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Stereoselective Modification of Circular Dichroism Spectra of Rat Lung β-Adrenoceptor Protein Preparation by Enantiomers of Epinephrine

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ABSTRACT The presence of β-adrenoceptor in a rat lung membrane preparation was confirmed by stereoselective competition of enantiomers of epinephrine with labeled iodocyanopindolol. The receptor-rich protein fraction, when combined with the pharmacologically active (−)-epinephrine, exhibited specific changes in the 205–220 nm region of circular dichroism spectra, indicating that the receptor helices may be perturbed. The (+)-epinephrine combined with the lung protein produced little or no change of the spectra. Bovine serum albumin, when combined with either enantiomer of epinephrine, produced nonstereoselective alterations of the spectra. Thus, the data provide important evidence for the higher intrinsic pharmacologic activity of the natural (−)-epinephrine over the unnatural (+)-enantiomer.

KEY WORDS: chirality of agonist-efficacy, β-adrenoceptor, stereoselective change of receptor, epinephrine enantiomers

Neurotransmitters and related agonists activate membrane receptor proteins so that specific conformational changes occur. These stimulant molecules are characterized by the "affinity" for the receptor and their "intrinsic activity."[1,2] The latter property of the agonist is in part quantitated in the pharmacologic bioassay.[3] The agonist-receptor interaction in terms of "affinity" and "intrinsic activity" or "intrinsic efficacy" obtained from pharmacologic experiments is stereoselective.[4,5] It has been rationalized that the initial conformational changes of the receptor by agonists must also be stereoselective. Direct evidence, however, is lacking. Circular dichroism spectroscopy, which is sensitive to changes of protein conformation in solution,[6,7] has been used in this study to detect the perturbation caused by the agonist-receptor interaction in the rat lung membrane, which contains a high density of β-adrenoceptor. A change in the secondary structure of the protein is observed on interaction with the natural (−)-epinephrine, whereas very little or no spectral perturbation is observed for the unnatural (+)-isomer. High intrinsic activity, as indicated by the specific spectral change after (−)-epinephrine, also correlates with its high affinity in ligand-binding experiments. These results were communicated at the FASEB meeting in Atlanta.[8]

METHODS

Rat Lung Membrane Protein Preparation

Several grams of lungs from freshly killed rats or lungs from Pel-Freez Biologicals (Roger, AR) were stored at −80°C until used. Tissues were thawed at room temperature in Tris-saline buffer (Tris-HCl 20 m*M*, pH 7.4, NaCl 0.154 *M*, and 1.1 m*M* ascorbic acid). Lungs were cleaned of connective tissue and minced with scissors, then homogenized in buffer with two bursts of a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY) at setting 5–7 for 10–15 sec per burst. Then, crude homogenates were centrifuged at 1000 *g* for 10 min to remove tissue clumps, unbroken cells, and nuclei. The supernatant was then centrifuged at 40,000 *g* for 15–20 min to pellet membrane fractions. Pellets were washed and resuspended three times in cold buffer and recentrifuged at 40,000 *g* for 20 min following each wash. Membrane pellets were immediately used or stored at −80°C until needed. The concentrations of protein in four different preparations varied between 3.75 and 9.81 mg/ml. Dilutions for use were made in the phosphate buffer. The presence of β-adrenoceptor in the preparation was confirmed by examining the competition of ^{125}I-cyanopindolol with enantiomers of epinephrine. The technical details of the method were identical to those published before.[9]

Chemicals and Instrumentation

Fat-free bovine serum albumin (BSA Sigma lot 11H0113) solution was prepared in a manner similar to that described for rat lung protein. Fresh solutions of (−)-epinephrine (+)-bitartrate (Sigma lot 14F-0438 and Sterling Winthrop lot N-185-JK) and (+)-epinephrine (−)-bitartrate (Sterling Winthrop lot R-033-BB or lot R-004-TN) were prepared in distilled water which contained 1 μ*M* of ethylene diamine tetraacetic (EDTA) to prevent spontaneous oxidation of the catecholamine. Small volumes, up to 60 μl, of the drug solution were added to the 3 ml cuvette which contained the protein solution (50 μg/ml).

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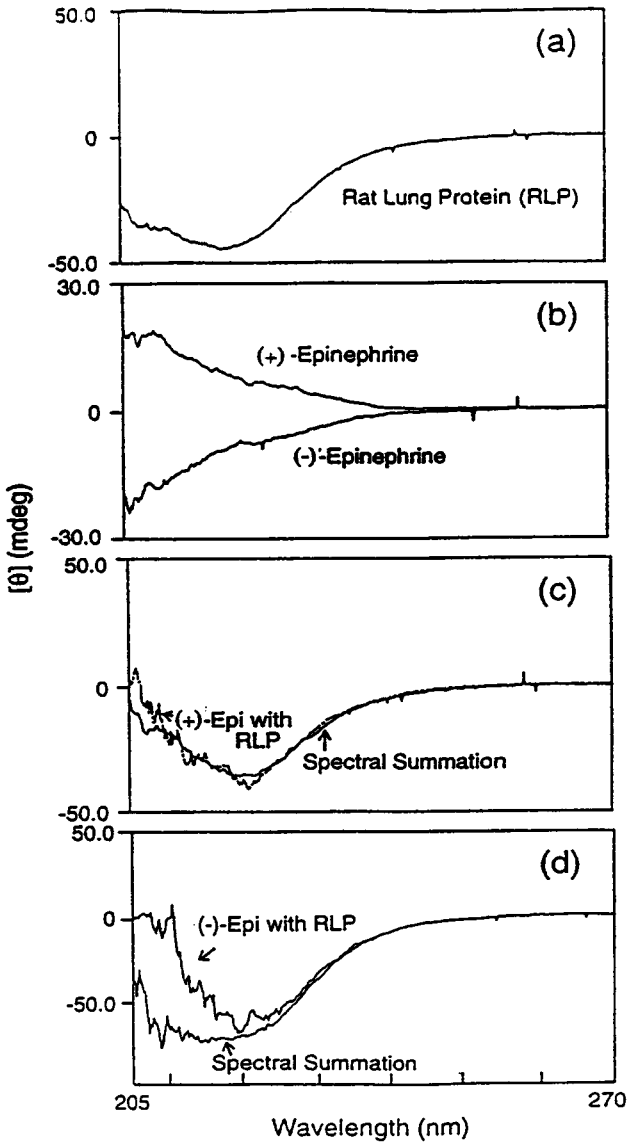

Fig. 1. Circular dichroism spectrum of **(a)** rat lung membrane protein (50 μg/ml) in phosphate buffer, **(b)** enantiomers of epinephrine (200 μ*M*) in phosphate buffer, **(c)** rat lung membrane protein (RLP, 50 μg/ml) in combination with (+)-epinephrine (200 μ*M*). The spectral summation of data obtained in (a) and (b) and corrected for dilution is also provided for comparison. **(d)** Rat lung membrane protein (50 μg/ml) in combination with (−)-epinephrine (200 μ*M*), along with spectral summation.

Circular dichroism spectra were obtained at room temperature (22–26°C). A Jasco-J 500C spectrometer was coupled to the visual as well as to the plotter output data acquisition computer system. Spectral scans were between 270 and 200 nm, and each spectral output represented an average of 10 scans. On a given day of the experiment, appropriate controls as well as the chemical combination spectra were obtained. Spectral summation analysis was automatically carried out by the computerized program.

RESULTS AND DISCUSSION

The presence of β-adrenoceptor in the preparation was confirmed by examining the competition of ^{125}I-cyanopindolol with either the (−) or the (+)-enantiomer of epinephrine. In the presence of 20–30 p*M* of labeled antagonist, the amount of specific binding decreased by various concentrations of either (−) or (+)-epinephrine with −log molar K_i values (n = 2) of 5.32 and 3.82, respectively. The affinity difference was about 30-fold. The maximum capacity for binding of the radiolabeled antagonist was ~300 fmol/mg protein.

Figure 1a shows the circular dichroism spectrum obtained with rat lung membrane protein and is characterized by peaks at 208 and 220 nm. Solutions of (−)-epinephrine and (+)-epinephrine (200 μ*M*) each exhibited expected negative and positive Cotton effects, as shown in Figure 1b. Upon mixing the (+)-epinephrine with the rat lung protein, the circular dichroism spectrum obtained is similar to the spectrum obtained by summation of the spectrum for the (+)-enantiomer and the protein (after correction for dilution). Figure 1c shows that there is very little effect on the conformation of the protein. However, as shown in Figure 1d, the interaction of the (−)-enantiomer with the rat lung protein results in a significant perturbation of ellipticity near the 205–215 nm region of peptide bond absorption, as compared to the mathematically summed spectra. The spectral alterations caused by binding of the (−)-enantiomer unambiguously demonstrate that there is a change in the secondary structure upon agonist-receptor interaction. Qualitatively, these can be explained by a decrease in α-helical content and an increase in random-coil conformation. Considering the complexity of the protein fraction, a quantitative analysis of these spectra is daunting and will require purer fractions and better signal-to-noise spectra. This is the future goal of this study.

In order to establish that we are observing receptor-relevant, enantiomer-dependent interaction, we also studied the binding of (+) and (−)-epinephrine to BSA. These data are shown in Figure 2. The combination of each enantiomer of epinephrine with BSA caused alterations in the ellipticity, indicating that there is no selectivity in binding of a particular enantiomer of the catecholamine with BSA. Non-stereoselective changes in the circular dichroism spectra of the BSA by enantiomers of epinephrine confirm previous observations on the catechol-specific, non-stereoselective interaction of catecholamines with the microsomal protein of rabbit aorta[10] and human serum albumin.[11] In contrast to this, we observed the stereoselective alterations of the spectra of the rat lung receptor protein in the region of 205–220 nm by the (−)-enantiomer. This observation reflects stereoselective complex formation of agonist-receptor interaction. Spectral changes by the (+)-epinephrine were either small or could not be recorded at the sensitivity of the instrumentation.

When compared at ED_{50} values of the concentration response data on tissues containing β-adrenoceptor, (−)-epinephrine was about 30 times more active than the (+)-form.[5] The radioligand competition study in the rat lung preparation also indicated 30-fold higher affinity of the (−)-enantiomer over the (+)-form. Equality of affinity and activity ratios[5] of the enantiomers in two types of experiment provides evidence

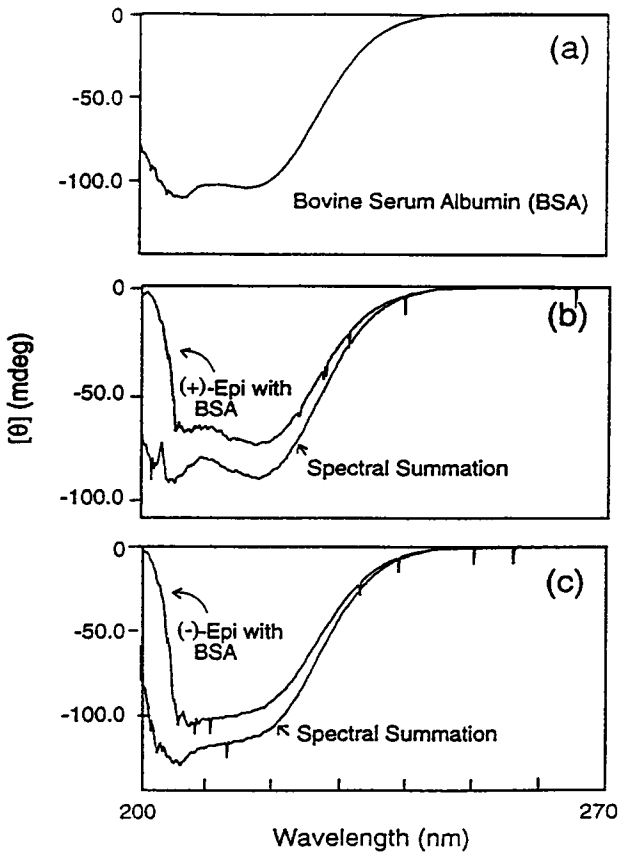

Fig. 2. Circular dichroism spectrum of **(a)** bovine serum albumin (BSA, 50 μg/ml) in phosphate buffer. **(b)** and **(c):** BSA (50 μg/ml) with enantiomers of epinephrine (200 μ*M*)in a phosphate buffer. Spectral summation in each case is also provided.

that the rat lung protein contains a β-adrenoceptor. Even though spectral changes of the lung protein by two isomers cannot be quantitated with great precision, the significant stereoselective changes due to the (−)-isomer can be attributed to activation of the β-adrenoceptor in the membrane preparation.

β-Adrenoceptor agonists activate the receptor protein to produce the "specific" conformational changes which trigger a series of biochemical and amplified mechanical coupling events leading to the response of the tissue. In pharmacologic experiments, so called intrinsic activity of the agonist is measured at the point distal to the initial activation of the receptor. The cellular transducer ratio determines the intensity of activity.[12] Radioligand binding experiments which provided an accurate dissociation constant of the agonist, do not measure the "intrinsic activity" of the agonist molecule. The measurement of ellipticity, and thereby the conformational change of the agonist-receptor protein complex by circular dichroism spectroscopy, can uncover the dynamics of the early events of receptor activation. In the present investigation, stereoselective alteration in the 205–220 nm region of the spectra reflects the agonist-mediated "specific" conformational changes of the lung β-adrenoceptor in the preparation. Spectral changes in this region are considered to be an indication of perturbation of receptor helices.[6,7,13] Thus, "intrinsic activity" of the agonist measured in the pharmacologic experiments as well as the initial conformational changes demonstrated by circular dichroism spectroscopy for enantiomers of epinephrine are stereoselective. The present data provide direct evidence for early events in the pharmacologic efficacy or the intrinsic activity of the agonist. It is quite fitting to quote Cushny,[14] who stated that in pharmacologic effects there can hardly be any question that the action is on a single receptor which embraces both isomers, though with unequal warmth.

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