Chloroethylclonidine Discriminates between α_{1A} - and α_{1B} -Adrenoceptors in the Presence of Guanosine 5'-Triphosphate in Rabbit Thoracic Aorta

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ABSTRACT—Studies on the displacement of [3 H]prazosin binding by the α_1 -agonist phenylephrine revealed the presence of at least high- and low-affinity binding sites in membrane preparations prepared from rabbit thoracic aorta. Although the low-affinity site was reduced by the pretreatment of tissues with chloroethylclonidine, this site was unaffected by the same pretreatment of membrane preparations that did not contain the GTP analog. However, in membrane preparations with the metabolically stable GTP analog GTP γ -S (10^{-5} M) and single cell preparations, the low-affinity site was completely eliminated by the chloroethylclonidine pretreatment. Displacement studies with the α_1 -antagonist WB4101 also revealed high- and low-affinity binding sites labeled by [3 H]prazosin. Displacement curves of WB4101 obtained from membrane preparations in the presence of GTP γ -S (10^{-5} M) did not differ from those in the absence of GTP γ -S. These results suggest that the low affinity phenylephrine binding site labeled by [3 H]prazosin was selectively bound by the chloroethylclonidine used to pretreat the tissues, membrane preparation containing GTP γ -S and single cells, and that chloroethylclonidine is able to recognize these two distinct subtypes of α_1 -adrenoceptors only when GTP γ -S is present.

Keywords: GTP γ -S, Chloroethylclonidine, Thoracic aorta (rabbit)

It is well known that chloroethylclonidine selectively alkylates α_{1B} -adrenoceptors, but has less effects on α_{1A} -receptors (1, 2). Chloroethylclonidine is used as a tool to distinguish between α_{1A} - and α_{1B} -adrenoceptors. Two distinct α_1 -adrenoceptor subtypes, α_{1A} and α_{1B} , were found to be involved in the norepinephrine-induced contraction of rabbit thoracic aorta (3-5). Recently, phenoxybenzamine was also found to interact preferably with the α_{1B} -subtype in the rabbit thoracic aorta (4). In the tissue and guanosine 5'-O-(3-thiotriphosphate) (GTP γ -S)-containing membrane fraction derived from the rabbit thoracic aorta, pretreatment with phenoxybenzamine eliminated the low-affinity phenylephrine binding sites (α_{1B}) labeled by [3 H]prazosin (6). The same treatment of the membrane preparations not containing GTP γ -S did not change the displacement curves. It was proposed that phenoxybenzamine interacts more selectively with the α_{1B} -subtype in the presence of guanosine 5'-triphosphate (GTP) or its analog but not in the absence of either of them, and GTP-binding regulatory proteins intracellularly modulate the

affinities or properties of α_1 -adrenoceptors (6).

Also, we previously proposed that there coexist propylbenzylcholine mustard (PrBCM)-sensitive and -resistant muscarinic cholinergic mechanisms in the guinea pig taenia caecum and longitudinal muscle of guinea pig ileum (7, 8). PrBCM can differentiate between PrBCM-sensitive and -resistant cholinergic receptors in the presence of guanine nucleotides such as GTP and GTP γ -S, but in their absence, PrBCM can not discern the two. In other words, one receptor mechanism is PrBCM-sensitive, irrespective of the presence or absence of the guanine nucleotide, and the other is PrBCM-resistant only when GTP or GTP γ -S is present (9).

In the present study, we determined if guanine nucleotide plays an essential role in the distinction between α_{1A} - and α_{1B} -adrenoceptors by chloroethylclonidine, since chloroethylclonidine, phenoxybenzamine and PrBCM have β -chloroethylamine as a common moiety in their chemical formulae.

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MATERIALS AND METHODS

Animals and experimental procedure

Male albino rabbits weighing 2.0–3.0 kg were stunned by a blow on the head and killed by bleeding from the neck. The thoracic aorta was quickly removed and dissected free of excess fat and connective tissue in oxygenated Krebs solution of the following composition: 118 mM NaCl, 1.2 mM MgCl₂, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 25 mM NaHCO₃ and 11.0 mM glucose dissolved in distilled water (pH 7.4 at 37°C). To determine the effects of chloroethylclonidine, an irreversible antagonist of α_1 -adrenoceptors, tissues were treated with chloroethylclonidine (10⁻⁴ M) for a total of 60 min; following the initial application, the antagonist was renewed every 10 min by washing the tissue with fresh Krebs solution containing 10⁻⁴ M chloroethylclonidine. Membrane preparations and single cell preparations were treated with chloroethylclonidine (10⁻⁴ M) for 30 min, and then these preparations were rinsed at least four times with 10 ml of fresh buffer.

Membrane preparations

Tissues were homogenized in 100 vol. of buffer (25 mM sucrose containing 10 mM Tris-HCl, pH 7.4) by a polytron (Kinematica, Lucerne, Switzerland) (setting 8, 15 sec $\times$ 2). The homogenates were filtered through four layers of cheesecloth and centrifuged at 5,000 $\times$ g for 20 min at 4°C. The supernatant was centrifuged at 100,000 $\times$ g for 60 min at 4°C. The pellets were resuspended in the same volume of assay buffer (Tris-HCl 50 mM, pH 7.4), incubated for 10 min at 37°C, and again centrifuged as above. The following procedures were carried out in ice-cold buffers. The final pellets were resuspended in assay buffer and used for the binding assay. Protein was measured by the method of Lowry et al. with bovine serum albumin as the standard (10).

Preparation of single cells

Single smooth muscle cells from rabbit thoracic aorta were prepared by the method of Momose and Gomi (11), with modifications. Slices of the tissue were incubated in a medium containing 0.2% collagenase (Amano), 0.05% elastase (Sigma Type IV) and 1.0% bovine serum albumin at 32°C for 60 min, followed by centrifugation at 1,000 $\times$ r.p.m. for 5 min. The pellets were resuspended in the incubation medium at 32°C for 15 min. Thereafter, the single cells could be readily separated from the muscle by gently pipetting the muscle strips in the same solution through a wide-pore Pasteur pipette. The suspension was filtered through nylon mesh. The viability of the single cells was assessed by the trypan blue exclusion test (12, 13). This involved the addition of 20 μ l of 0.4% trypan

blue solution into a Burkner-Turk hemocytometer. The total number of viable single smooth muscle cells was then determined. The single cells prepared from rabbit thoracic aorta had a viability of $91.25 \pm 1.61\%$.

Binding assay

The membrane preparations were incubated with 0.2 nM [³H]prazosin in the presence or absence of GTP γ -S (10 μ M) in 50 mM Tris buffer for 30 min at 32°C. The reaction was terminated by rapid filtration (Cell Harvester; Brandel Co., Gaithersburg, MD, USA) through Whatman GF/B glass fiber filters, and the filters were rinsed 3 times with 4 ml of ice-cold buffer. Membrane bound radioactivity was extracted from the filters overnight in scintillation fluid and the level determined by a liquid scintillation counter. Specific [³H]prazosin binding was determined experimentally from the difference between the counts in the absence and presence of 10 μ M phentolamine. All assays were conducted in duplicate. The apparent dissociation constant (K_D) and maximum binding site density (B_{max}) for [³H]prazosin were estimated by Scatchard analysis of the saturation data over a concentration range of 0.01 to 1.0 nM (14). The ability of phenylephrine to inhibit specific [³H]prazosin binding was expressed as the IC₅₀ value, which was the molar concentration of unlabeled drug necessary to displace 50% of the specific binding. Data obtained from competition studies were analyzed by the weighted least-squares iterative curve fitting program LIGAND (15). The data were first fitted to a one- and then a two-site model; and if the residual sums of squares were statistically less for a two-site fit of the data than for a one-site fit, as determined by an *F*-test comparison, then the two-site model was accepted. The value of the inhibition constant (K_i) was calculated from the equation $K_i = IC_{50}/(1 + L/K_D)$ (16), where *L* equals the concentration of [³H]prazosin used. The Hill coefficients for the saturation data of [³H]prazosin and inhibition by a drug were obtained by Hill plot analysis.

Statistical analyses

All numerical data are expressed as means $\pm$ S.E. with the number of observations. Values were considered significant when the *P* value was less than 0.05 by Student's *t*-test or Duncan's new multiple range test as appropriate.

Drugs

Drugs used were phenylephrine (Wako-Junyaku, Osaka); collagenase (Amano, Nagoya); elastase and guanosine 5'-3-*O*-(thio)triphosphate (GTP γ -S) (Sigma, St. Louis, MO, USA); WB4101 and chloroethylclonidine (Research Biochemicals Inc., Natick, MA, USA), all in powder form; and [³H]prazosin (specific activity 82 Ci/mmol; NEN, Boston, MA, USA). Other chemicals used were of analytical grade.

RESULTS

Effects of chloroethylclonidine on displacement of the binding of [3 H]prazosin by phenylephrine in membrane fractions

Specific binding of [3 H]prazosin (0.01–1.0 nM) in the membrane preparations was saturable, forming a plateau between 0.5 and 0.6 nM [3 H]prazosin. The Scatchard plot was linear, suggesting a single population of binding sites with a K_D value of 0.17 ± 0.05 nM and a $B_{\max}$ of 49.2 ± 1.64 fmol/mg protein. The inhibition curves for the agonist phenylephrine are shown in Fig. 1A. The Hill coefficient for the drug obtained from the Hill plot (Fig. 1B) was 0.28 ± 0.09 ($n=6$) for phenylephrine, suggesting that this agent interacts with high- and low-affinity sites

labeled by [3 H]prazosin. The K_i values obtained by computer analysis of the competition curves are shown in Table 1. The Hill coefficient was increased, and the low affinity site of the displacement curve was reduced by chloroethylclonidine (10^{-4} M) pretreatment of the tissues that contained sufficient activating factors such as GTP (Fig. 1). The pretreatment with 10^{-4} M chloroethylclonidine decreased the [3 H]prazosin specifically bound to approximately 42% of the control level determined in the absence of chloroethylclonidine.

Effects of GTP γ -S on the displacement [3 H]prazosin binding by phenylephrine in membrane fractions treated or untreated with chloroethylclonidine

Chloroethylclonidine (10^{-4} M) pretreatment of mem-

Fig. 1. Effects of chloroethylclonidine in tissue on phenylephrine displacement curves for [3 H]prazosin binding sites in microsomal membranes prepared from rabbit aorta. A, Inhibition of the binding of 0.2 nM [3 H]prazosin to the membrane prepared from thoracic aorta by phenylephrine. B, Pseudo-Hill plots obtained from their displacement curves. I, Inhibitory percentage of each point. ●, tissue not treated with chloroethylclonidine; ○, 10^{-4} M chloroethylclonidine-pretreated tissue. Data shown are the means $\pm$ S.E. of at least three separate experiments.

Table 1. Effects of GTP γ -S (10 μ M) on inhibition of the [3 H]prazosin binding by chloroethylclonidine in the membrane fractions of rabbit thoracic aorta

	n	pIC ₅₀	nH	High (α _{1A})		Low (α _{1B})		B _{max} Low (%)
				pK _i	B _{max} (fmol/mg protein)	pK _i	B _{max} (fmol/mg protein)	
Tissue								
Untreated	6	5.14±0.15	0.28±0.04*	5.55±0.22	10.07±1.25	4.46±0.18	38.56±1.09	78.38±2.22
CEC	4	5.09±0.10	0.74±0.08*	5.55±0.17	8.26±0.84	4.49±0.26	16.37±0.63 [†]	67.79±3.48 [#]
Membrane								
CEC	3	5.11±0.05	0.29±0.03*	5.83±0.29	8.50±1.40	4.23±0.39	38.77±1.80	80.78±3.74
CEC & GTPγ-S	3	5.21±0.31	1.02±0.02	5.65±0.31	13.42±1.93	—	—	0

Membrane preparations were incubated with 0.2 nM [3 H]prazosin and different concentrations of phenylephrine. The IC₅₀ value is the molar concentration of unlabeled drug necessary to displace 50% of the specific binding. pIC₅₀, negative logarithm of the IC₅₀ value. Negative logarithm of the K_i value (pK_i) and B_{max} for the high- and low-affinity binding sites were calculated from the IC₅₀ values according to Cheng and Prusoff (16). Percentage of low-affinity binding sites (B_{max} Low (%)) was calculated from the equation $B_{\max} \text{ Low (\%)} = B_{\max} \text{ Low} / (B_{\max} \text{ High} + B_{\max} \text{ Low}) \times 100$. n, number of experiments; nH, pseudo Hill coefficient; CEC, chloroethylclonidine treatment. The values represent means $\pm$ S.E. *: significant difference from unity ($P < 0.05$). † , $^{\#}$: significantly different from the value in untreated membranes at $P < 0.05$.

Fig. 2. Effects of chloroethylclonidine in the membrane preparation on phenylephrine displacement curves for [3 H]prazosin binding sites in microsomal membranes prepared from rabbit aorta. A, Inhibition of the binding of 0.2 nM [3 H]prazosin to the membrane prepared from thoracic aorta by phenylephrine. B, Pseudo-Hill plots obtained from their displacement curves. I, Inhibitory percentage of each point. ●, membrane preparation not treated with chloroethylclonidine; ○, 10^{-4} M chloroethylclonidine-pretreated membrane preparation. Data shown are the means $\pm$ S.E. of at least three separate experiments.

Fig. 3. Effect of chloroethylclonidine on phenylephrine displacement curves for [3 H]prazosin binding sites in microsomal membranes prepared from rabbit aorta, where GTP γ -S was added. A, Inhibition of the binding of 0.2 nM [3 H]prazosin to the membrane prepared from thoracic aorta by phenylephrine. B, Pseudo-Hill plots obtained from their displacement curves. I, Inhibitory percentage of each point. ●, 10^{-5} M GTP γ -S; ○, 10^{-4} M chloroethylclonidine-pretreated membrane preparation with 10^{-5} M GTP γ -S. Data shown are the means $\pm$ S.E. of at least three separate experiments.

Fig. 4. Effect of chloroethylclonidine on phenylephrine displacement curves for [3 H]prazosin binding sites in single cells prepared from rabbit aorta. A, Inhibition by phenylephrine of the binding of 0.2 nM [3 H]prazosin to the single cells prepared from thoracic aorta. B, Pseudo-Hill plots obtained from their displacement curves. I, Inhibitory percentage of each point. ●, single cells not treated with chloroethylclonidine; ○, 10^{-4} M chloroethylclonidine-pretreated single cells. Data shown are the means $\pm$ S.E. of at least three separate experiments.

brane preparations that did not contain activating substances such as GTP had no effect on the displacement curve of phenylephrine, and the value of the Hill coefficient was the same as that obtained in the preparations not treated with chloroethylclonidine (Fig. 2). Effects of GTP γ -S on the displacement curve of phenylephrine were determined. In the chloroethylclonidine-pretreated membrane preparations to which GTP γ -S (10 μ M) had been added, however, the Hill coefficient for the displacement curve was not different from unity, and the low-affinity sites of the curve were completely eliminated (Fig. 3, Table 1). In 10 μ M GTP γ -S-containing membrane fraction, the pretreatment with chloroethylclonidine decreased the [3 H]prazosin specifically bound to approximately 31% of the control level determined in the absence of chloroethylclonidine. However, the same treatment of the membrane preparation not containing GTP γ -S did not change the amount of specific binding so that it remained the same as that of the untreated preparation.

Effect of chloroethylclonidine on inhibition of the binding of [3 H]prazosin by phenylephrine in single cells

Furthermore, in single cells prepared from rabbit thoracic aorta, which retain receptor-second messenger systems such as intracellular receptor-G protein coupling in the intact condition, for which the inhibition curves for phenylephrine are shown in Fig. 4A, the Hill coefficient was significantly different from unity ($nH=0.39\pm0.14$), suggesting that phenylephrine interacted with both the high- and low-affinity sites in this preparation. Pretreatment of the single cells with 10^{-4} M chloroethylclonidine selectively eliminated the low affinity sites for phenylephrine (Fig. 4 and Table 2), and it decreased the specifically bound [3 H]prazosin to approximately 26% of the control level determined in the absence of chloroethylclonidine.

Inhibition curves of the binding of [3 H]prazosin to membrane preparations by the competitive inhibitor WB4101

The inhibition curves for the competitive inhibitor

Table 2. Effects of chloroethylclonidine on [3 H]prazosin binding in single cells prepared from rabbit thoracic aorta

	n	pIC ₅₀	nH	High (α_{1A})		Low (α_{1B})		B _{max} Low (%)
				pK _i	(10 ⁻⁴ B _{max} fmol/cell)	pK _i	(10 ⁻⁴ B _{max} fmol/cell)	
Single cell								
Untreated	3	5.41 $\pm$ 0.18	0.39 $\pm$ 0.14*	5.82 $\pm$ 0.16	0.98 $\pm$ 0.43	4.59 $\pm$ 0.19	2.43 $\pm$ 0.43	71.30 $\pm$ 12.6
CEC	3	5.58 $\pm$ 0.24	1.04 $\pm$ 0.28	5.91 $\pm$ 0.24	0.89 $\pm$ 0.18	—	—	0

Single cells were incubated with 0.2 nM [3 H]prazosin and different concentrations of phenylephrine. The IC₅₀ value is the molar concentration of unlabeled drug necessary to displace 50% of the specific binding. pIC₅₀, negative logarithm of the IC₅₀ value. Negative logarithm of the K_i value (pK_i) and B_{max} for the high- and low-affinity binding sites were calculated from the IC₅₀ values according to Cheng and Prusoff (16). Percentage of low-affinity binding sites (B_{max} Low (%)) was calculated from the equation B_{max} Low (%) = B_{max} Low / (B_{max} High + B_{max} Low) $\times$ 100. n, number of experiments; nH, pseudo Hill coefficient; CEC, chloroethylclonidine treatment. The values represent means $\pm$ S.E. *: significant difference from unity (P < 0.05).

Fig. 5. Displacement curves of WB4101 for [3 H]prazosin binding sites in microsomal membranes prepared from rabbit aorta in the absence (●) and the presence (○) of GTP γ -S. A, Inhibition by WB4101 of the binding of 0.2 nM [3 H]prazosin to the membrane prepared from thoracic aorta. B, Pseudo-Hill plots obtained from their displacement curves. I, Inhibitory percentage of each point. Data shown are the means of triplicate determinations in representative experiments.

Table 3. Inhibition of the [3 H]prazosin binding by WB4101 in the membrane fractions of rabbit thoracic aorta in the absence and the presence of GTP γ -S (10 μ M)

	n	pIC ₅₀	nH	High (α_{1A})		Low (α_{1B})		B _{max} Low (%)
				pK _i	B _{max} (fmol/mg protein)	pK _i	B _{max} (fmol/mg protein)	
WB4101	3	8.13 $\pm$ 0.13	0.62 $\pm$ 0.02*	9.04 $\pm$ 0.44	8.48 $\pm$ 1.13	7.43 $\pm$ 0.29	45.20 $\pm$ 1.13	87.20 $\pm$ 2.10
GTP γ -S & WB4101	3	8.07 $\pm$ 0.14	0.60 $\pm$ 0.01*	9.31 $\pm$ 0.67	8.59 $\pm$ 2.17	7.50 $\pm$ 0.20	42.82 $\pm$ 2.17	83.47 $\pm$ 4.23

Membrane preparations were incubated with 0.2 nM [3 H]prazosin and different concentrations of WB4101. The IC₅₀ value is the molar concentration of unlabeled drug necessary to displace 50% of the specific binding. pIC₅₀, negative logarithm of the IC₅₀ value. Negative logarithm of the K_i value (pK_i) and B_{max} for the high- and low-affinity binding sites were calculated from the IC₅₀ values according to Cheng and Prusoff (16). Percentage of low-affinity binding sites (B_{max} Low (%)) was calculated from the equation B_{max} Low (%) = B_{max} Low / (B_{max} High + B_{max} Low) $\times$ 100. n, number of experiments; nH, pseudo Hill coefficient; GTP γ -S, 10 μ M GTP γ -S. The values represent means $\pm$ S.E. *: significant difference from unity (P < 0.05).

WB4101 in the membrane fractions of rabbit thoracic aorta in the absence and the presence of GTP γ -S (10 μ M) are shown in Fig. 5A. The Hill coefficient for the drug obtained from the Hill plot (Fig. 5B) was 0.62 $\pm$ 0.02 for WB4101, suggesting that this agent interacts with both the high- and low-affinity sites labeled by [3 H]prazosin. The K_i values and maximum binding sites (B_{max} values) obtained by computer analysis of the competition curves are shown in Table 3; The Hill coefficient, pK_i values and B_{max} values obtained from the membrane preparation in the presence of GTP γ -S did not differ significantly from those obtained in the absence of GTP γ -S. In the GTP γ -S containing membrane fraction, the pretreatment with chloroethylclonidine completely eliminated the low-affinity sites of the curve, and the level of [3 H]prazosin specifically bound was reduced to approximately 28% of the control level determined without chloroethylclonidine pretreatment (data not shown).

DISCUSSION

α_1 -Adrenoceptors in membrane prepared from rabbit thoracic aorta were selectively labeled by [3 H]prazosin, and then the labeled receptors were displaced by the α_1 -agonist phenylephrine. The computer analysis of the displacement curve showed the presence of high-affinity and low-affinity sites (Fig. 1A, Table 1). The low-affinity sites were selectively eliminated by the pretreatment with chloroethylclonidine of the tissue preparation, and the displacement curves obtained from the chloroethylclonidine-pretreated membrane in the absence of GTP γ -S did not differ from those obtained from the untreated preparations. However, in membrane preparations containing GTP γ -S, the low-affinity sites were completely eliminated by chloroethylclonidine pretreatment. Furthermore, in single cell preparations, the pretreatment with chloroethylclonidine also eliminated the low-affinity sites for phenylephrine. These results show that phenylephrine interacts

with at least two different α_1 -adrenoceptor binding sites in the membrane preparations and single cell preparations from rabbit thoracic aorta. The low-affinity sites are α_{1B} -subtypes and the other are α_{1A} -subtypes, because chloroethylclonidine selectively inactivated the low-affinity sites for phenylephrine. There were no differences between the drug-receptor interactions of [3 H]prazosin in the membrane preparations and single cell preparations.

Johnson et al. (17) reported that the α_1 -adrenergic agonist phenylephrine stimulated GTP γ -S binding to G_s and G_i in rat aortic membranes, and the degree of stimulation was reduced in desensitized vessels. It is known that the guanine nucleotide-induced conversion of drug binding sites was typically from the high-affinity to the low affinity state. The alterations in binding states in the presence of guanine nucleotides reflect an interaction between the receptors and G-protein. In the presence of a high concentration of GTP γ -S or GTP, the phenylephrine competition curve was shifted to the right (18). In the present studies, a low concentration of GTP γ -S (10 μ M) did not affect the phenylephrine competition curve, and the irreversible antagonist chloroethylclonidine selectively eliminated the low-affinity sites of α_1 -adrenoceptors in the presence of GTP γ -S or GTP and in the single cell preparations. Kokubu et al. (6) reported that in the tissue and GTP γ -S-containing membrane fraction derived from rabbit thoracic aorta, the pretreatment with the irreversible antagonist phenoxybenzamine selectively eliminated the low-affinity sites, but the same treatment of the membrane preparation not containing GTP γ -S did not affect the low-affinity sites of the displacement curves. They proposed that phenoxybenzamine interacts more selectively with the α_{1B} -subtype in the presence of GTP or its analog and in the single cell preparations, but not in the absence of either of them. Their findings support the present results that chloroethylclonidine selectively eliminated the α_{1B} -adrenoceptor subtype in the presence of GTP γ -S or GTP. Our findings obtained from the present study sug-

gest that GTP-related regulatory proteins intracellularly modulate the affinities or properties of α_1 -adrenoceptors, and they also show that GTP plays an important role in the specificity of α_1 -adrenoceptor subtypes and that GTP γ -S causes some populations of the receptor sites to become sensitive to chloroethylclonidine.

The competitive antagonist WB4101 discriminates between two subtypes (α_{1A} and α_{1B}) (19). The high-affinity site for WB4101 is an α_{1A} -subtype and the low-affinity site is an α_{1B} -subtype eliminated irreversibly by chloroethylclonidine pretreatment, while the high-affinity site is unaffected by chloroethylclonidine. In contractile experiments with thoracic aorta strips, Takayanagi et al. (4) reported that WB4101 is a competitive antagonist of the two sites for the full agonist phenylephrine. Morrow and Creese (20) and Kinami et al. (21) also reported that WB4101 clearly distinguishes between the two different binding sites in membrane preparations. These findings demonstrate that WB4101 acts as a competitive antagonist of a drug-receptor interaction with different affinities toward two distinct α_1 -adrenoceptors (α_{1A} and α_{1B}). In the present experiments, the competitive binding studies with WB4101 also revealed the high- and low-affinity binding sites labeled by [3 H]prazosin (Fig. 5 and Table 3), and the displacement curves of WB4101 obtained from membrane preparations containing GTP γ -S did not differ from those in the absence of GTP γ -S. These findings suggest that mechanisms of the inhibitory action, including the G-protein, are different between β -haloethylamines and competitive inhibitors. The present studies indicate that rabbit thoracic aorta contains two distinct α_1 -adrenoceptor subtypes, and one of them, the α_{1B} -subtype, mainly couples with the G-protein. Although further studies on the molecular basis of the drug-receptor interaction and its modulation by guanine nucleotides are needed, our experiments illustrate a pharmacological approach of general applicability for resolving the mechanisms involved in the discrimination of receptors in tissues containing heterologous populations of receptor subtypes.

In conclusion, chloroethylclonidine distinguishes between α_{1A} - and α_{1B} -adrenoceptors only when GTP γ -S is present.

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