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Distribution of α_{1L}-Adrenoceptors in Canine Prostate: Characterization by Quantitative Autoradiography

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Abstract

Our aim was to determine the distribution of α_{1L}-adrenoceptors in canine prostate by an autoradiographic technique using [^{3}H]JTH-601 (an α_{1L}-adrenoceptor antagonist) and [^{3}H]JTH-601-G1 (an active metabolite of JTH-601).

Prostates were removed from three male beagle dogs. Several slices of the specimens were incubated with 5 nM of [^{3}H]JTH-601, [^{3}H]JTH-601-G1 and [^{3}H]tamsulosin (an α_{1A}-adrenoceptor antagonist). For macroscopic autoradiography, visualization was performed using an imaging plate and image-analyser. To examine microscopic localization of binding sites, preparations were exposed, developed and fixed.

Specific binding of [^{3}H]JTH-601 and [^{3}H]JTH-601-G1 was observed diffusely throughout the entire interstitium on macroscopic autoradiography. Specific binding of [^{3}H]tamsulosin was also recognized although the binding was weaker than that of [^{3}H]JTH-601. On microscopic autoradiograms, the grains of each ligand were mainly distributed on smooth muscle.

These results indicate morphologically that specific binding sites of JTH-601 and JTH-601-G1 exist in canine prostate, suggesting the distribution of α_{1L}-adrenoceptors in this tissue, in addition to α_{1A}-adrenoceptors.

Currently, α_1-adrenoceptors have been classified into three subtypes (α_{1A}, α_{1B} and α_{1D}) and each corresponding cDNA has been cloned (α_{1a}, α_{1b} and α_{1d}, respectively) (Bylund et al 1994; Hieble et al 1995). Alternatively, α_1-adrenoceptors can be divided into α_{1H}- and α_{1L}-adrenoceptor subtypes based on their high and low affinity for prazosin, respectively (Flavahan & Vanhoutte 1986; Muramatsu et al 1990; Oshita et al 1991; Ohmura et al 1992). All three cloned α_1-adrenoceptors are included in the α_{1H}-adrenoceptor subtype. Although the identity of the α_{1L}-adrenoceptor is uncertain because the receptor has not yet been cloned, many researchers have reported the existence of α_1-adrenoceptors with low affinity for prazosin (Bylund et al 1994; Muramatsu et al 1995; Graham et al 1996).

Smooth muscle contraction of human prostate is reportedly mediated by α_1-adrenoceptors (Lepor & Shapiro 1984). Since the α_{1A}-adrenoceptor is preferentially expressed in prostate (Price et al 1993), it is suggested that this subtype plays a role in mediating the prostatic contractile response to α_1-adrenoceptor activation. However, compounds have been identified that have high affinity for the α_{1A}-adrenoceptor and yet are very weak functional antagonists of noradrenaline-induced contraction of human prostate (Marshall et al 1992; Ford et al 1996). Evidence from both radioligand binding and functional studies has indicated that the putative α_{1L}-adrenoceptors may be predominantly involved in contraction of prostatic smooth muscle (Takeda et al 1993; Muramatsu et al 1994).

JTH-601 (3-{*N*-[2-(4-hydroxy-2-isopropyl-5-methylphenoxy)ethyl]-*N*-methylaminomethyl}-4-methoxy-2,5,6-trimethylphenol hemifumarate; Figure 1), a novel α_1-adrenoceptor antagonist, has been reported to show high affinity for the α_{1L}-adrenoceptor and to selectively antagonize α_1-adrenoceptor agonist-induced contraction of human and animal prostate both in-vitro and in-vivo (Muramatsu et al 1996; Suzuki et al 1999, 2000; Takahashi et al 1999). JTH-601-G1 (JTH-601 β-D-glucopyranosyl uronic acid; Figure 1) is an active metabolite of JTH-601

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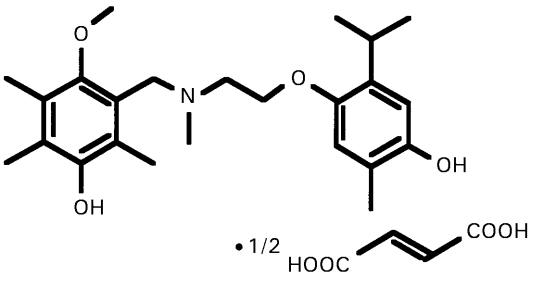
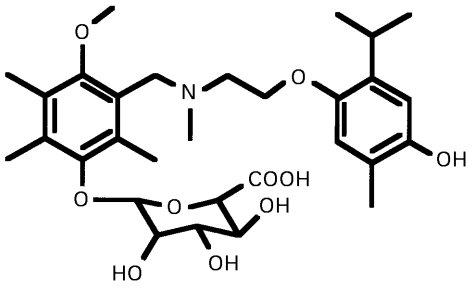


JTH-601



JTH-601-G1

Figure 1. Chemical structures of JTH-601 and JTH-601-G1.

(Takahashi et al 1999), an α_{1A}-adrenoceptor antagonist (Michel & Insel 1994). The purpose of this study was to determine the distribution of the α_{1L}-adrenoceptor in canine prostate based on a comparison of the binding of [^{3}H]JTH-601 and [^{3}H]JTH-601-G1 with that of [^{3}H]tamsulosin.

Materials and Methods

Preparation of canine prostates

This experiment was performed in accordance with the guidelines for animal experimentation set by the ethics committee for animal use at Japan Tobacco Inc. Three male beagle dogs (Keari, Osaka, Japan), $10{\cdot}5 \pm 0{\cdot}4$ kg, were used. Dogs were anaesthetized with sodium pentobarbital (30 mg kg^{-1}, i.v.) and killed by bleeding. The prostate was isolated and embedded in O.C.T Compound (Sakura einetechnical Co. Ltd, Tokyo, Japan), immediately frozen in liquid nitrogen, and stored at -80°C until experiments were performed. Specimens were then cut into slices, 15–20 μm thick, with a cryostat-microtome at -20°C. The slices were thaw-mounted onto poly-L-lysine-coated glass slides.

Autoradiography

The methods for autoradiography were modified following those of Moriyama et al (1994). Two sets of consecutive sections of prostate were first pre-incubated in phosphate-buffered saline (PBS, pH 7·4, 10 mM) for 30 min at 25°C. One section was incubated with 100 μM of phentolamine (for non-specific binding) and the other was incubated without phentolamine (for total binding) in PBS for 30 min at 25°C. Sections were then incubated with 5 nM of [^{3}H]JTH-601, [^{3}H]JTH-601-G1 or [^{3}H]tamsulosin in the presence or absence of phentolamine (100 μM) for 60 min at 25°C. After incubation, the slices were rinsed three times in ice-cold PBS and flushed with ice-cold distilled water.

For macroscopic autoradiography, the dried sections were exposed to [^{3}H]-sensitive imaging plates (BAS imaging plate TR, Fuji Photo Film, Tokyo, Japan) with a [^{3}H]-microscale (Amersham Int. PLC, Bucks, UK) for 1 week at room temperature. Autoradiograms were processed by a computerized image analysis system (Bio-imaging analyzer BAS3000, Fuji Photo Film). Total and non-specific binding were both registered according to the [^{3}H]-microscale density. Then, specific binding was determined by subtracting non-specific binding from total binding. Specific-binding images were prepared in the same manner. Binding of radio-ligand was also quantified using the BAS3000. In at least 3 regions of each preparation, binding (total and paired non-specific) of radio-ligand was measured and subtracted values (total−non-specific) averaged to express specific binding.

For microscopic autoradiography, the specimens consecutive to those for macroscopic autoradiography were coated with emulsion (NR-M2, Konica, Tokyo, Japan). After exposure for 12–20 weeks, specimens were developed, fixed and counterstained with hematoxylin and eosin for observation of the microscopic localization of binding sites.

Materials

[^{3}H]JTH-601 (1·15 TBq mmol^{-1}) and [^{3}H]JTH-601-G1 (1·37 TBq mmol^{-1}) were synthesized by Amersham Int. PLC. Other chemicals were purchased from the following sources: [^{3}H]tamsulosin ([^{3}H]YM617, 2·08 TBq mmol^{-1}) from DuPont NEN (Boston, MA); phentolamine mesylate (Regitin injection) from Ciba-Geigy (Takarazuka, Japan); sodium pentobarbital from Dainabot (Osaka, Japan).

Results

Specific binding of [^{3}H]JTH-601 was observed diffusely throughout the entire canine prostate interstitium (Figure 2). The quantified specific binding of [^{3}H]JTH-601 was $8{\cdot}80 \pm 1{\cdot}15$ nCi

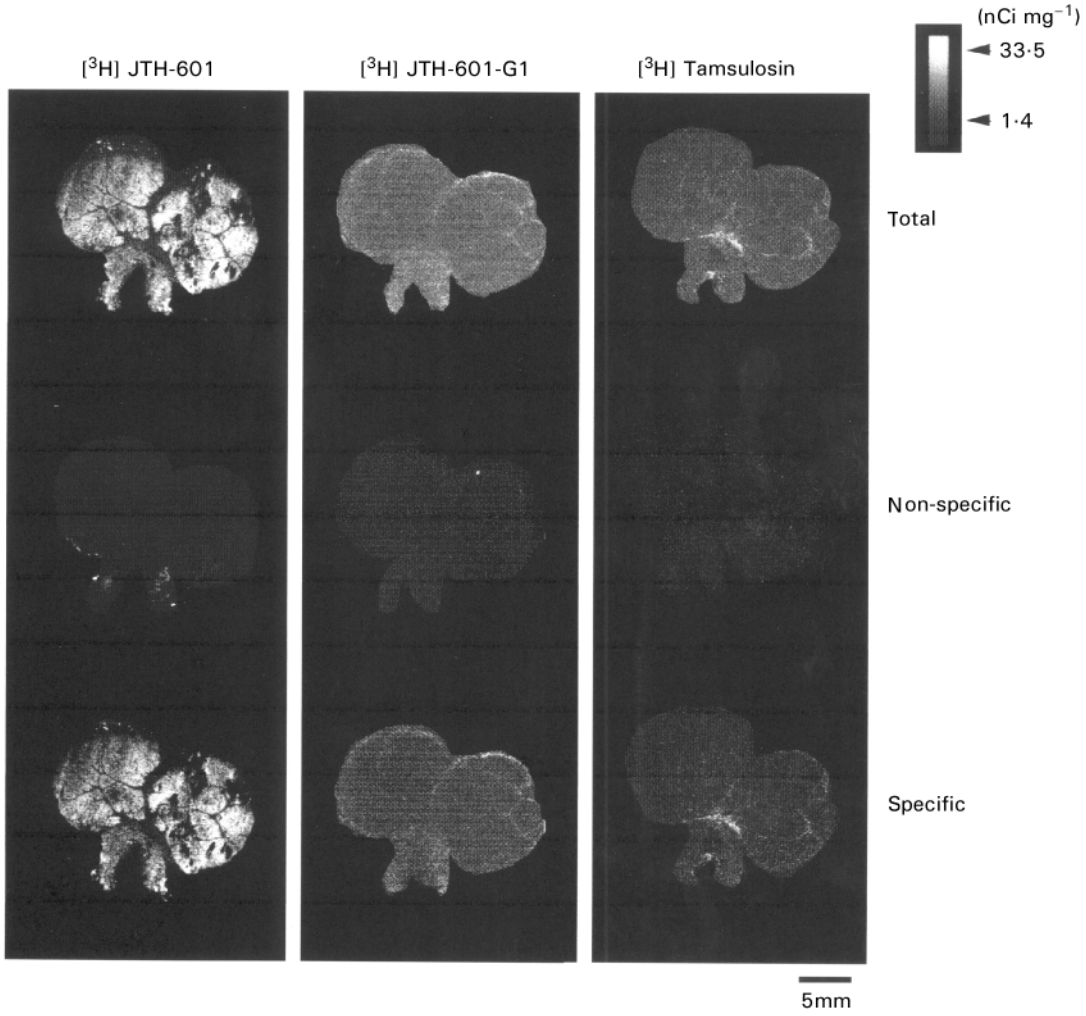


Figure 2. Macroscopic autoradiograms of [^{3}H]JTH-601, [^{3}H]JTH-601-G1 and [^{3}H]tamsulosin in canine prostate. Each ligand was evaluated at 5 nM. Total: binding of [^{3}H]ligand without treatment with phentolamine (100 μM); non-specific: binding of [^{3}H]ligand after treatment with phentolamine; specific: autoradiogram of non-specific subtracted from total.

(mg tissue)$^{-1}$. Specific binding of [^{3}H]JTH-601-G1 and [^{3}H]tamsulosin was also noted (Figure 2), although the binding was weaker than that of [^{3}H]JTH-601 ($0{\cdot}63 \pm 0{\cdot}24$ and $0{\cdot}77 \pm 0{\cdot}07$ nCi (mg tissue)$^{-1}$, respectively). Very little non-specific binding was observed in prostate sections treated with phentolamine (Figure 2).

Microscopic autoradiography revealed grains distributed over the interstitium (mainly corresponding to smooth muscle) in the canine prostate specimens treated with [^{3}H]JTH-601, [^{3}H]JTH-601-G1 and [^{3}H]tamsulosin (Figure 3). For each ligand, few grains were observed in specimens treated with 100 μM phentolamine (non-specific binding, data not shown).

Discussion

α_1-Adrenoceptors have been classified into three subtypes (α_{1A}, α_{1B} and α_{1D}) (Bylund et al 1994; Hieble et al 1995). The α_{1L}-adrenoceptor is now noted as a fourth subtype of α_1-adrenoceptor (Flavahan & Vanhoutte 1986; Muramatsu et al 1990; Oshita et al 1991; Ohmura et al 1992). JTH-601 and JTH-601-G1 have been shown to possess a high affinity for the α_{1L}-adrenoceptor (Muramatsu et al 1996; Takahashi et al 1999).

In a previous functional study using isolated canine prostate and common carotid artery, both JTH-601 and JTH-601-G1 antagonized α_1-adrenoceptor agonist-induced contractions in both prostate and artery, their inhibitory activity in the prostate being 9–27 times that in the artery (Suzuki et al 1999, 2000; Takahashi et al 1999). These findings suggested that functional α_{1L}-adrenoceptors are of importance in prostatic smooth muscle contraction. This data demonstrates morphologically that specific binding sites of JTH-601 and JTH-601-G1 exist in the canine prostate. The distribution of α_{1L}-adrenoceptors in the smooth muscle of canine

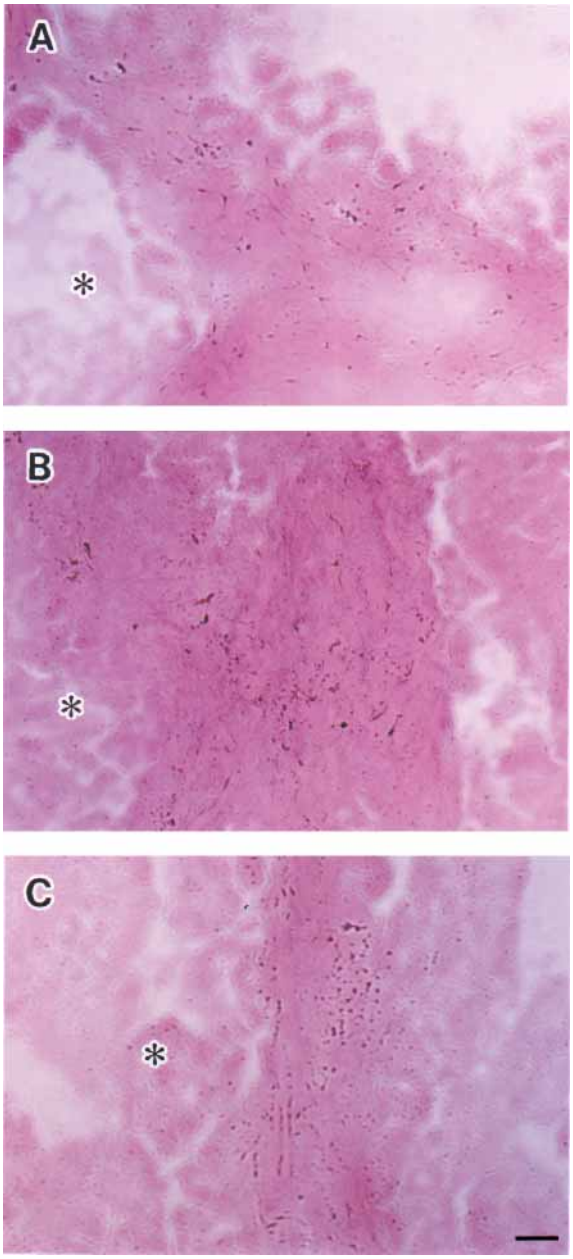


Figure 3. Microscopic autoradiograms of [^{3}H]JTH-601 (A), [^{3}H]JTH-601-G1 (B) and [^{3}H]tamsulosin (C), in canine prostate at 5 nM, show grains distributed over the interstitium (mainly corresponding to smooth muscle) in the specimens. Asterisk represents the glands. Specimens were counterstained with haematoxylin and eosin. Bar = 10 μm.

prostate is suggested, supporting the hypothesis that these compounds play a functional role in this tissue.

In this study, specific binding of both JTH-601 and JTH-601-G1 was observed in canine prostate, though binding of JTH-601-G1 was approximately 14-times weaker than JTH-601. Although we did not examine α_1-adrenoceptor binding of these compounds using membrane fraction of canine prostate, Takahashi et al (1999) reported that the affinity of JTH-601-G1 (pKi = 8·24) was approximately 57-times less potent than that of JTH-601 (pKi = 10·0). It therefore seems that the differing affinity for α_{1L}-adrenoceptors between these compounds is one reason for the weaker binding of JTH-601-G1 compared with JTH-601 in canine prostate observed in the present study.

Other α_1-adrenoceptor antagonists such as prazosin and bunazosin, which are non-selective, have been reported to show specific binding to α_1-adrenoceptors in human prostate (Kawabe et al 1990; Moriyama et al. 1991). In this study, specific binding of [^{3}H]tamsulosin was also observed. Moriyama et al (1994) also demonstrated the presence of specific binding sites of tamsulosin in human prostate using autoradiography. These findings suggest that the α_{1A}-adrenoceptor is distributed in the smooth muscle of canine prostate.

In a receptor binding study using membrane fractions of human prostate and recombinant human α_1-adrenoceptors, JTH-601 and JTH-601-G1 were found to have high affinity for $\alpha_{1A(a)}$-adrenoceptors in addition to α_{1L}-adrenoceptors (Muramatsu et al 1996; Takahashi et al 1999). It is therefore thought that specific binding of [^{3}H]JTH-601 and [^{3}H]JTH-601-G1 indicates the distribution of both α_{1L}- and α_{1A}-adrenoceptors, but without a specific ligand to distinguish between the two, their respective distributions can not yet be determined.

The existence of a distinct α_1-adrenoceptor with low affinity for prazosin has been widely accepted, especially considering evidence from functional experiments, but since the α_{1L}-adrenoceptor has not yet been cloned, its identity has not been fully established. Ford et al (1996, 1997) and Williams et al (1996) proposed that the receptor is an altered conformational state of the α_{1A}-adrenoceptor, suggesting that environmental factors around cells (whole-cell or homogenate, and temperature) influence the state of the receptor. In this study, it is unknown how the experimental conditions reflect the physiological state. To clarify the function of an α_{1L}-adrenoceptor, it is first necessary to elucidate its structure and its selective ligands.

In conclusion, specific binding sites of JTH-601 and JTH-601-G1 were morphologically demonstrated in the smooth muscle of canine prostate, suggesting the distribution of α_{1L}-adrenoceptors in this tissue, in addition to α_{1A}-adrenoceptors.

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