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β-ADRENERGIC RECEPTORS IN GUINEA-PIG LIVER PLASMA MEMBRANES AND THERMAL LABILITY OF [^{3}H]DIHYDROALPRENOLOL BINDING SITES

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Abstract—β-Adrenergic receptors in guinea-pig liver plasma membranes were characterized by radioligand binding, using *l*-[^{3}H]dihydroalprenolol ([^{3}H]DHA), *l*-3-[^{125}I]iodocyanopindolol ([^{125}I]CYP) and *dl*-[^{3}H]4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-one hydrochloride ([^{3}H]CGP-12177). The binding of both [^{125}I]CYP and [^{3}H]CGP-12177 to membranes exhibited high affinity (K_d = 3.5 ± 0.2 pM for [^{125}I]CYP and 0.75 ± 0.10 nM for [^{3}H]CGP-12177) and stereospecificity; the maximal binding sites were 130 ± 15 and 137 ± 8 fmoles/mg protein respectively. Catecholaminergic agonists competed for these binding sites in the order *l*-isoproterenol > *l*-epinephrine > *l*-norepinephrine, which is typical for β_2-adrenergic receptors. The binding data are supported by parallel experiments on adenylate cyclase activation by catecholamines, and on antagonism of this activation by β_1- and β_2-selective blockers. The binding of [^{3}H]DHA was excessive (B_{max} = 21.4 pmoles/mg protein), exhibited low affinity (K_d = 34.6 nM), and lacked stereospecificity. When liver membranes were incubated at 50° for 40 min in the presence of an agonist, *l*-isoproterenol, the binding of [^{3}H]DHA to the heat-treated membranes exhibited high affinity (K_d = 1.07 ± 0.17 nM) and the B_{max} was reduced to 139 ± 22 fmoles/mg protein. In such membranes, as opposed to native membranes, stereospecificity was evident and catecholaminergic agonists competed for the binding sites in the order typical for β_2-adrenergic receptors. However, agonist competition of the binding to the heat-treated membranes could not be modulated by guanine nucleotides, indicating a loss of communication between the receptor and the guanine nucleotide regulatory protein.

Catecholamines elicit a wide variety of important physiological responses in mammalian tissues, and their actions are mediated by α- and β-adrenergic receptors. That α_1-receptors rather than β-receptors mediate catecholamine regulation of glycogenolysis in adult rat liver is well established [1, 2]. However, studies with guinea-pig and rabbit hepatocytes show that the glycogenolytic effects of catecholamines in these species are mediated by the β_2-receptor, and are quite insensitive to α-blockade [3, 4]. These findings corroborate the studies of Arnold *et al.* [5] using intact dogs and emphasize that species differences occur in the mechanism by which catecholamines modulate this metabolic process.

We have postulated that the molecular basis for the species differences in agonist response may be related to the density of α- and β-receptors or subtypes thereof in livers of various species [3]. In rat liver for which this measurement is available, there are fewer binding sites for β-adrenergic ligands than for α-adrenergic ligands [6]. In metabolic studies on guinea-pig hepatocytes metabolizing endogenous glycogen, we indicated that the stimulatory effects of catecholamines are mediated by β- rather than α-receptors [3]. We have now quantified these β-receptors, using [^{125}I]iodocyanopindolol, [^{3}H]CGP-12177, and [^{3}H]dihydroalprenolol as radioligand β-receptor probes. Although it appears technically feasible to measure β-receptors in liver membranes with [^{3}H]DHA† using the mild heat-treatment protocol suggested by Baker and Potter [7], the approach is not desirable since it results in a loss of guanine nucleotide-dependent modulation of receptor function.

MATERIALS AND METHODS

Animals and materials. Hartley strain male guinea pigs (400–500 g) were obtained from Camm Laboratory Animals, Wayne, NJ, U.S.A. *l*-[^{3}H]DHA (36–42 Ci/mmole) was purchased from the New England Nuclear Corp., Boston, MA, U.S.A. [^{125}I]CYP (1963 Ci/mmole), [^{3}H]CGP-12177 (38–42 Ci/mmole) and [α-^{32}P]ATP (410 Ci/mmole) were obtained from the Amersham Corp., Arlington Heights, IL, U.S.A. *l*-Alprenolol, *l*-isoproterenol, *d*-isoproterenol, *l*-epinephrine, *l*-norepinephrine, Gpp(NH)p, and cholera toxin were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. Pertussis toxin was purchased from List Biological Laboratories, Campbell, CA, U.S.A. Phentolamine and atenolol were provided by Ciba-Geigy, Summit, NJ, U.S.A., and Imperial Chemical Industries, Wilmington, DE, U.S.A., respectively. IPS 339 was a

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† Abbreviations: [^{3}H]DHA, *l*-[^{3}H]dihydroalprenolol; [^{125}I]CYP, *l*-3-[^{125}I]iodocyanopindolol; [^{3}H]CGP-12177, *dl*-[^{3}H]4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-one hydrochloride; IPS 339, (tertiarybutylamino-3-ol-2-propyl)oximino-9-fluorene hydrochloride; Gpp(NH)p, guanyl-5′-yl imidodiphosphate; SDS, sodium dodecyl sulfate.

gift from Dr. G. Leclerc, Université Louis Pasteur, Strasbourg, France. Other chemicals were obtained from commercial sources.

Preparation of liver plasma membranes. Guinea-pig liver plasma membranes were prepared essentially according to the procedure devised by Neville [8] up to the sucrose density gradient centrifugation step (step 11). Protein was determined by the method of Lowry *et al.* [9] using bovine serum albumin as standard, after solubilization of membrane fractions [4].

Heat-treatment of membranes. Plasma membranes (2 mg/ml) were incubated with 50 μM *l*-isoproterenol and 0.1% ascorbic acid in 75 mM Tris/HCl, pH 7.5, containing 12.5 mM $MgCl_2$ and 1.5 mM EDTA (incubation buffer), first at 25° for 15 min, and then at 50° for 40 min. At the end of the incubation, ice-cold incubation buffer was added and the mixture was centrifuged at 26,000 *g* for 10 min. The resulting pellets were washed four times and then resuspended in the incubation buffer to yield 3–4 mg of protein/ml. The suspension was divided into small aliquots and stored frozen at −80° until used for binding assays.

Binding assays. Unless otherwise stated, plasma membranes (100 μg protein) were incubated with a 1 nM concentration of [^{3}H]DHA or [^{3}H]CGP-12177 for 30 min at 30° in a final volume of 0.2 ml. For [^{125}I]CYP binding, membranes (10 μg protein) were incubated with 5 pM [^{125}I]CYP at 37° for 5 hr in a final volume of 1 ml. The incubation buffer was 75 mM Tris/HCl, pH 7.5, containing 12.5 mM $MgCl_2$, 1.5 mM EDTA, and 1 mM ascorbic acid. For [^{3}H]DHA binding, phentolamine (50 μM) was included in all assay tubes to reduce non-specific binding. The binding assays were stopped by addition of 2 ml of cold 50 mM Tris/HCl buffer, pH 7.5, containing 10 mM $MgCl_2$ (wash buffer), and the samples were rapidly filtered through Whatman GF/C filters, using a Millipore filtration manifold. Each filter was washed with 15 ml of cold wash buffer, and the radioactivity was determined in a Packard Tri-Carb 4530 liquid scintillation counter for ^{3}H or in a Packard Auto-Gamma 5650 counter for ^{125}I. Non-specific binding was determined in the presence of 100 μM *l*-isoproterenol, and this value was subtracted from the total binding to obtain specific binding. All binding assays were performed at protein concentrations within the linear range. At ligand concentrations near K_d values, non-specific binding was less than 5% of total binding for [^{125}I]CYP and [^{3}H]CGP-12177.

Assay of adenylate cyclase. Adenylate cyclase assays were performed in duplicate as previously described [10]. Each assay tube contained 100 μM GTP and <30 μg of partially purified membrane protein. The [^{32}P]cyclic AMP formed was isolated and assayed by the method of Salomon *et al.* [11].

[^{32}P]*ADP-ribosylation of membrane proteins by cholera toxin or pertussis toxin.* Immediately before use, cholera toxin (400 μg/ml) and pertussis toxin (200 μg/ml) were preactivated by incubation with 40 mM dithiothreitol in 10 mM potassium phosphate buffer, pH 7.8, for 15 min at 30°. Unless otherwise stated, membranes (100 μg protein) were incubated for 60 min at 30° with the preactivated cholera toxin (20 μg/ml) or pertussis toxin (10 μg/ml) in a final volume of 100 μl containing 20 μM [^{32}P]NAD$^+$ (1 μCi/nmole), 1 mM ATP, 0.5 mM GTP, 15 mM thymidine, 5 mM dithiothreitol, 1 mM $MgCl_2$, 10 mM creatine phosphate, 36 units/ml of creatine phosphokinase, 10 mM isonicotinic acid hydrazide (isoniazid), 0.75 mM $NADP^+$, and 100 mM potassium phosphate, pH 7.5. The reaction was stopped by the addition of 500 μl of cold 10 mM Tris/HCl buffer, pH 7.5, containing 1 mM EDTA. The membranes were recovered by centrifugation at 40,000 *g* for 15 min and subsequently washed once with the same buffer. The washed membranes were solubilized in 50 μl of Laemmli's sample buffer [12] at room temperature overnight. Samples were then analyzed by SDS-polyacrylamide gel electrophoresis as described by Laemmli [12], using 10% acrylamide in the separating gel and 3% acrylamide for the stacking gel. The gels were stained with Coomassie blue, destained, and dried on filter paper using a Bio-Rad gel dryer. Autoradiography was performed at −80° for 1–2 days with Kodak X-Omat XAR film in the presence of intensifying screens.

Data analysis. Curves plotted were generated by nonlinear least squares curve-fitting methods using a Digital Pro 350 computer and the RS/1 data analysis system obtained from the Digital Equipment Corp., Nashua, NH, U.S.A. Data for inhibition of binding and activation of adenylate cyclase were analyzed individually using a logistic equation [13].

RESULTS

Binding of [^{125}I]*CYP,* [^{3}H]*CGP-12177 and* [^{3}H]*DHA to liver plasma membranes.* Figure 1a shows a typical saturation experiment for [^{3}H]CGP-12177 binding to partially purified guinea-pig liver membranes. Scatchard plots of the binding data were linear, suggesting a single class of binding sites. The density of binding sites (B_{max}) was 137 ± 8 fmoles/mg of protein (mean ± SE, N = 6 experiments). The equilibrium dissociation constant (K_d) determined from these experiments was 0.75 ± 0.10 nM. With [^{125}I]CYP as ligand, specific binding was also saturable and exhibited a B_{max} of 130 ± 15 fmoles/mg protein but extremely low K_d values (3.5 ± 0.2 pM; N = 4 experiments). Whereas binding of [^{3}H]CGP-12177 equilibrated within 20 min, that of [^{125}I]CYP was extremely slow even at 37° and required 4–5 hr to equilibrate at 5 pM [^{125}I]CYP (Fig. 1b). On the basis of these data, we routinely terminated the assays for [^{125}I]CYP binding after 5 hr.

Figure 2a shows that it is possible to demonstrate saturability of [^{3}H]DHA binding in membranes that were not heat treated. The B_{max} displayed in Fig. 2a was, however, much higher than other hepatic β-receptor systems [4, 6]. Moreover, it represents binding to low affinity sites (i.e. high K_d) and did not exhibit stereospecificity.

Baker and Potter [7] reported that an agonist or an antagonist can protect β-receptors from heat lability, presumably in an analogous manner as some enzymes are protected from heat inactivation or degradation by their substrates. Based on their report, we reasoned that the high ligand-acceptor sites in Fig. 2a might be heat sensitive and that mild

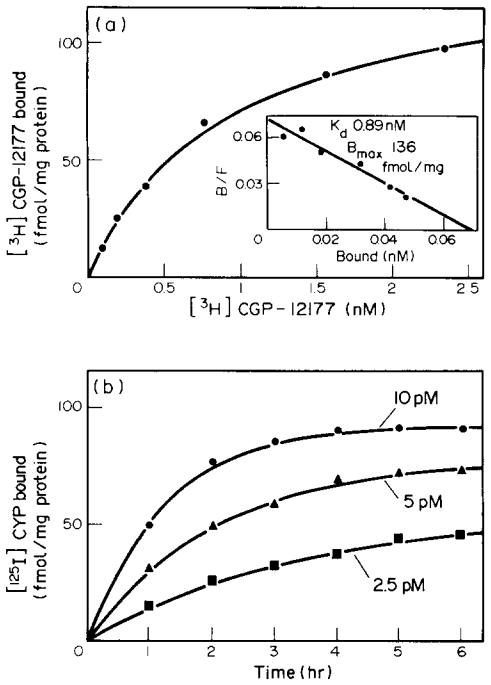

Fig. 1. (a) Saturability of [^{3}H]CGP-12177 binding to guinea-pig liver plasma membranes. Membranes (100 μg protein) were incubated at 30° for 30 min with the indicated concentrations of [^{3}H]CGP-12177. Non-specific binding was determined in the presence of 100 μM *l*-isoproterenol, and this value was subtracted from total binding to obtain specific binding. At all concentrations, specific binding was greater than 80% of total binding. The data shown are representative of six experiments. *Inset:* Scatchard plot of the same data. (b) Time course of specific binding of [^{125}I]CYP. Membranes (10 μg protein) were incubated with 2.5, 5, and 10 pM [^{125}I]CYP at 37° for up to 6 hr as indicated. Specific binding was determined as in Fig. 1a, and at all three concentrations was greater than 90% of total binding. The data shown are representative of two experiments.

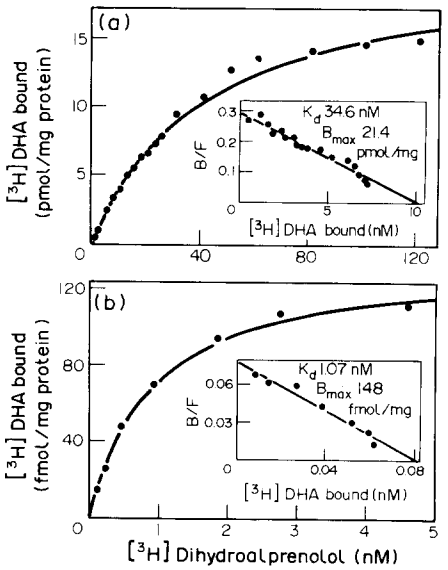

Fig. 2. Saturability of [^{3}H]DHA specific binding to guinea-pig liver plasma membranes. In panel a, membranes (100 μg protein) were incubated at 25° for 20 min with the indicated concentrations of [^{3}H]DHA. Non-specific binding was determined in the presence of 10 μM *dl*-propranolol, and this value was subtracted from total binding to obtain specific binding. Points plotted are means for six experiments. *Inset:* Scatchard plot of the same data. In panel b, membranes (100 μg protein) heat-treated in the presence of *l*-isoproterenol, as described under Materials and Methods, were incubated at 30° for 30 min with the indicated concentrations of [^{3}H]DHA. Non-specific binding was determined in the presence of 100 μM *l*-isoproterenol. The data shown are representative of five experiments. *Inset:* Scatchard plot of the same data.

heat-treatment should make it possible to measure the cryptic β-receptor in the membrane which is otherwise not possible when [^{3}H]DHA is used as ligand. When the heat-treatment protocol (50° for 40 min with 50 μM *l*-isoproterenol) was applied to the membranes (Fig. 2b), saturability of [^{3}H]DHA binding was routinely observed at [^{3}H]DHA concentration of 4–5 nM, a dramatic contrast to the 100 nM observed in the experiments with non-heated membranes (Fig. 2a). Binding equilibrium was reached within 15 min; the binding remained stable for at least 50 min (data not shown). Scatchard analysis of the data indicated a single class of binding sites with a Hill coefficient of 1.03, suggesting the absence of cooperativity. The B_{max} was 139 ± 22 fmoles/mg protein (mean ± SE, five experiments, range 91–206 fmoles/mg protein); the K_d was 1.07 ± 0.17 nM (range 0.47 to 1.49 nM), indicating high affinity binding. These values agreed well with those obtained by using [^{3}H]CGP-12177 and [^{125}I]CYP, as ligands, in native (i.e. non-heated) membranes.

B_{max} and K_d values determined from [^{3}H]CGP-12177 binding to the heat-treated membranes were similar to those shown in Fig. 1a (cf. Fig. 5). If heat-treatment was performed in the absence of isoproterenol, there were no measurable β-receptors as judged by either [^{3}H]DHA or [^{3}H]CGP-12177 binding as described previously [7]. Thus, the presence of the agonist was necessary during the heat-treatment protocol to protect the β-receptor sites from heat inactivation.

Specificity of binding. In competition experiments using [^{3}H]CGP-12177 as ligand, catecholaminergic agonists competed for [^{3}H]CGP-12177 binding sites in the order *l*-isoproterenol > *l*-epinephrine > *l*-norepinephrine (Fig. 3a), a potency order which is typical for β_2-adrenergic receptors [14, 15]. The binding of [^{3}H]CGP-12177 was stereospecific in that the *l*-isomer of isoproterenol was clearly three orders of magnitude more potent than its corresponding *d*-isomer in displacing bound [^{3}H]CGP-12177 (Fig. 3a). Using [^{125}I]CYP as ligand, stereospecificity and agonist potency order similar to that depicted in Fig. 3a were also obtained; therefore, these data were not reproduced here. The β_2-selective antagonist IPS 339 was at least three orders of magnitude more potent in competing for the binding than was atenolol, a β_1-selective antagonist. A typical competition experiment using [^{3}H]CGP-12177 as ligand is shown in Fig. 3b. These competition curves were

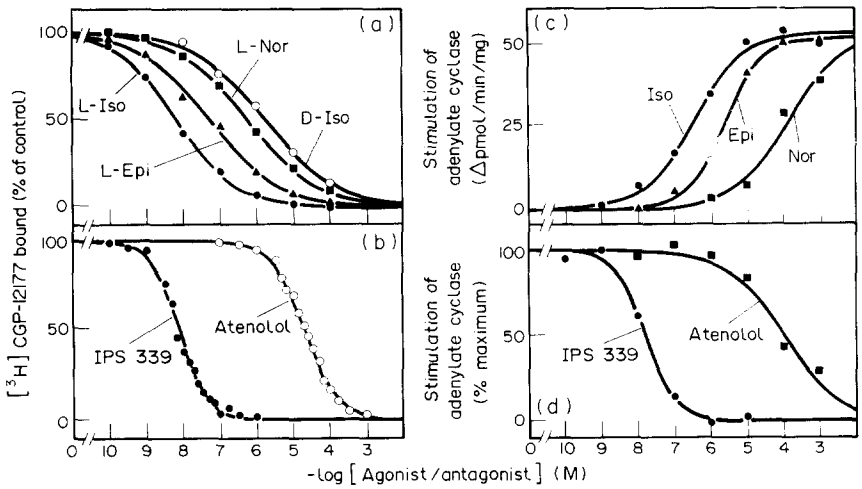

Fig. 3. (a) Inhibition of [^{3}H]CGP-12177 binding to liver plasma membranes by adrenergic agonists. Membranes (100 μg protein) were incubated with 1 nM [^{3}H]CGP-12177 in the presence of various concentrations of *d*- and *l*-isomers of isoproterenol (Iso), and of *l*-isomers of epinephrine (Epi), and norepinephrine (Nor). The data shown are representative of two experiments. Specific binding in the absence of inhibitors (control) was 61 fmoles/mg protein and was taken to be 100%. For the various agonists the concentrations required to achieve 50% inhibition of binding (EC_{50}) were: *l*-Iso, 6.3×10^{-9} M; *l*-Epi, 5.0×10^{-8} M; *l*-Nor, 5.6×10^{-7} M; and *d*-Iso, 1.7×10^{-6} M. (b) Inhibition of [^{3}H]CGP-12177 binding by β_1- and β_2-selective antagonists. Specific binding was determined with 1 nM [^{3}H]CGP-12177 in the presence of various concentrations of the β_1-antagonist, atenolol, and the β_2-antagonist, IPS 339. The data shown are representative of two experiments. Binding in the control experiment was 74 fmoles/mg protein. (c) Activation of adenylate cyclase by catecholamines. Membranes (<30 μg) were incubated at 30° for 5 min in the presence of 100 μM GTP and the indicated concentrations of *l*-isoproterenol (Iso), *l*-epinephrine (Epi) or *l*-norepinephrine (Nor). Stimulation of adenylate cyclase activity is expressed as the increase over basal activity (69.4 ± 12.5 pmoles/min/mg protein). Points plotted are means of four experiments. (d) Antagonism of isoproterenol-stimulated adenylate cyclase by β_1- and β_2-selective blockers. In the absence of any antagonist, the maximum stimulation of adenylate cyclase by 1×10^{-6} M *l*-isoproterenol (44.6 ± 8.6 pmoles/min/mg protein) was taken to be 100%. Points plotted are means of two experiments.

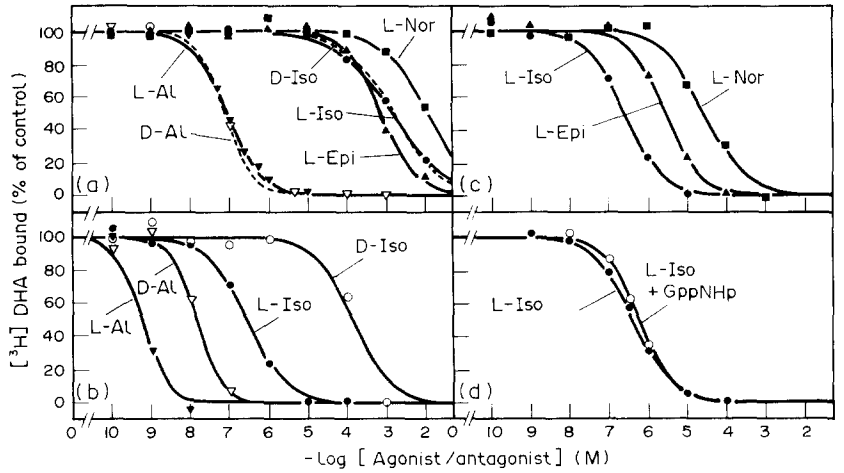

Fig. 4. (a) Inhibition of [^{3}H]DHA binding to non-heated membranes by adrenergic agonists/antagonists. Membranes (60 μg protein) were incubated with 5 nM [^{3}H]DHA, as described in the legend to Fig. 2a, in the presence of various concentrations of *d*- and *l*-isomers of isoproterenol (Iso) and alprenolol (Al), and *l*-isomers of epinephrine (Epi) and norepinephrine (Nor). Points plotted are means of two experiments. Binding in the control experiment was 1.8 ± 0.4 pmoles/mg protein. (b and c) Inhibition of [^{3}H]DHA binding to heat-treated membranes by adrenergic agonists/antagonists. Heat-treated membranes (100 μg protein) were incubated with 1 nM [^{3}H]DHA, as described in the legend to Fig. 2b, in the presence of various concentrations of inhibitors. Abbreviations are the same as in Fig. 4a. Points plotted are means of two experiments. Binding in the control experiment was 71 ± 13 fmoles/mg protein. (d) Effect of Gpp(NH)p on the inhibition of [^{3}H]DHA binding. Heat-treated membranes (100 μg protein) were incubated with 1 nM [^{3}H]DHA and increasing concentrations of *l*-isoproterenol in the absence (●) or presence (○) of 200 μM Gpp(NH)p. Points plotted are representative of two experiments. Binding in the absence and presence of Gpp(NH)p was 59 and 60 fmoles/mg protein respectively.

best fitted to a one-site model [16], suggesting the predominance of the β_2-subtype.

Hormone-dependent activation of adenylate cyclase can be used as reflecting receptor occupancy by hormone. Therefore, specificity determined from ligand binding data should be confirmable by agonist-specific effects on adenylate cyclase. Figure 3c shows that the rank order of potency for catecholamine-dependent activation of the cyclase was isoproterenol > epinephrine > norepinephrine, the same potency order as in the binding experiments. Further evidence that these interactions are mediated by β_2-receptors is presented in Fig. 3d. In these experiments, IPS 339 was at least three orders of magnitude more effective in inhibiting isoproterenol-dependent activation of the cyclase than atenolol.

In competition experiments using non-heated membranes, inhibition of [^{3}H]DHA binding by *l*-alprenolol was not distinguishable from that of *d*-alprenolol. A similar lack of stereospecificity was observed for *d*- and *l*-isoproterenol; furthermore, adrenergic agonists did not compete for [^{3}H]DHA binding sites in the order characteristic of either β_1- or β_2-receptors (Fig. 4a). These data are similar to those reported for membranes from rat liver and rat adipocyte [17, 18] as well as for cultured HeLa and Chang liver cells [19], and can be interpreted to imply that the specific β-receptor binding sites were masked by presumably high numbers of apparently specific acceptor sites. The purpose of applying mild heat-treatment to membranes was to unmask these sites. With membranes subjected to mild heat-treatment in the presence of *l*-isoproterenol, the *l*-isomers of both alprenolol and isoproterenol were clearly more potent than their corresponding *d*-isomers (by one order of magnitude for alprenolol and at least two orders of magnitude for isoproterenol) in displacing bound [^{3}H]DHA (Fig. 4b). Only in heated membranes did catecholaminergic agonists compete for [^{3}H]DHA binding sites in the potency order typical for β_2-receptors.

Effect of Gpp(NH)p on the binding of [^{3}H]*DHA to heat-treated membranes.* In several receptor systems, agonist competition curves against antagonist ligands are shifted to the right (to lower affinities) and become steep in the presence of guanine nucleotides [20]. This shift to lower affinity has generally been used as a monitor of communication between the receptor (R) and the guanine nucleotide regulatory protein (G-protein) [21]. This communication is a biological function of the β-receptor-adenylate cyclase system. It was therefore important to know whether ligand binding in heat-treated membranes responds appropriately to guanine nucleotide-dependent modulation. Figure 4d shows the effect of Gpp(NH)p on *l*-isoproterenol competition of [^{3}H]DHA binding to heat-treated membranes; the two competition curves were superimposable, indicating abolition of guanine nucleotide modulation. The slope factors for both competition curves were close to unity, indicating the presence of homogeneous, probably low affinity, binding sites even in the absence of the nucleotides. The effect of heat-treatment on guanine nucleotide-dependent modulation of binding of another ligand (i.e. [^{3}H]CGP-12177) is shown in Fig. 5. When the membranes

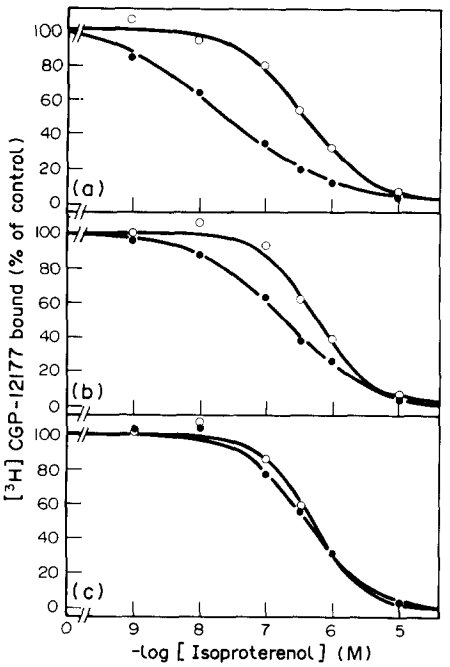

Fig. 5. Effect of Gpp(NH)p on the inhibition of [^{3}H]CGP-12177 binding by *l*-isoproterenol. Native (a) and heat-treated (at 37° in b and at 50° in c) liver membranes (100 μg protein) were incubated with 1 nM [^{3}H]CGP-12177 and increasing concentrations of *l*-isoproterenol in the absence (●) and presence (○) of 200 μM Gpp(NH)p. Points plotted are representative of two experiments. Control values were 88, 71 and 93 fmoles/mg protein in panels a, b and c, respectively.

were heated at 50° for 40 min in the presence of *l*-isoproterenol, guanine nucleotide-dependent modulation was also completely lost (Fig. 5c). This effect resembled the results with [^{3}H]DHA binding (cf. Fig. 4d). When the membranes were incubated at 37° under the same conditions, only a partial loss of R-G communications was observed (Fig. 5b) as opposed to control (i.e. non-heated membranes, Fig. 5a). Taken together these results show that the β-receptor in the heated membranes has lost the capacity to be modulated by guanine nucleotides.

Effect of heat-treatment on bacterial toxin-dependent [^{32}P]*ADP-ribosylation of membranes.* In other experiments, we [^{32}P]ADP-ribosylated [22] the heat-treated membranes in the presence of either cholera toxin or pertussis toxin in an attempt to label the guanine nucleotide regulatory proteins which mediate stimulation (N_s) and inhibition (N_i), respectively, of adenylate cyclase. As seen in Fig. 6, N_s (45 kDa), the cholera toxin substrate, and N_i (41 kDa), the pertussis toxin substrate, were clearly evident in native (i.e. non-heated) membranes (lanes 2 and 3). In heated (50°) membranes, however, there was practically no detectable bacterial toxin-dependent ^{32}P-labeling (lanes 8 and 9). With membranes that were heated at 37°, both N_s and N_i were detectable (lanes 5 and 6), but the densities of the bands were decreased substantially. If the heat treatments of membranes were performed in the presence of

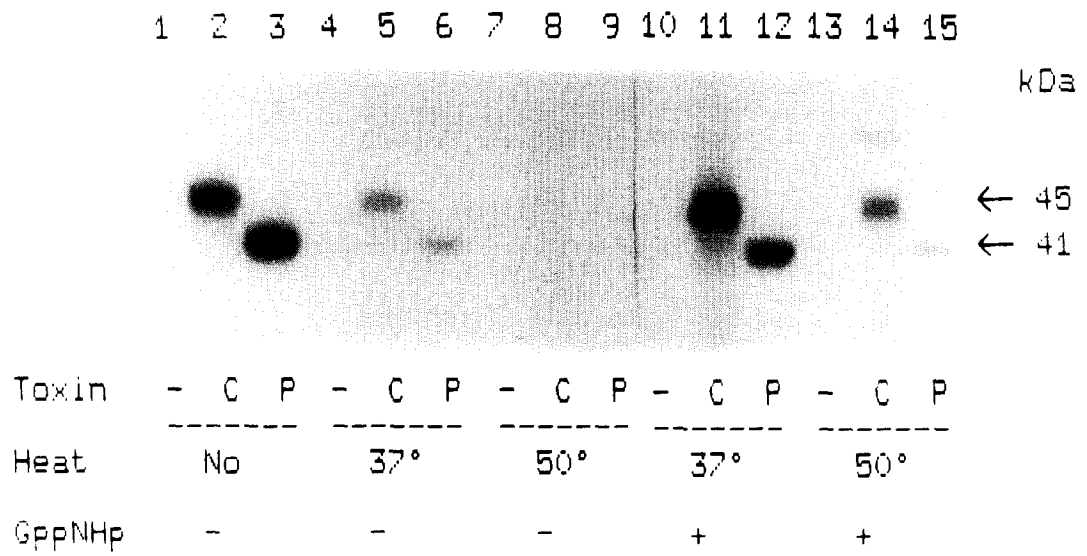

Fig. 6. [^{32}P]ADP-ribosylation of cholera toxin and pertussis toxin substrates in liver plasma membranes. Membranes were heat-treated at 37° (lanes 4–6 and 10–12) or at 50° (lanes 7–9 and 13–15) in the absence (lanes 4–9) and presence (lanes 10–15) of 200 μM Gpp(NH)p. Native (lanes 1–3) and heat-treated membranes (100 μg protein) were then ADP-ribosylated in the absence (−) or presence of cholera toxin (C) or pertussis toxin (P) as described under Materials and Methods. A representative autoradiogram of three experiments is shown.

Gpp(NH)p (200 μM), the nucleotide appeared to prevent the loss of ^{32}P-labeling, especially for N_s (lanes 11 and 14 vs lanes 5 and 8). Although other interpretations are possible, these results suggest that the loss of R-G communication upon heat-treatment may result from heat inactivation of G-unit or the GTP-binding site(s) on this unit.

DISCUSSION

The β-adrenergic receptor can be quantified in a wide variety of tissues by use of appropriate radiolabeled ligands such as [^{3}H]DHA, [^{125}I]CYP, [^{125}I]hydroxybenzylpindolol and [^{3}H]CGP-12177. However, for some tissues even the use of such radioligands results in saturable binding that sometimes fails to display stereospecificity [17–19], a fundamental criterion for validating measurements of biologically relevant receptors [23, 24]. Although the problem of lack of stereoselectivity in receptor measurements is not usually reported by many investigators, it would appear that crude rat liver membranes prepared from whole livers are notorious in this respect [17], and the phenomenon may be more widespread than is commonly acknowledged [18, 19].

Baker and Potter [7] have suggested that β-receptors in canine cardiac membranes can be stabilized to heat inactivation by the agonist, *l*-isoproterenol, or the antagonist, *dl*-alprenolol. Their rationale was that the agonist or the antagonist would protect specific hormone receptor sites from heat lability. This idea is not unlike that developed in enzymology, that certain enzymes are protected from heat lability or degradation by their substrates. We inferred from the Baker and Potter [7] paper that, in membranes containing high numbers of ligand acceptor sites, non-specific acceptor sites for the β-receptor ligand might be inactivated by the mild heat-treatment in the presence of an agonist, *l*-isoproterenol, while specific β-receptor binding sites are protected. In the studies described in this paper, we have explored this approach to unmask non-β-receptor binding sites for [^{3}H]DHA in partially purified guinea-pig hepatic plasma membranes. As we expected, non-specific acceptor sites for [^{3}H]DHA were inactivated by the heat-treatment, while specific β-receptor binding sites were protected. However, the heat-treatment causes loss of communication between the receptor and the guanine nucleotide regulatory protein as judged by the effect of guanine nucleotides on agonist competition of ligand binding to membranes and by the extent of bacterial toxin-catalyzed ADP-ribosylation of G-proteins. This phenomenon resembles the thermal inactivation of the guanine nucleotide regulation of α_2-receptor–agonist interactions in platelet membranes [25]. We show in this paper that heat-treatment of membranes is not necessary to measure β-receptors when [^{3}H]CGP-12177 and [^{125}I]CYP are used as ligands. With these ligands, the binding to native membranes readily exhibited high affinity and stereospecificity. Thus, although it appears technically feasible to measure β-receptors in liver membranes with [^{3}H]DHA using the mild heat-treatment protocol suggested by Baker and Potter [7], the results presented here indicate that it is not a desirable or practical approach, especially since other radioligands are now available which appear to offer clear advantages.

The β-adrenergic receptor in guinea-pig liver plasma membranes is predominantly of the β_2-subtype. This conclusion is consistent with the results

of metabolic studies [3] which demonstrate that the glycogenolytic response induced in guinea-pig hepatocytes by catecholamines is mediated primarily by the β_2-adrenergic receptor. Taken together with the results discussed elsewhere [26], it seems clear that mammalian liver β-receptors are predominantly of the β_2-subtype.

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