Agonist versus antagonist binding to α -adrenergic receptors

(α_1 and α_2 /[^3H]dihydroergocryptine/[^3H]epinephrine/liver/platelet)

BRIAN B. HOFFMAN*, THOMAS MICHEL*, DEBRA MULLIKIN KILPATRICK*, ROBERT J. LEFKOWITZ*,
MARGARET E. M. TOLBERT†, H. GILMAN†, AND JOHN N. FAIN†

*Departments of Medicine (Cardiovascular Division) and Biochemistry, Howard Hughes Medical Research Laboratory, Duke University Medical Center, Durham, North Carolina 27710; and †Section of Physiological Chemistry, Brown University, Providence, Rhode Island 02912

Communicated by Henry A. Lardy, May 5, 1980

ABSTRACT The binding properties of two α -adrenergic radioligands, [^3H]epinephrine (an agonist) and [^3H]dihydroergocryptine (an antagonist), were compared in two model systems—membranes derived from human platelets and membranes from rat liver. The platelet contains exclusively α_2 and the liver mostly ($\approx 80\%$) α_1 receptors. Agonists induce the formation of a guanine nucleotide-sensitive high-affinity state of α_2 but not α_1 receptors. [^3H]Dihydroergocryptine labels all the α receptors, whereas [^3H]epinephrine at low concentrations labels predominantly the high-affinity form of the α_2 receptor in both platelet and liver. However, in the liver, α -adrenergic effects such as glycogen phosphorylase activation are shown to be mediated via α_1 receptors. Thus, in liver membranes the endogenous “physiological” agonist may not label the physiologically relevant α_1 receptors in typical radioligand binding assays using low concentrations of [^3H]epinephrine.

A wide variety of radioligands, both agonists and antagonists, has been utilized to label α -adrenergic receptors in various tissues (1–4). In analogy with β -adrenergic receptors, α -adrenergic receptors have recently been subclassified into two subtypes (5). α_1 receptors, which mediate many typical postsynaptic effects, are characterized by much greater affinity for the antagonist prazosin than for yohimbine (6). In contrast, α_2 receptors, which may mediate pre- or postsynaptic effects and which are coupled in an inhibitory fashion to adenylate cyclase in some systems (7), are pharmacologically distinct, generally having higher affinity for yohimbine than for prazosin (6).

The complex patterns of α -adrenergic radioligand binding data have been in many cases difficult to resolve. These complexities relate to: (i) whether the radioligand is an agonist or an antagonist (2); (ii) the preferential affinity of different ligands for one or the other α -adrenergic receptor subtype (α_1 or α_2 receptor) (5, 6); and (iii) the modulation of agonist but not antagonist binding affinities by guanine nucleotides at α_2 but not α_1 receptors (7–9). α_2 receptors, in the absence of guanine nucleotides, exhibit two states of affinity for agonists: one of higher affinity (α_{2H}) and one of lower affinity (α_{2L}) (9). The α_{2H} state is apparently abolished by guanine nucleotides; this explains the overall loss of affinity for agonists produced by guanine nucleotides in α_2 receptor populations (7, 8). Failure to appreciate these complexities may confound the interpretation of α -adrenergic binding data and lead to erroneous conclusions about the physiological relevance of binding data. Thus, it is possible for a particular ligand to label an α receptor binding site in a tissue that is nonetheless not the relevant α receptor for all physiological responses in that tissue.

In the present studies we examine the nature of α -adrenergic

receptor binding of an α -adrenergic ^3H -labeled agonist and a ^3H -labeled antagonist, as exemplified by the important ligands [^3H]epinephrine and [^3H]dihydroergocryptine ([^3H]DHE), respectively, in two model systems—the human platelet (containing exclusively α_2 receptors) (6) and rat liver plasma membranes (containing predominantly α_1 and fewer α_2 receptors). It is demonstrated that [^3H]DHE labels the entire α receptor population, whereas [^3H]epinephrine, under the assay conditions used, labels predominantly the α_{2H} state of the α_2 receptor population in each system. Because physiological α -adrenergic responses in liver cells are of the α_1 subtype, the use of the ^3H -labeled form of the naturally occurring catecholamine epinephrine to label α_1 receptors may not be an effective way to study the physiologically relevant α receptors in a tissue such as liver. A general framework for interpreting α -adrenergic receptor binding data in physiological terms is presented and discussed.

METHODS

Materials. [^3H]DHE (39 Ci/mmol) and (–)-[^3H]epinephrine (41 Ci/mmol) were obtained from New England Nuclear (1 Ci = 3.7×10^{10} becquerels). Yohimbine-HCl was from Nutritional Biochemicals. Prazosin-HCl was a gift from Pfizer. Other chemicals were from commercial sources.

Radioligand Binding Assays. Human platelets. Blood was obtained from normal human subjects and the platelets were isolated as described (10). The membrane preparation and assay conditions were similar to previously described methods with some modification (10). The platelets were washed three times in buffer (150 mM NaCl/50 mM Tris-HCl/20 mM EDTA, pH 7.35) with intervening centrifugations at $16,000 \times g$ for 10 min at 4°C . The platelets were then suspended in a hypotonic buffer (5 mM Tris-HCl/5 mM EDTA, pH 7.5) and homogenized with 30 strokes of a Teflon-tipped pestle in an ice bath. The pestle was driven by a motor drive (3431-J70, Thomas) controlled by an autotransformer (Type 116B, Thomas), at setting 90. The platelet lysate was obtained by centrifugation at $39,000 \times g$ for 10 min and was resuspended in an incubation buffer containing 10 mM MgCl_2 , 50 mM Tris-HCl, 1 mM catechol, 10 μM dithiothreitol, and 0.1 mM EDTA (pH 7.7) to a protein concentration of ≈ 4 mg/ml. Assay conditions for [^3H]DHE and [^3H]epinephrine were identical. Assays were in a total volume of 1.0 ml, which consisted of 0.025 ml of radioligand and 0.875 ml of drug in incubation buffer. Incubations (60 min at 25°C) were started by the addition of 0.1 ml of membranes. α receptor binding was stable for greater than 2 hr. Incubations were terminated by vacuum filtration with a 20-ml wash with ice-cold buffer (50 mM Tris-HCl/10 mM MgCl_2 /0.1% ascorbic acid/1 mM catechol, pH 7.7). Specific binding of [^3H]epinephrine was generally 40–60% of total binding.

Abbreviation: DHE, dihydroergocryptine.

Rat liver. Membranes were prepared as described (11, 12) from the fresh livers of 250- to 300-g male Sprague-Dawley rats (Charles River Breeding Laboratories, C.D. strain). [3 H]DHE binding studies were done as previously described (11), except that the final assay volume was 0.15 ml. [3 H]epinephrine binding studies were done as described by El-Refai *et al.* (13) in a final volume of 0.15 ml, except that (–)-[3 H]epinephrine (rather than (±)-[3 H]epinephrine) was used throughout. For the concentrations of [3 H]epinephrine utilized, specific binding was 35–65% of total binding.

Physiological Studies in Rat Hepatocytes. Hepatocytes were isolated from rats by the procedure of Birnbaum and Fain (14). Rat hepatocytes (approximately 3×10^6 per ml) were incubated for 20 min without or with adrenergic antagonists prior to the addition of 0.1 μ M epinephrine. One minute later the stopping solution was added and the cells were processed for glycogen phosphorylase assay as described by Malbon *et al.* (15). The values are expressed as percent increases in phosphorylase due to epinephrine over the values for controls incubated either without or with the adrenergic antagonists, which had no detectable effect on phosphorylase.

Data Analysis. Computer modeling of the competition and saturation curves was done as described (6, 16). In brief, a fit of the data to two states of binding was accepted as appropriate if and only if the fit was significantly improved ($P < 0.05$) over that for a one-state fit. The modeling provides values for the affinity constants of the competing ligand for the sites and the proportions of the sites present when a two-state fit is involved.

Computer resolution of “shallow” competition curves into distinct components has been used in two very different contexts. Antagonists agents do not discriminate different affinity states of α -receptors that are apparently interconverted by nucleotides (7). Thus, heterogeneity of binding curves of subtype-selective antagonists such as prazosin can be used to quantitate the proportions of α_1 and α_2 receptor subtypes (Fig. 3B) (6). A second and quite distinct application of these methods has been to quantitate different affinity states of a single subtype (α_2) distinguished by agonists such as epinephrine (9) (Fig. 2).

The curves in Figs. 1 and 3A were analyzed by a four-parameter logistic equation (17) that provides 50% inhibitory concentrations (IC_{50}) and slope factors (pseudo Hill coefficients) for each competitor.

RESULTS

Human Platelet. The platelet's α -adrenergic receptors are exclusively of the α_2 subtype (6). Platelet membranes are therefore an ideal system for characterizing α_2 receptor interactions. [3 H]epinephrine binding to platelet membranes has the characteristics of binding to α receptors, as has been demonstrated previously (18). In competition curves the potency series of epinephrine > norepinephrine > isoproterenol and stereospecificity (–)-epinephrine > (+)-epinephrine is typical of α receptor binding (data not shown). Moreover, yohimbine is far more potent than prazosin, confirming an α_2 receptor pattern of binding in the platelet previously demonstrated with [3 H]DHE (6) (Fig. 1). Propranolol did not displace [3 H]epinephrine (Fig. 1), indicating that [3 H]epinephrine was not bound to β -adrenergic receptors.

We have previously demonstrated that competition curves of agonists with [3 H]DHE indicate that agonist binding to the platelet's α_2 receptors may be resolved into two states of binding—one of higher affinity (α_{2H}) and one of lower affinity (α_{2L}) (9). This effect is specific to agonists, because antagonists yield steep curves modeling to a single state of binding (6). The

FIG. 1. Competition curves of various antagonists with [3 H]epinephrine in human platelet membranes. $\square$, Yohimbine; Δ , phentolamine; $\bullet$, prazosin; $\circ$, propranolol. The results of a typical experiment (replicated two or three times), performed in duplicate, are illustrated. [3 H]epinephrine was at a concentration of 5 nM. The curves were drawn by the computer, utilizing a four-parameter logistic equation (17). The antagonists showed a potency series yohimbine > phentolamine > prazosin, confirming that the platelet's α receptor has the characteristics of an α_2 receptor (6). Little, if any, of the [3 H]epinephrine binding was displaced by propranolol, indicating that [3 H]epinephrine was not binding to β -adrenergic receptors.

full agonist epinephrine (Fig. 2) is found to yield a competition curve with [3 H]DHE (mean of nine experiments) significantly better fit ($P < 0.001$) to a two-state rather than to a one-state model. The curve has a slope factor of 0.65. The α_{2H} state for epinephrine constituted 55% of the α_2 receptors and had a dissociation constant (K_d) of 11 nM, whereas the α_{2L} state for epinephrine constituted the remaining 45% of the α_2 receptors and had a K_d of 450 nM. Whereas competition curves of epinephrine with [3 H]DHE modeled to a two-state fit, competition curves of epinephrine with [3 H]epinephrine modeled to a

FIG. 2. Competition curve of [3 H]DHE with (–)-epinephrine in human platelet membranes. The competition curve is the mean of nine experiments, each performed in duplicate at a [3 H]DHE concentration of 3 nM. The curve was significantly ($P < 0.001$) better fit to a two-state than to a one-state model of binding. The higher-affinity state (α_{2H}) constituted 55% of the α_2 receptors in the platelet, the remainder of the sites being in the lower-affinity form (α_{2L}). (Inset) Saturation curves of [3 H]epinephrine ($\square$) and [3 H]DHE ($\bullet$) in platelet membranes. A typical experiment is shown. In five such experiments [3 H]epinephrine labeled 64% of the sites labeled by [3 H]DHE, with saturation binding of 158 and 247 fmol/mg of protein, respectively. The [3 H]epinephrine sites correspond to the α_{2H} state. The mean K_d of [3 H]DHE for the α receptor was 3 nM, and that of [3 H]epinephrine was 5 nM.

one-state fit with a K_d of 9 nM (data not shown), which is similar to the α_{2H} K_d of epinephrine determined in the [3 H]DHE competition curve (11 nM). This suggested that [3 H]epinephrine at concentrations used in these assays was binding exclusively to receptors in the α_{2H} state, leading to competition curves of a single binding state of high overall affinity.

This possibility was further evaluated by constructing [3 H]epinephrine saturation curves in platelet membranes and comparing them with [3 H]DHE saturation curves in the same membrane preparations. A typical experiment is illustrated in Fig. 2 *inset*, where it is seen that [3 H]epinephrine labels fewer sites than does [3 H]DHE. In a mean of five experiments, the [3 H]epinephrine sites constituted 64% of the [3 H]DHE sites. This difference was significant ($P < 0.01$). However, the proportion of [3 H]epinephrine sites was not significantly different from the proportion of the platelet's α_2 receptors existing in the high affinity state determined by competition curves of epinephrine with [3 H]DHE (55%) as previously indicated (Fig. 2). Also, the mean K_d of [3 H]epinephrine determined by saturation curves in these five experiments was 5 nM, also in good agreement with the K_d of the α_{2H} state of epinephrine in competition with [3 H]DHE (from Fig. 2).

It is known that in platelet membranes agonist affinity at [3 H]DHE sites is reduced by guanine nucleotides, probably due to the abolishment of the α_{2H} state because agonist competition curves in the presence of guanine nucleotides model to a single state of binding of low affinity (7, 9). The notion that [3 H]epinephrine was binding exclusively to the α_{2H} state in platelet membranes was consequently further tested by attempting to construct [3 H]epinephrine saturation curves in the presence of high concentrations (0.1 mM) of guanosine 5'-[β , γ -imido]triphosphate. In three such experiments no specific [3 H]epinephrine binding was present at [3 H]epinephrine concentrations up to 15 nM; this is the expected result because agonist binding is of uniformly low affinity under these conditions (7).

In summary, these data demonstrate that [3 H]epinephrine binds to fewer sites than [3 H]DHE in platelet membranes and that these [3 H]epinephrine sites have characteristics that suggest they are the α_{2H} state of the platelet's α_2 receptor, which was also identified by competition curves with [3 H]DHE.

Rat Liver. With this background on agonist binding to the exclusively α_2 receptor population found in platelets, we next examined the more complex α receptors found in liver membranes, which are found to be composed of both α_1 and α_2 receptors, which the α_1 receptors predominating.

In an effort to unravel the pharmacological properties of the hepatic α -adrenergic receptor subtypes, which are not only involved in mediating characteristic physiological responses but also may be differentially labeled by [3 H]DHE and [3 H]epinephrine, we utilized the α -adrenergic antagonists prazosin and yohimbine. Prazosin has generally been found to have considerably higher potency at α_1 than α_2 receptors in both physiological and radioligand binding sites (6, 19, 20). Conversely, yohimbine has been found to be α_2 selective, although it has not demonstrated the same marked α -adrenergic subtype selectivity as prazosin (6).

Catecholamine activation of phosphorylase in rat liver is mediated by α receptors (21, 22). In Fig. 3A are illustrated dose-response curves for the inhibition of epinephrine-stimulated glycogen phosphorylase by prazosin and yohimbine. Prazosin exhibits high potency in inhibiting this response, with an apparent K_d of 0.3 nM, whereas yohimbine is considerably less potent in inhibiting this response, with an apparent K_d of 1000 nM. This shows that phosphorylase activation occurs via α_1 receptors. We have also found prazosin to be several orders

FIG. 3. Inhibitions by prazosin (●) and yohimbine (□). (A) Inhibition of epinephrine-stimulated glycogen phosphorylase in isolated hepatocytes. The data (mean of three experiments) were analyzed by a four-parameter logistic equation (17). The stimulating concentration of epinephrine was 100 nM which gave half-maximal stimulation. Because prazosin is far more potent than yohimbine in inhibiting phosphorylase activation, this response is demonstrated to occur via α_1 receptors. (B) Inhibition of [3 H]DHE binding in liver membranes by prazosin and yohimbine. The biphasic prazosin competition curve with [3 H]DHE modeled to a two-site fit ($P < 0.01$); the majority of the sites were α_1 receptors with high affinity for prazosin. The experiment was replicated 11 times. The overall affinity of yohimbine was much weaker, confirming that the majority of the [3 H]DHE sites are α_1 receptors. The [3 H]DHE concentrations in these experiments was ≈ 5 nM. (C) Inhibition of [3 H]epinephrine binding in liver membranes by prazosin and yohimbine. [3 H]Epinephrine concentration was 10 nM. It is evident that yohimbine is slightly more potent than prazosin at the [3 H]epinephrine sites. This finding is quite different from the relative affinities of prazosin and yohimbine in inhibiting phosphorylase activation and [3 H]DHE binding, suggesting that [3 H]epinephrine labels α_2 receptors, which do not activate glycogen phosphorylase.

of magnitude more potent than yohimbine in inhibiting epinephrine-stimulated phosphatidylinositol turnover in isolated hepatocytes (data not shown; ref. 23).

In Fig. 3B are illustrated typical competition curves of [³H]DHE by prazosin and yohimbine in liver membranes. The prazosin curve is biphasic, with computer modeling indicating the presence of two receptor types (α_1 and α_2) having, respectively, higher and lower affinity for prazosin. The α subtype proportions are: $\alpha_1 = 83 \pm 3\%$ and $\alpha_2 = 17 \pm 3\%$ (mean $\pm$ SEM, $n = 11$). The K_d s of prazosin at α_1 and α_2 sites labeled by [³H]DHE are:

$$\alpha_1 K_d = 0.8 \pm 0.1 \text{ nM and } \alpha_2 K_d = 89 \pm 13 \text{ nM } (n = 11).$$

Computer modeling of such biphasic prazosin displacement curves of [³H]DHE provides a reliable means of quantitating the α_1 and α_2 subtype proportions in a membrane preparation, because of the great selectivity of prazosin for α_1 as opposed to α_2 receptors.

The yohimbine competition curve (Fig. 3B) modeled to one site with an overall $K_d = 350 \pm 110 \text{ nM}$ ($n = 4$). The apparent inability of yohimbine to discriminate between α_1 and α_2 receptors when determined by computer modeling of [³H]DHE curves probably relates to its modest α_2 selectivity's being at the limits of the sensitivity of the computer modeling techniques under these conditions (see also Table 1 legend). The good agreement between the affinities of prazosin and yohimbine determined by inhibition of phosphorylase activity and inhibition of [³H]DHE binding is evident (Table 1).

We next examined some of the properties of [³H]epinephrine binding in the liver. [³H]Epinephrine has been previously shown to bind to α receptors in these membranes (13). [³H]-Epinephrine binding curves in liver (0–35 nM) were compared with [³H]DHE saturation curves in the same membrane preparation. In 11 such experiments the [³H]epinephrine sites apparently saturated and were $69 \pm 6 \text{ fmol/mg}$ of protein and the [³H]DHE sites were $867 \pm 116 \text{ fmol/mg}$ of protein. Thus, the [³H]epinephrine sites constitute only 8% of the number of [³H]DHE sites. This result is similar to the 10% ratio of the high affinity [³H]epinephrine sites to [³H]DHE sites previously reported by El-Refai *et al.* (13). To further characterize the nature of these sites labeled by [³H]epinephrine, competition curves of [³H]epinephrine (10 nM) with prazosin and yohimbine were constructed (Fig. 3C). The prazosin competition curve with [³H]epinephrine modeled to one site with a K_d of $30 \pm 13 \text{ nM}$ ($n = 6$) (Table 1), indicating that [³H]epinephrine was binding predominantly to one class of α receptor subtype (as compared to the two α receptor subtypes evident in the biphasic prazosin competition curve of [³H]DHE in Fig. 3B). The K_d of prazosin at the [³H]epinephrine site (30 nM) is in reasonable agreement with the K_d of prazosin at the α_2 receptor determined with [³H]DHE (89 nM), suggesting that [³H]epinephrine was binding largely to α_2 receptors. This was further substantiated by the yohimbine competition curve with [³H]epinephrine, which yields a K_d for yohimbine of $11 \pm 3 \text{ nM}$ ($n = 6$) (Table 1). Thus, in marked contrast with the results obtained when [³H]DHE is the ligand, yohimbine is found to be more potent than prazosin at the sites labeled with low (10 nM) concentrations of [³H]epinephrine, again suggesting that [³H]epinephrine is labeling predominantly α_2 receptors in this concentration range.

In 11 separate experiments, a prazosin competition curve of [³H]DHE was constructed and the number of [³H]epinephrine and [³H]DHE sites was also determined by saturation curves. The ratio of [³H]epinephrine to [³H]DHE sites was 8%, as noted above. Using the prazosin competition curves of [³H]DHE, the

Table 1. Dissociation constants of prazosin and yohimbine for inhibition of α receptor-stimulated phosphorylase activity in isolated hepatocytes and inhibition of binding of [³H]DHE and [³H]epinephrine to α receptors in liver plasma membranes

	K_d , nM	
	Prazosin	Yohimbine
Phosphorylase activation	0.3 ($n = 3$)	1000 ($n = 3$)
[³ H]DHE sites		
α_1	0.8 ($n = 11$)	
α_2	89 ($n = 11$)	350* ($n = 4$)
[³ H]Epinephrine sites	30 ($n = 6$)	11 ($n = 6$)

The assays were performed and the K_d s were determined by computer modeling techniques, as described in *Methods*. The K_d s for inhibition of phosphorylase activation were calculated according to the equation of Cheng and Prusoff (24):

$$K_d = \frac{IC_{50}}{1 + S/K_m},$$

in which IC_{50} is the 50% inhibitory concentration of drug, K_m is the half-maximal stimulatory concentration of epinephrine determined in separate experiments (100 nM), and S is the concentration of epinephrine used in these experiments, also 100 nM. Agreement is evident between the K_d s for prazosin determined by the inhibition of [³H]DHE binding at α_1 receptors (0.8 nM) and inhibition of phosphorylase activation (0.3 nM); similarly, agreement is found for yohimbine K_d s (350 nM and 1000 nM, respectively). However, at [³H]epinephrine sites, yohimbine is more potent than prazosin; this is opposite to the clear α_1 potency order found with both [³H]DHE binding and phosphorylase activation. n , Number of experiments.

* Yohimbine competition curves with [³H]DHE consistently modeled to one site rather than the two sites evident with prazosin and [³H]DHE. Because the majority of the [³H]DHE sites ($\approx 80\%$) are α_1 receptors, the single yohimbine affinity at the [³H]DHE sites predominantly reflects yohimbine's affinity at α_1 receptors. We presume that our inability to detect two sites with yohimbine in [³H]DHE competition curves is due to the modest selectivity of yohimbine's being beyond the sensitivity of our methods to directly detect in this system. This modest selectivity is suggested by noting that the affinity of yohimbine at [³H]epinephrine sites (α_2 receptors) is only ≈ 30 -fold higher than yohimbine's overall affinity at [³H]DHE sites (predominantly α_1 receptors).

proportion of α_2 receptors was determined to be significantly greater than this ratio ($P < 0.02$), with the α_2 receptors constituting 17% of the total number of α receptors. Thus the [³H]epinephrine sites were only $\approx 50\%$ of the total α_2 receptors determined with prazosin and [³H]DHE. This suggests that under these assay conditions [³H]epinephrine is labeling only $\approx 50\%$ of the α_2 receptors in the liver membranes, which is similar to the situation with [³H]epinephrine in the pure α_2 system of the platelet as indicated above.

DISCUSSION

We have demonstrated in human platelets that [³H]epinephrine labels a subfraction of the total α_2 receptor population. These sites are identified as the guanine nucleotide-sensitive α_{2H} state. Furthermore, high affinity [³H]epinephrine sites appear to be predominantly α_2 receptors in liver. Indeed, it is likely that the fraction of α_2 receptors labeled by [³H]epinephrine is the high-affinity state of the α_2 receptor in liver, because these sites contribute $\approx 50\%$ of the α_2 receptor population and are also guanine nucleotide sensitive (13).

The metabolic effects of catecholamines appear to be mediated through α_1 receptors in rat hepatocytes. It is shown here that prazosin is orders of magnitude more potent than yohimbine in inhibiting glycogen phosphorylase activation. Also, prazosin is potent in inhibiting gluconeogenesis (25) and phosphatidylinositol turnover (23), suggesting that these responses are also mediated via α_1 receptors.

These results clarify the nature of the sites of binding of [^3H]DHE and [^3H]epinephrine in liver membranes. [^3H]DHE labels the entire α receptor population, including both the α_1 (83%) and α_2 (17%) receptors, whereas [^3H]epinephrine seems to bind with high affinity only to a fraction of the α_2 receptor population. Aggerbeck *et al.* (26) also found that [^3H]DHE labels α_1 receptors in liver. It was claimed by El-Refai *et al.* (13) that [^3H]epinephrine, and not [^3H]DHE, labeled with high affinity the physiologically relevant α receptors in liver. This seems to be an untenable hypothesis because physiological responses in isolated hepatocytes appear to be mediated via α_1 receptors rather than via the predominantly α_2 receptors labeled here by [^3H]epinephrine. The physiological function of the α_2 receptors detected in these liver membrane preparations is unknown. They may possibly reside in nerve terminals in the liver or be located on the hepatocytes themselves and perform some other function, the nature of which remains obscure. In favor of the possibility that the α_2 receptors are localized on the hepatocytes is the fact that in two experiments, when membranes for binding were prepared from isolated hepatocytes (rather than whole livers), the usual fractions of α_2 receptors and [^3H]epinephrine sites were present (data not shown). It is also possible that these α_2 sites play a minor role in phosphorylase activation in view of the fact that prazosin, even at a concentration up to 1000 nM, did not completely block epinephrine activation of phosphorylase.

These results highlight the caution that must be used in interpreting the results of α -adrenergic receptor binding studies. Thus, while one might imagine that an endogenous ligand (such as [^3H]epinephrine) would be entirely reliable for labeling physiologically relevant sites, this appears not to be the case in the liver. The presence of receptors in the α_{2H} state, which accumulate in membrane preparations that are washed free of endogenous guanine nucleotides, confounds the interpretation of ligand binding data when the actual physiological response is mediated by α_1 receptors such as those in liver. It is likely that at much higher concentrations [^3H]epinephrine labels both α_1 and α_2 receptors. Indeed, recent experiments indicate that, when higher concentrations of [^3H]epinephrine are used, α_1 receptors are also labeled. This is evident by the fact that prazosin competition curves tend to become biphasic with discernible high- and low-affinity components when there is competition with high concentrations of [^3H]epinephrine (unpublished observations).

These findings may have general relevance for the understanding of ligand binding data, because significant analogies to these findings for the α receptors exist in a number of other receptor systems. Such systems as, for example, dopaminergic and serotonergic (27) receptors may each be characterized by the existence of discrete receptor subtypes, with one but not the other subtype appearing to be regulated by guanine nucleotides. In all such systems the conclusion that a particular ligand is labeling the physiologically relevant receptor subtype found in a particular tissue will depend on application of the types of analysis described here.

Ms. C. Wood kindly prepared isolated hepatocytes from which purified membranes were made. B.B.H. is a Fellow of the Medical Research Council of Canada. T.M. is a student in the Medical Scientist Training Program (National Institutes of Health Grant GM07171). R.J.L. is an Investigator of the Howard Hughes Medical Institute. This work was supported in part by National Institutes of Health Grants HL16037, HL20339, and AM21470.

- Williams, L. T. & Lefkowitz, R. J. (1976) *Science* **192**, 791–793.
- Greenberg, D. A., U'Prichard, D. C. & Snyder, S. H. (1976) *Life Sci.* **191**, 69–76.
- U'Prichard, D. C. & Snyder, S. H. (1977) *Life Sci.* **20**, 527–533.
- Greengrass, P. & Brenner, R. (1979) *Eur. J. Pharmacol.* **55**, 323–326.
- Berthelson, S. & Pettinger, W. A. (1977) *Life Sci.* **21**, 595–606.
- Hoffman, B. B., De Lean, A., Wood, C. J., Schocken, D. D. & Lefkowitz, R. J. (1979) *Life Sci.* **24**, 1739–1746.
- Tsai, B. S. & Lefkowitz, R. J. (1979) *Mol. Pharmacol.* **16**, 61–68.
- U'Prichard, D. C. & Snyder, S. H. (1978) *J. Biol. Chem.* **253**, 3444–3452.
- Hoffman, B. B., Mullikin Kilpatrick, D. & Lefkowitz, R. J. (1980) *J. Biol. Chem.* **255**, 4645–4652.
- Newman, K. D., Williams, L. T., Bishopric, N. H. & Lefkowitz, R. J. (1978) *J. Clin. Invest.* **61**, 395–402.
- Clarke, W. R., Jones, L. R. & Lefkowitz, R. J. (1978) *J. Biol. Chem.* **253**, 5975–5979.
- Neville, D. M., Jr. (1968) *Biochim. Biophys. Acta* **154**, 540–552.
- El-Refai, M. F., Blackmore, P. F. & Exton, J. H. (1979) *J. Biol. Chem.* **254**, 4375–4386.
- Birnbaum, M. J. & Fain, J. N. (1977) *J. Biol. Chem.* **252**, 528–535.
- Malbon, C. C., Si, S.-Y. & Fain, J. N. (1978) *J. Biol. Chem.* **253**, 8820–8825.
- Hancock, A. A., De Lean, A. L. & Lefkowitz, R. J. (1979) *Mol. Pharmacol.* **16**, 1–9.
- De Lean, A. L., Munson, P. J. & Rodbard, D. (1978) *Am. J. Physiol.* **235**, E97–E102.
- Reynolds, W. H., Alexander, R. W. & Handin, R. I. (1978) *Circulation* **58**, 20 (abstr.).
- Doxey, J. C., Smith, C. F. C. & Walker, J. M. (1977) *Br. J. Pharmacol.* **60**, 91–96.
- U'Prichard, D. C., Charness, D., Robertson, D. & Snyder, S. H. (1978) *Eur. J. Pharmacol.* **60**, 87–89.
- Fain, J. N. (1978) in *Receptors and Recognition*, eds Cuatrecasas, P. & Greaves, M. F. (Chapman & Hall, London), Vol. A6, pp. 1–62.
- Exton, J. H. (1979) *J. Cyclic Nucleotide Res.* **5**, 277–287.
- Tolbert, N. E. M., White, A. C., Aspry, K., Cutts, J. & Fain, J. N. (1980) *J. Biol. Chem.* **255**, 1938–1944.
- Cheng, Y. C. & Prusoff, W. H. (1973) *Biochem. Pharmacol.* **22**, 3099–3108.
- Kneer, N. M., Wagner, M. J. & Lardy, H. A. (1979) *J. Biol. Chem.* **254**, 12160–12168.
- Aggerbeck, M., Guellaen, G. & Hanoune, J. (1980) *Biochem. Pharmacol.* **29**, 643–645.
- Peroutka, S. J., Lebovitz, R. M. & Snyder, S. H. (1979) *Mol. Pharmacol.* **16**, 700–708.