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Practical and Effective Method for the Solubilization and Characterization of Mammalian β-Adrenergic Receptor

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Abstract—In order to understand the machanism of action and regulation of β-adrenergic receptor in terms of molecular level, the purification of receptor protein has a fundamental importance. Moreover, species differences among avian, amphibian and mammalian β-adrenergic receptors make it more important to purify mammalian β-adrenergic receptor. Because β-adrenergic receptor is an integral membrane protein, it must be solubilized from the membrane for the purification. The purpose of the present study was to solubilize and characterize the mammalian β-adrenergic receptor from guinea pig lung in quantities by more efficient and practical method eventually to purify receptor. Guinea pig lung membrane preparation was solubilized by sequential treatment of buffers containing low and high concentration of digitonin which are 0.2 and 1.2% respectively. About 50% of the total receptor pool was released by this double extraction procedure. The β-adrenoceptors in the digitonin extract were identified using the β-adrenergic antagonist, (−)-[^{3}H]-dihydroalprenolol ([^{3}H]DHA). The solubilized receptor retained all of the essential characteristics of membrane-bound receptor, namely saturability; stereoselectivity; high affinity to β-adrenergic drugs. For the measurement of soluble receptor activity, Sephadex G-50 chromatography method has been widely used. Inspite of its accuracy and wide acceptance, this technique employed troublesome column work which required long time to assay the activity of receptor. We employed another methods to measure receptor activity. When using 0.5% polyethylenimine pretreated GF/B glass fiber filter, filtration technique could be used to measure soluble receptor activity. This technique enabled us to reduce the total amount of time to assay by a factor of 4 as well as to detect soluble receptor. In the present study, we could establish more efficient and practical solubilization method of mammalian β-adrenergic receptor. The rapidity and high yield of this solubilization scheme, together with the favorable recovery of the receptor activity, are significant steps toward the ultimate purification of the mammalian β-adrenergic receptor. The result of this study together with more convenient purification method could provide large amount of purified receptor with ease for various research purposes.

Keyword □ guinea pig, lung, β-adrenergic receptor, solubilization, [^{3}H]-dihydroalprenolol, polyethylenimine.

Of the many dozens of receptors coupled to G-proteins, more is known about the family of adrenergic receptors than perhaps any of the others. All of these receptor subtypes have been purified to homogeneity and reconstituted with G-proteins in phospholipid vesicles (Levitzki, 1985; Shreeve et al., 1985; Lefkowitz and Caron, 1986); their functional regulation by covalent modification has been extensively studied (Sibley et al., 1987); and recently, the genes and/or cDNAs for three of the four subtypes, have been isolated and sequenced (Dohlman et al., 1987; Frielle et al., 1987). In addition catecholamines acting through β-adrenergic receptors regulate a wide range of metabolic activities in mammalian tissues.

In many cases, receptor studies require prior purification of receptor to exclude of interfering substances and to concentrate receptor molecule. Moreover, species differences among amphibian, avian and mammalian β-adrenergic receptors make it more important to purify mammalian β-adrenergic receptor. Studies of

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β-adrenergic receptor, however, have been largely confined to a description of nonmammalian tissue. Since β-adrenergic receptor is an integral membrane protein, it must be solubilized from the membrane to be purified. Solubilization and characterization of receptors has been reported for mammalian β-adrenergic receptors (Homcy et al.,1983; Smith and Lee, 1981; Graziano et al., 1985), but their procedures were suffered from the very low yield making subsequent purification more difficult. Moreover, in those studies detection of receptor activity in solubilized state was very laborious and time-consuming. Accordingly, it seems worth while to solubilized and characterize the β-adrenergic receptor from mammalian tissues in higher yield and more rapidly.

Guinea pig lung has been widely used for the study of mammalian β-adrenergic receptors (Benovic et al., 1983). Quantitative analysis of β-adrenergic receptor subtypes has shown that membrane prepared from guinea pig lung possesses a relatively large and homogeneous population of β-adrenergic receptors (Benovic et al., 1984). Due to the well-defined pharmacological properties and the amount with which quantities of membrane can be prepared, guinea pig lung represents an excellent source to purify mammalian β-adrenergic receptors.

The purpose of the present study is to solubilize and characterize the mammalian β-adrenergic receptor from guinea pig lung in quantities by more efficient and less laborious method for the eventual receptor purification.

Elucidating the nature of mammalian β-adrenergic receptors has been the focus of intense research. For this reason, relatively easy method to provide mammalian β-adrenergic receptor in large quantities is indispensible. More practical method for the solubilization of mammalian β-adrenergic receptor through rapid and efficient step to detect soluble receptor activity will make it easier to purify mammalian β-adrenergic receptor in large quantities for the various research purposes.

Materials and Methods

Materials— (−)-oalprenolol ([^{3}H]DHA, 76 Ci/mmol) was purchased from Amersham (Aylesbury, UK), Sephadex G-50 (fine) from Pharmacia (Uppsala, Sweden), and Atomlight scintillation cocktail from New England Nuclear (Boston, MA). Digitonin was obtained from Gallard-Schlessinger (Carle Place, NY). Polyethylenimine (50% aqueous solution) was obtained from Sigma (St. Louis, MO).

All other reagents were obtained from Sigma Chemicals (St. Louis, MO) and were of the highest commercially available grade.

Preparation of the Membrane Particulate Fraction from Guinea Pig Lung

Guinea pig lung (1 g of tissue in 10 m*l* of buffer) was thoroughly minced and disrupted with a Tekmar Tissumizer (Cincinnati, OH) at a maximum speed (three 10-sec bursts on ice interrupted by two 20-sec rests on ice). The buffer contained 50 mM HEPES, 5 mM EDTA, 2 mM EGTA, pH 7.2 (pH determined at 25℃), 10 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, leupeptin (10 μg/m*l*), aprotinin (10 μg/m*l*) and pepstatin (5 μg/m*l*). The homogenate was centrifuged at 48,000×g in a Ti 45 rotor in a Beckman L-80 centrifuge (High Wycombe, UK) for 20 min at 4℃. The pellet was resuspended in 10 volume of buffer using motor driven teflon glass homogenizer and the homogenate was filtered through 2 sheets of cheese cloth. The filtrate was centrifuged and washed three times in 5 volume of buffer by successive resuspension and centrifugation step. Finally the pellet was resuspended by Teflon-glass homogenizer in 5 volume of buffer.

Solubilization of the Guinea Pig β-Adrenergic Receptor

The material pelleted following washing procedure in previous experimental step and derived from 1 g of lung, wet weight, was homogenized with 10 up and down strokes in a tight fitting motor-driven Teflon-glass homogenizer held in ice in 5 m*l* of buffer containing 0.25% digitonin, 10 mM HEPES, 100 mM NaCl, 5 mM EDTA, 2 mM EGTA, 10 mM benzamidine, 10 U/m*l* trasylol, leupeptin (10 μg/m*l*), pH 7.2. After homogenization, the suspension was gently stirred on ice for 30 min, and then centrifuged at 48,000×g in a Ti 70 rotor in Beckman L-80 Ultracentrifuge for 40 min to yield the pre-solubilized particulate preparation.

The resulting precipitate was again solubilized by the homogenization and stirring procedure described above with 5 m*l* of buffer containing 1.2% digitonin, 10 mM HEPES, 100 mM NaCl, 5 mM EDTA, 2 mM EGTA, 10 mM benzamidine, 10 U/m*l* trasylol, and leupeptin (10 μg/m*l*) pH 7.2. The digitonin-containing extract was centrifuged for 40 min at 48,000×g to yield the digitonin-solubilized preparations typically contained 1.7 pmol [^{3}H]DHA binding/mg total protein.

Determination of [^{3}H]DHA Binding in Particulate and Detergent-Solubilized Preparations

Membrane binding assays were performed in 0.5

m*l* incubations containing 0.1~0.2 pmol receptor, 50 mM HEPES, 100 mM NaCl, 5 mM EDTA, 2 mM EGTA, pH 7.2 and 2 nM [^{3}H]DHA for 30 min at 23℃. The incubation was terminated by filtration through Whatman GF/B filters and three washes with 5 m*l* each of ice-cold 10 mM HEPES, 100 mM NaCl, 5 mM EDTA, 2 mM EGTA, pH 7.2. The filters were dried prior to counting in a Pharmacia Wallac Liquid Scintillation Counter in 10 m*l* of toluene-Triton X-100 scintillation fluid at 40~45% efficiency. Nonspecific binding was defined as that binding not inhibited by 10 μM *dl*-propranolol and represented up to 25% of the total binding to particulate preparations.

Binding to detergent-solubilized preparations was performed for 30 min at 23℃ in a final volume of 0.5 m*l* typically containing 0.1% digitonin, 10 mM HEPES, 100 mM NaCl, 5 mM EDTA, 2 mM EGTA, pH 7.2 and 2 nM [^{3}H]DHA. This concentration of radioligand is approximately Kd estimated for solubilized receptors and thus reflects a half-saturating concentration of ligand. Receptor-bound ligand was separated from free ligand by chromatography at 4℃ on 0.8×5 cm Sephadex G-50 columns equilibrated and eluted with 0.05% digitonin, 10 mM HEPES, 100 mM NaCl, 5 mM EDTA, 2 mM EGTA, pH 7.4. The value representing the void volume of blue dextran (0.7 m*l*) was collected and counted in 10 m*l* of Atomlight Scintillation Fluor at 35~40% efficiency. Nonspecific binding was defined as that binding not competed for by 10 μM *dl*-propranolol and was generally less than 5% of total binding in digitonin-solubilized preparations. Alternatively, the receptor ligand complex was separated by filter technique using Whatman GF/B filters pretreated with 0.5% polyethylenimine (PEI) as described for membrane particulate binding assay. There was no difference between specific binding from the two techniques employed.

Conditions for saturation, kinetic, and competition experiments were given in Result section in detail. The binding data were analyzed by LIGAND computer program (the iterative nonlinear curve fitting program; Munson and Rodbard, 1980).

Protein Determinations

Content of protein was estimated in particulate and detergent-solubilized preparations by the procedure of Bradford (1976), using bovine serum albumin as the standard. All samples and standards were adjusted to equal concentrations of digitonin.

Results

Solubilization of β-Adrenergic Receptors from Guinea Pig Lung Membranes

Treatment of the membrane particulate preparations of guinea pig lung with the nonionic detergent, digitonin, released the membrane-bound [^{3}H]DHA binding sites in a solubilized form to a degree dependent on the concentration of digitonin (Table I). Solubilization of the [^{3}H]DHA binding site by digitonin appeared to be quite specific for this detergent since other detergents were ineffective in solubilizing the binding sites in an active form. The detergents tested were Triton

Table I. Solubilization of the β-adrenergic receptor from guinea pig lung membrane

preparation	protein(mg/m*l*)[b]	specific[^{3}H]DHA Binding[d] (pmol/mg protein)	receptor content (pmol/m*l*)[a]	step yield (%)[e]
crude homogentate	10.1± 0.76	0.35± 0.04	3.53± 0.35	100
membrane particulate fraction				
without protease inhibitors	2.62± 0.36	1.01± 0.18	2.64± 0.23	75
with protease inhibitors[c]	2.53± 0.12	1.28± 0.03	3.24± 0.13	92
double extraction[c]				
(1.2% digitonin after 0.2% digitonin treatment)	0.73± 0.04	2.32± 0.27	1.71± 0.28	53
single extraction				
0.5% digitonin	0.80± 0.07	0.98± 0.12	0.76± 0.12	23
1.0% digitonin	1.18± 0.07	0.88± 0.11	1.02± 0.09	31
1.5% digitonin	1.26± 0.04	1.02± 0.09	1.28± 0.08	40

[a]1 g of guinea pig lung was solubilized with 5 m*l* of the indicated concentration of digitonin.
[b]As mesured by Bradford method.
[c]Binding data was generated from satutration experiments.
[d][^{3}H]DHA binding activiry was measured at a single concentration of 20 nM. Cold ligand for nonspecific binding was *dl*-propranolol (10 μM). Each value represents the mean± S.E.M. of data from three separate experiment, each carried out in triplicate.
[e]Average values, which varied≤15% between preparation.

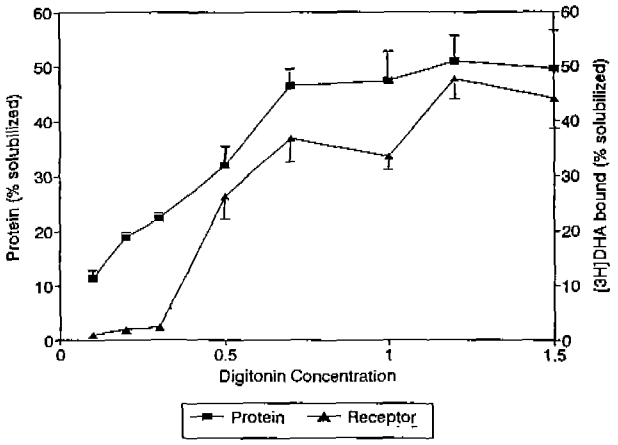

Fig. 1. Solubilization of protein and receptor activity from guinea pig lung membrane preparation. Guinea pig lung membrane was homogenized with increasing concentration of digitonin containing buffers. Solubilized receptor activity was measured by PEI filter assay. Each point represents the mean of data from three separate experiments each carried out in triplicate. Vertical line represents standard error.

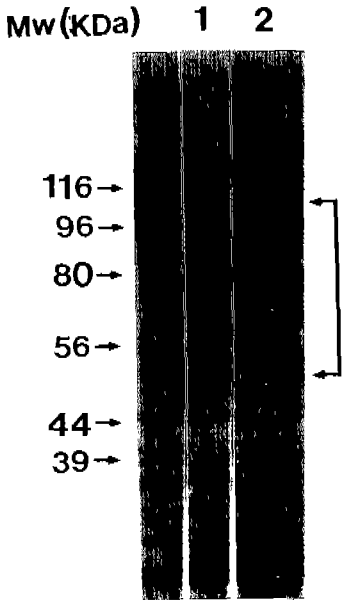

Fig. 2. SDS-PAGE of membrane particulate and soluble receptor preparation. 30 μl of each fraction was combined with 2X SDS sample buffer. The gel was visualized by coomassie blue staining. lane 1: membrane particulate fraction lane 2: soluble receptor preparation more effectively solubilized portion was indicated by a line.

X-100 and sodium cholate at 0.2, 0.5, and 1%; Tween 80, sodium dodecylsulfate, and sodium deoxycholate at 1%. These data do not rule out the possibility that certain detergents did solubilize the sites but then interfered with binding assay (Nevidi and Schramm, 1984). At a digitonin concentration less than 0.3%, virtually no receptor activity was solubilized but significant amount of nonspecific protein was solubilized. As digitonin concentration were increased both receptor and nonspecific protein activity was increased in solubilized fraction (Fig. 1). From the result of this experiment, we presolubilized membrane particulate fraction with 0.25% digitonin containing buffers to exclude some nonspecific protein. Resulting pellet obtained from presolubilization was homogenized in 1.2% digitonin containing buffers to solubilize receptor activity.

This double extraction method of the [^{3}H]DHA binding sites afforded a relatively high-yield (approximately 53% of receptor extracted) and effective (specific activity was increased about 1.8 fold) procedure for solubilizing β-adrenergic receptors from guinea pig lung membrane particulate preparations when compared with direct extraction into 1.5% digitonin-containing buffers (Table I). As shown in Fig. 2. proteins of Mw range 50~105 kDa were solubilized more effectively. This Mw range encompasses integral membrane protein range, therefore, this double extraction method can be used with other integral membrane protein. The inclusion of protease inhibitors in the preparation buffer gave about 1.3 fold increase of specific activity to membrane particulate preparation (Table I). Based on these results, membrane preparation was performed in buffers containing protease inhibitors (5 mM EDTA, 10 μM Leupeptin, 10 μM Trasylol, 10 mM Benzamidine, 10 μM PMSF, 5 μM Pepstatin). During solubilization, PMSF and pepstatin were not used.

To verify the soluble state of the β-adrenergic receptor released by treatment with digitonin, solubilized preparations were subjected to experimental tests, namely, ultracentrifugation and Millipore filtration. When digitonin-solubilized preparations were subjected to further ultracentrifugation at 100,000×g for 60 min, no sedimented material was observable and [^{3}H]DHA binding remained unchanged. In addition, after passage of the soluble preparations through 0.22-μm filters no decrease in binding was observed.

The [^{3}H]DHA binding activity present in the solubilized preparations was stable for storage for at least 4 months at −70℃ and for up to 8 hours at 4℃. Binding activity assessed by chromatographic techniques was linearly related to the amount of protein over the range tested (from 0.1 to 0.4 mg/ml).

Equilibrium Studies of [^{3}H]DHA Binding in Particulate and Soluble Preparations

Under equilibrium conditions (30 min incubation at

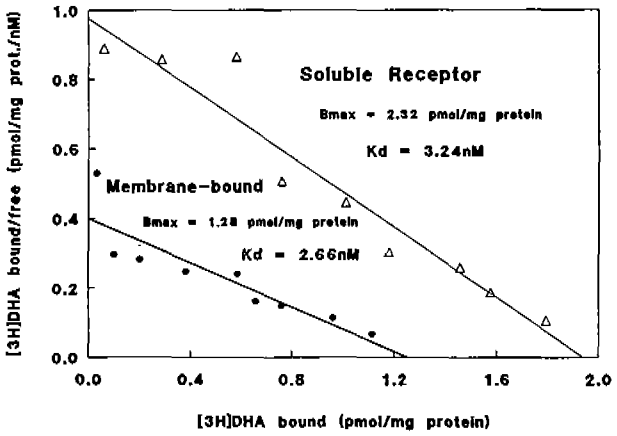

Fig. 3. Scatchard analysis of equilibrium binding study with the particulate and soluble receptor preparation of guinea pig lung. [^{3}H]DHA (0.1-20 nM) was used as the radioligand. Bound concentration was determined as the fraction of total binding inhibitable by 10 μM unlabeled *dl*-propranolol. Each point represents the mean of data from three separate experiments each carried out in triplicate.

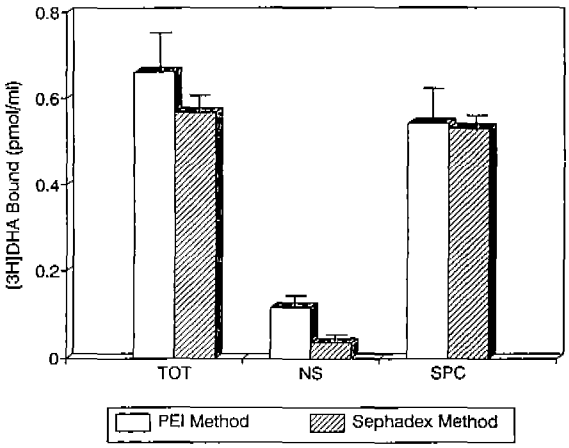

Fig. 4. Comparison of PEI filtration assay and Sephadex G-50 chromatography assay. [^{3}H]DHA (2 nM) was used as the radioligand. Bound concentration was determined as the fraction of total binding inhibitable by 10 μM unlabeled dl-propranolol. Each point represents the mean of data from three separate experiments each carried out in triplicate. Vertical line represents the standard error. TOT; total binding, NS; nonspecific binding, SPC; specific binding.

23℃) specific binding of [^{3}H]DHA to the particulate preparations was a saturable process. Scatchard plots of the data(Fig. 3) were linear over the concentration range employed(0.1~20 nM). An equilibrium dissociation constant (K_d) of 2.7± 0.4 nM(mean± SE; n=3) was observed, with an apparent maximum number of sites bound(B_{max}) of 1.3± 0.03 pmol/mg protein. The slope of Hill plot (Hill coefficient, nH) was 1.00± 0.03 suggesting an interaction of the ligand with a single population of sites.

Binding of [^{3}H]DHA to the solubilized preparations was also saturable (B_{max}=2.3± 0.27 pmol/mg protein; n=3), and of similar affinity (K_d=3.2± 0.8 nM) to that determined for the membrane receptor. Hill coefficient was also close to unity (n_H=0.98± 0.05)

For the more rapid and convenient measurement of soluble receptor activity, we examined the applicability of polyethylenimine filtration assay as a alternative of conventional Sephadex G-50 chromatography method. As shown in Fig. 4, there is no difference between specific binding from the two techniques. Thus we usually employed PEI filtration assay for the activity of soluble receptor thereafter.

Kinetic Studies of [^{3}H]DHA Binding in Soluble Preparations

Association of [^{3}H]DHA to the membrane particulate fraction was rapid with a $t_{1/2}$ of 2.57 min (Table II). After reaching equilibrium a slight decrease in binding was observed that could conceivably be due to either radioligand or receptor degradation. The observed association rate constant(K_{obs}) for the single site

Table II. Kinetic Parameters of [^{3}H]DHA Binding to membrane and Soluble Receptor Preparation

	Membrane	Soluble
K_d(Scatchard)	2.66 nM	3.24 nM
K_{+1}	7.99×10^7/M·min	4.98×10^7/M·min
$t_{1/2}$(Association)	2.57 min	3.12 min
(Dissociation)	5.30 min	5.70 min
k_{-1}	0.11/min	0.12/min
K_d(Kinetic)	1.38 nM	2.45 nM

[^{3}H]DHA (2 nM) was used as a radioligand. $t_{1/2}$ was calculated from corresponding rate constant. Each value represents the mean of data from two separate experiments each carried out in triplicate. For detail, see text.

was found to be 0.270 min^{-1}. The association reaction of [^{3}H]DHA is a pseudo-first order reaction. Thus association constant can be calculated by the equation of $k_{+1}=(K_{obs}-k_{-1})/F$ (F; free ligand concentration). Given a radioligand concentration of 2 nM, actual association rate constants can be calculated to be 7.99×10^7 M^{-1} min^{-1}.

Binding of [^{3}H]DHA to the membrane particulate fraction was essentially reversible. The half-life for dissociation at 23℃ is about 6.3 min. The calculated dissociation rate constant from the mono exponential model of the dissociation experiment was 0.11 min^{-1}. Thus, an estimate of the dissociation constant ($K_d=k_{-1}/k_{+1}$) of 1.38 nM can be obtained from the kinetic rate constants. The kinetically derived dissociation constant

is in reasonable agreement with the estimate of Kd obtained from equilibrium studies.

Association of [^{3}H]DHA binding to the solubilized preparation was reached equilibrium by 15 min at 23℃ with a $t_{1/2}$ of 3.12 min (Table II). The observed association rate constant (K_{obs}) for the single site was found to be 0.222 min^{-1}. Given a radioligand concentration of 2 nM, actual association rate constants can be calculated to be 4.98×10^7 M^{-1} min^{-1}. The half-life for dissociation at 23℃ is about 5.7 min. The calculated dissociation rate constant from the mono exponential model of the dissociation experiment was 0.122 min^{-1}. Thus, an estimate of the dissociation constant ($K_d=k_{-1}/k_{+1}$) of 2.45 nM can be obtained from the kinetic rate constants. This value is in reasonable agreement with the estimate of Kd obtained from equilibrium studies.

Table II summarizes kinetic parameters from membrane particulate and soluble receptor preparation. All kinetic parameters of the two preparation had very close values.

Specificity of [^{3}H]DHA Binding to the Solubilized Receptor

Table III demonstrates the ability of several adrenergic agents to compete with [^{3}H]DHA for binding to membrane-bound and solubilized receptors. As in the intact membrane, the unlabeled β-adrenergic antagonists, (−)-alprenolol and (−)-propranolol, appear to be equipotent in inhibiting the binding of [^{3}H]DHA. The data also indicate that the solubilized sites retain their stereospecificity in binding β-adrenergic ligands. The (−)-isomers of propranolol and isoproterenol are about 2 orders of magnitude more potent than the respective (+)-isomers. The potency series of the various agonists in inhibiting binding of [^{3}H]DHA was (−)-isoproterenol、(−)-epinephrine>(−)-norepinephrine, which is typical of the β-adrenergic receptor. It is also apparent from these data that the solubilized receptor sites retain the characteristics of a β-adrenergic receptor. This is demonstrated by the fact that (−)-isoproterenol is 2 to 3 orders of magnitude more potent than (−)-epinephrine in competing for the binding sites. The solubilized binding sites show no affinity for the β-adrenergic antagonist phentolamine. The dissociation constants (K_i) calculated from competition experiments correlate very well with those for the binding sites in intact guinea pig lung membranes.

In general, the affinities of adrenergic ligands for solubilized receptor sites correlate reasonably with the affinities of the binding sites of the same ligands in membrane preparations.

Table III. Inhibitor of [^{3}H]DHA binding by various adrenergic agents in the guinea pig lung

Ligand	K_i, nM	
	membrane	soluble
Antagonists		
1. (−)-alprenolol	10.0 ± 3.15	3.04± 0.41
2. (−)-propranolol	9.54± 3.22	3.43± 0.57
3. (+)-propranolol	4650± 420	149± 84
Agonists		
4. (−)-Isoproterenol	4.85± 0.42	5.36± 0.59
5. (+)-Isoproterenol	3477± 946	3740± 626
6. (−)-Epinephrine	2239± 669	1334± 373
7. (−)-Norepinephrine	4557± 757	8407± 998

K_i values were calculated from competition assay data using IC_{50} values determined by LIGAND computer program and Cheng and Prusoff analysis.
K_d values for [^{3}H]DHA of 2.66 nM(membrabe) 3.24 nM(soluble) were used these calculations. The concentrations of [^{3}H]DHA were 2 nM for the competition studies.

Discussion

Purification and characterization of receptors have been a difficult task because of the extremely small quantities of these macromolecules present in most cell types. Although extensive progress has been made in the characterization of β-adrenergic receptors isolated from many avian, amphibian and mammalian tissues (Strosberg, 1987), these preparation had some drawbacks such as lowered affinity and nonmammalian characteristics. Moreover, the purification methods employed in these studies were not efficient enough in terms of yield, experimental expenses, convenience and labor for experiment. Solubilization is an initial step for the purification of membrane embedded proteins.

As reported by others (Graziano et al., 1985; Homcy et al., 1983), only in the presence of the detergent, digitonin, plant sterol, direct assay of the soluble species was possible but the yield of solubilized receptors was very low. Solubilization of the guinea pig lung β-adrenergic receptor in the present study was efficiently achieved by the double extraction with low and high concentration of digitonin. At a low digitonin concentration, virtually no receptor activity was solubilized but substantial amount of protein was solubilized. Accordingly nonspecific protein can be partly removed by presolubilization of membrane particulate fraction with low digitonin buffers. By this methods the specific activity of solubilized receptor can be increased about 1.8 fold. Nonspecific protein can interfere adsorption of receptor to affinity matrix. Therefore this double

extraction method may aid following purification step. Solubilized receptor we obtained displayed all of the characteristics expected of the β-adrenergic receptor. It demonstrated saturability and retained its high affinity for (−)-alprenolol, which was identical with the membrane-bound form. Stereospecificity retained following solubilization. The rank order of potency for agonists (isoproterenol、epinephrine>norepinephrine) was maintained following solubilization and was consistent with a β_2-adrenergic subtype (Harden and McCarthy, 1982). There are some discrepancies, however, in the apparent absolute affinities of β-adrenergic ligands. The K_d values determined by the procedure of Cubero and Malbon (1984) is higher than those of the present study. A major difference between the two procedures is the 10~20 fold higher [^{3}H]DHA concentration used in the former procedure. The present values are very similar to the K_d values reported by Vauquelin et al. (1977) for [^{3}H]DHA binding to turkey erythrocyte. As noted above, the membrane and solubilized receptor preparations from guinea pig lung consistently demonstrated the binding characteristics of the β_2-subtype.

The rapidity and high yield of this solubilization scheme, together with the favorable recovery of the receptor activity, are significant steps toward the ultimate purification of the mammalian β-adrenergic receptor.

Conventional filter binding assay which uses glass fiber filters under reduced pressure has the advantage of rapidity and convenience. But this technique cannot be used with soluble receptor preparation, since the soluble receptor freely pass the glass fiber filters.

For the assay of solubilized receptor activity Sephadex G-50 chromatography method has been used for many years by various investigators. Although this technique shows low nonspecific binding component and is relatively accurate, it is not ideal to apply for soluble receptor assay. It requires much troublesome column work prior to experiment and very long time with many laborious steps. Such disadvantages would be eliminated to some extent by using glass fiber filter soaked with polyethylenimine. Polyethylenimine is shown to possess positive charge (Bruns et al., 1983) which may be useful to extract protein with negative charges from incubation mixture. As shown in result the two receptor assay methods, one is we used and the other the conventional Sephadex G-50 chromatography, show identical detectability of soluble receptor activity. By employing polyethylenimine filter technique more rapid method of receptor activity measurement during receptor purification can be set.

In the present study, modification in solubilization step by using double extraction with digitonin plus that in detection step using polyethylenimine for receptors could provide an efficient and rapid method to prepare large amount of receptors with purity useful for various research.

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