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SHORT COMMUNICATION

Agonist-Specific Alterations in Receptor Binding Affinity Associated with Solubilization of Turkey Erythrocyte Membrane *Beta* Adrenergic Receptors

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SUMMARY

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The *beta* adrenergic receptor binding sites of turkey erythrocyte membranes, identified by (−)-[^{3}H]dihydroalprenolol binding, were solubilized with digitonin. The binding sites in particulate and soluble preparations displayed appropriate characteristics of $beta_1$ adrenergic specificity, stereospecificity, high affinity and saturability. The affinity of all agonists tested was significantly (2-50-fold) greater in the soluble than in the particulate preparations. The affinities of antagonists, however, were identical in the soluble and particulate preparations. Agonist-specific alterations in receptor binding affinity were also found to be mediated by assay temperature. Thus agonist but not antagonist affinities increased as the temperature was lowered from 37° to 4°. Possible molecular mechanisms responsible for the observed shifts are discussed.

INTRODUCTION

We have previously reported that the *beta* adrenergic receptor of the frog erythrocyte could be effectively solubilized with the plant glycoside digitonin (1). The affinities of the solubilized and membrane-bound receptors were essentially identical for a wide variety of agonist and antagonist drugs.

We now report a strikingly different result observed when the *beta* adrenergic receptors of turkey erythrocyte membranes are solubilized with digitonin. The solubilized receptor displays considerably higher affinity for agonists than does the membrane-bound receptor. The binding affinity for antagonists, however, is unaltered in the solubilized preparations. Changes in temperature are also shown to modulate agonist-specific alterations in receptor affinity.

Turkey particulate fractions were prepared by hyposmotic lysis (2), and resuspended for assay in 75 mM Tris-HCl-25 mM $MgCl_2$, pH 7.9. Solubilization was accomplished by gently homogenizing the membrane in a solution containing 75 mM Tris-1% digitonin (w/v), pH 7.9, at 4° at a ratio of 4-5 mg of protein per milliliter of digitonin-containing buffer. The suspension was placed on ice with constant stirring for 30 min and then centrifuged for 30 min at 40,000 × *g*. This simple centrif-

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ugation procedure was used routinely for preparation of the soluble receptor, since prolonged centrifugation at higher speeds (100,000 × *g*) failed to sediment additional material.

Before binding assays were performed, catechol and ascorbate were added to both particulate and soluble preparations at final concentrations of 0.1 and 1 mM, respectively. [^{3}H]Dihydroalprenolol binding in particulate fractions at 37° and 4° was assayed by the vacuum filtration method described previously (3). A column chromatographic technique (1) was employed for the assay of soluble turkey *beta* adrenergic receptors at 4°. In both assays, specific binding was defined as that portion of the total [^{3}H]dihydroalprenolol bound which was displaceable by 10 μM (±)-propranolol.

The binding of [^{3}H]dihydroalprenolol to the turkey erythrocyte particulate fractions exhibited characteristics appropriate for *beta* adrenergic receptors and similar to those previously found by other investigators using other radioligands (4–7). Specific binding was rapid and reversible upon addition of 10 μM propranolol. The binding of [^{3}H]dihydroalprenolol was saturable, and Scatchard analysis of the data indicated the presence of a single class of sites with a K_D of 2.8 nM and a capacity of 0.1 pmole/mg of protein (data not shown). Catecholamines competed with [^{3}H]dihydroalprenolol for these sites with a potency order (isoproterenol > epinephrine ≅ norepinephrine) characteristic of a $beta_1$ adrenergic receptor subtype. Both the catecholamine agonists and specific *beta* adrenergic antagonists competed stereospecifically for these sites, with the (−) isomers being more potent than the (+) isomers by two or more orders of magnitude.

Approximately 30% of the membrane-bound [^{3}H]dihydroalprenolol binding sites were released in soluble form by treatment of the membranes with 1% digitonin. Since no binding activity could be detected in the pellet, the remainder of the sites were either degraded or inactivated by the solubilization procedure. Other detergents, including Lubrol PX, Triton X-305, Tween 80, and deoxycholate, either inactivated the receptors or failed to release them from the membranes.

The soluble [^{3}H]dihydroalprenolol binding sites exhibited characteristics similar to those of the *beta* adrenergic receptor identified in particulate fractions. The binding was saturable at a level of 60 fmoles/mg of protein and possessed a K_D of 3.6 nM for a single class of sites as determined by Scatchard analysis (Fig. 1A and B). Catecholamines and *beta* adre-

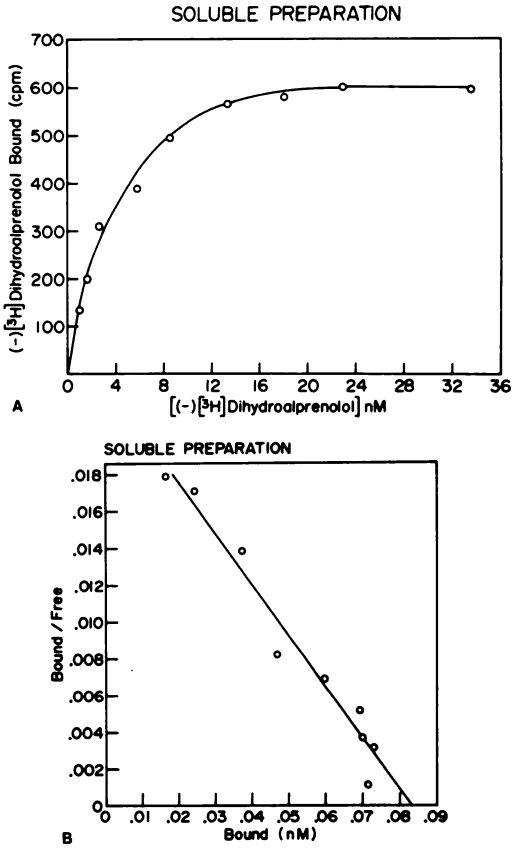

FIG. 1. *(−)-[^{3}H]Dihydroalprenolol binding to soluble preparations derived from turkey erythrocyte particulate fractions*

A. Binding as a function of the concentration of (−)-[^{3}H]dihydroalprenolol. Assays were performed as described in the text, with incubations carried out for 2 h at 4°. Each value is the mean of duplicate determinations.

B. Scatchard plot for (−)-^{3}H]dihydroalprenolol binding to soluble preparations. The value for the slope ($-1/K_D$) was determined by regression analysis with r = 0.98.

nergic antagonists competed stereospecifically for the soluble sites. Among the agonist drugs the order of potency in competing for [^{3}H]dihydroalprenolol binding was isoproterenol > epinephrine ≅ norepinephrine (Fig. 2A and B), which was

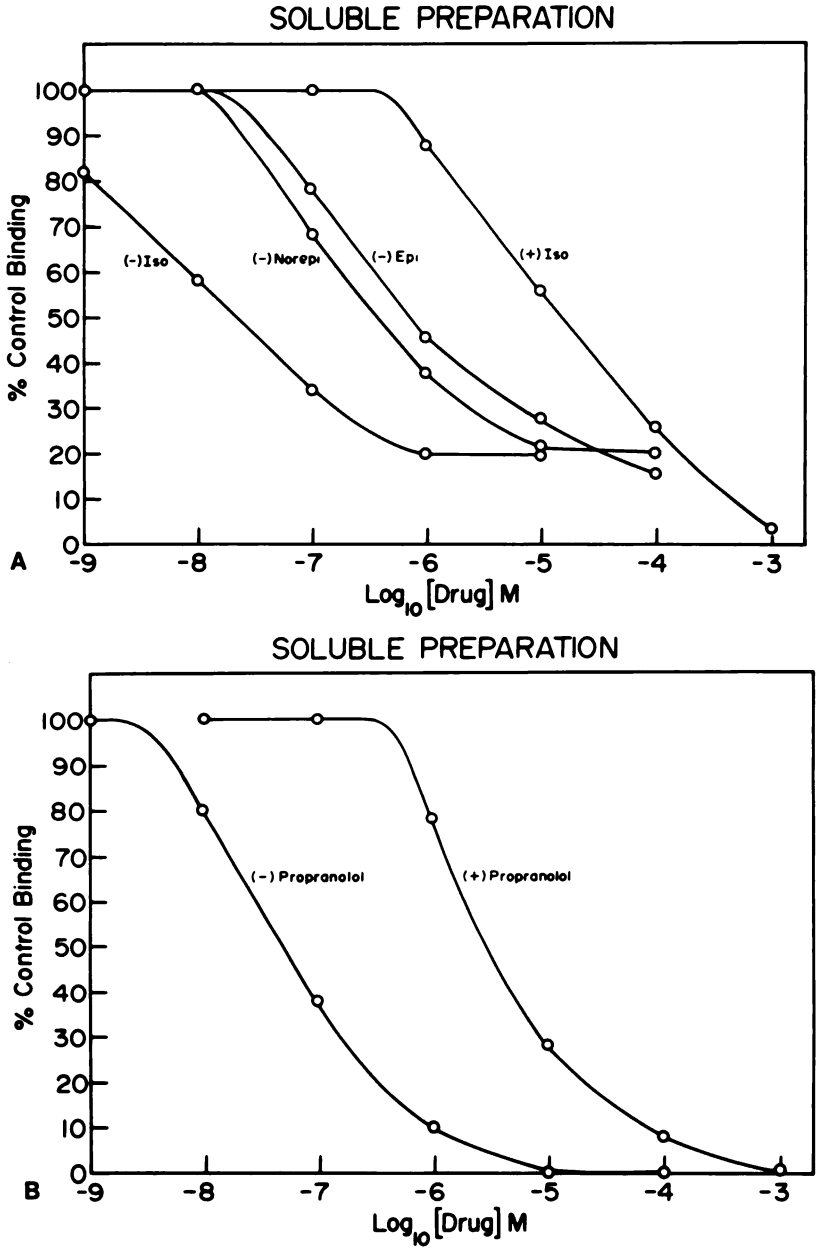

FIG. 2. *Inhibition of specific (−)-[^{3}H]dihydroalprenolol binding to soluble preparations by (−) and (+) isomers of beta adrenergic agonists (A) and antagonists (B)*

Assays were performed at 4° for 2 hr. [^{3}H]Dihydroalprenolol binding was determined in the presence of various concentrations of a number of *beta* adrenergic agents in an incubation mixture containing 25 nM [^{3}H]dihydroalprenolol. On the ordinates, 100% refers to no inhibition of specific [^{3}H]dihydroalprenolol binding. Each value is the mean of duplicate determinations. The data shown are representative of the curves generated in four to nine separate experiments. Iso, isoproterenol; Norepi, norepinephrine; Epi, epinephrine.

identical with the order observed for these sites in the native membranes. Because the characteristics of the particulate and soluble [^{3}H]dihydroalprenolol binding sites correspond so closely, it seems likely that the binding activity released from the membranes by digitonin represents a solubilized form of the turkey erythrocyte *beta* adrenergic receptor.

While these findings are quite analogous to those previously reported for the frog erythrocyte *beta* adrenergic receptor (1), one striking difference was observed. Whereas solubilized and particulate preparations of frog erythrocyte *beta* adrenergic receptor displayed identical affinities for a wide variety of agonist and antagonist drugs, soluble preparations from turkey erythrocytes consistently exhibited a higher apparent affinity for agonist drugs than did particulate turkey erythrocyte preparations. However, a portion of this apparent increase in agonist affinity appeared to be mediated by changes in temperature. This agonist-specific increase in affinity is illustrated in Fig. 3A and B.

Under the three assay conditions tested, the affinity of the receptor for the antagonist dichlorisoproterenol remained unchanged. However, the dose-response curve of the agonist isoproterenol in particulate preparations shifted approximately 8-fold to the left as the temperature was lowered from 37° to 4°. This phenomenon of changes in affinity with changes in temperature is referred to as the temperature shift. A further 7-fold increase in the affinity of isoproterenol for the binding sites was observed upon solubilization. We refer to the change in affinity between the membrane-bound sites at 4° and the soluble sites at 4° as the solubilization shift.

The observed alterations in agonist affinity did not appear to be due to differences in degradation of the catecholamines at the different assay temperatures, for several reasons. First, possible catecholamine degradation was minimized by routinely adding catechol (0.1 mM) and ascorbate (1 mM) to the membranes as indicated previously. Furthermore, when the catecholamines in the binding assay supernatants were tested for bioactivity in an adenylate cyclase assay, no detectable loss of activity was observed, indicating that no significant degradation of the catechol-

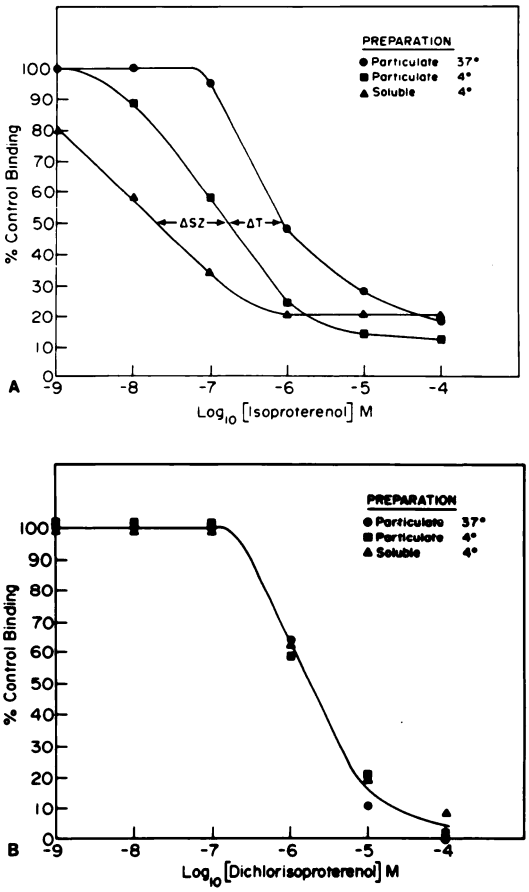

FIG. 3. *Agonist-specific solubilization (ΔSZ) and temperature (ΔT) shifts of turkey beta adrenergic receptor*

A. (−)-[^{3}H]Dihydroalprenolol competition dose-response curves for the agonist (−)-isoproterenol. Incubations were carried out for 10 min at 37° and 2 hr at 4°. Assays were performed as indicated in the text. *ΔT* refers to the temperature shift, defined as the difference in EC_{50} values between the particulate receptor at 4° and 37°. *ΔSZ* refers to the solubilization shift, defined as the difference in EC_{50} values between the soluble receptor at 4° and the particulate receptor at 4°. Each point was determined in duplicate in a single experiment. Data shown are representative of the results of nine separate experiments.

B. Competition dose-response curves for the antagonist (±)-dichlorisoproterenol. Data shown are representative of the results of four separate experiments.

amine had occurred under the assay conditions employed.

Since both temperature and solubilization shifts appeared to be agonist-specific, we determined whether the ability of an agonist to induce these shifts was a function of its intrinsic activity. The data in Table 1 indicate that both full and partial agonists exhibited solubilization shifts. Any compound capable of stimulating adenylate cyclase showed some increase in affinity. Conversely, those compounds that did not stimulate the enzyme showed no change in affinity. However, the magnitude of these shifts did not correlate directly with the intrinsic activity of the compounds.

Also shown in Table 1 are the temperature-dependent increases in affinity observed for these compounds. Only full agonists exhibited temperature shifts. Partial agonists and antagonists showed no change in affinity as the temperature was decreased from 37° to 4°. The magnitude of the shift varied considerably among the catecholamines and obviously did not correlate with the intrinsic activity of these drugs.

A number of molecular mechanisms could be postulated to account for the occurrence of the solubilization shift in agonist affinity in the turkey erythrocyte *beta* adrenergic receptor system. One possibility is that two distinct classes of [^{3}H]dihydroalprenolol binding sites, possessing identical affinities for antagonists but different affinities for agonists, are present in the membranes. Treatment with digitonin might preferentially solubilize the sites exhibiting higher affinity for agonists. Although the 30% recovery of receptors in soluble form makes it difficult to exclude this explanation with confidence, we feel that it is an unlikely one, since detailed competition dose-response curves with agonist drugs in membrane preparations at both 4° and 37° failed to give any indication of two different classes of sites (e.g., no intermediate plateau in the saturation curve).

A more likely explanation is that solubilization of the receptors causes a confor-

TABLE 1

Solubilization and temperature shifts for a number of beta adrenergic drugs exhibiting different intrinsic activities

Intrinsic activities were determined in standard adenylate cyclase assays by comparing the maximal cyclase stimulation produced by the drug with the maximal stimulation of the enzyme produced by isoproterenol. Values are means (and standard errors, where shown) of at least four separate experiments. The solubilization shift was calculated by dividing the K_D of a given compound in particulate preparations at 4° by its K_D in soluble preparations at 4°. Likewise, the temperature shift was determined by dividing the K_D of a drug in particulate preparations at 37° by its K_D in particulate preparations at 4°.

Compound	Adenylate cyclase stimulation	Solubilization shift	Temperature shift
	%	*-fold*	*-fold*
Full agonists			
(±)-Hydroxybenzylisoproterenol	100	10 ± 1[a]	2
(−)-Isoproterenol	100	8 ± 2[a]	7 ± 0.8[a]
(+)-Isoproterenol	100	5 ± 1[a]	5
(−)-Epinephrine	100	6 ± 0.6[a]	7 ± 0.8[a]
(−)-Norepinephrine	100	3 ± 0.2[a]	23 ± 0.4[a]
Partial agonists			
(−)-Soterenol	56	56 ± 6[a]	1
Dobutamine	24	41 ± 4[a]	1
(−)Phenylephrine	38	4	1
Ephedrine	0-10	2	1
Antagonists			
(±)-Dichlorisoproterenol	0	1	1
(−)-Propranolol	0	1	1
(−)-Alprenolol	0	1	1

[a] $p < 0.02$.

mational change in the protein that results in increased binding affinity for agonists. Such an alteration in protein structure could be due solely to the release of nonspecific membrane constraints originating from either lipids, proteins, or a combination of the two, or could be the result of the removal of specific regulatory influences (e.g., coupling factors). The shift is presumably not due merely to the uncoupling of the receptor from the adenylate cyclase during solubilization, however, since treatment of turkey erythrocyte membranes with filipin, which "uncouples" the system, did not result in an increase in affinity of the membrane-bound receptors for isoproterenol.[2]

Similar alterations in agonist affinity upon solubilization have been reported for the nicotinic cholinergic receptor of *Torpedo* and *Electrophorus*. In *Torpedo* the results were interpreted as suggesting the existence of a heterogeneous population of sites (8, 9), while in *Electrophorus* the increase in affinity was thought to result from "release of the receptor protein from its membrane environment" (8, 10, 11).

Possible mechanisms for the molecular basis of the temperature shift include temperature-induced changes in protein conformation, lipid alterations such as changes in fluidity or phase transitions, and differences in lipid-protein interactions.

It should be emphasized that neither the agonist-specific solubilization nor the temperature shift observed with the turkey *beta* adrenergic receptor occurs in the frog erythrocyte *beta* adrenergic receptor system. This difference in properties must be added to a growing list of differences between the *beta* receptors in these two model systems, which includes nucleotide effects (12, 13) and the presence or absence of densensitization (14).[3] Whether the differences are related phenomena and whether they are due to intrinsic differences in the receptors themselves or to differences in the type of membranes remains to be determined.

[2] L. J. Pike and R. J. Lefkowitz, unpublished observations.

[3] C. Mukherjee and R. J. Lefkowitz, unpublished observations.

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