Tizanidine May Discriminate between Imidazoline-Receptors and α_2 -Adrenoceptors

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ABSTRACT—The selectivity of tizanidine to the imidazoline-receptor and α_2 -adrenoceptor recognized by ^3H -*p*-aminoclonidine was examined in rat kidney membranes and was compared with those of other imidazoline compounds. Tizanidine bound to the imidazoline-receptors with approximately 20 times higher affinity than the α_2 -adrenoceptors. The order of relative selectivity to imidazoline receptor was tizanidine > oxymetazoline > clonidine > naphazoline, where clonidine showed an equal affinity to both receptors. Tizanidine may act more potently on the imidazoline-receptors than the α_2 -adrenoceptors.

Keywords: Tizanidine, Imidazoline-receptor, α_2 -Adrenoceptor, Kidney (rat)

Tizanidine is an α_2 -adrenoceptor agonist which has an imidazoline ring in its structure and is clinically used as an antispastic drug. Its pharmacological features are similar to those of clonidine, although some differences have been reported (1, 2). Recently, clonidine and other imidazoline compounds have been found to act on not only α_2 -adrenoceptors but also imidazoline-receptors; their hypotensive action is in part associated with the imidazoline-receptor located in the medulla oblongata (3–5). Imidazoline-receptors are widely distributed in central and peripheral tissues, and an endogenous clonidine-like substance has been found to have a high affinity to the imidazoline-receptors (5–7). Therefore, it is supposed that the imidazoline-receptors may play an important role in many physiological functions. In the present study, we examined the selectivity of tizanidine to the imidazoline-receptor and α_2 -adrenoceptor of rat kidney membranes and compared it with those of clonidine and other compounds.

MATERIALS AND METHODS

Membrane preparation

Male Wistar rats (300–350 g) were decapitated and the kidneys were rapidly removed. The kidneys were minced in ice-cold 50 mM Tris-HCl, 5 mM EDTA (pH 7.7) and then homogenized by a polytron. The homogenate was filtered through 4 layers of silk gauze

and centrifuged at $40,000 \times g$ for 13 min. The pellet was resuspended in 50 mM Tris-HCl, 25 mM NaCl buffer (pH 7.7), incubated for 30 min at 25°C, and recentrifuged as described above. The final pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.7) and used for the binding assay.

Binding assays

Incubations were performed in duplicate at 25°C for 40 min in a total volume of 1 ml. The incubate consisted of ^3H -*p*-aminoclonidine (^3H -PAC), a suspension of kidney membranes (approximately 0.55 mg/ml) and either buffer alone or buffer containing a test compound. Non-specific binding was defined by parallel incubations containing 10 μM phentolamine. In experiments using adrenaline, all samples contained ascorbic acid in a final concentration of 0.01%. Incubations were terminated by vacuum filtration over Whatman GF/C filters by using a cell harvester. The filters were washed with ice-cold Tris-HCl; then the filter-bound radioactivity was determined using a scintillation counter (Aloka, Japan). All binding data were analyzed by the weighed least-squares iterative curve fitting program LIGAND, and protein was assayed by the method of Bradford, as shown previously (8).

Drugs

Drugs were obtained from the following sources: tiza-

nidine hydrochloride (Sandoz, Tokyo, Japan), clonidine hydrochloride, oxymetazoline hydrochloride, naphazoline hydrochloride, *l*-adrenaline bitartrate (Nacalai, Kyoto, Japan) and phentolamine mesylate (Ciba-Geigy, Tokyo, Japan). ^3H -*p*-Aminoclonidine (^3H -PAC, specific activity 2153.4 GBq/mmol) was purchased from New England Nuclear (Boston, USA).

RESULTS

The specific binding of ^3H -PAC (0.1–20 nM) was concentration-dependent and saturated at 15–20 nM. Scatchard plots of the binding data showed a straight line, suggesting a single class of binding sites ($K_D = 2.17 \pm 0.21$ nM, $B_{\text{max}} = 66.5 \pm 2.7$ fmol/mg protein, n

= 4).

Clonidine monophasically displaced the specific binding of 1 nM ^3H -PAC, resulting in a single affinity ($K_i = 9.20 \pm 1.23$ nM) (Fig. 1). However, tizanidine showed a shallow displacement curve (Fig. 1). Computerized analysis revealed that tizanidine subdivided the binding sites into two populations; the proportion of low affinity sites was 71%. Oxymetazoline and naphazoline also discriminated the two distinct affinity sites with a similar proportion. On the other hand, adrenaline partially displaced the ^3H -PAC binding (Fig. 1), revealing the occurrence of adrenaline-sensitive and insensitive sites (Fig. 1). According to the relative proportions, the affinities of two different sites discriminated by each competing drug are summarized in Table 1.

Fig. 1. Inhibition of ^3H -*p*-aminoclonidine (PAC) binding to rat kidney membranes by tizanidine (●), clonidine (○) and adrenaline (△). ^3H -PAC (1 nM) was incubated with various concentrations of competing drugs under the assay conditions described in Methods. The figure represents a single experiment for each drug, where each point is the mean of duplicate determinations.

Table 1. Inhibition of ^3H -*p*-aminoclonidine binding to rat renal membranes by competing drugs

Drug	n	Imidazoline-receptors K_i (nM)	α_2 -Receptors K_i (nM)	Ratio	% α_2
Tizanidine	4	4.17 ± 1.81	91.5 ± 9.1	0.046	71
Clonidine	4	9.20 ± 1.23	9.20 ± 1.23	1.0	— ^a
Oxymetazoline	3	2.28 ± 0.36	25.9 ± 5.5	0.088	70
Naphazoline	4	309 ± 26	2.13 ± 0.33	145.0	81
Adrenaline	4	> 10000	3.18 ± 0.41	> 3000	82

Ratio represents the K_i value for imidazoline-receptor divided by that for α_2 -adrenoceptor. % α_2 means the proportion of α_2 -adrenoceptors in the total specific binding sites. ^a: not discriminated.

DISCUSSION

The present study confirms the previous observations that ^3H -PAC binding sites of rat kidney membranes consist of two distinct populations (9). Both sites can be discriminated by their different affinities to adrenaline or imidazoline compounds. One type of site is α_2 -adrenoceptors which are sensitive to adrenaline and make up 70–80% of the total ^3H -PAC binding sites, while another population corresponds to imidazoline-receptors insensitive to adrenaline.

Tizanidine and oxymetazoline show higher affinity to imidazoline-receptors as compared with α_2 -adrenoceptor. On the other hand, clonidine cannot discriminate between the two receptor types, and naphazoline shows a high selectivity to α_2 -adrenoceptors. Therefore, tizanidine is the most selective ligand for the imidazoline-receptor among the compounds tested.

Wide distribution of imidazoline-receptors has been demonstrated in the central nervous system and the periphery (5–7). The physiological functions are still unclear, although the possible involvement of the receptors of the medulla oblongata in hypotensive response has been demonstrated. Recently, prejunctional inhibitory action through imidazoline-receptors has been found in the sympathetic nerve terminals of the rabbit pulmonary artery and thoracic aorta (10). Imidazoline-receptors are also highly sensitive to an endogenous clonidine-displacing substance (6, 9). These evidence suggest that imidazoline-receptor-selective drugs such as tizanidine may cause unique pharmacological actions in addition to their actions on α_2 -adrenoceptors (5).

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