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MAPPING OF MAMMALIAN β-ADRENORECEPTORS BY USE OF MACROMOLECULAR ALPRENOLOL DERIVATIVES

A COMPARISON WITH AMPHIBIAN ERYTHROCYTE RECEPTORS

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Abstract—The interaction of macromolecular alprenolol derivatives with β-adrenoreceptors of rat heart, lung, and erythrocytes and frog erythrocytes has been studied. Macromolecular derivatives were prepared by covalently coupling alprenolol to dextrans containing a homologous series of spacer arms of various lengths. The affinity of these macromolecules for frog erythrocyte membranes increased with increasing length of spacer arm. In contrast, the affinity of these macromolecules for all mammalian membrane preparations was weak and insensitive to the length of the spacer arm. The inhibition of [^{3}H]dihydroalprenolol binding to rat heart, lung, and erythrocyte membrane preparations by these macromolecular derivatives was more than 1000-fold less potent than inhibition by alprenolol. The results suggest different structural characteristics between mammalian and amphibian β-adrenoreceptors; however, apparently only small differences between mammalian receptors could be distinguished with these probes.

β-Adrenergic receptors have been studied extensively in amphibian, avian, and mammalian systems [1–3]. Attempts have been made to purify and characterize β-receptors from different species. Caron *et al.* [4] and Shorr *et al.* [5] have utilized affinity and ion exchange chromatography to purify receptors from frog erythrocytes. They estimated the molecular weight of the hormone binding subunit to be approximately 58,000 and to have a pI of 5.8. Fraser and Venter [6] utilized anti-β-receptor monoclonal antibodies attached to Sepharose in the purification of the turkey erythrocyte β-receptor. They reported a primary molecular weight product of 71,000 and a 31,000 molecular weight subunit for this receptor preparation. This laboratory also reported [7] optimal conditions for solubilizing canine cardiac and hepatic β-receptors which is an important consideration in the purification and characterization of β-receptors from different mammalian tissues. At the present time very little is known about the molecular characteristics of mammalian β-receptors. However, subclasses of mammalian β-adrenergic receptors, termed β_1 and β_2, have been delineated based upon differential sensitivities to agonists and antagonists [8, 9], and direct binding studies by Rugg *et al.* [10] have suggested the co-existence of varying proportions of β_1 and β_2 receptors in mammalian lungs. Recent review articles have discussed the significance of β-adrenoceptor subtypes and their properties [11–13].

The immobilization of adrenergic ligands on synthetic or natural polymers has been attempted and may prove useful in delineating differences between receptor subtypes and modes of action of adrenergic compounds. In this regard, Verlander *et al.* [14] have synthesized polymeric derivatives of isoproterenol using a soluble copolymer of hydroxylpropyl-glutamine and *p*-aminophenylalanine. They showed that these compounds were biologically active in a heart perfusion assay and only about 2-fold less potent than *l*-isoproterenol. Most importantly, they rigorously demonstrated that the activity of these polymers was due to covalently attached isoproterenol and not to free isoproterenol.

For the present work, a series of polymeric derivatives of a β-adrenergic antagonist, alprenolol, was developed. (±)Alprenolol was linked covalently to dextran (average molecular weight 40,000) by means of a homologous series of spacer arms 4, 8, 11, and 13 atoms in length (see Fig. 1). This dextran, due to its large size relative to alprenolol, provides a considerable amount of steric hindrance to the interaction of alprenolol with β-receptors. It had been shown previously that similar but non-homologous derivatives inhibit the binding of (−)-[^{3}H]dihydroalprenolol to particulate and solubilized frog erythrocyte β-receptors [15]. The inhibitory potencies of these non-homologous derivatives increase with increasing length of the spacer arm, while their affinities for antibody to receptor–drug complex are uniformly high (all in the nanomolar range). These previous results indicated that the derivatives with shorter spacer arms are sterically hindered from interacting with the alprenolol binding site and, further, that the alprenolol portion is not buried within the dextran molecule, since the short

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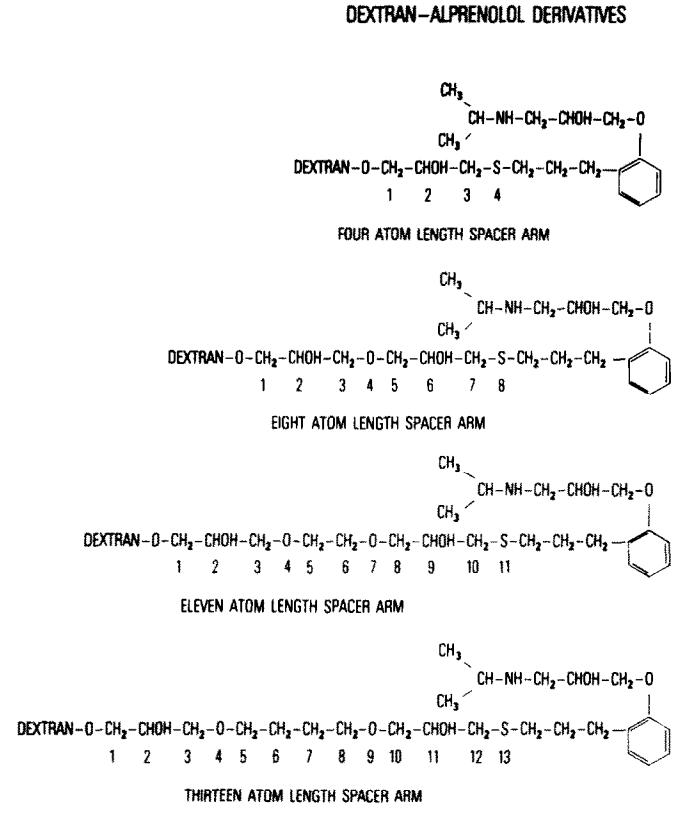

Fig. 1. Chemical structures of the dextran–alprenolol derivatives.

spacer arm derivatives interact with antibody to receptor–drug complex with an affinity equal to that of the longer spacer arm derivatives.

Thus, by studying the interactions of this series of derivatives with β-adrenergic receptors of other species and tissues, it should be possible to estimate the accessibility and steric constraints of various β-receptors and perhaps provide an explanation, at the molecular level, for differences in subclasses of β-receptors. The synthesis of these compounds has been altered to obtain an expanded and completely homologous series of spacer arms linking the dextran and alprenolol, and we have eliminated the use of the spacer arm with the *N*-acetyl side chain. In this report we have attempted to compare the affinities of these derivatives in three different mammalian tissues since it is well known from pharmacological studies that heart and lung, for example, contain primarily β_1- and β_2-type receptors respectively. We have also compared mammalian and amphibian receptor preparations using this expanded series of homologous derivatives. We wanted to address the questions of whether these derivatives might be useful in discriminating between receptor subtypes in mammalian systems or whether they could distinguish differences in receptor topography between species. The results suggest that these derivatives may be useful in exploring accessibility of receptors for β-adrenergic compounds in different animal species.

METHODS

(±)Alprenolol was from Hässle (Mölndal, Sweden) and (−)[propyl-2,3-^{3}H]dihydroalprenolol hydrochloride* from the New England Nuclear Corp. (Boston, MA, U.S.A.). Dextran T40 was from Pharmacia (Uppsala, Sweden). All other chemicals were of analytical grade and were used without further purification.

Synthesis of macromolecular alprenolol derivatives with spacer arms 4 and 13 atoms in length was described previously [15] under the names dihydroalprenolol-SA-dextran and dihydroalprenolol-LA-dextran respectively. The derivatives with spacer arms 8 and 11 atoms in length were synthesized using the procedure described for the preparation of dihydroalprenolol-LA-dextran, the only difference being that, in place of 1,4-butanediol diglycidyl ether, either diglycidyl ether or 1,2-ethanediol diglycidyl ether was used. The former compound was prepared by epoxidation of allyl glycidyl ether (Aldrich Chemical Co., Milwaukee, WI) with *m*-chloroperbenzoic acid [16]; the latter was purchased from Polyscience, Inc., Warrington, PA. All dextran derivatives were purified by repeated extensive dialysis and the final preparations contained the following amounts of drug per mg: derivative with spacer arm 4 atoms long, 0.078 μmole alprenolol; derivative with spacer arm 8 atoms long, 0.026 μmole

* The abbreviation used is [^{3}H]DHA, levo[propyl-2,3-^{3}H]dihydroalprenolol hydrochloride.

alprenolol; derivative with spacer arm 11 atoms long, 0.031 μmole alprenolol; and derivative with spacer arm 13 atoms long, 0.053 μmole alprenolol (see Fig. 1).

The following experiments were performed to ascertain the stability and purity of these derivatives. Dextran and each dextran derivative, equivalent to 1 μmole alprenolol, were dialyzed at 25° for 0.5 hr (to approximate our assay conditions) and for a further 8 hr at 4°. The dialysates were lyophilized and dissolved in 1 ml of Tris buffer (50 mM Tris, 10 mM $MgCl_2$, pH 7.4), and 50-μl aliquots were assayed for inhibition of (−)[^{3}H]DHA binding in our standard assay system (see below). If alprenolol or an alprenolol derivative were released from the dextran complexes, significant displacement of (−)-[^{3}H]DHA from receptors would be measurable under our assay conditions when more than 0.01% of the alprenolol were released (in this case the final concentration of "released" alprenolol would be approximately 2×10^{-8} M). In the second experiment, dextran and the dextran alprenolol derivatives (equivalent to about 0.2 μmole (±)alprenolol) were dissolved in 2 ml of 3 N HCl and hydrolyzed for 1 hr at 100°. Aliquots of each were taken before and after hydrolysis and chromatographed by thin-layer chromatography on silica gel in 1-butanol–ethanol–water (4:3:3). D-Glucose and (±)alprenolol were also chromatographed with these samples. The hydrolyzates were diluted with water and lyophilized. The lyophilizates were dissolved in 2 ml of Tris buffer and 50-μl aliquots were assayed for inhibition of binding. This experiment was designed to show that no free (±)alprenolol or (±)alprenolol derivative was present in the intact dextran–alprenolol complex and that only upon acid hydrolysis was free (±)alprenolol or, more likely, an (±)alprenolol derivative released from the complex.

Male, Sprague–Dawley rats, 200–250 g, were purchased from Taconic Farms (Germantown, NY) and unsexed Southern Grass Frogs were obtained from the Carolina Biological Supply Co. (Burlington, NC).

Rat lung membranes were prepared exactly as described by Morishige *et al.* [17]. Rat heart membranes were prepared according to the method of Baker and Potter but with the omission of the density gradient centrifugation step [18], and frog and rat erythrocyte membranes were prepared as described by Caron and Lefkowitz [1].

Binding of (−)[^{3}H]DHA to membrane fractions and inhibition of this binding by various macromolecular (±)alprenolol derivatives were performed as follows. Substrate (5 nM final concentration, 44.9 Ci/mmole) was incubated with membranes (100–800 μg protein for rat membranes and approximately 50 μg protein for frog erythrocyte membranes) in a total volume of 250 μl of Tris buffer. Incubations were done in triplicate for 20 min at 30°. Reactions were stopped by rapid dilution with 4 ml of ice-cold Tris buffer and immediate filtration using reduced pressure through Whatman GF/C filters. The reaction tubes and filters were washed an additional three times with 4 ml of the same buffer. Dried filters were then counted in a Beckman LS-250 Liquid Scintillation System. Specific binding represented the difference between binding in the absence and presence of 1×10^{-5} M (±)alprenolol. Saturation experiments were also performed by varying the (−)[^{3}H]DHA concentration between 0.5 and 15 nM. The specific binding was then evaluated by the method of Scatchard [19]. The results of the mammalian tissues are the averages of three separate experiments. Inhibitory potencies of macromolecular (±)alprenolol derivatives were assessed by mixing aliquots of serial 1 : 10 dilutions of the various derivatives with substrate prior to the addition of membranes in the assay mixtures and measuring the reduction in specific binding. IC_{50} Values were determined graphically and K_D values were calculated according to the method of Cheng and Prusoff [20].

RESULTS

Specific binding of (−)[^{3}H]DHA to rat heart, lung, and erythrocyte and frog erythrocyte membranes represented 79 ± 11, 92 ± 4.2, 95 ± 2.8, and 98 ± 1.9% of the total (−)[^{3}H]DHA bound to these membrane preparations respectively (average percent ± standard deviation, N = 3). Equilibrium dissociation constants and β_{max} values calculated from Scatchard analyses of binding data were as follows: frog erythrocytes 2 nM, 2000 fmoles/mg [21]; rat heart 2.7 nM, 47 fmoles/mg; rat lung 5.5 nM, 514 fmoles/mg; and rat erythrocyte 3.3 nM, 37 fmoles/mg. The binding of (−)[^{3}H]DHA was inhibited by (±)alprenolol with K_D values of 15 nM for rat heart, 17 nM for rat lung, 12 nM for rat erythrocyte, and 4.8 nM for frog erythrocyte membranes. However, the K_D values for macromolecular dextran–alprenolol derivatives were from 200- to 6000-fold greater than for (±)alprenolol. Neither dextran itself nor intermediates in the synthesis of the derivatives [15], a sulfhydryl and a thiosulfate containing dextran, inhibited the binding of (−)[^{3}H]DHA at concentrations ten times greater than the highest concentration of the dextran–alprenolol derivatives.

We have confirmed a previous report [15] that the potency of macromolecular derivatives of (±)-alprenolol in inhibiting the binding of (−)[^{3}H]DHA increases with increasing chain length using amphibian erythrocyte membranes as a source of β-adrenergic receptors (Table 1). In contrast, all mammalian receptor preparations examined failed to show this differential sensitivity to the macromolecular (±)alprenolol derivatives (Table 1). The derivatives varied somewhat in their potencies (approximately 480-fold to 2300-fold less potent than (±)alprenolol) in inhibiting the binding of (−)-[^{3}H]DHA to rat lung membrane preparations. These derivatives were also more uniformly insensitive with rat heart membranes (all approximately 1800-fold less potent than (±)alprenolol) and rat erythrocyte membranes (all approximately 3500-fold less potent). One set of experiments with a rat reticulocyte-enriched membrane preparation (data not shown) also indicated weak interaction of these macromolecular derivatives with the β-receptors of these cells. The K_D values were in a range similar to those obtained with rat erythrocyte membranes. However, when examined on frog erythrocyte membrane preparations, the derivative with the longest

Table 1. Equilibrium dissociation constants (K_D, nM) for (±)alprenolol–dextran derivatives and (±)alprenolol*

Spacer arm (# atoms)	Membrane preparation			
	Frog erythrocyte	Rat heart	Rat lung	Rat erythrocytes
4	14,100 ± 5,500	31,000 ± 12,000	38,400 ± 9,200	41,400 ± 4,200
8	5,690 ± 900	19,300 ± 6,900	11,200 ± 3,700	34,200 ± 3,500
11	8,140 ± 800	24,600 ± 9,500	29,800 ± 8,600	33,000 ± 11,000
13	1,000 ± 180	30,900 ± 12,600	8,220 ± 2,900	57,200 ± 2,300
(±)Alprenolol	4.8 ± 0.14	15 ± 0.88	17 ± 0.81	12 ± 0.76

* Inhibition of binding of (−)[^{3}H]DHA by macromolecular derivatives was assessed by including in the standard assay derivatives at concentrations from 1×10^{-9} M to 1×10^{-4} M with respect to the alprenolol moiety. K_D values were calculated as described in Methods. The results are the averages of at least three separate experiments ± standard deviation.

spacer arm (13 atoms) was about 200-fold less potent while the derivative with the shortest spacer arm (4 atoms) was 3000-fold less potent than (±)alprenolol (Table 1), indicating a higher degree of inhibition of (−)[^{3}H]DHA binding with the longer spacer arm.

To establish the stability of the dextran–alprenolol derivatives, we dialyzed dextran and dextran derivatives for 0.5 h at 25° (a time and temperature approximating the assay conditions) and for a further 8 hr and assayed the dialysates for inhibition of (−)-[^{3}H]DHA binding to rat heart and lung membranes. As shown in Table 2, dialysis under the conditions of our assay did not cause the release of material capable of inhibiting (−)[^{3}H]DHA binding. Only after 8 hr of dialysis was material released from the 4, 8, and 13 arm dextran (±)alprenolol derivatives capable of inhibiting (−)[^{3}H]DHA binding to a small extent. Furthermore, thin-layer chromatography of purified dextran–alprenolol derivatives was unable to detect (±)alprenolol or (±)alprenolol-like material comigrating with (±)alprenolol and only upon hydrolysis of these derivatives was (±)-alprenolol-like material observed by thin-layer chromatography (Fig. 2). The release of (±)alprenolol from the dextran derivatives only by acid hydrolysis was confirmed by assaying the hydrolysates for their abilities to inhibit the binding of (−)-[^{3}H]DHA to rat heart and lung membranes. As can be seen in Table 2, the hydrolysates of the dextran–alprenolol derivatives were able to effect between 80 and 90% inhibition of binding in rat lung and between 70 and 80% inhibition of binding in rat heart, while the dextran hydrolysate inhibited only about 15–20%.

DISCUSSION

β-Adrenoreceptors are present in many organs, and the regulation of the functioning of these receptors by agonists and antagonists has important pharmacological considerations. In this regard we prepared an improved series of macromolecular derivatives of alprenolol and have used these derivatives in uncovering steric differences among β-receptors of different species and in subclasses of mammalian β-receptors. That there are subclasses of mammalian receptors, which also seem to differ from receptors of other organisms, has been shown by differential sensitivities of the receptors to agonists and antagonists as well as by kinetic differences in the binding of antagonists [8–10, 22–24]. Mammalian heart is primarily composed of β_1- and mammalian (excepting rabbit) lung and liver primarily of β_2-type receptors. Minneman *et al.* [23] have shown that the receptor of turkey erythrocytes behaves differently from these mammalian subtypes. A recent report by Dickinson and Nahorski [25] has demonstrated that

Table 2. Percentage inhibition of specific (−)[^{3}H]DHA binding by dialysates and hydrolysates of dextran and dextran–alprenolol derivatives*

Derivative	Dialysates				Hydrolysates	
	0.5 hr		8 hr			
	Heart	Lung	Heart	Lung	Heart	Lung
Dextran	0	0	0	0	15.3 ± 13.4	22.7 ± 2.5
4 Arm (±)Alp-Dextran	0	0	0	2	83.0 ± 12.2	87.7 ± 10.2
8 Arm (±)Alp-Dextran	0	0	6	0	71.0 ± 9.9	81.0 ± 4.2
11 Arm (±)Alp-Dextran	0	0	0	0	75.0 ± 10.2	89.0 ± 8.9
13 Arm (±)Alp-Dextran	0	0	0	4	80.3 ± 3.8	89.0 ± 9.5

* Inhibition of binding was measured as described in Methods and represents the average of duplicate experiments for the dialysates and the average ± standard deviation of three experiments for hydrolysates.

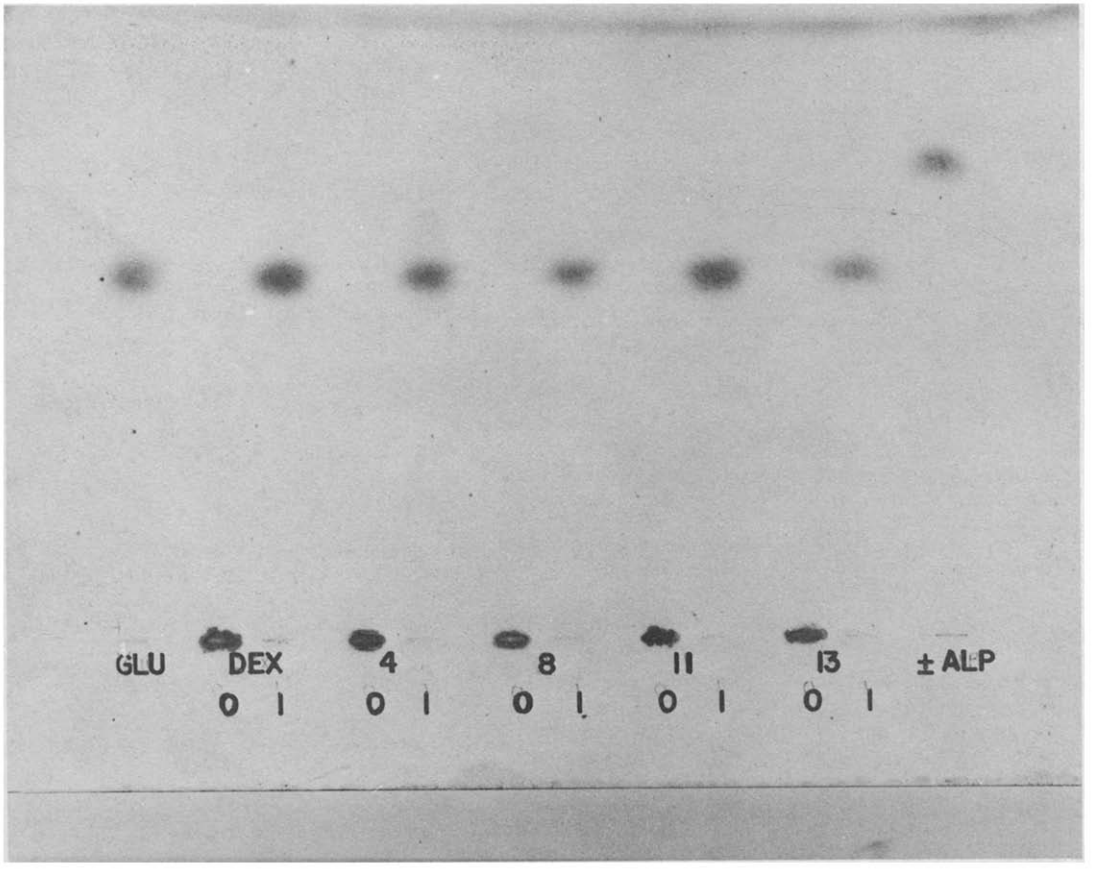

Fig. 2. Thin-layer chromatogram of dextran and dextran–alprenolol hydrolysates. The far left lane is D-glucose and the far right lane is (±)alprenolol. The pairs of lanes from left to right are unhydrolyzed and hydrolyzed dextran, dextran-4 atom arm (±)alprenolol, dextran-8 atom arm (±)alprenolol, dextran-11 atom arm (±)alprenolol, and dextran-13 atom arm (±)alprenolol.

chick erythrocyte (β_1) and frog erythrocyte (β_2) receptors differ significantly from mammalian β_1 and β_2 receptor subtypes in their affinities for β_1 and β_2 selective antagonists. Their data also imply different structural features of the receptors in different species.

We believe that these dextran–alprenolol derivatives represent valid tools for use in delineating receptor subtypes. The dialysis and hydrolysis experiments strongly suggest that (±)alprenolol was covalently attached to dextran, and that this attachment was stable under our assay conditions. First, we noted no inhibition after 0.5 hr and only minor inhibition of (−)[^{3}H]DHA binding after 8 hr of dialysis. This inhibition represented less than 0.01% release of (±)alprenolol from the derivatives. A concentration of (±)alprenolol of 2×10^{-8} M would lead to a 20–30% inhibition of (−)[^{3}H]DHA binding in our assay system, and this concentration of (±)-alprenolol would represent 0.01% of the (±)alprenolol content of the dialyzed derivatives. Since the measured inhibition (2–6%) was much less than 20–30%, we feel that minor amounts of these derivatives were released only after 8 hr of dialysis which was much longer than the 20-min assay incubation where we noted no inhibition of (−)[^{3}H]DHA binding of the dialysates. Second, our data show that there was no trapped (±)alprenolol or (±)alprenolol derivatives since thin-layer chromatography (Fig. 2) and the hydrolysis experiments (Table 2) demonstrated that (±)alprenolol-like material was only released from the derivatives after hydrolysis by HCl for 1 hr at 100°. The small amount of inhibition by hydrolyzed dextran itself may represent effects of glucose or traces of HCl that remained in the lyophilizates.

The macromolecular (±)alprenolol derivatives that were utilized in the present study were clearly able to differentiate between amphibian and mammalian receptors, but not between different mammalian receptors (see Fig. 3). The small shift in affinities of the derivatives for the frog erythrocyte membranes in the previous report and the present study probably reflect slight differences in the preparations used. While the derivative with a spacer arm 13 atoms in length interacted with the frog erythrocyte β-receptor with a K_D of 1 μM, the K_D for rat heart was about thirty-one times larger and for rat lung about eight times larger, clearly showing a difference between amphibian and mammalian receptors. However, these ligands could not distinguish between different mammalian receptors since the K_D values were relatively consistent regardless of the length of the spacer arm and, in addition, they

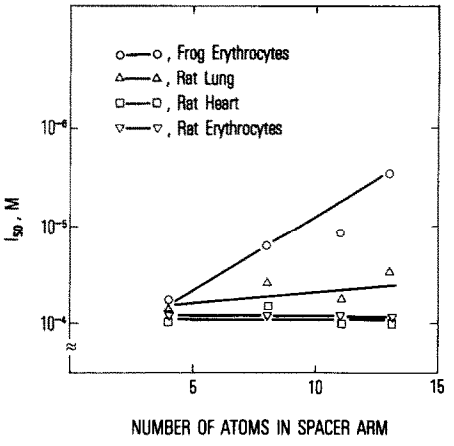

Fig. 3. Interaction of macromolecular (±)alprenolol derivatives with various β-adrenergic receptor preparations. IC_{50} Values are plotted versus the number of atoms in the spacer arm between dextran and (±)alprenolol.

interacted only weakly. We also have observed these same results using a rat reticulocyte-enriched membrane preparation in which the β-receptor is tightly coupled to adenylate cyclase. This implied similar inaccessibility for most mammalian β-adrenergic receptors. In contrast to the results obtained with antibody to drug–receptor complex where the affinity of all of the macromolecular derivatives was very high, the affinity of macromolecular derivatives for mammalian receptors was very low.

There may be several reasons for this weak interaction with mammalian receptors. The binding site may be in a very deep pocket in the receptor that precludes interaction with any macromolecular form of alprenolol. However, the lung β-receptor may be slightly more accessible in that the (±)alprenolol–dextran derivative with a spacer arm 13 atoms in length is somewhat more potent than the other shorter arm derivatives. Also, other membrane components, including other proteins, glycoproteins, or phospholipids, may exist in close proximity to the receptor shielding it from interaction with the derivatives. In this regard, we are synthesizing irreversible blockers of alprenolol in an attempt to label either the receptor protein or other molecules in the vicinity of the receptor.

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REFERENCES

1. M. G. Caron and R. J. Lefkowitz, *J. biol. Chem.* **251**, 2374 (1976).
2. E. M. Brown, S. A. Fedak, C. J. Woodard, G. D. Aurbach and D. Rodbard, *J. biol. Chem.* **251**, 1239 (1976).
3. O. Durieu-Trautmann, C. Delavier-Klutchko, G. Vauquelin and A. D. Strosberg, *J. supramolec. Struct.* **13**, 411 (1980).
4. M. G. Caron, Y. Srinivasan, J. Pitha, K. Kociolek and R. J. Lefkowitz, *J. biol. Chem.* **254**, 2923 (1979).
5. R. G. L. Shorr, R. J. Lefkowitz and M. G. Caron, *J. biol. Chem.* **256**, 5820 (1981).
6. C. M. Fraser and J. C. Venter, *Proc. natn. Acad. Sci. U.S.A.* **77**, 7034 (1980).
7. W. L. Strauss, G. Ghai, C. M. Fraser and J. C. Venter, *Archs Biochem. Biophys.* **196**, 566 (1979).
8. A. M. Lands, A. Arnold, J. P. McAuliff, F. P. Luduena and T. G. Brown, *Nature, Lond.* **214**, 597 (1967).
9. K. P. Minneman, L. R. Hegstrand and P. B. Molinoff, *Molec. Pharmac.* **16**, 21 (1979).
10. E. L. Rugg, D. B. Barnett and S. R. Nahorski, *Molec. Pharmac.* **14**, 996 (1978).
11. S. R. Nahorski, *Trends pharmac. Sci.* **2**, 95 (1981).
12. G. LeClerc, B. Rouot, J. Velley and J. Schwartz, *Trends pharmac. Sci.* **2**, 18 (1981).
13. K. P. Minneman, R. N. Pittman and P. B. Molinoff, *A. Rev. Neurosci.* **4**, 419 (1981).
14. M. S. Verlander, J. C. Venter, M. Goodman, N. O. Kaplan and B. Saks, *Proc. natn. Acad. Sci. U.S.A.* **73**, 1009 (1976).
15. J. Pitha, J. Zjawiony, R. J. Lefkowitz and M. G. Caron, *Proc. natn. Acad. Sci. U.S.A.* **77**, 2219 (1980).
16. H. Wittcoff, J. R. Roach and S. E. Miller, *J. Am. chem. Soc.* **71**, 2666 (1949).
17. W. K. Morishige, C-A. Uetake, F. C. Greenwood and J. Akaka, *Endocrinology* **100**, 1710 (1977).
18. S. P. Baker and L. T. Potter, *Membr. Biochem.* **3**, 185 (1980).
19. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
20. Y-C. Cheng and W. H. Prusoff, *Biochem. Pharmac.* **22**, 3099 (1973).
21. R. J. Lefkowitz, L. E. Limbird, C. Mukherjee and M. G. Caron, *Biochim. biophys. Acta* **457**, 1 (1976).
22. K. P. Minneman, A. Hedberg and P. B. Molinoff, *J. Pharmac. exp. Ther.* **211**, 502 (1979).
23. K. P. Minneman, G. A. Weiland and P. B. Molinoff, *Molec. Pharmac.* **17**, 1 (1980).
24. N. Bieth, B. Rout, J. Schwartz and J. Velly, *Br. J. Pharmac.* **68**, 563 (1980).
25. K. E. J. Dickinson and S. R. Nahorski, *Eur. J. Pharmac.* **94**, 43 (1981).