Characterization of Adrenergic Receptors in Membranes from the Rat Seminal Vesicle

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Received August 5, 1992 Accepted November 10, 1992

ABSTRACT—Characterization of adrenergic receptors in membranes from the rat seminal vesicle was studied by radioligand binding assay. Seminal vesicle membranes contained saturable and high affinity binding sites for the β -adrenergic receptor antagonist ^3H -dihydroalprenolol (^3H -DHA) and for the α -adrenergic antagonist ^3H -prazosin. The observed order of potency for adrenergic agonists in competing for the ^3H -DHA binding sites: isoproterenol > epinephrine $\approx$ salbutamol > norepinephrine indicates that these membrane receptors have the properties of β_2 -adrenergic receptors. α_1 -Adrenergic receptors were defined mainly as α_{1A} subtypes by demonstrating their insensitivity to pretreatment with chlorethylclonidine and the different rank orders of antagonist affinities. No significant binding sites for the α_2 -adrenergic receptor agonist ^3H -clonidine were observed. The GTP-induced reduction in the affinity of α_1 -adrenergic receptors for epinephrine was significantly reversed by the muscarinic cholinergic agonist carbachol. Atropine effectively antagonized this effect of carbachol on the competitive inhibition of ^3H -prazosin binding by epinephrine in the presence of GTP, which suggests that muscarinic cholinergic receptors regulate the affinity of α_1 -adrenergic receptors by modulating the effect of guanine nucleotides. The effect of GTP on decreasing the affinity of β_2 -adrenergic receptors was not influenced by the addition of carbachol.

Keywords: Adrenergic receptor, Seminal vesicle, Cholinergic regulation

Several reports have suggested the presence of α -adrenergic receptors in the rat seminal vesicle (1, 2). Adeneken (3) has reported evidence for α_1 -adrenergic receptor predominance in the rat seminal vesicle. A previous study (4) demonstrated that rat seminal vesicle membranes contained α_1 - and β -adrenergic receptors regulated inversely by androgens.

In rat ventral prostate membrane preparations, β -adrenergic agonists stimulated adenylate cyclase activity with the observed properties characteristic of a β_2 -subtype receptor (5–7). As for α_1 -adrenergic receptors, it has become increasingly clear that there are at least two pharmacologically distinct subtypes of α_1 -adrenergic receptors: α_{1A} -subtypes that are not inactivated by the site-directed alkylating agent chlorethylclonidine (CEC) and α_{1B} -subpopulations that are sensitive to CEC (8–11). In the present study, I investigated the properties of α - and β -adrenergic receptors of rat seminal vesicle membranes and the regulation of adrenergic receptors by guanine nucleotides and muscarinic cholinergic agonists.

MATERIALS AND METHODS

Seminal vesicles from male Wistar rats weighing 300 to 350 g (Nippon Rats Co., Ltd.) were homogenized in 20 mM Tris-HCl buffer, pH 7.4, containing 0.25 mM sucrose, 4 mM MgCl_2 , 1 mM EDTA and 3.25 mM 2-mercaptoethanol by a Potter-Elvehjen homogenizer. The homogenate was centrifuged at $600 \times g$ for 10 min. The supernatant was centrifuged at $20,000 \times g$ for 20 min. Crude membrane preparations were incubated for 20 min with or without 100 μM CEC and washed three times. The membrane preparations were assayed for β -adrenergic receptor binding with ^3H -dihydroalprenolol (^3H -DHA, 52.3 Ci/mmol; New England Nuclear, Boston, MA, USA), as previously described (12–15). Membranes (0.2–0.3 mg of protein) were incubated with ^3H -DHA (1–20 nM) in a buffer (40 mM Tris-HCl, pH 7.4, 10 mM MgCl_2) for 10 min at 37°C. The α_1 - and α_2 -adrenergic receptor binding study was performed with ^3H -prazosin (0.1–5.0 nM, 30 Ci/mmol, New England Nuclear, Boston, MA, USA) or ^3H -clonidine (0.5–5.0 nM,

28.3 Ci/mmol, New England Nuclear, Boston, MA, USA), respectively. Incubations of the membranes (0.1–0.2 mg of protein) with ^3H -prazosin or ^3H -clonidine in 40 mM Tris-HCl buffer, pH 7.4, containing 10 mM MgCl_2 , 1 mM sodium *l*-ascorbate and 1 mM pyrocatechol were carried out for 30 min at 37°C, as previously described (4, 16, 17). For the experiments of CEC pretreatment, incubations of the membranes with or without 100 μM CEC for 20 min were followed by washing with the medium, 3 times, before the binding assay. Incubations for the binding assay were terminated by a rapid dilution of the assay mixture, followed by filtration through Whatman GF/B glass fiber filters. Specific binding to receptors, defined as the difference between the radioactivity bound in the presence or absence of 20 μM propranolol, 10 μM phentolamine or 5 μM clonidine, was approximately 65 to 75% of the total binding. The concentrations of protein were measured by the method of Lowry et al. (18).

The drugs used were: *l*-propranolol (Japan ICI Pharma), phentolamine (Japan Ciba Geigy), *l*-isoproterenol (Nikken Chemicals), salbutamol (Sankyo Pharmaceutical), *l*-epinephrine (Dainippon Seiyaku), norepinephrine (Wako Chemicals), carbachol (Sigma Chemical), chlor-ethylclonidine (CEC, Research Biochemicals), atropine (Sigma Chemical Co.), 2,2,6-dimethoxy-phenoxyethyl-aminomethyl-1,4-benzodioxane (WB4101, Amersham) and dihydroergocryptine (DHE, Sandoz).

RESULTS

The saturable and specific binding of ^3H -DHA to seminal vesicle membranes was demonstrated by the linearity of the Scatchard analysis, indicating a single class of receptors, and the number of binding sites was calculated to be 93.8 ± 1.8 fmol/mg protein with an apparent affinity, K_d , of 6.3 ± 0.3 nM (Fig. 1). The agonists competed for the receptor binding sites in the following order of potency: *l*-isoproterenol > epinephrine > salbutamol > *l*-norepinephrine; graphical estimation of the concentration resulting in half maximal inhibition gave IC_{50} values for isoproterenol, epinephrine, salbutamol and norepinephrine of 0.01, 0.61, 0.82, and 2.10 μM , respectively (Fig. 2). Affinity of the β -adrenergic receptor for isoproterenol was decreased by the guanine nucleotide (GTP, 50 μM), as demonstrated by an increase of more than one order of magnitude in the IC_{50} value for isoproterenol in the presence of GTP (Fig. 3). Addition of the cholinergic agonist carbachol (10 μM) had no effect on the decreased affinity of the β -adrenergic receptor for isoproterenol by the guanine nucleotide (Fig. 3).

The binding of ^3H -prazosin, α_1 -adrenergic antagonist, to seminal vesicle membranes was also to a single, saturable population of sites, with a linear Scatchard plot, and the number of binding sites was 46.8 ± 0.3 fmol/mg protein with a high affinity K_d of 0.36 ± 0.01 nM (Fig. 4). Pretreatments with 100 μM CEC for 20 min caused no significant decrease in the density of specific ^3H -prazosin binding sites in seminal vesicle membranes (Fig. 5).

Fig. 1. Representative saturation and Scatchard analysis of ^3H -dihydroalprenolol (^3H -DHA) binding to the membrane fraction of the seminal vesicle. Crude membranes were incubated with various concentrations (1–20 nM) of ^3H -DHA. Specific binding was determined as described in Materials and Methods. Each value plotted is the mean of four determinations.

Fig. 2. Displacement curves of ^3H -dihydroalprenolol (^3H -DHA) binding to the membrane fraction of the seminal vesicle by various β -adrenergic agonists. Membranes of the seminal vesicle were incubated with 5 nM ^3H -DHA and increasing concentrations of the indicated adrenergic agonists (● isoproterenol, ○ epinephrine, △ salbutamol, □ norepinephrine) for 10 min. Results are expressed as a percent of the amount bound in the absence of catecholamines. Each value is the mean of four determinations.

Fig. 3. Isoproterenol competition for ^3H -dihydroalprenolol (^3H -DHA) binding sites. Membranes of the seminal vesicle were incubated with 5 nM ^3H -DHA in the presence of the indicated concentrations of isoproterenol with (●) or without (○) 50 μM GTP and in the presence of 10 μM carbachol (△) for 10 min. Maximum specific binding to membranes in the absence of isoproterenol was taken as 100%. Values shown represent the means of four determinations.

Figure 6 shows the competition curves for the antagonists with a rank order of affinity of WB4101 > phentolamine > DHE.

Guanine nucleotide (GTP, 100 μM) significantly decreased the affinity of α_1 -adrenergic receptors for epinephrine, causing an increase of more than one order of magnitude in the IC_{50} value for epinephrine in the presence of GTP (Fig. 7). The GTP-induced reduction in the affinity of α_1 -adrenergic receptors for epinephrine was

Fig. 4. Representative saturation and Scatchard analysis of ^3H -prazosin binding to the membrane fractions of the seminal vesicle. Crude membranes were incubated with various concentrations (0.1–5 nM) of ^3H -prazosin. Specific binding was determined as described in Materials and Methods. Each value plotted is the mean of four determinations.

Fig. 5. Effects of chlorethylclonidine (CEC) pretreatment on ^3H -prazosin binding sites in membranes of the seminal vesicle. Membrane preparations were incubated for 20 min with (●) or without (○) 100 μM CEC and washed three times. ^3H -Prazosin binding was determined as described in Materials and Methods. Each value is the mean of four determinations.

significantly reversed by addition of carbachol (10 μM) (Fig. 7). Carbachol had no effect on the specific ^3H -prazosin binding either alone or in the presence of GTP (data

Fig. 6. Displacement curves of the binding of ^3H -prazosin to the membrane fraction of the seminal vesicle by various α -adrenergic antagonists. Membranes of the seminal vesicle were incubated with 1 nM ^3H -prazosin and increasing concentrations of the indicated α -adrenergic antagonists (● WB4101, ○ phentolamine, △ dihydroergocryptine) for 10 min. Results are expressed as a percent of the amount bound in the absence of antagonists. Each value is the mean of four determinations.

Fig. 7. Epinephrine competition for ^3H -prazosin binding sites. Membranes of the seminal vesicle were incubated with 1 nM ^3H -prazosin in the presence of the indicated concentrations of epinephrine with (●) or without (○) 50 μM GTP and in the presence or absence of (△) 10 μM carbachol and (□) 0.1 μM atropine for 10 min. Maximum specific binding to membranes in the absence of epinephrine was taken as 100%. Values shown represent the means of four determinations.

not shown). Atropine, an antagonist for muscarinic cholinergic agonists, effectively reversed the effects of carbachol on the competitive inhibition of ^3H -prazosin binding by epinephrine in the presence of GTP (Fig. 7). No significant binding sites for ^3H -clonidine were observed (data not shown).

DISCUSSION

The observed order of potency for agonist in competing for the β -adrenergic binding sites was *l*-isoproterenol > epinephrine $\approx$ salbutamol > *l*-norepinephrine, indicating that these receptors have properties characteristic of a typical β_2 -adrenergic receptor system. Guanine nucleotides have been reported to modulate receptor binding function (19–22). Membranes from seminal vesicles responded to GTP with decreased β -adrenergic receptor-ligand affinity. Carbachol, a muscarinic cholinergic agonist, had no effect on the GTP regulation. Membranes from the rat seminal vesicle showed saturable and high affinity binding sites for the α_1 -adrenergic receptor antagonist, confirming the previous study (4). No specific binding for the α_2 -adrenergic receptor agonist ^3H -clonidine was observed (data not shown), indicating a predominance of α_1 -adrenergic receptors in the rat seminal vesicle (3). Guanine nucleotides have been demonstrated to decrease the affinity of α_1 - and α_2 -adrenergic receptors for epinephrine (23, 24). The effect of GTP on decreasing the affinity of the α_1 -adrenergic receptor bind-

ing was apparent in rat seminal vesicle membranes. The effect of GTP reducing the affinity of α_1 -adrenergic receptors for epinephrine was reversed by addition of carbachol, and atropine, a muscarinic cholinergic receptor antagonist, effectively antagonized this effect of carbachol on the competitive inhibition of ^3H -prazosin by epinephrine in the presence of GTP, suggesting a mediation by muscarinic cholinergic receptors in the regulatory components of guanine nucleotides for α_1 -adrenergic receptors. The presence of muscarinic cholinergic receptor in the rat seminal vesicle has been suggested by the contractile response of the organ to muscarinic cholinergic agonists (25).

Yamada et al. (26) have reported a muscarinic cholinergic agonist regulating the affinity of cardiac α_1 -adrenergic receptors by modulating the effect of guanine nucleotides.

Recently, the irreversible alkylating agent, CEC, a reactive derivative of clonidine has been reported to inactivate only a subpopulation of α_1 -adrenergic receptor binding sites with the characteristics of the CEC-sensitive subtype (α_{1B}) (8–10). The distribution of α_1 -adrenergic receptors of the CEC-insensitive α_{1A} -subtype and CEC-sensitive α_{1B} -subtype has been reported in various tissues such as hepatocytes (9, 11, 27–29), renal cells (11), thyroid cells (30, 31) brain (8, 9, 28, 32), aorta (27, 29), spleen and vas deferens (9). More recently, Lomansney et al. (33) have stated that the α_{1A} -subtype in COS-7 cell membranes appears to be less sensitive to inactivation by CEC (~15%) than the α_{1B} (70%) and α_{1C} (80%) subtypes. Pretreatment of membranes with 10 μM CEC had no effect on ^3H -prazosin binding, suggesting that α_1 -adrenergic receptors of rat seminal vesicles are mostly of the α_{1A} -subtype. Moreover, the types of ^3H -prazosin binding in this assay exhibited the pharmacological profile of antagonists, $\text{WB4101} > \text{phentolamine} > \text{DHE}$, which were against the α_{1A} -subtype of adrenergic receptor (32).

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