Biochimica et Biophysica Acta 861 (1986) 345–352
Elsevier

BBA 72937

Purification and characterization of the β_2-adrenergic receptor from calf lung

Robert Insoft, David G. Sawutz and Charles J. Homcy

Cellular and Molecular Research Laboratory, Cardiac Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114 (U.S.A.)

(Received 3 June, 1986)

Key words: β-Adrenergic receptor; Membrane protein; Isoelectric focusing; (Calf lung)

Improved methods for the solubilization and purification of the mammalian β_2-adrenergic receptor have allowed this protein to be characterized further. In the present study, the β_2-adrenergic receptor has been solubilized from calf lung membranes using a 0.4% digitonin/0.08% cholate-Tris buffer with multiple proteinase inhibitors. This solubilization buffer produced 60–75% solubilization of the receptor, which retained complete ligand-binding activity as determined by Scatchard analysis. Subsequent receptor purification employed a modified acebutolol-agarose affinity resin. The eluate from the affinity resin was then purified further by HPLC-gel exclusion chromatography on a Spherogel TSK-3000 column. The receptor, detected by [^{3}H]dihydroalprenolol or [^{125}I]iodocyanopindolol binding, eluted with a retention time identical to that of IgG (Stokes radius 49 Å). Autoradiography following SDS-PAGE of the purified iodinated receptor clearly demonstrated two distinct bands: a major band of 67 kDa and a minor band of 53 kDa. With the addition of leupeptin to the proteinase inhibitor regimen, the 53-kDa band became less apparent. Two-dimensional gel electrophoresis indicated that the 67-kDa peptide behaved as a predominantly single species with a p*I* of 6.0 ± 0.2. The purified receptor protein recognized adrenergic ligands with a specificity identical to that of the membrane-bound β_2-adrenergic receptor.

Introduction

The pharmacological properties of the β-adrenergic receptor have been extensively investigated over the last several years. Certain groups have successfully explored the molecular characteristics of receptors solubilized from frog and turkey erythrocytes [1–3]. We previously reported the conditions allowing for the solubilization and characterization of the mammalian β_2-adrenergic receptor from canine lung [4]. At that time, the purified receptor was reported to have a molecular weight of approximately 53000, based on SDS gel electrophoresis [4]. Purification of the receptor from rat, hamster and guinea pig lung was subsequently reported by Benovic et al. [5]. They demonstrated that the β_2-receptor purified from mammalian lung had a molecular weight of 64000, with a minor contaminating band of 45000. When these authors treated β_2 receptor purified from hamster lung with chymotrypsin, they found a major polypeptide of 55 kDa. This suggested that the 53-kDa polypeptide that had been previously reported was probably a degradation product of the 64-kDa protein, likely the result of proteolysis during purification of the receptor. Here we describe modified procedures and conditions that allow for the solubilization, purification and characterization of the β_2 receptor from calf lung. A preliminary communication of this work has been previously published [6].

Abbreviation: PMSF, phenylmethylsulfonyl fluoride.

Correspondence address: Charles Homcy, M.D., Cardiac Unit, Massachusetts General Hospital, Fruit Street, Boston, MA 02114, U.S.A.

Materials and Methods

Bovine γ-globulin, poly(ethylene glycol) 8000, Tris base, (−)- and (+)-alprenolol tartrate, (−)-norepinephrine bitartrate, (−)-epinephrine bitartrate, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride were obtained from Sigma. The acebutolol amine was donated by Dr. Wooldridge of May and Baker, Ltd. U.K. We obtained Sepharose 4B, Sephadex G-50 and Pharmalyte (pH 5–8 and pH 3–10) from Pharmacia. [^{3}H]Dihydroalprenolol and (−)-[^{125}I]iodocyanopindolol were purchased from New England Nuclear. Bovine serum albumin, ferritin, dextran blue, IgG (bovine) and ovalbumin (employed as standards for HPLC, SDS-PAGE and desalting chromatography) were obtained from either Sigma or Pharmacia. Proteinase inhibitors, including bacitracin, PMSF, sodium azide, soybean trypsin inhibitor and leupeptin, were also purchased from Sigma, as was the digitonin (80% pure).

Membrane preparation. Calf lungs were excised immediately (within 2 min) from commercially killed calves and stored in 2 liters of ice-cold, lactated Ringer's solution in the presence of proteinase inhibitors (1.0 mM bacitracin, 1.0 mM PMSF, 0.2% sodium azide, 10 μg/ml leupeptin, 5 mM EDTA and 10 μg/ml soybean trypsin inhibitor). The lung parenchyma was cut and placed in ice-cold buffer consisting of 1 mM $KHCO_3$, 1 mM $MgCl_2$ and 0.3 M sucrose (pH 8.5), with the same proteinase inhibitors (buffer A). All bronchi, hilar vessels, connective tissue and pleura were discarded. The total mixture was then homogenized in a Polytron tissue grinder at setting 3 for 5 s and then at setting 8 for 5 s. The homogenate was diluted and filtered through one layer of Japanese silk screen. This material then underwent two successive 10-min spins at 3000 rpm (Sorvall, SS34 rotor); the supernatants were combined and respun at 17500 rpm for 30 min. The pellet was subsequently washed and respun three times in 100 mM Tris, 5 mM EDTA and 1 mM $MgCl_2$ with proteinase inhibitors, pH 7.5 (buffer B).

Solubilization of receptors. Solubilization was achieved by immediately adding prefiltered 0.4% digitonin/0.08% cholic acid in buffer B plus proteinase inhibitors to the washed pellets. The optimal digitonin/protein ratio was found to be 2 : 1 by weight (i.e. 300 ml of 0.4% digitonin/0.08% cholic acid to 600 mg). Gentle homogenization was done in a Dounce homogenizer. The resulting mixture was slowly rotated at room temperature (23°C) for 1.5 h. This was followed by centrifugation at 18000 rpm for 30 min. No additional precipitation of receptor protein was observed in an aliquot of supernatant after a 45-min spin at 90000 rpm in a Beckman ultracentrifuge. The solubilized receptor was assayed for binding activity with [^{125}I]iodocyanopindolol according to the previously published protocol [4]. Saturation binding studies on both the fresh membranes and the purified, solubilized material were also performed.

Synthesis of affinity resin. The acebutolol amine-agarose was prepared according to the method of Homcy et al. [4], with some modifications. Acebutolol amine was allowed to react for 12 h in the presence of water-soluble carbodiimide. Next 6 g of 2-aminoethanol were added and allowed to react for 1 h at pH 4.8. Finally, the resin was thoroughly washed with 1 liter of distilled H_2O, a second liter of 1 M NaCl, and 1 liter of distilled H_2O, then allowed to equilibrate in 0.1% digitonin buffer prior to use.

Desalting column. A 1.0 m × 2.0 cm (400 ml) Kontes column was packed with washed Sephadex G-50 (medium) equilibrated in 0.1% digitonin/0.02% cholic acid in buffer B at 4°C. The column was washed with 3 column volumes prior to use. The receptor preparation was applied to the column, and 3-ml fractions were collected over a 2.0 h period at a flow rate of 60 ml/h.

HPLC gel filtration. A TSK-3000 Spherogel column attached to a Beckman (model 332) HPLC was equilibrated with prefiltered 0.1% digitonin/0.02% cholic acid buffer. The HPLC flow rate used was 6.0 ml/min. Dextran blue (void volume), IgG, hemoglobin, ferritin and alprenolol tartrate (salt volume) were all used as standards. Between each injection, the column was thoroughly washed with 1 column volume of buffer. The use of a higher-ionic-strength buffer (100 mM Tris, 200 mM NaCl) was found to separate unbound alprenolol more effectively, which, to some extent, interacted with the Spherogel resin at lower salt concentrations.

Purification outline. The solubilized material and

the affinity resin were allowed to shake at room temperature (22°C) for 3 h. The mixture was spun at 2000 rpm for 5 min and the resin was washed with 3 × 200 ml ice-cold 0.1% digitonin/0.02% cholic acid in buffer B. The resin was then eluted with 25 ml of 0.1 mM (−)-alprenolol for 2 h at 4°C, centrifuged as above, then washed with 10 ml of the ice-cold buffer. The elutions were combined and immediately desalted over the Sephadex G-50 column (4°C). One ml of 1% dextran blue was added as a column marker. The peak fractions were then combined. Each preparation yielded approx. 20 ml of desalted material. This step was necessary, since HPLC alone did not completely remove free alprenolol, which could still be detected by radioimmunoassay. The HPLC protocol was performed immediately thereafter, utilizing 2-ml injection volumes. HPLC elution with a higher-ionic-strength buffer (buffer B with 200 mM NaCl) was found to prevent adsorption of free alprenolol to the gel matrix. Such nonspecific adsorption had resulted in the alprenolol contamination of fractions that had been sequentially injected onto the column. Further purification was achieved by combining all HPLC elutions and reincubating them with the affinity resin, then eluting and desalting them again.

Two-dimensional gel electrophoresis. Purified β_2 receptor, iodinated by the chloramine-T method, was analyzed in the first dimension by isoelectric focusing (IEF) according to the method of O'Farrell et al. [7] as described by Jones [8], followed by SDS-PAGE in the second dimension, according to the method of Laemmli [9]. Briefly, 25 μl of ^{125}I-labeled β receptor ($4 \cdot 10^5$ cpm) in IEF sample buffer consisting of 9 M urea, 2% Nonidet P-40, 5% 2-mercaptoethanol and 1.6% Pharmalyte (pH 5–8) were layered onto IEF tube gels (2.5 × 100 mm) that had a final gel composition of 4% polyacrylamide, 2% ampholines, 2% Nonidet P-40 and 9.2 M urea. The gels were overlayed with 10–20 μl cathode solution and electrophoresed at room temperature for 15 h at 300 V. The anode solution was 0.02 M H_3PO_4, and the cathode solution was 0.02 M NaOH. Ferritin, whale skeletal myoglobin and bovine hemoglobin were used as visual standards. Under these IEF conditions, the respective p*I* values of the standards were 4.8, 8.0 and 7.0. These p*I* values are in close agreement with previously published values for the proteins [10,11]. Bovine serum albumin coupled to fluorescein was used as a non-visual standard (p*I* = 5.3). The gels were carefully removed from the tubes (see Jones [8]), and equilibrated in SDS sample buffer (60 mM Tris, 10% glycerol, 2.3% SDS and 5% 2-mercaptoethanol) for 2 h before electrophoresis in the second dimension. The pH determination of 5-mm gel slices was done according to the method of O'Farrell [12] by placing the gel slices in 2 ml of degassed, deionized water for 2 h and measuring the final pH at room temperature with an Orion digital pH meter.

For electrophoresis in the second dimension, 3.0-mm-thick SDS-PAGE gels (10% acrylamide) were overlayed with 1 ml 1% agarose heated in SDS sample buffer. The IEF tube gels were carefully layered onto the warm agarose and additional agarose was applied to totally surround the gel. Iodinated molecular weight standards were run in a separate lane next to the IEF gel, but at a distance of approximately 10 mm to prevent any overlap during electrophoresis. The gels were electrophoresed in a Tris-glycine-SDS buffer (pH 8.3) for 16 h at 10°C, with a constant current of 60 mA and then dried under vacuum at 60°C. Autoradiography was done by exposing the gels to Kodak XA-5 film for up to 48 h at −70°C.

Protein determination. Membrane and starting solubilized preparations were measured for protein content by the method of Lowry et al. [13]. Purified preparations were kindly analyzed for amino acid content by Dr. John Smith (Department of Molecular Biology, Massachusetts General Hospital).

Results

The solubilization and purification to homogeneity of calf lung β_2-adrenergic receptors is described here. The best solubilization was obtained in a 0.4% digitonin/0.08% cholic acid–buffer B mixture with a digitonin : protein ratio of 2 : 1. Previously reported experiments showed that higher digitonin (0.8%) and various other cholate concentrations masked specific receptor binding.

A modified acebutolol-agarose resin that employed an additional reaction with 2-aminoethanol

TABLE I

PURIFICATION OF THE β_2-ADRENERGIC RECEPTOR FROM CALF LUNG

These data represent three separate receptor purifications. The receptor concentration was determined by saturation binding studies utilizing (−)-[^{125}I]iodocyanopindolol at each step. Protein content was determined by the method of Lowry et al. [13].

	Total protein (mg)	Total receptors (pmol)	Yield receptor (%)	Spec. act. (pmol/mg)	Purification (fold)
Starting lung homogenate	650	143.0	100.0	0.22	–
Digitonin-solubilized	473	92.2	64.5	0.19	–
1st affinity elution + HPLC	0.78	38.0	23.0	48.40	220
2nd affinity elution	0.58 μg [a]	4.4	3.0	7559.00	34090

[a] Based on amino acid analysis.

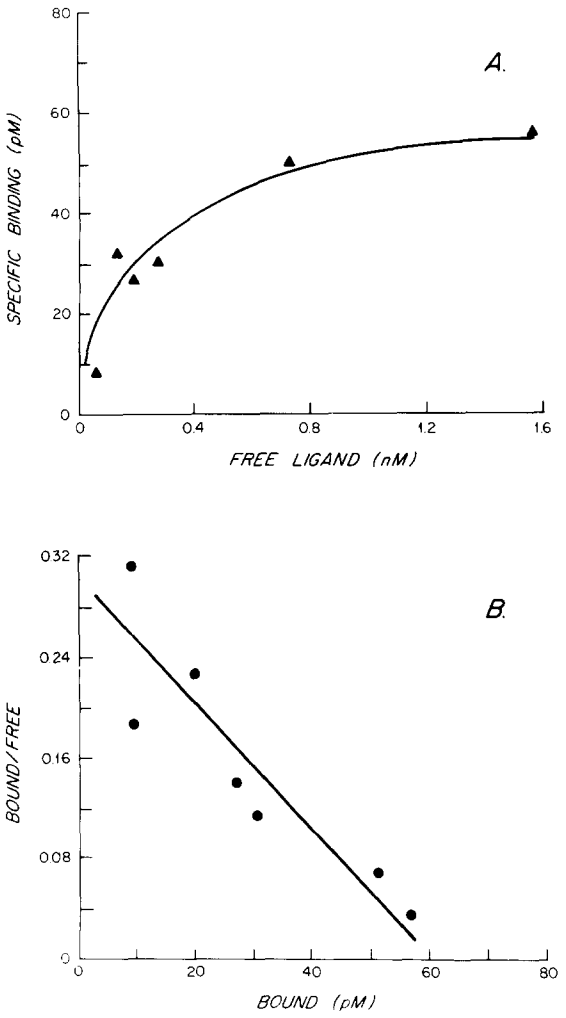

Fig. 1. Saturation binding experiments were performed by incubating lung membranes (50 μg) with increasing concentrations of [^{125}I]iodocyanopindolol (0.05–1.6 nM) at 37°C for 1 h. Alprenolol (10^{-5} M) was used to determine non-specific binding. The binding experiments were terminated by vacuum filtration through glass-fiber filters and each filter was washed with 15 ml of buffer B. The binding data were analyzed with LIGAND on a Digital VAX computer system [14].

(0.5 M, pH 4.8, 2 h at 23°C) to block unreacted carboxyl groups reduced non-specific absorption to the resin, and improved both the yield and the degree of purification at this step. Quantification by radioimmunoassay, employing an antibody raised against acebutolol, indicated consistent ligand substitution in the 1–5 mM range. The binding capacity of the resin was assayed by comparing the activity of the starting solubilized material with that of the supernatant after incubation.

We report the results of multiple large-scale purifications (Table I). The entire procedure, including HPLC purification, now only requires 18–24 h, as opposed to the 3–5 days required by the previously reported protocol [4]. When adequate receptor binding activity was detected after HPLC, a second affinity purification was carried out, which required an additional 4 h. Initially, 625–675 mg of membrane protein was solubilized in 315 ml of 0.4% digitonin/0.08% cholic acid–buffer B. The membrane receptor concentration was calculated to be 220 fmol/mg and exhibited a K_d of 100 pM for [^{125}I]iodocyanopindolol, as determined by equilibrium binding experiments and a computer-assisted, non-linear, iterative curve-fitting procedure (Fig. 1) [14].

To lower the concentration of free alprenolol, material eluted from the resin was subsequently desalted over a Sephadex G-50 column prior to

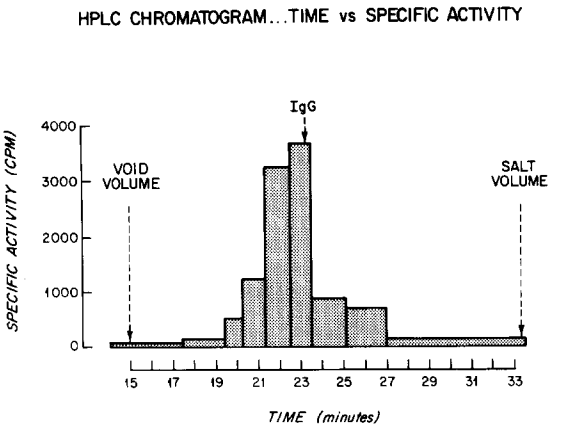

Fig. 2. Elution pattern of affinity-purified β-adrenergic receptor assayed with [^{125}I]iodocyanopindolol from a TSK-3000 Spherogel column eluted at 6 ml/min in 0.1% digitonin-buffer B. The receptor activity elutes with an apparent Stokes radius identical to that of IgG.

HPLC. The eluant was purified with repeated 2-ml injections over a TSK-3000 Spherogel column. Peak binding activity, assayed using [^{125}I]iodocyanopindolol, eluted with a retention time similar to that of IgG (Stokes radius = 49 Å, Fig. 2). The peak fractions were then reincubated with 10 ml of acebutolol-agarose resin for 2 h, eluted with 0.1 mM (−)-alprenolol, and dialyzed against 0.1% digitonin in buffer B for 24 h. Final

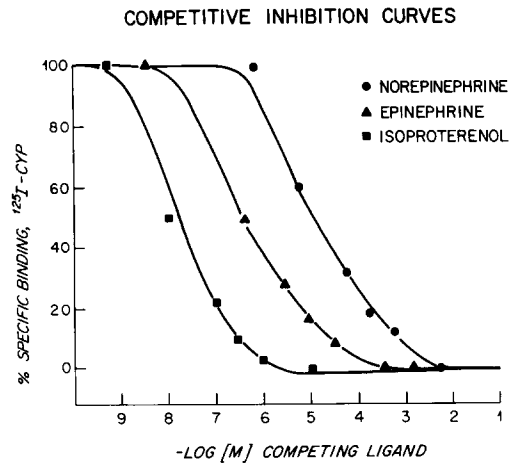

Fig. 3. Agonist competitive inhibition curves of purified β-adrenergic receptor demonstrating a typical β_2-subtype order of potency. Binding assays employed [^{125}I]iodocyanopindolol at a concentration of 100 pM and were done as previously described [4].

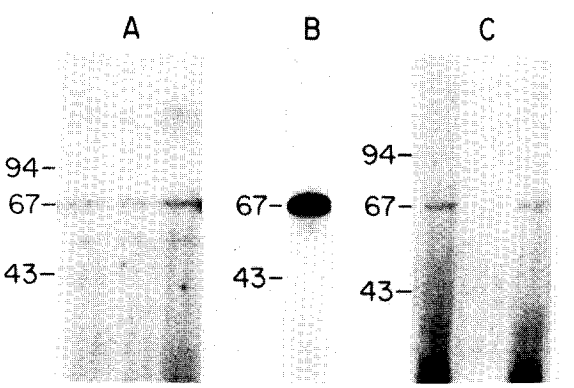

Fig. 4. Autoradiograms of SDS-PAGE gels of three different purified receptor preparations. (A) Single affinity and HPLC-purified receptor demonstrating a principal band of 67 kDa and a lesser band at 53–55 kDa. (B) Single affinity and HPLC-purified receptor with leupeptin added to proteinase inhibitor regimen. The lower-molecular-weight band (53–55 kDa) is now less apparent in comparison to the 67000 band. (C) Affinity and HPLC-purified receptor was reapplied to the affinity resin. A single 67-kDa band is apparent.

binding activity was determined prior to dialysis against distilled water and lyophilization. One affinity-HPLC pass and a subsequent second affinity step resulted in a β-receptor purification of approximately 34000-fold with an overall yield of

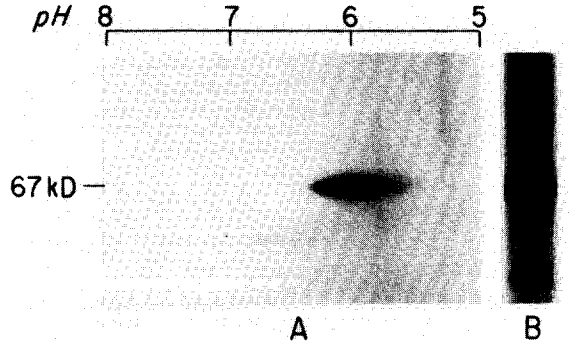

Fig. 5. Two-dimensional gel pattern of ^{125}I-labeled β_2 receptor. (A) Purified β_2 receptor radiolabeled with ^{125}I by a chloramine-T method, was electrophoresed by IEF in the first dimension followed by SDS-PAGE in the second dimension as described in the Methods section. Following autoradiography (48 h), a major protein was evident at a molecular weight of 67000 and a pH value of approximately 6.0 ± 0.2 pH units. Identical results were obtained in three separate experiments run under the same conditions. (B) ^{125}I-labeled β-adrenergic receptor used for (A) was analyzed by SDS-PAGE on a 10% polyacrylamide gel.

3% (4.35 pmol β receptor). Agonist competitive inhibition curves of [^{125}I]iodocyanopindolol binding to purified receptor revealed a rank order of potency of isoproterenol > epinephrine > norepinephrine, which is characteristic of the β_2-adrenergic receptor (Fig. 3).

Samples from both single and double affinity-HPLC purifications were iodinated with chloramine-T and sodium metabisulfite. Subsequent SDS-PAGE of the ^{125}I-labeled proteins from both purification protocols yielded two distinct bands having molecular weights of 67 and 53–55 kDa (Fig. 4). In repeated experiments, the 67-kDa band was always the most prevalent. The lower-molecular-weight band essentially disappeared after the addition of leupeptin (Fig. 4, end lanes). The primary difference between the single and double affinity purified preparations was the loss of lower-molecular-weight contaminants which were inconsistently present in the single affinity purified material.

Preparations containing the 67-kDa species were subjected to further isoelectric focusing and second-dimension SDS-PAGE. The receptor migrated as a single species with a pI of 6.0 ± 0.2 (Fig. 5).

Discussion

There are now reports on the size of the hormone-binding subunit of the β-adrenergic receptor, of both β_1 and β_2 subtypes. Several groups have employed photoaffinity ligands to label the membrane-bound receptor prior to SDS-PAGE [15–18]. Shorr et al. [2] initially purified the β_1 receptor from frog erythrocytes and reported a subunit molecular weight of 58 000 for the receptor based on photoaffinity labeling of the protein. The same group then purified the β_1 receptor from turkey erythrocyte membranes and reported that the purified, iodinated receptor was composed of two subunits having molecular weights of 40 000 and 45 000. Similar results were also reported by Hekman et al. [25]. These values were slightly higher than those previously reported by Atlas and Levitzki (41 and 37 kDa) [22], which were based on photoaffinity labeling of the receptor. Cubero and Malbon subsequently purified the β_1 receptor from rat fat cells [19]. Iodination and SDS-PAGE of the purified receptor protein revealed that it migrated with a molecular weight of 67 000.

The mammalian β_2-adrenergic receptor was purified to apparent homogeneity initially by this group from canine lung [4]. A 53-kDa protein was identified as the receptor peptide following SDS-PAGE and staining with Coomassie blue. Benovic et al. [5] then purified the β_2 receptor from hamster, guinea pig and rat lungs and reported a molecular weight of 64 000 for the purified peptides from each tissue. Recently, Graziano et al. [21] purified the β_2 receptor from rat hepatic tissue and also reported a subunit molecular weight of 67 000 for the receptor.

The earlier reports suggest that multiple-molecular-weight forms exist with heterogeneity among the different subtypes [19,20]. Recently, however, several investigators have emphasized that proteolysis may partly or perhaps completely explain the observed heterogeneity in the molecular weights [5,16,24]. In a previous report, we identified a 53-kDa peptide after the β_2 receptor from canine lung had been purified to apparent homogeneity [4]. Although we had chosen canine lung as the source for β_2 receptor, our inability to obtain this tissue immediately after death, and the absence of a wide spectrum of proteinase inhibitors during the purification protocol, may have resulted in the cleavage of a higher-molecular-weight form during the processing, solubilization and purification steps. Even in these earlier experiments, relatively high concentrations of EDTA (5 mM) did not prevent proteolysis. This is in contrast to the reports of Cerione et al. [16] and Benovic et al. [5], who present evidence that a metalloproteinase is the major enzyme species responsible for the appearance during membrane preparation and processing of lower-molecular-weight forms of the receptor.

We believe that three factors are responsible for the higher-molecular-weight form of the β_2-adrenergic receptor from calf lung observed in the present study. First, we were able to obtain fresh calf lung immediately following death and to complete the purification protocol without freezing. Second, the use of HPLC gel exclusion chromatography allows purification of the receptor to be completed within 18–24 h. Third, the addition of multiple

proteinase inhibitors, including leupeptin, largely brings about the disappearance of the 53-kDa species. Despite the evidence of a higher-molecular-weight species from SDS-PAGE following the addition of multiple proteinase inhibitors, the non-denatured receptor still eluted during gel filtration chromatography in a fraction coincidental with that of IgG, a globular protein with a Stokes radius of approximately 49 Å. This is identical to our previous finding [4], and suggests that the cleaved fragments are probably constrained within the protein's tertiary structure by non-covalent interactions. Furthermore, despite its migration as a higher-molecular-weight species by gel filtration, the specific activity of the purified receptor preparation suggests that 1 mol of peptide binds 1 mol of ligand, as recently argued by Shorr et al. [26]. It is likely that its migration pattern on gel filtration chromatography is related to the fact that it does not behave as a globular protein in solution as well as the fact that it binds detergent. This is in agreement with the molecular weight determined by sedimentation equilibrium analysis for purified lung β_2-adrenergic receptor, which was found to be 64000 [26].

The purified β_2 receptor protein from calf lung, identified by autoradiography following iodination, appears as a relatively discrete band on SDS-PAGE. This pattern is similar to that described for the β_2 receptor from rat liver [21]. In contrast, the β_1 receptor from amphibian erythrocytes migrates as a broad band on SDS-PAGE [23]. It is possible that this pattern of a broad band is due to heterogeneity in the glycosylated state of the receptor. The discrete nature of the purified receptor from calf lung is further underscored by its behavior on two-dimensional gels. The 67-kDa peptide appears to behave as a single species after isoelectric focusing, with a p*I* of 6.0. This finding suggests that the mammalian β_2-receptor polypeptide is a relatively discrete species in terms of its carbohydrate composition. Determining interspecies differences in the receptor will depend on the purification of the protein in a quantity sufficient to allow detailed structural analysis.

Acknowledgements

We thank G. DeMatteo for excellent technical assistance and K. Taylor for assisting in the preparation of the manuscript. These studies were supported in part by grant HL-19259, a grant from the R.J. Reynolds Co., and were completed during the tenure of C.J.H. as an Established Investigator of the American Heart Association (AHA 80-148).

References

1 Limbird, L.E. and Lefkowitz, R.J. (1978) Proc. Natl. Acad. Sci. USA 75, 228–232
2 Shorr, R.G.L., Lefkowitz, R.J. and Caron, M.G. (1981) J. Biol. Chem. 256, 5820–5826
3 Cerione, R.A., Strulovici, B., Benovic, J.L., Lefkowitz, R.J. and Caron, M.G. (1984) Nature 306, 562–566
4 Homcy, C.J., Rockson, S.G., Countaway, J. and Egan, D.A. (1983) Biochemistry 22, 660–668
5 Benovic, J.L., Shorr, R.G.L., Caron, M.G. and Lefkowitz, R.J. (1984) Biochemistry 23, 4510–4518
6 Insoft, R. and Homcy, C.J. (1984) Fed. Proc. 43: 6 (Abstr. 1580)
7 O'Farrell, P.Z., Goodman, H.M., and O'Farrell, P.H. (1977) Cell 12, 1133
8 Jones, P.P. (1980) in Selected Methods in Cellular Immunology (Mishell, B.B. and Shiigi, S.M., eds.), pp. 398–437, W.H. Freeman and Co., San Francisco
9 Laemmli, U.K. (1970) Nature (London) 227, 680–685
10 Malamud, D. and Drysdale, J.W. (1978) Anal. Biochem. 86, 620–647
11 Righetti, P.G. and Caravaggio, T. (1976) J. Chromatogr. 127, 1–28
12 O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007–4021
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
14 Munson, P.J. and Rodbard, D. (1980) Anal. Biochem. 107, 220–239
15 Burgermeister, W., Hekman, M. and Helmreich, E.J.M. (1982) J. Biol. Chem. 257, 5306–5311
16 Stiles, G.L., Strasser, R.H., Lavin, T.N., Jones, L.R., Caron, M.G. and Lefkowitz, R.J. (1982) J. Biol. Chem. 258, 8443–8449
17 Rashidbaigi, A., Ruoho, A.E., Green, D.A. and Clark, R.B. (1983) Proc. Natl. Acad. Sci. USA 80, 2849–2853
18 Lavin, T.N., Heald, S.L., Jeffs, P.W., Shorr, R.G.L., Lefkowitz, R.J. and Caron, M.G. (1981) J. Biol. Chem. 256, 11944–11950
19 Cubero, A. and Malbon, C.C. (1984) J. Biol. Chem. 259, 1344–1350
20 Lavin, T.N., Nambi, P., Heald, S.L., Jeffs, P.W., Lefkowitz, R.J. and Caron, M.G. (1982) J. Biol. Chem. 257, 12332–12340

21 Graziano, M.P., Moxham, C.P. and Malbon, C.C. (1985) J. Biol. Chem. 260, 7665–7674
22 Atlas, d. and Levitzki, A. (1978) Nature (London) 272, 370–371
23 Shorr, R.G.L., Heald, S.L., Jeffs, P.W., Lavin, T.N., Strohsacker, M.W., Lefkowitz, R.J. and Caron, M.G. (1982) Proc. Natl. Acad. Sci. USA 79, 2778–2782
24 Jurss, R., Hekman, M. and Helmreich, E.J.M. (1985) Biochemistry 24, 3349–3354
25 Hekman, M., Feder, D., Keenan, A.K., Gal, A., Klein, H.W., Pfeuffer, T., Levitzki, A. and Helmreich, E.J.M. (1984) EMBO J. 3, 3339–3345
26 Shorr, R.G.L., McCaslin, D.R., Strohsacker, M.W., Alianell, G., Rebar, R., Stadel, J.M. and Crooke, S.T. (1985) Biochemistry 24, 6869–6875