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PHOTOAFFINITY LABELING OF β-ADRENERGIC RECEPTORS IN MAMMALIAN TISSUES

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Abstract—Photoaffinity labeling of β_1- and β_2-adrenergic receptors in plasma membranes from various mammalian tissues has been been performed utilizing the recently developed β-adrenergic antagonist probe [^{125}I]para-azidobenzylcarazolol. Tissues studied and their proportions of β_1 and β_2 receptors were: rat lung (18% β_1, 82% β_2), rabbit lung (72% β_1, 28% β_2), guinea pig lung (15% β_1, 85% β_2), dog lung (20% β_1, 80% β_2) and rabbit skeletal muscle (10% β_1, 90% β_2). As assessed by autoradiograms of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, two to three specifically protected bands of M_r 62,000–65,000, 50,000–55,000 and 38,000–42,000 were observed in each tissue system. In each case, β-adrenergic agonists and antagonists protected against photolabeling with appropriate β_1 and β_2 selectivity. Thus, in rat lung the β_2 selective antagonist ICI-118,551 was more potent in blocking incorporation than the β_1 selective antagonist betaxolol, whereas in rat, dog and guinea pig lung and rabbit skeletal muscle epinephrine was more potent than norepinephrine in blocking labeling, indicating a β_2 specificity in these tissues. Conversely, in rabbit lung membranes, norepinephrine was approximately equipotent with epinephrine in blocking photoincorporation, indicating a β_1 selectivity. In some systems protease inhibitors, especially those specific for metalloproteases (EDTA, EGTA), markedly diminished the amount of the smaller M_r peptides. For example, in rat lung the ratio of M_r 62,000:47,000:36,000 peptides changed from 30:40:30 to 60:35:5 in the presence of inhibitors. These results demonstrate the applicability of using [^{125}I]para-azidobenzylcarazolol to covalently label mammalian β-adrenergic receptors and suggest that mammalian β_1 and β_2 receptor binding sites primarily reside on peptides of M_r 62,000–65,000 and that smaller ligand binding fragments may arise by proteolysis.

Adrenergic catecholamines initiate their physiological and biochemical effects by interacting with specific receptor proteins located on the plasma membranes of their target cells. It is now recognized that adrenergic receptors are divisible into α and β types [1]. Each of these categories can be further subdivided into α_1 and α_2 [2] and β_1 and β_2 [3] subcategories respectively. The β-adrenergic receptors have attracted a great deal of interest due to their ubiquity and role in activating the enzyme adenylate cyclase. Elucidation of the structure and molecular properties of these receptors is an important goal in understanding the mechanism of receptor-mediated adenylate cyclase activation.

A recently developed approach for studying the molecular structure of β-adrenergic receptors is the covalent incorporation of photoaffinity probes derived from high-affinity β-adrenergic antagonists. Several photoaffinity probes such as [^{125}I]para-azidobenzylpindolol [4], [^{125}I]para-azidocyanopindolol [5] and [^{125}I]para-azidobenzylcarazolol have been developed [6]. Using primarily amphibian and avian erythrocyte model systems, all of these ligands have been shown to incorporate, in a photodependent manner, into β-adrenergic receptor peptides [4–6]. The extremely high affinity (pM range) and high specific activity of these probes, however, also enable the direct visualization of β_1- and β_2-adrenergic receptors in mammalian tissues where the receptor concentration is often quite low. Indeed, we have recently reported preliminary photoaffinity labeling studies of mammalian β-adrenergic receptors primarily from tissues containing both receptor subtypes [6]. In every system thus far examined, however, heterogeneous peptides of various molecular weights have been visualized. The relationship of these heterogeneous peptides to the pharmacologically defined β_1 and β_2 receptor subtypes has, as yet, not been explained or clarified.

In this paper, we report the photoaffinity labeling of the ligand binding subunits of β_1- and β_2-adrenergic receptors from a variety of mammalian tissues using a radioiodinated derivative of para-azidobenzylcarazolol. We show that photoincorporation into receptor-specific peptides can be blocked with appropriate β_1 and β_2 selective drugs, thus revealing the pharmacological identity of the peptides under study. These results shed additional light on the structural and molecular properties of mammalian β_1- and β_2-adrenergic receptor subtypes and demonstrate the utility of photoaffinity labeling for their direct visualization.

MATERIALS AND METHODS

Membrane preparations. Rat, rabbit, and dog (fresh lungs) and guinea pig (frozen lungs) membranes were prepared in ice-cold buffer containing 50 mM Tris–HCl, pH 7.4, at 25°, in the presence

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or absence of various protease inhibitors (5 μg/ml soybean trypsin inhibitor, 100 μM benzamidine, 5 μg/ml leupeptin, 5 mM EDTA and 1 mM PMSF*). After cleaning and mincing, the tissue was homogenized in 20 vol. of buffer for 2 × 10 sec using a Brinkman polytron at maximum speed. The homogenate was centrifuged at 500 *g* for 10 min. The resulting supernatant fraction was centrifuged at 40,000 *g* for 10 min. The membrane pellet was washed two more times by resuspension and centrifugation at 40,000 *g*. The membranes were stored at −70° at receptor concentrations between 0.5 and 1 nM. Protein was determined by the method of Lowry *et al.* [7] using bovine serum albumin as standard.

Radioligand binding assays. Radioligand binding assays were performed as previously described [6]. Briefly, competing ligands and membranes were incubated with [^{125}I]CYP in 75 mM Tris–HCl, pH 7.4, 25 mM $MgCl_2$ buffer for 60 min at 25°. Bound ligand was separated from free by filtering the incubation mixture through Whatman GF/C filters under vacuum and washing the filters with 75 mM Tris–HCl, 25 mM $MgCl_2$, pH 7.4 (3 × 5 ml). Specific binding was determined by subtracting the amount of radioactivity not displaced by 10^{-4} M (−)isoproterenol or 10^{-5} M alprenolol from the total amount of radioactivity bound and was usually between 80 and 90% of total binding. Agonist/[^{125}I]CYP competition experiments were performed in the presence of 0.1 mM Gpp(NH)p.

Data analysis. Data were quantitatively analyzed with a weighted, non-linear least-squares curve fitting program using a general model for complex ligand–receptor binding systems. All computations were performed on a PDP 11/45 computer. The exact treatment of experimental data has been described previously in detail by De Lean *et al.* [8]. Briefly, competition curves were analyzed according to a model for the mass-action binding of the radioligand and competing drug to one or multiple binding sites based on the law of mass action. Deviation of the observed points from the predicted values were weighted according to the reciprocal of the predicted variance. Testing for statistical difference between models was accomplished by comparing their residual variances of fits to the data. A model for two binding sites was retained only when it fitted the data significantly better ($P < 0.05$) than a model for a single binding site.

Membrane photoaffinity labeling. Photoaffinity labeling of the various tissues was done following the procedure of Lavin *et al.* [6]. Membranes were diluted in buffer containing either 25 mM Tris–HCl, 2 mM $MgCl_2$ or 75 mM Tris–HCl, 25 mM $MgCl_2$, pH 7.4, at 25° with or without protease inhibitors (see above) to obtain a final receptor concentration of 30–50 pM. The photoaffinity ligand ([^{125}I]pABC) in equal concentration (30–50 pM) was then added under dim light, and the incubation was carried out for 90 min at 25°. After the incubation, the tubes were filled with appropriate buffer containing 0.5% fatty acid free BSA and centrifuged at 40,000 *g* for 10 min. This washing was repeated two more times and one final wash was done with incubation buffer to remove BSA. The sample was then photolyzed for 90 sec as described [6]. After photolysis, the suspension was again centrifuged at 40,000 *g* and resuspended in a smaller volume (1.3 ml). This was centrifuged at 13,000 *g* prior to resuspension in SDS-PAGE sample buffer. All photoaffinity labeling experiments were performed two to three times with identical results. The percent of covalent incorporation of specifically bound [^{125}I]pABC varied with the tissue examined but was generally 3–15%.

SDS-Gel electrophoresis. Gel electrophoresis was performed according to the method of Laemmli [9] using 8% acrylamide gels. After denaturing the samples at room temperature for 30 min in 10% SDS, 10% glycerol, 5% β-mercaptoethanol, 50 mM Tris–HCl, pH 6.8 (SDS-PAGE sample buffer), samples were electrophoresed and then dried prior to autoradiography at −80° with Kodak XAR-5 film with intensifying screens (Cronex Lightening Plus, Dupont).

Materials. *p*-Azido-*m*-[^{125}I]iodobenzylcarazolol ([^{125}I]pABC) and [^{125}I]cyanopindolol ([^{125}I]CYP) were obtained from the New England Nuclear Corp., Boston, MA. Alprenolol hydrocholoride was a gift from the Hassle Pharmaceutical Co. of Sweden. Premixed SDS-polyacrylamide gel electrophoresis standards (phosphorylase b, M_r = 94,000; albumin, M_r = 67,000; ovalbumin, M_r 43,000; carbonic anhydrase, M_r 30,000; soybean trypsin inhibitor, M_r 20,100; α-lactalbumin, M_r 14,400) were from Pharmacia and radioiodinated according to the method of Greenwood *et al.* [10]. Electrophoresis reagents were from Bio-Rad Laboratories. Specially pure SDS was obtained from BHD, Poole, England. X-Ray film (XAR-5) and developing solutions were from Kodak. Intensifying screens (Cronex Lightening Plus) were from DuPont. Adrenergic compounds were from sources previously described [11]. ICI-118,551 was a gift from Imperial Chemical Industries, Macclesfield, Cheshire, United Kingdom. Betaxolol was a gift of Synthelabo, Paris, France. EDTA was from Mallinckrodt. Leupeptin, soybean trypsin inhibitor, benzamidine and PMSF were from the Sigma Chemical Co., St. Louis, MO. Other chemical reagents were from Sigma. Rats (Sprague–Dawley) were from the Charles River Breeding Laboratories, Wilmington, MA. New Zealand white rabbits were from a local supplier. Guinea pig lungs were from Pel-Freez. Rabbit skeletal muscle sarcolemma was a gift from Dr. Peter Smith, Bowman Gray School of Medicine, Winston-Salem, NC.

RESULTS

Our first approach was to use radioligand binding methods to quantitatively delineate the relative proportions of β_1 and β_2 receptors in the mammalian

* Abbreviations: PMSF, phenylmethylsulfonyl fluoride; [^{125}I]pABC, [^{125}I]para-azidobenzylcarazolol; [^{125}I]CYP, [^{125}I]cyanopindolol; BSA, bovine serum albumin; Betax, betaxolol; ICI, ICI-118, 551; Iso, isoproterenol; Alp, alprenolol; Epi, epinephrine; NE, norepinephrine; Gpp(NH)p, guanylyl-5′-imidodiphosphate; and SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

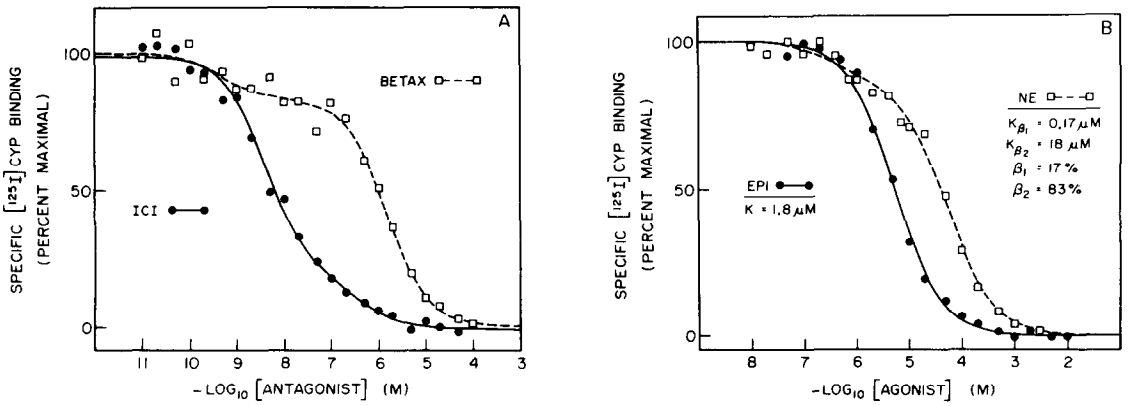

Fig. 1. Competition of [^{125}I]CYP binding with adrenergic antagonists and agonists in rat lung membranes. The symbols represent the experimentally determined data points while the drawn lines represent the computer modeled best fit to the data. (A) The competition curves for both ICI-118,551 and betaxolol were best explained by assuming the presence of two receptor binding sites (i.e. β_1 and β_2). The computer modeled parameters for the antagonist curves are as follows: ICI-118,551, K_{β_1} = 150 nM, K_{β_2} = 1.3 nM, % β_1 = 19, % β_2 = 81; betaxolol, K_{β_1} = 0.4 nM, K_{β_2} = 490 nM, % β_1 = 18, % β_2 = 82. When the two curves were analyzed simultaneously, the distribution of β_1 and β_2 receptors was 19 and 81% respectively. (B) The competition curve for epinephrine was best explained by assuming a single binding site whereas the curve for norepinephrine was resolved into two components representing 17% β_1 and 83% β_2 receptors. Both curves were generated in the presence of 0.1 mM Gpp(NH)p.

tissues under study. Figure 1A demonstrates the pharmacological characteristics of the β-adrenergic receptors in rat lung membranes as assessed by direct ligand binding using [^{125}I]cyanopindolol and the subtype selective antagonists ICI-118,551 and betaxolol. ICI-118,551 has high affinity for β_2-adrenergic receptors with lower affinity for β_1-adrenergic receptors [12]. In contrast, betaxolol has high affinity for β_1-adrenergic receptors and low affinity for β_2 receptors [12]. As seen in Fig. 1A, ICI-118,551 was more potent than betaxolol in competing for [^{125}I]CYP binding, indicating that the rat lung membranes contain a preponderance of β_2-adrenergic receptors. Moreover, it is apparent that the antagonist competition curves are biphasic, each possessing a high- and a low-affinity component reflecting the distribution of β_1- and β_2-adrenergic receptor subtypes in this tissue. When both curves were analyzed simultaneously, the distribution of β_1 and β_2 receptors in these membranes was found to be 19 and 81% respectively. Shown in Fig. 1B are similar experiments employing the agonists epinephrine and norepinephrine. Epinephrine exhibits about equal affinity for β_1 and β_2 receptors and thus cannot be used to discriminate them. Nevertheless, epinephrine clearly demonstrated an overall potency greater than norepinephrine in competing for [^{125}I]CYP binding. This is consistent with the presence in this tissue of a majority of β_2 receptors since norepinephrine is less potent at β_2 receptors. Norepinephrine, however, did discriminate between β_1 and β_2 receptors and exhibited a competition curve with a high-affinity phase, indicating 17% β_1 receptors and a low-affinity phase representing 83% β_2 receptors. These

Table 1. β-Adrenergic receptor subtypes determined by radioligand binding studies and polypeptides identified by photoaffinity labeling techniques*

Species	Tissue	Receptor subtype	Peptides identified (M_r)
Rat	Lung	β_1 (18%) β_2 (82%)	62,000; 47,000; 36,000
Rabbit	Lung	β_1 (72%) β_2 (28%)	65,000; 45,000; 38,000
Guinea pig	Lung	β_1 (15%) β_2 (85%)	63,000; 56,000; 49,000
Dog	Lung	β_1 (20%) β_2 (80%)	52,000; 40,000
Rabbit	Skeletal muscle	β_1 (10%) β_2 (90%)	54,000; 48,000; 44,000

* The proportions of β_1- and β_2-adrenergic receptors were determined as explained in Fig. 1. The data represent the means from two to three independent experiments.

β_1 and β_2 proportions determined with the agonists are in good agreement with those found in Fig. 1A determined with the subtype selective antagonists.

Similar experiments were conducted with membranes derived from a number of mammalian tissues using both agonists and antagonists; the results are summarized in Table 1. Rat, dog and guinea pig lung and rabbit skeletal muscle membranes were found to contain predominantly β_2-adrenergic receptors whereas rabbit lung contained mostly β_1 receptors. These values for the relative proportions of β_1 and β_2 receptors agree well with previously published results [13].

With the precise receptor-subtype pharmacology established in each tissue, the next step was the direct visualization of the β_1 and β_2 receptors via photoaffinity labeling with [^{125}I]para-azidobenzylcarazolol. This ligand binds to both β_1 and β_2 receptors with high and equal affinity [6]. Figure 2 presents the results of photoaffinity labeling of β-adrenergic receptors in rat lung membranes. Three peptides were labeled, with apparent molecular weights of 62,000, 47,000 and 36,000. All three bands were protected by the β-adrenergic agonist isoproterenol. Densitometric scanning of the gels indicated that the ratio of the three bands was 30:40:30 respectively. These results are similar to those previously reported by Lavin *et al.* [6]. Most importantly, all three bands were demonstrated to be β_2-adrenergic in nature (Fig. 2). ICI-118,551 was more potent than betaxolol in blocking the photoincorporation of [^{125}I]pABC into these three bands. At a concentration of 10^{-9} M, ICI-118,551 blocked more than 60% of the photoincorporation, whereas betaxolol did not have any effect at this concentration.

To investigate the possibility that the lower molecular weight bands might represent degradation products of the 62,000 molecular weight peptide,

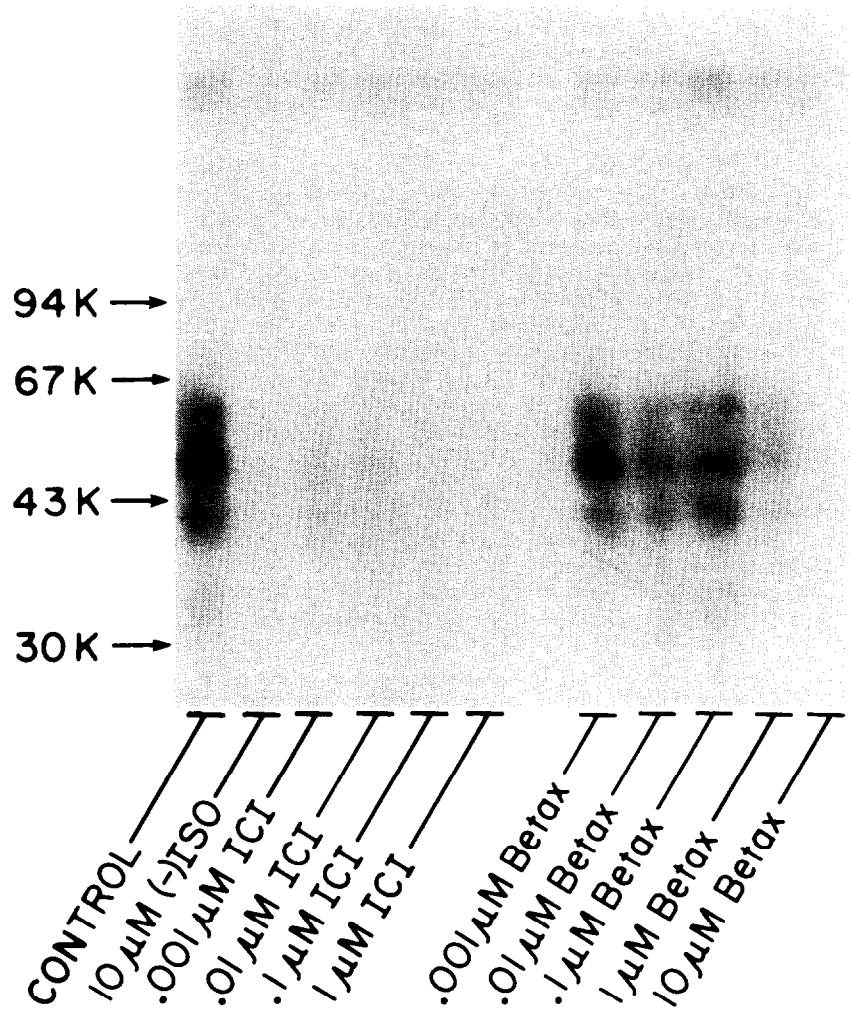

Fig. 2. Photoaffinity labeling and pharmacological specificity of incorporation of [^{125}I]pABC in rat lung membranes in the absence of protease inhibitors. Membranes were incubated at 25° for 90 min with [^{125}I]pABC alone or with 10 μM (−)isoproterenol or various concentrations of ICI-118,551 or various concentrations of betaxolol. At the end of the incubation, membranes were washed and photolyzed as explained under Methods. After denaturing the membrane pellets with SDS, the samples were electrophoresed on an 8% acrylamide gel. Molecular weight shown × 1000 (K).

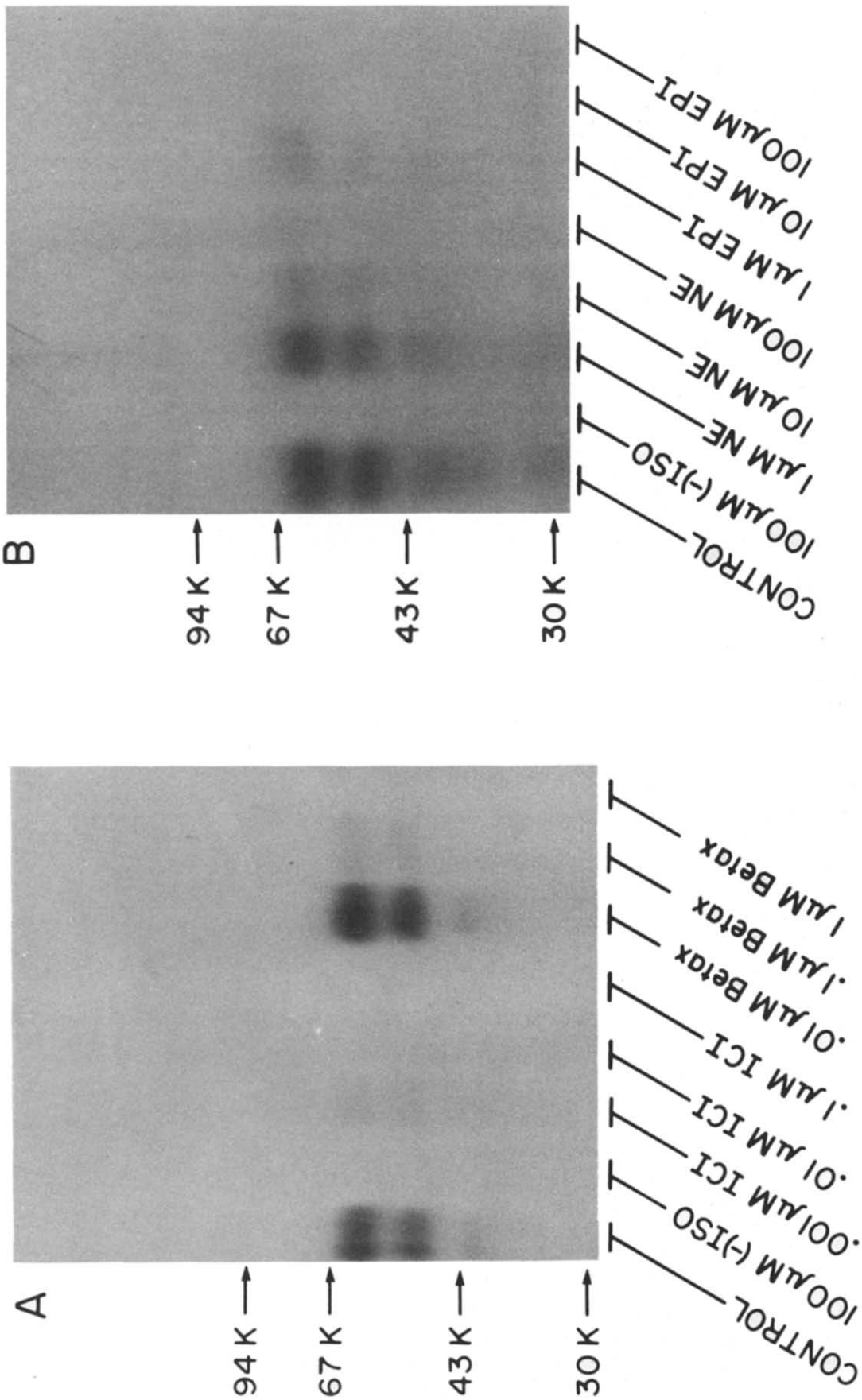

Fig. 3. Photoaffinity labeling and pharmacological specificity of incorporation of [^{125}I]pABC in rat lung membranes in the presence of protease inhibitory. The preparation of the membranes and the photoaffinity labeling were done in the presence of various protease inhibitors as explained in Methods. Aliquots of membranes were incubated with [^{125}I]pABC alone or in the presence of the indicated concentrations of antagonists (A) or agonists (B) and photolabeled. The samples were solubilized and electrophoresed on an 8% acrylamide gel, and autoradiography was obtained as described in Methods.

attempts were made to inhibit the degradation by including various protease inhibitors during the membrane preparation. A variety of protease inhibitors such as soybean trypsin inhibitor, benzamidine, PMSF, leupeptin, and EDTA, which has been shown to inhibit metalloproteases, were included in the homogenization buffer as described in Methods. The same buffer was used in the photoaffinity labeling experiment. The results of such an experiment using rat lung are shown in Fig. 3, panels A and B. In contrast to Fig. 2 where the three bands appeared to be in a ratio of 30:40:30, the ratio of the three bands in Fig. 3A and B was approximately 60:35:5. Similar results were obtained when experiments were done in which all the other protease inhibitors were eliminated except the chelating agent EDTA (data not shown).

As shown in Fig. 3A, these bands were still protected by ICI-118,551 and betaxolol in a β_2-adrenergic fashion. ICI-118,551, at a concentration of 10^{-9} M prevented more than 70% of the incorporation, whereas betaxolol was ineffective even at 10^{-8} M. Protection experiments with the agonists epinephrine and norepinephrine confirmed these findings and are shown in Fig. 3B. At a concentration of 10^{-6} M, epinephrine prevented more than 90% of the photoincorporation into the three peptides whereas norepinephrine was much weaker at this concentration. These results suggest that all the bands that are labeled in rat lung membranes are of the β_2 subtype, that the smaller peptides may be derived from the larger one by proteolysis, and that each of these peptides contains the intact ligand binding site.

Unlike rat lung membranes where inclusion of protease inhibitors during the membrane preparations made a dramatic difference in the distribution of the specific receptor peptides, rabbit lung membrane receptors did not show any change when the membranes were prepared in the presence

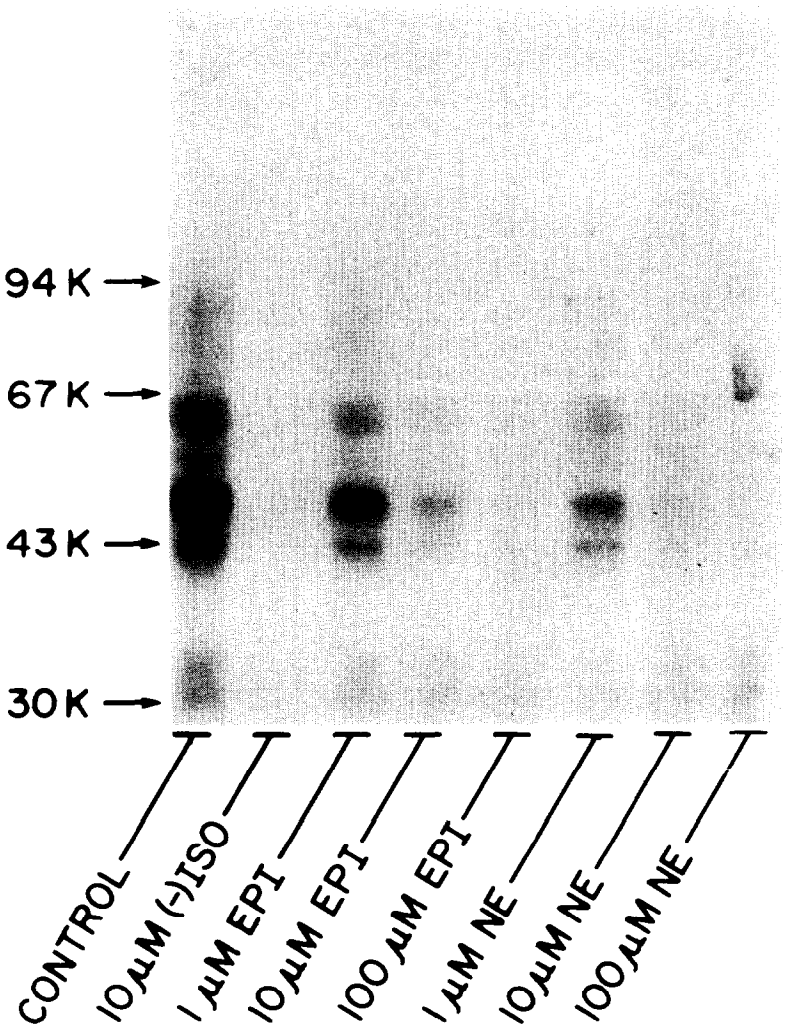

Fig. 4. Photoaffinity labeling and pharmacological specificity of incorporation of [^{125}I]pABC in rabbit lung membranes. The preparation of the membranes and the photoaffinity labeling were done in the presence of various protease inhibitors. Aliquots of membranes were incubated with [^{125}I]pABC alone or in the presence of the indicated concentrations of competing ligands and photolabeled as explained under Methods.

or absence of these protease inhibitors. Figure 4 shows the results of photoaffinity labeling and the pharmacological specificity of [^{125}I]pABC incorporation into rabbit lung membrane preparations. The membranes were prepared in the presence of benzamidine, leupeptin, soybean trypsin inhibitor, PMSF and EDTA as described under Methods. Three major peptides with molecular weights of 65,000, 45,000 and 38,000 were specifically labeled in the ratio 35:50:15 and were protected by the β-adrenergic agonist isoproterenol. When pharmacological experiments were performed using various concentrations of epinephrine and norepinephrine, it was found that norepinephrine was slightly more potent than epinephrine in blocking the photoincorporation into these three peptide bands (Fig. 4). Several other peptide bands could be observed which were blocked by a high concentration of isoproterenol; however, no distinct pharmacological pattern for blockade by epinephrine and norepinephrine could be observed for these bands, suggesting that they may not be receptor peptides. This suggests that the three major bands labeled are of the β_1 subtype. Identical results were obtained when the photoaffinity labeling was done in membranes prepared in the absence of protease inhibitors (data not shown).

Figure 5 shows the photoaffinity labeling of guinea pig lung membranes prepared and photolabeled in the presence of protease inhibitors. These membranes which have a β_1–β_2 ratio of 15:85 showed three labeled peptide bands with apparent molecular weights of 63,000, 56,000 and 49,000 in the ratio 48:34:17. All three bands were protected by alprenolol, a β-adrenergic antagonist, and isoproterenol, a β-adrenergic agonist. The figure also shows that covalent labeling of these peptides displayed appropriate stereoselectivity since the (−)isomer of isoproterenol was more effective than its (+)isomer in blocking the incorporation. This figure also establishes that the labeling of these bands in guinea pig lung membranes is typically β_2-specific since epinephrine was more effective than norepinephrine in blocking labeling when both agents were tested at the same concentration.

The pattern obtained when rabbit skeletal muscle

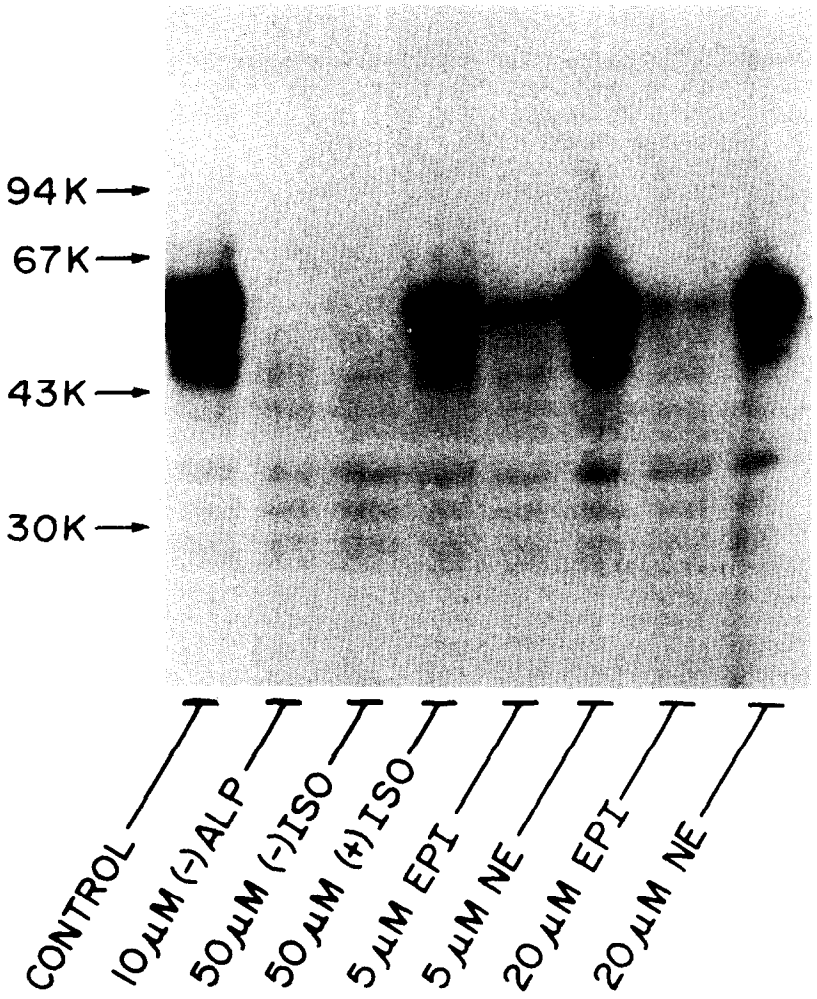

Fig. 5. Photoaffinity labeling and pharmacological specificity of incorporation of [^{125}]pABC in guinea pig lung membranes. Aliquots of membranes were incubated with [^{125}I]pABC alone or in the presence of indicated concentrations of competing ligands and photolabeled as explained under Methods.

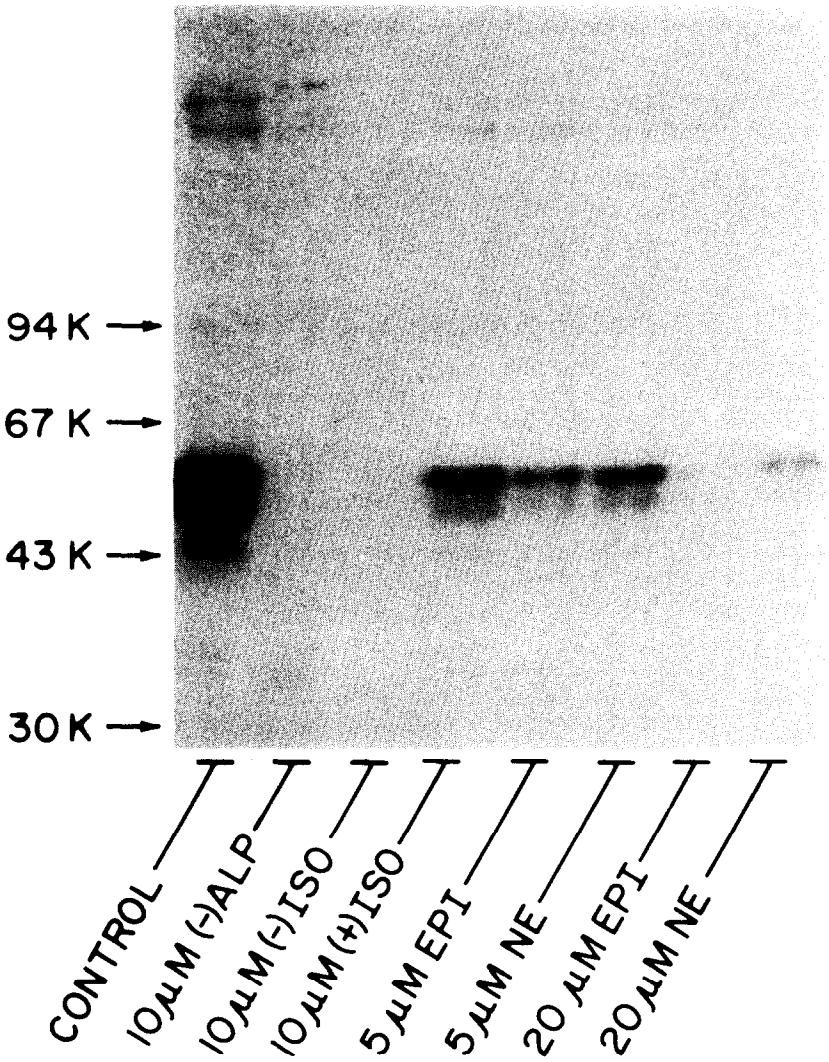

Fig. 6. Photoaffinity labeling and pharmacological specificity of incorporation of [^{125}I]pABC in rabbit skeletal muscle membranes. Aliquots of membranes were incubated with [^{125}I]pABC alone or in the presence of indicated concentrations of competing ligands and photolabeled as explained under Methods.

membranes were photolabeled is presented in Fig. 6. Rabbit skeletal muscle sarcolemma contained predominantly β_2 adrenergic receptors (90% β_2 and 10% β_1). Two major peptides (mol. wt 54,000 and 48,000) and one minor peptide (mol. wt 44,000) were labeled (Fig. 6). All three bands were protected by alprenolol and (−)isoproterenol. The stereoisomer (+)isoproterenol was less effective in blocking labeling as compared to (−)isoproterenol. Epinephrine was more potent than norepinephrine in blocking the incorporation in all bands at both concentrations tested, indicating the β_2 specificity of these peptides.

Dog lung, which is another system containing predominantly β_2 adrenergic receptors, shows a pattern similar to guinea pig lung membranes (Fig. 7). Two peptides were labeled with mol. wt of 52,000 and 40,000. Incorporation into both peptide bands was blocked by the β-adrenergic antagonists alprenolol. (−)Isoproterenol was more potent than (+)isoproterenol. Epinephrine and norepinephrine protected the bands with a typical β_2-adrenergic specificity in that epinephrine was found to be more potent than norepinephrine.

DISCUSSION

Beta-adrenergic receptors have been classified into β_1 and β_2 receptor subtypes on the basis of differing pharmacological properties and physiological responses. It has become feasible recently to delineate and quantitate these receptor subtypes using radioligand binding techniques and subtype selective drugs. Heretofore, however, the structural similarities and/or differences between β_1- and β_2-adrenergic receptors have not been identified. We have now demonstrated that the ligand binding subunits of mammalian β_1- and β_2-adrenergic receptors can be directly visualized *and* pharmacologically classified using photoaffinity labeling methods. This is demonstrated by the appropriate agonist and antagonist

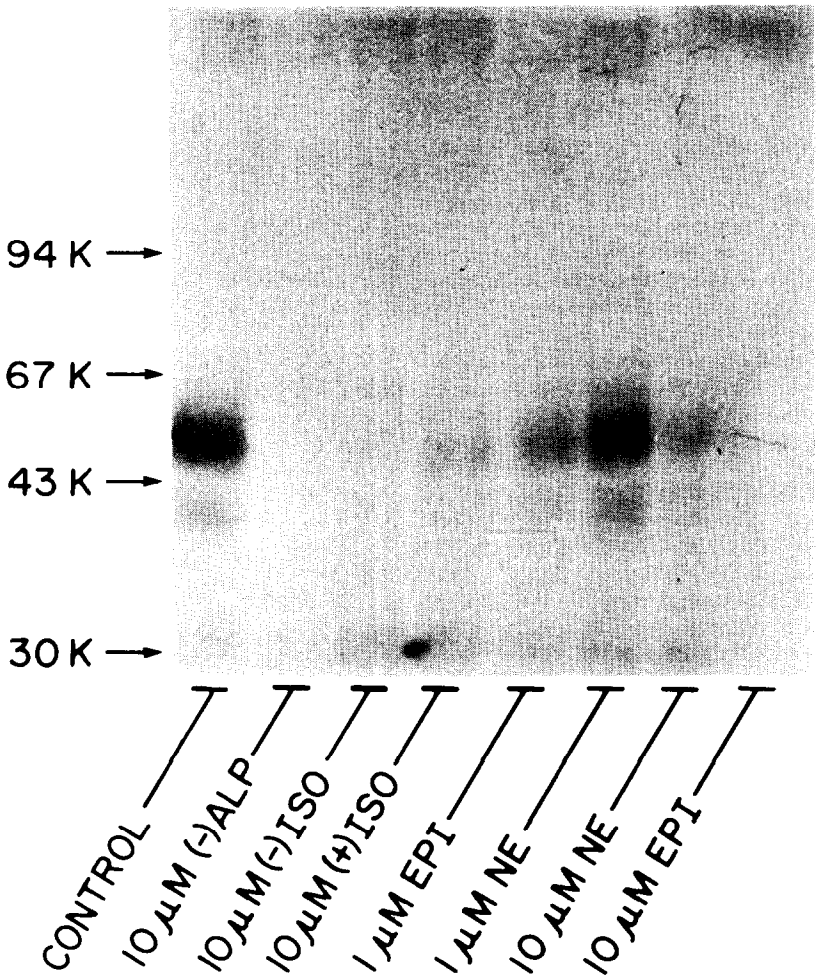

Fig. 7. Photoaffinity labeling and pharmacological specificity of incorporation of [^{125}I]pABC in dog lung membranes. Aliquots of membranes were incubated with [^{125}I]pABC alone or in the presence of indicated concentrations of competing ligands and photolabeled as explained under Methods.

potencies and stereospecificity in protecting the receptor peptides from photoincorporation.

It is of interest that the ligand binding subunits from a variety of mammalian tissues containing both β_1 and β_2 subtypes in different proportions displayed similar banding patterns on SDS-PAGE (Table 1). This phenomenon is best exemplified by comparing the rat and rabbit lung membrane systems which displayed inverse proportions of β_1 and β_2 receptors (Table 1) yet showed almost identical peptide bands after labeling and SDS-PAGE (Fig. 2–4 and Table 1). This suggests that the ligand binding subunits of mammalian β_1- and β_2- adrenergic receptors have similar molecular weights. The absence of a 62,000 M_r peptide in the dog lung and rabbit skeletal muscle membrane systems could be due to the absence of protease inhibitors during their preparation (see below). Homcy *et al.* [14] have recently shown, using purification and photoaffinity labeling, in the absence of protease inhibitors that the molecular weight of the dog lung β_2 receptor is 52,000–53,000, in good agreement with our present results.

Stiles *et al.* [15] have shown that mammalian heart β_1 receptors and frog heart β_2 receptors exhibit two photoaffinity labeled peptides of $M_r = 62{,}000$ (major component) and $M_r = 55{,}000$ (minor component), additionally suggesting similarities in the size of the receptor peptides between mammalian *and* non-mammalian β_1 and β_2-adrenergic receptors.

In all the mammalian tissues studied thus far, multiple peptide bands have been identified by photoaffinity labeling. This heterogeneity cannot be due to the presence of both β_1 and β_2 receptor subtypes since in each case all of the peptides demonstrate the appropriate β_1 or β_2 specificity. A likely explanation for the presence of these multiple bands is proteolysis. Recently Benovic *et al.* [16] have shown that the ratio of the three bands that are labeled in rat lung membranes could be changed from 30:40:30 to 72:28:6 by including protease inhibitors during the membrane preparation, in good agreement with our data shown in Figs. 2 and 3. The less dramatic change in the proportion of the 47,000 peptide may indicate that this peptide might be deri-

ved from the 64,000 peptide by another mechanism or by a proteolytic activity which was only partially inhibited.

While rat lung receptors displayed differences in their banding patterns in the presence and absence of protease inhibitors, rabbit lung membrane receptors were unaffected by the presence of these inhibitors (data not shown). This suggests that either proteolysis is not the only mechanism which is responsible for the multiple peptides or that the appropriate conditions for its blockade in rabbit lung membranes have not yet been found. The different peptides observed might also be a reflection of physiological processing of the receptors prior to or after their insertion into the plasma membrane.

When pharmacological studies were conducted to assess the proportion of β_1 and β_2 receptors labeled with [^{125}I]pABC, it was always found that only the predominent subtype that is present in the particular tissue was labeled. For example, all three peptides that were labeled in rat and rabbit lung membranes showed β_2 and β_1 specificity, respectively, even though rat and rabbit lungs contain about 20% β_1 and β_2 receptors respectively. The reason for not detecting the minor subtype component is likely due to their low density and similar molecular weights compared to the predominate subtype such that any labeling to which they contributed was readily obscured.

In summary, we have been able to directly visualize and pharmacologically characterize by photoaffinity labeling mammalian β_1- and β_2-adrenergic receptor peptides. These studies suggest similarities in the size of the ligand binding peptides of β_1 and β_2 receptors while demonstrating the usefulness of [^{125}I]pABC as a covalent probe of both mammalian β-adrenergic receptor subtypes.

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