[2[ethoxyring(n)-^{3}H](*o*-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide HCl

Fig. 1 Structure of [^{3}H]YM617

7.4) with 0.25 M sucrose in a Polytron homogenizer. The homogenates were centrifuged at 500 *g* for 15 min at 4 °C. The supernatant was filtered through gauze and centrifuged at 50,000 *g* for 20 min at 4 °C. The pellets were washed twice with ice-cold 50 mM Tris-HCl buffer (pH 7.4), after which the protein concentration was determined by the Bradford method [2]. The homogenate was incubated in a 50 mM Tris-HCl buffer (pH 7.4) with [^{3}H]YM617 (0.05–5 nM) at 25 °C for 30 min. After incubation the reaction mixture was filtered through glassfiber filters (Whatman GF/C) under vacuum. The filters were rinsed three times with 3 ml of ice-cold buffer. Tissue-bound radioactivity was extracted from the filters in 10 ml of a scintillation fluid, and the radioactivity was determined in a liquid scintillation counter. All assays were performed in duplicate. Specific binding for [^{3}H]YM617 was defined as the difference in binding determined in the absence and presence of 10 μM of phentolamine.

The affinity (K_d) and the maximum number of binding sites (B_{max}) were determined by Scatchard analysis of saturation data.

Morphometric examination

Slices of frozen sections (5 μm) were thaw-mounted onto standard glass slides, then stained with hematoxylin-eosin and by the Mallory-Azan method. The lengths and widths of muscle cells were calculated at random in ten areas using a computerized image analysis system (Photron, Tokyo, Japan). As muscle cells were assumed to be spindle-shaped (i.e. like two cones tip to tip), the muscle volume was determined by the following formula:

$$\text{muscle volumes} = \frac{\pi}{3}\left[\text{muscle length} \times \left(\frac{\text{muscle width}}{2}\right)^2\right]$$

The percentage area density of the muscle area was also calculated by the above methods and defined as follows:

$$\text{muscle percentage area density} = \frac{\text{muscle area}}{\text{other area}} \times 100$$

Statistical analysis

Results are expressed as the mean±SE. Statistical significance (5%) of K_d, B_{max}, and muscle morphometric data for each rat group (8, 52 and 104 weeks of age) was calculated by the multiple comparison test (Scheffer's method).

Results

Binding experiments using membrane preparations

In the dorsolateral lobes of the rat prostate the affinities (K_d) of [^{3}H]YM617 for each group of rats were 115.8±9.1 pM in 8-week-old rats, 100.5±5.8 in 52-week-old rats and 116.4±9.8 in 104-week-old rats. Densities (B_{max}) were 37.0±4.3 fmol/mg protein in 8-week-old rats, 37.2±3.4 fmol/mg protein in 52-week-old rats and 11.0±1.2 fmol/mg protein in 104-week-old rats. The B_{max} for 104-week-old rats was significantly lower than for the other two groups ($P< 0.01$). The difference in B_{max} between 8-week-old rats and 52-week-old rats was not significant (Fig. 2).

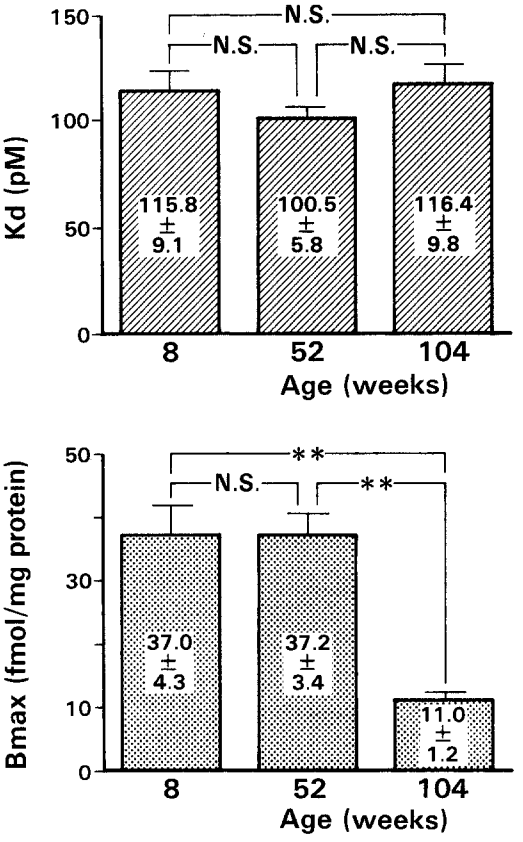


Fig. 2 Affinity (K_d) and density (B_{max}) of [^{3}H]YM617 binding to rat prostate membranes (n=6). *N.S.*, not significant; $^{**}P< 0.01$

Morphometric examination

The mean volume of muscle cells of the prostate of 8-week-old rats (3.7±1.1 μm^3) was significantly smaller than that of 52-week-old rats (30.0±6.2 μm^3) ($P< 0.01$) and 104-

Table 1 Morphometric data of muscle cells of the rat prostate (n=6 in each group). [a] $P< 0.05$; [b] $P< 0.01$ (difference from volume in 8-week-old rats)

Age	Muscle cells	
	Volume (μm^3)	Area density (%)
8 weeks	$3.7 \pm 1.1 \times 10^3$	20.1 ± 2.2
52 weeks	$30.0 \pm 6.2 \times 10^{3b}$	27.3 ± 2.9
104 weeks	$18.6 \pm 8.2 \times 10^{3a}$	20.3 ± 3.4

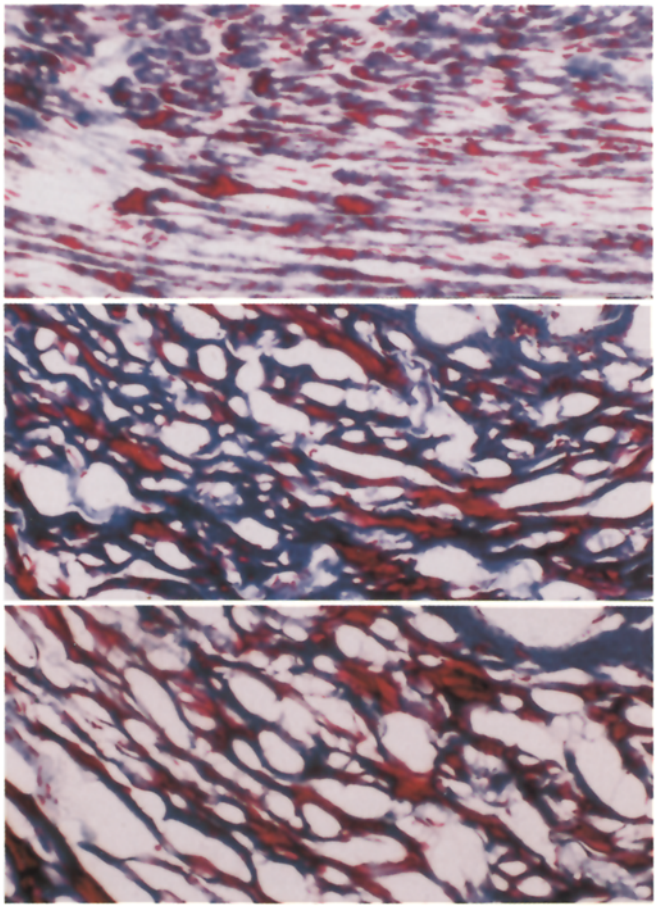


Fig 3 Microscopic findings in prostate muscle from individual rats of different ages, stained with Mallory-Azan method (*top*, 8 weeks; *middle*, 52 weeks; *bottom*, 104 weeks)

week-old rats (18.6±8.2 μm^3) ($P<0.05$). However, there was no difference in muscle volume between 52-week-old rats and 104-week-old rats. The area density of the muscle cells was very similar in all three groups (no significant difference); 20.1±2.2% in 8-week-old rats, 27.3±2.9% in 52-week-old rats and 20.3±3.4% in 104-week-old rats (Table 1, Fig. 3).

Discussion

YM617 was used as a ligand against alpha$_1$-adrenoceptors in this study. This reagent has been established as a specific alpha$_1$-adrenergic blocker with extremely low nonspecific binding [24]. It also shows an excellent clinical effect on the obstruction caused by BPH [6]. Thus, YM617 is considered a satisfactory ligand for analyzing the prostatic alpha$_1$-adrenoceptor.

Localization of alpha$_1$-adrenoceptor in the hypertrophied human prostate has been well studied [9, 13]. The effect of age-related changes in sex hormones on the prostate has also been well studied [3, 5, 10]. Age-related changes in prostatic adrenoceptor content, however, have not been examined.

The present results showed that the density of alpha$_1$-adrenoceptors was decreased in older rats but their binding affinity did not change. Kobatake et al. [8] previously reported that the B_{max} of alpha$_1$-adrenoceptors in rat epididymal fat cells was significantly decreased in 20-week-old rats compared with 6-week-old rats, but that K_d values remained unchanged. Kimball et al. [7] also showed that the age-related decline in alpha$_1$-adrenergic responsiveness in rat ventricular muscle in rats aged 4, 14, and 25 months is mediated partly by a decrease in cardiac alpha$_1$-adrenoceptor density. It has also been reported that the senescent cardiac muscle has diminished responsiveness to norepinephrine [18, 23]. Our results are consistent with these reports in that the density of alpha$_1$-adrenoceptor decreased in older rats. Taken together, these results invite the speculation that senescent humans also show decreased responsiveness to alpha$_1$-adrenoceptor blockers. The present results may also mean that an increased dose of these drugs is required to achieve the same effect in older patients as in younger patients.

Quantification of the smooth muscle content of the prostate has not been reported for rats. With regard to muscle density, Lepor et al. [12] demonstrated that the percentage area density of smooth muscle of the prostate in dogs older than 3 years was 6.5 to 24.4 in several prostatic regions. In humans, Shapiro et al. [16], using double staining with prostatic acid phosphatase and desmin to determine muscle density, reported that the histological composition of the smooth muscle in the human hypertrophied prostate was 21.6±4.1%. Siegel et al. [17] reported a morphometric analysis of BPH using a point system and a 7% mean area density of the smooth muscle. In the present study, muscle was identified using the conventional Mallory-Azan method, which stains muscle red. This method is very convenient and can be utilized in computerized image analyzing systems. Our study showed that the muscular density of rats was almost the same as that of dogs and humans [12, 16]. The percentage muscle density showed no significant change throughout life in the rats. In contrast, the muscle cell volume of 52- and 104-week-old rats was significantly greater than that of 8-week-old rats. The density of YM617 binding sites (alpha$_1$-adrenoceptors) was significantly lower in 104-week-old rats than in rats aged 8 and 52 weeks. No correlation was seen between alpha$_1$-adrenoceptor density and muscle volume or muscle density. The muscle volume may increase with age up to a point (52 weeks in this study) and then decrease, probably due to atrophy and/or degeneration. Muscle density revealed the same tendency, but not significantly. In this atrophic and/or degenerative phase the alpha$_1$-adrenoceptor might be considered to show decreased density.

Although there are some anatomical differences to the human prostate, the rat model was chosen for this study of age-related changes in the prostate. This model is important in comparing the functions of alpha$_1$-adrenoceptor and/or other substances in rat BPH model [19] and other

organs [7, 8, 18]. Furthermore, aged animals such as dogs which are frequently used as a BPH model [1], were difficult to obtain.

The correlation between human and rat life spans is not clear. There are no exact data indicating the human equivalent ages for 52-week-old and 104-week-old rats. Unger and Schmidt [22] used 3-month-old rats for young adults and 20-month-old rats for late middle age to study age-related changes in galamin immunoreactivity in the nucleus basalis of Meynert in rats. In any case, it seems likely that the age of 104 weeks in rats may correspond to moderately old age in humans.

In summary, the density of YM617 binding sites (alpha$_1$-adrenoceptors) was significantly lower in 104-week-old rats than in 8- and 52-week-old rats. The muscle volume in the prostate was larger in 52- and 104-week-old rats than in 8-week-old rats. However, no correlation was seen between alpha$_1$-adrenoceptor density and muscle volume or muscle density. The muscle volume in rat prostate may increase with age up to a point and then decrease again, probably due to atrophy and/or degeneration with decreased alpha$_1$-adrenoceptor density.

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References

1. Berry SJ, Coffey DS, Strandberg JD, Ewing LL (1986) Effect of age, castration, and testosterone replacement on the development and restoration of canine benign prostatic hyperplasia. Prostate 9:295
2. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal Biochem 72:248
3. Coffey DS, Walsh PC (1990) Clinical and experimental studies of benign prostatic hyperplasia. Urol Clin North Am 17:461
4. Collier WL, Iacopino L, Amenta F (1984) Changes of [^{3}H]spiroperidol binding in the renal artery of aged rabbits. Mech Aging Dev 28:41
5. Habenicht UF, el-Etreby MF (1991) Rationale for using aromatase inhibitors to manage benign prostatic hyperplasia. Experimental studies. J Androl 12:395
6. Kawabe K, Ueno A, Takimoto Y, Aso Y, Kato H, YM617 Clinical study group (1990) Use of an α1-blocker, YM617, in the treatment of benign prostatic hypertrophy. J Urol 144:908
7. Kimball KA, Cornett LE, Seifen E, Kennedy RH (1991) Aging: changes in cardiac alpha 1-adrenoceptor responsiveness and expression. Eur J Pharmacol 208:231
8. Kobatake T, Watanabe Y, Matsuzawa Y, Tokunaga K, Fujioka S, Kawamoto T, Keno Y, Tarui S, Yoshida H (1991) Age-related changes in adrenergic alpha 1, alpha 2, and beta receptors of rat white fat cell membranes: an analysis using [^{3}H]bunazosin as a novel ligand for the alpha 1 adrenoceptor. J Lipid Res 32:191
9. Kobayashi S, Demura T, Nonomura K, Koyanagi T (1991) Autoradiographic localization of alpha 1-adrenoceptors in human prostate: special reference to zonal difference. J Urol 146:887
10. Kyprianou N, Isaacs JT (1987) Quantal relationship between prostatic dihydrotestosterone and prostatic cell content: critical threshold concept. Prostate 11:41
11. Lepor H (1990) Role of alpha-adrenergic blockers in the treatment of benign prostatic hyperplasia. Prostate [Suppl] 3:75
12. Lepor H, Tang R, Meretyk S, Hartanto V, Shapiro E (1992) Binding and functional properties of alpha 1-adrenoceptors and area density of smooth muscle in the canine prostate. J Urol 148:1310
13. Moriyama N, Kawabe K, Hamada K, Kimura K, Murahashi I, Aso Y (1991) Demonstration of α_1-adrenoceptors in human hypertrophied prostate with tritiated bunazosin. Acta Histochem Cytochem 24:421
14. Ordway GA, Kolta MG, Gerald MC, Wallace LJ (1986) Age-related change in α-adrenergic responsiveness of the urinary bladder of the rat is regionally specific. Neuropharmacology 25:1335
15. Ricci A (1990) The renal dopaminergic system in aging. J Auton Pharmacol 10 [Suppl]: 19
16. Shapiro E, Hartanto V, Lepor H (1992) Quantifying the smooth muscle content of the prostate using double-immunoenzymatic staining and color assisted computer image analysis. J Urol 147: 1167
17. Siegel YI, Zaidel L, Hanmel I (1990) Morphometric evaluation of benign prostatic hyperplasia. Eur Urol 18:71
18. Simpkins JW, Field FP, Ress RJ (1983) Age-related decline in adrenergic responsiveness of the kidney, heart and aorta of male rats. Neurobiol Aging 4:233
19. Snyder BW, Winneker RC, Batzold H (1989) Endocrine profile of Win 49596 in the rat: a novel androgen receptor antagonist. J Steroid Biochem 33:1127
20. Takayanagi I, Koike K (1991) Effects of aging on postsynaptic alpha 1-adrenoceptor mechanisms in rat aorta. Gen Pharmacol 22:211
21. Takayanagi I, Moriya M, Koike K (1992) Effect of ageing on alpha 1A-adrenoceptor mechanisms in rabbit isolated bronchial preparations. J Smooth Muscle Res 28:63
22. Unger JW, Schmidt Y (1993) Galanin-immunoreactivity in the nucleus basalis of Meynert in the rat. Neurosci Lett 153:140
23. Vestal RE, Wood AJJ, Shand DG (1979) Reduced β-adrenoceptor sensitivity in the elderly. Clin Pharmacol Ther 26:181
24. Yazawa H, Takanashi M, Sudoh K, Inagaki O, Honda K (1993) Characterization of [^{3}H]YM617, R-(−)-5-[2-[[2[Ethoxyring(*n*)-^{3}H](*o*-ethoxyphenoxy)ethyl]amino]-propyl]-2-methoxybenzensulfonamide HCl, a potent and selective alpha-1 adrenoceptor radioligand. J Pharmacol Exp Ther 263:201