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PRELIMINARY COMMUNICATIONS

SOLUBILIZATION OF ACTIVE ALPHA-2 ADRENERGIC RECEPTOR FROM RAT BRAIN: REGULATION BY CATIONS AND GTP

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The regulation of α_2-adrenergic receptor binding by GTP and cations (Mn^{2+} or Na^+) in membrane-bound receptor preparations has been studied (1,2). Several authors have attempted to obtain the α_2-receptor in a soluble form to elucidate the mechanisms of receptor regulation at the molecular level. Human platelet α_2-receptor has been solubilized and used as a model system (3,4), but there has apparently been no report on the regulation of solubilized α_2-receptor in the brain. In this communication, we describe the first successful solubilization, using 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPS) (5) as detergent, and partial characterization of solubilized α_2-adrenergic receptor in rat brain. We also examined the regulation of solubilized receptor by Mn^{2+}, Na^+ and GTP.

MATERIALS AND METHODS

Chemicals. The sources of chemicals used were: [^{3}H]clonidine (66.8 Ci/mmole) from New England Nuclear, clonidine-HCl from Tokyo Kasei, GTP and polyethylenimine (PEI) from Sigma, and CHAPS from Calbiochem. All other chemicals used were analytical grade.

Brain membrane preparation and solubilization of α_2-receptor. Whole brain (w...us cerebellum) of male Wistar rat was homogenized in 20 vol. of ice-cold 50 mM Tris-HCl buffer (pH 7.4) and then centrifuged at 50,000 g for 10 min. The pellet was suspended in the same buffer and recentrifuged at 50,000 g for 10 min. The final pellet (crude brain membranes) was stored at -80°. Frozen brain membranes were thawed and homogenized in 4 vol. of ice-cold Tris-buffer (pH 7.4). CHAPS was added to a final concentration of 6 mM. The suspension was gently homogenized and stirred for an additional 20 min at 4°.

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Then the suspension was centrifuged at 100,000 g for 60 min. The supernatant fraction was saved, and the pellet was extracted a second time with CHAPS by the same procedure. The first and second extracts were combined. This solution was denoted the solubilized fraction and was used for binding assays.

[^{3}H]Clonidine binding assay. [^{3}H]Clonidine binding was measured by the method of U'Prichard et al. (6). Crude membranes (0.3 to 0.7 mg protein) or solubilized fractions (0.3 to 0.5 mg protein) were incubated with [^{3}H]clonidine at 25° for 30 min in 1 ml of Tris-HCl buffer (pH 7.4) in the presence or absence of 10 μM clonidine. Incubations were terminated by vacuum filtration through Whatman GF/B glass filters, which were treated with 0.3% PEI as described in Ref. 7. The filters were washed three times with 5 ml of cold Tris-HCl buffer (pH 7.4) and dried; then the retained radioactivities were measured at 44% efficiency. Specific [^{3}H]clonidine binding was defined as the radioactivity bound after subtraction of the non-specific binding assayed in the presence of 10 μM clonidine.

Other methods. Protein was measured by the method of Lowry et al. (8); bovine serum albumin was used as standard. Student's t-test was used for statistical analysis. Scatchard plots were analyzed using nonlinear least-squares computer programs (9).

RESULTS AND DISCUSSION

Solubilization of [^{3}H]clonidine binding sites from rat brain. In this study, we demonstrate the first successful solubilization of rat brain α_2-adrenergic receptors. Preservation of binding properties in solubilized receptors appeared to depend on the choice of an appropriate detergent. Among the detergents we tested (Triton X-100, cholate, deoxycholate, chenodeoxycholate and digitonin) (0.125 to 2%), CHAPS (5) was the most effective in solubilizing [^{3}H]clonidine binding sites. Selection of an appropriate technique for measuring the binding to solubilized receptors was also critical. For instance, in our laboratory, binding assays that use conventional techniques, such as Sephadex column gel filtration, charcoal binding and the ammonium sulfate precipitation method (10), were unsuccessful. In addition, the polyethylene glycol precipitation method (11) was relatively less effective than the filtration method using PEI-treated GF/B filters (7). Therefore, we adopted this filtration method to measure [^{3}H]clonidine binding in solubilized preparations. About 40% of the original [^{3}H]clonidine binding sites in the membranes were solubilized with 6 mM CHAPS. This value was relatively lower than that reported for human platelet α_2-receptors, where about 85% of the original binding sites were solubilized by digitonin (3).

Properties of the solubilized [^{3}H]clonidine binding sites. Specific [^{3}H]clonidine binding in CHAPS-solubilized preparations was saturable after incubation for 30 min at 25°, as previously reported for membrane-bound α_2-receptors (6). The pH optima for [^{3}H]clonidine binding in solubilized and membrane preparations were around pH 7.4 and 7.6 respectively. In a typical experiment with 1 nM [^{3}H]clonidine, total binding was about 1470 cpm, and non-specific binding measured in the presence of 10 μM clonidine was about 100 cpm. We examined the inhibition of 1 nM [^{3}H]clonidine binding by adrenergic drugs in soluble and membrane preparations. In both preparations, the most potent agent was clonidine itself (IC_{50} = 3.5 nM for soluble, IC_{50} = 5.0 nM for membrane), and the order of agonist potencies was: clonidine > epinephrine > norepinephrine >> isoproterenol.

The order of antagonist potencies was: yohimbine >>> prazosin. These results agree with the competition studies on rat brain α_2-adrenergic receptors (6,12) and indicate that the solubilized [^{3}H]clonidine binding sites had the characteristics of α_2-adrenergic receptors.

Scatchard plots of [^{3}H]clonidine binding (0.25 to 16 nM) to both solubilized and membrane-bound α_2-receptors were curvilinear, indicating the existence of two distinct binding components, as previously reported (12). The results are summarized in Table 1, assuming a two-site model. There was no difference between the solubilized and the membrane-bound α_2-receptors. These results also indicate that the solubilized α_2-receptors retained the characteristics of the membrane-bound α_2-receptors.

Table 1. Scatchard analysis of [^{3}H]clonidine binding to membrane-bound and CHAPS-solubilized α_2-adrenergic receptors in rat brain*

Receptor preparation	KH (nM)	RH (fmoles/mg protein)	KL (nM)	RL (fmoles/mg protein)
Membrane-bound	1.42 ± 0.11	48.7 ± 4.0	56.3 ± 4.4	187 ± 10.0
CHAPS-solubilized	0.97 ± 0.07	35.1 ± 6.7	60.9 ± 4.0	195 ± 5.5

* Curvilinear Scatchard plots (0.25 to 16 nM [^{3}H]clonidine) were analyzed by non-linear least-squares computer programs. Data are the mean ± S.E. of three independent experiments, each of which was carried out in triplicate. There was no significant difference between membrane-bound and CHAPS-solubilized receptor preparations ($P < 0.05$). Abbreviations: KH, affinity for high-affinity component; KL, affinity for low-affinity component; RH, number of the high-affinity component; and RL, number of the low-affinity component.

Effects of GTP, Na^+ and Mn^{2+} on solubilized α_2-receptor binding. Data are summarized in Table 2. Solubilized receptors retained sensitivity to GTP. At concentrations of 1-5 μM, GTP inhibited [^{3}H]clonidine binding to both solubilized and membrane preparations by about 50%. These results indicate that the receptor-GTP binding protein complex remained intact throughout the solubilization. Na^+ (10-100 mM), a representative monovalent cation, inhibited [^{3}H]clonidine binding in both preparations. In the membrane preparations, Mn^{2+}, a representative divalent cation, enhanced [^{3}H]clonidine binding, as previously reported (1,2). If the divalent cations exert their effects through interaction with the GTP binding protein (1,2,13), effects on solubilized α_2-receptors would be expected. However, no enhancement was observed. These results are similar to those described for solubilized adenosine receptors (14). Recently, GTP binding protein was reported to consist of several subunits (α and β) (13). If distinct subunits of the GTP binding protein were to bind cations and GTP (13), it is possible that our solubilized preparations retained the GTP binding, but not the divalent cation binding, subunits.

This work should provide the basis for further studies, such as purification of α_2-adrenergic receptors in the brain.

Table 2. Effects of various drugs on [^{3}H]clonidine binding to membrane-bound and solubilized α_2-adrenergic receptors in rat brain*

Drugs	Binding at molar concentrations (% of control)						
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
	Membrane-bound receptors						
GTP	94	72	28	26			
$MnCl_2$	98	103	150	165	169		
NaCl				98	90	73	27
	Solubilized receptors						
GTP	99	93	37	26			
$MnCl_2$	101	103	96	87	57		
NaCl				100	99	65	34

* Data are the percentage of control specific 1 nM [^{3}H]clonidine binding in the presence of various concentrations of drugs. Values are the means of three or more determinations, each in triplicate, which varied by less than 10%. Control binding in both preparations was about 26 fmoles/mg protein.

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