Binding and uptake of [³H]adrenaline by perfused rat liver

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The binding and uptake of [³H]adrenaline by the intact perfused rat liver was investigated. We showed that the administration of [³H]adrenaline to liver resulted in the rapid uptake of the radioligand, and that such uptake was independent of any Ca²⁺ redistributions induced by the hormone. At low adrenaline concentrations (<50 nM) uptake was inhibited by prazosin, whereas at higher hormone concentrations a significant proportion of total [³H]adrenaline uptake could not be inhibited by this antagonist. [³H]Adrenaline uptake could be directly correlated with adrenaline-induced responses such as an increased rate of respiration and glycogenolysis. The partial inhibition (approx. 25%) of [³H]adrenaline uptake by antagonists was sufficient for the total inhibition of hormone-induced responses. The effect of various pharmacological agents on [³H]adrenaline uptake was investigated, and the contribution of tissue-related factors to α -adrenergic agonist–antagonist interactions *in vivo* is discussed.

Liver plasma-membrane preparations have been shown to contain at least two types of α -adrenergic receptors, classified as α_1 and α_2 , on the basis of pharmacological potency series (Wikberg, 1979; Wood *et al.*, 1979; El-Refai & Exton, 1980; Hoffman *et al.*, 1981; Graham *et al.*, 1982). Most α -adrenergic-agonist-induced responses appear to be mediated through binding of the agonist to α_1 -receptors (El-Refai & Exton, 1979; Garcia-Sainz *et al.*, 1980; Aggerbeck *et al.*, 1980; Hoffman *et al.*, 1980a,b; Reinhart *et al.*, 1982a,b), whereas binding to α_2 -receptors has been implicated with an inhibition of adenylate cyclase activity (Jard *et al.*, 1981; Limbird, 1983). Furthermore, competition binding assays *in vitro* have revealed that rat liver contains a high proportion of α_1 -receptors, that a single subtype may exist in more than one form (Hoffman *et al.*, 1980a,b; Geynet *et al.*, 1981), and that binding data obtained for α -antagonists such as [³H]dihydroergocryptine differ from data obtained for α -agonists such as [³H]adrenaline (El-Refai & Exton, 1979; Hoffman *et al.*, 1980a; Geynet *et al.*, 1981).

However, the full understanding of agonist–receptor interactions *in vivo* requires knowledge about drug-related factors such as the efficacy and affinity of agonists and antagonists, as well as tissue-related factors such as the coupled-receptor number, and the rate of agonist or antagonist removal from the receptor compartment (Kena-

kin, 1983). In liver there has been much interest in examining drug-related factors, by using isolated fragments enriched in plasma membranes (see Schmelck & Hanoune, 1980; Hanoune *et al.*, 1981; Exton, 1982), despite the fact that virtually nothing is known about tissue-related factors. Hence we have attempted to extend the information obtained from binding studies *in vitro* by examining the [³H]adrenaline–receptor interaction in the intact perfused liver.

Our studies show that [³H]adrenaline is rapidly taken up by the liver, and that this uptake can be inhibited by α -adrenergic antagonists such as prazosin or yohimbine, agents previously used to distinguish between binding to α_1 - and to α_2 -adrenergic receptors respectively (Doxey *et al.*, 1977; Hoffman *et al.*, 1979).

Inhibition of [³H]adrenaline uptake by α -adrenergic antagonists is paralleled by inhibition of hormone-induced responses. The dynamic nature of α -adrenergic-agonist–receptor interactions and possible applications of receptor-binding studies *in situ* are discussed.

Methods

Animals and perfusions

Male Wistar-strain albino rats weighing 230–270 g and having free access to food were used for all experiments. Rats were anaesthetized with

sodium pentobarbitone (50 mg/kg body wt.), and the livers were perfused with Krebs–Henseleit (1932) bicarbonate medium equilibrated with O₂/CO₂ (19:1) as described previously (Reinhart *et al.*, 1982a). Briefly, livers were perfused at a flow rate of 3.5 ml/min per g wet wt. of liver, at 35°C. All livers were pre-perfused for 15 min with medium containing 1.3 mM added CaCl₂. In some experiments the perfusate CaCl₂ concentration was decreased to approx. 6 µM at 15 min (Reinhart *et al.*, 1982b). All experiments were performed between 08:00 h and 12:00 h to minimize diurnal fluctuations of basal metabolism.

[³H]Adrenaline-uptake assay

[³H]Adrenaline (approx. 2×10^6 c.p.m./ml) was prepared just before each experiment in degassed Krebs–Henseleit buffer containing 250 µM catechol. The hormone was infused into either the inflow cannula or the outflow cannula by using a foil-covered glass/glass infusion syringe (EVA Superglass µ-Micromatic) mounted on a Braun Perfusor IV infusion pump. Perfusate samples were collected at the times indicated in the legends to Figures, and the radioactivity was determined by adding 200 µl perfusate samples to scintillation vials containing 10 ml of scintillant [6 g of 5-(biphenyl-4-yl)-2-(4-*t*-butylphenyl)-1-oxa-3,4-diazole in 400 ml of 2-methoxyethanol and 600 ml of toluene] and counting to within 0.1% error in a Beckman LS-330 liquid-scintillation counter. All counts obtained were corrected for background radioactivity.

For all experiments, catechol (0.6 mM) was also infused 3 min before [³H]adrenaline administration, since this was found to decrease significantly the non-specific uptake of [³H]adrenaline by the liver (results not shown).

The difference between the radioactivity in the perfusate after infusion into the inflow and outflow cannulae respectively was taken to represent the [³H]adrenaline taken up by the liver.

Perfusate glucose and oxygen determinations

Glucose release by the liver was determined by the glucose oxidase/peroxidase method as previously described (Reinhart *et al.*, 1982b). Perfusion-circuit lag times, from the point of hormone or ionophore infusion to the sampling point, were routinely determined by using [³H]inulin. All data shown have been corrected for this lag time. The O₂ consumption by the liver was calculated from the difference between influent and effluent O₂ concentrations measured with a Clark-type O₂ electrode modified for a flow-through mode of operation as described previously (Reinhart *et al.*, 1982a). The linearity of the electrode response was established by using the colorimetric O₂ assay of

Hamlin & Lambert (1971). Perfusion-circuit lag times were determined by using sodium dithionite, and data shown were corrected for this lag time.

Materials

DL-[7-³H(n)]Adrenaline hydrochloride was obtained from New England Nuclear (Boston, MA, U.S.A.), prazosin from Pfizer (Brooklyn, NY, U.S.A.) and yohimbine and antimycin A from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Tri-fluoperazine and R24571 were gifts from Smith, Kline and French and Janssen Pharmaceutical (French's Forest, N.S.W., Australia) respectively. Verapamil was obtained from Knoll A.G., Munich, West Germany. All other chemicals used were of analytical grade.

Expression of data

All experiments were performed at least three times, and the data are expressed as means $\pm$ S.E.M. for the numbers of experiments described.

Results

Time course of [³H]adrenaline uptake by perfused rat liver

The data in Fig. 1 show changes in the perfusate radioactivity (a) and O₂ concentration (b) owing to the administration of 60 nM [³H]adrenaline to the intact perfused liver. The specific radioactivity of [³H]adrenaline was determined by infusing [³H]adrenaline into the outflow cannula (Fig. 1a) between 6.5 and 11.5 min of perfusion. The subsequent infusion, at 23 min, of [³H]adrenaline through the liver results in a significant decrease in the perfusate radioactivity, indicative of a continuous uptake of the α -agonist by the tissue (Fig. 1a). From the initial plateau of radioactivity observed at between 2 and 4 min of [³H]adrenaline administration, the rate of uptake can be estimated at 82.1 ± 2.5 pmol/min per g of liver ($n = 11$). This rate remains virtually constant for at least 15 min. The onset of [³H]adrenaline uptake is rapid, since maximal rates are observed at the earliest time point measurable (approx. 2 min). The addition of prazosin (2 µM) at 27 min rapidly increases the perfusate radioactivity, indicating a decrease in the rate of α -adrenergic agonist uptake by the liver. Half-maximal effects are observed after approx. 3 min, and maximal effects of 65–70% inhibition occur between 8 and 10 min after prazosin infusion.

The decrease in concentration of perfusate [³H]adrenaline was interpreted as being due to uptake rather than binding to extracellular sites, since the addition of a 50-fold excess (1 µM) of unlabelled adrenaline did not significantly alter the

Fig. 1. [^3H]Adrenaline uptake by perfused rat liver

Livers of fed rats were perfused as described in the Methods section. Between 6.5 and 11.5 min of perfusion, [^3H]adrenaline was infused into the outflow cannula and the specific radioactivity of the perfusate determined. At 20 min of perfusion, catechol (fine arrows; 0.6 mM final concn.) was infused through the liver, and 3 min later [^3H]adrenaline (60 nM final concn.) was administered (bold arrows). The rate of [^3H]adrenaline uptake was usually calculated by determining the radioactivity of 12 samples collected between 3 and 4 min after adrenaline administration (see 'a'). In some experiments prazosin (2 μM final concn.) was administered at 27 min (●), while other animals served as controls (○). The proportion of [^3H]adrenaline uptake inhibited by prazosin is calculated by determining the radioactivity of 12 samples collected between 9 and 10 min after prazosin administration (see 'b'). Perfusate radioactivities (a) and O_2 uptake (b) were determined as described in the Methods section. The data shown are from a typical experiment from five independent experiments performed for prazosin and three performed as controls.

rate of [^3H]adrenaline (20 nM) removal from the perfusate (results not shown).

The rate of O_2 uptake by the liver during the same experiment is shown in Fig. 1(b). Although the infusion of catechol induces a small stimulation in the rate of O_2 uptake, the previously reported stimulation of respiration by adrenaline (Scholz & Schwabe, 1980; Reinhart *et al.*, 1982a) is also observed in the present study.

The addition of prazosin, however, rapidly and completely inhibits the adrenaline-induced respiratory response, with a time course very similar to that observed on the effect on [^3H]adrenaline uptake. The adrenaline-stimulated glucose output was similarly inhibited by prazosin (results not shown).

Effect of α -adrenergic antagonists on [^3H]adrenaline uptake

The data in Fig. 2 show the effect of changing the concentration of either prazosin or yohimbine on the rate of [^3H]adrenaline uptake (a, b), O_2 uptake and glucose output (c, d). Prazosin inhibition of [^3H]adrenaline uptake is significant at 1 nM, half-maximal at 80 nM and maximal at 2 μM (Fig. 2a). Prazosin concentrations as high as 10 μM do not inhibit more than 70% of the [^3H]adrenaline uptake. Prazosin also inhibits adrenaline-induced responses such as the stimulation of respiration and of glycogenolysis by up to 90% over the same concentration range (Fig. 2c).

The effects of yohimbine are more complex, since [^3H]adrenaline uptake is inhibited in a bi-

Fig. 2. Effect of α -adrenergic antagonists on $[^3\text{H}]$ adrenaline uptake, glucose output and O_2 uptake. Livers of fed rats were perfused as described in the Methods section. The specific radioactivity of $[^3\text{H}]$ adrenaline in the perfusate was determined as described in the legend to Fig. 1. At 20 min of perfusion, catechol (0.6 mM) was infused, and at 23 min $[^3\text{H}]$ adrenaline (60 nM) was administered. Then 4 min later either prazosin or yohimbine was infused at the concentrations shown. The rate of $[^3\text{H}]$ adrenaline uptake (a, b) was determined by calculating the average radioactivity in samples collected both between 3 and 4 min of $[^3\text{H}]$ adrenaline administration, and between 9 and 10 min of antagonist administration (see Fig. 1). The rates of adrenaline-stimulated O_2 uptake (c, d; ●) and glucose output (c, d; ○) were determined as described in the Methods section. Each data point shown was obtained from a separate experiment.

phasic manner (Fig. 2b). A relative plateau of approx. 25% inhibition is observed at between 0.1 μM - and 10 μM -yohimbine. At higher concentrations of the α -antagonist, the rate of $[^3\text{H}]$ adrenaline uptake is further inhibited, until the maximal inhibition of 70% is observed at approx. 100 μM -yohimbine. Maximal inhibitory effects of yohimbine on α -adrenergic-agonist-induced respiration and glucose output (Fig. 2d) are observed at concentrations of the antagonist which inhibit only approx. 25% of $[^3\text{H}]$ adrenaline uptake. Hence

inhibition of α -adrenergic-agonist-induced rates of glucose output and respiration in the intact organ is induced by similar concentrations of prazosin and yohimbine.

To determine whether or not yohimbine and prazosin are acting at separate receptor sites, we investigated the inhibition of $[^3\text{H}]$ adrenaline uptake by the sequential addition of these antagonists.

The data in Fig. 3(a) show that the administration of yohimbine after 10 min of prazosin treatment results in virtually no further inhibition of

Fig. 3. Additivity of prazosin- and yohimbine-mediated inhibition of $[^3\text{H}]$ adrenaline uptake

Livers of fed rats were perfused as described in the legend to Fig. 2. After $[^3\text{H}]$ adrenaline administration, either prazosin (closed arrow) or yohimbine (open arrow) was infused at $2\text{ }\mu\text{M}$ (a). In addition, some livers were then treated with either yohimbine (open arrow) or prazosin (closed arrow) (b), while others served as controls ($\circ$, in a and b). $[^3\text{H}]$ -Adrenaline uptake was measured as described in the Methods section. Each data point shown represents means for either three (a) or four (b) independent experiments. S.E.M. values have been omitted for the sake of clarity, but $\pm 7.6\%$ was never exceeded.

$[^3\text{H}]$ adrenaline uptake than that observed in the presence of prazosin alone. Similarly the addition of prazosin after yohimbine (Fig. 3b) does not in-

Fig. 4. Effect of the perfusate adrenaline concentration on the rate of $[^3\text{H}]$ adrenaline uptake

Livers of fed rats were perfused as described in the legend to Fig. 1, except that the adrenaline concentration was varied as shown in the Figure. The rate of $[^3\text{H}]$ adrenaline uptake ($\circ$) was measured between 3 and 4 min after adrenaline administration, as shown in Fig. 1. The percentage of this rate inhibited by the infusion of $2\text{ }\mu\text{M}$ -prazosin ($\circ$) was calculated as described in Fig. 1. Data points shown are the means of 12 samples collected between 3 and 4 min after $[^3\text{H}]$ adrenaline administration ($\bullet$), or the means of three to six independent experiments performed in the presence of prazosin ($\circ$).

crease the inhibition of $[^3\text{H}]$ adrenaline uptake above 70%. In addition, the time course of yohimbine-induced $[^3\text{H}]$ adrenaline uptake is very similar to that for prazosin, where maximal effects were observed after 8–10 min.

Effect of adrenaline concentration in $[^3\text{H}]$ adrenaline uptake

The rate of $[^3\text{H}]$ adrenaline uptake is directly proportional to the perfusate $[^3\text{H}]$ adrenaline concentration over the range 4–90 nM (Fig. 4). This proportionality remained virtually unchanged even at a perfusate $[^3\text{H}]$ adrenaline concentration of $1\text{ }\mu\text{M}$ (results not shown). Higher concentrations of adrenaline were not used, since deleterious effects of the agonist were evident. However, the proportion of $[^3\text{H}]$ adrenaline uptake that is inhibited by prazosin decreases from 80% at 10 nM to less than 50% at 80 nM, a concentration that induces near-maximal metabolic effects. Thus, at high hormone

Fig. 5. Effect of multiple hormone administrations on the rate of $[^3\text{H}]$ adrenaline uptake

Livers of fed rats were perfused with medium containing 1.3 mM added Ca^{2+} as described in the Methods section. At 14 min of perfusion the perfusate Ca^{2+} concentration was decreased to approx. $6\ \mu\text{M}$ (as determined by atomic absorption spectroscopy). At 10 min and 64 min of perfusion, $[^3\text{H}]$ adrenaline was administered into the outflow cannula for 4 min to allow determination of the specific radioactivity of perfusate $[^3\text{H}]$ adrenaline. At 19, 28, 37, 46 and 55 min of perfusion, $[^3\text{H}]$ adrenaline was infused through the liver for 4 min and the perfusate radioactivity determined as described in the Methods section. Data shown are from a typical experiment chosen from three independent experiments.

Table 1. Effect of pharmacological agents on the rate of $[^3\text{H}]$ adrenaline uptake

Livers of fed rats were perfused as described in the legend to Fig. 1. At 23 min of perfusion $[^3\text{H}]$ adrenaline was administered, and 4 min later the agents shown below were infused for 10 min. At this time yohimbine ($2\ \mu\text{M}$) was administered for 10 min. Rates of $[^3\text{H}]$ adrenaline uptake were determined between 9 and 10 min of infusing either the pharmacological agent or the agent plus yohimbine. Control experiments indicated that yohimbine alone inhibited 26.7 ± 1.3 ($n = 8$)% of $[^3\text{H}]$ adrenaline uptake. Hence, by subtracting the inhibition by the agent from that by the agent plus yohimbine (Δ) an assessment of the additivity of these two agents was made. The numbers of independent experiments performed for each condition are shown in parentheses in the Table.

Agent infused	Final concn. (μM)	Inhibition (%) of $[^3\text{H}]$ adrenaline uptake by			Inhibition of yohimbine sites (%)
		Agent	Agent + yohimbine	Δ	
Dinitrophenol	30	1.2 ± 2.2 (3)	29.3 ± 3.2 (3)	28.1	≈ 0
Insulin	0.016	4.5 ± 1.5 (3)	31.8 ± 4.0 (3)	27.3	≈ 0
R24571	10	38.0 ± 2.7 (4)	70.2 ± 4.2 (4)	32.2	≈ 0
Antimycin A	5	43.6 ± 1.8 (3)	71.5 ± 3.1 (3)	27.9	≈ 0
Trifluoperazine	10	49.1 ± 2.9 (4)	56.0 ± 3.3 (4)	6.9	≈ 75
Verapamil	100	74.1 ± 2.9 (3)	81.4 ± 4.3 (3)	7.3	≈ 73

concentrations, a large proportion of $[^3\text{H}]$ adrenaline uptake occurs independently of α -adrenergic receptor interactions.

Lack of a role for Ca^{2+} in $[^3\text{H}]$ adrenaline uptake

Further experiments were designed to examine the Ca^{2+} -dependence of $[^3\text{H}]$ adrenaline uptake, since previous work has indicated that all other α -adrenergic-agonist-induced responses are obligatorily dependent on the redistribution of cellular

Ca^{2+} (Reinhart *et al.*, 1982b, 1984). The data in Fig. 5 show that the repeated administration of $[^3\text{H}]$ adrenaline to liver perfused with media containing only micromolar Ca^{2+} concentrations does not decrease the rate of $[^3\text{H}]$ adrenaline uptake. We have previously shown that this experimental regimen depletes the hormone-sensitive pool of cellular Ca^{2+} , and hence the expression of all Ca^{2+} -dependent responses to α -agonists (Reinhart *et al.*, 1982b, 1984). Thus it appears that neither the

interaction between adrenaline and α -receptors nor the internalization of the agonist is dependent on a redistribution of cellular Ca^{2+} .

Effect of pharmacological agents on [^3H]adrenaline uptake

Previous studies have indicated that numerous pharmacological agents, not usually regarded as α -adrenergic antagonists, can inhibit binding of [^3H]adrenaline to receptors *in vitro* (Blackmore *et al.*, 1978, 1981; Reinhart *et al.*, 1981). Hence further experiments were designed to examine the effects of several pharmacological agents both on the rate of [^3H]adrenaline uptake and on hormone-induced metabolic effects. The data in Table 1 show that, with the exceptions of dinitrophenol and insulin, all other agents tested significantly inhibit the rate of [^3H]adrenaline uptake. However, only trifluoperazine and verapamil appear to inhibit at coupled receptor sites, as judged by the ability to compete with yohimbine in blocking the rate of [^3H]adrenaline uptake. Hence the inhibitory effect of these two agents on adrenaline-induced respiration does appear to be related to their α -adrenergic antagonist properties, whereas the inhibitory effect of antimycin A on adrenaline-induced respiration cannot be interpreted in this way. Neither insulin nor R24571, a potent inhibitor of calmodulin-mediated effects (Van Belle, 1981), appears to inhibit [^3H]adrenaline binding to coupled α -adrenergic receptors.

Discussion

The data presented above reveal new information about α -adrenergic-agonist-antagonist interactions *in situ*. The most striking difference between data obtained from previous binding assays *in vitro*, and the administration of [^3H]adrenaline to the intact perfused liver, is the rapid, large and prolonged uptake of the radioligand *in situ*. The lack of any observable recycling of radiolabel indicates that [^3H]adrenaline and/or the labelled portion of metabolites formed are not released over the 10–20 min duration of the experiment. Furthermore, the uptake of [^3H]adrenaline appears to progress by at least two distinct routes. The first of these occurs through the binding of adrenaline to α -adrenergic receptors, as judged by the effectiveness of prazosin in inhibiting uptake, and may involve the internalization of the agonist-receptor complex (see Goldstein *et al.*, 1979; Schlessinger, 1980; Brown & Orci, 1983; Lienhard, 1983). This route appears to predominate at low adrenaline concentrations, whereas uptake of adrenaline not inhibited by prazosin, and hence possibly independent of receptor binding, pre-

dominates at agonist concentrations greater than 60 nM.

A second major observation made in the present work is that the inhibition of receptor-mediated adrenaline uptake can be directly correlated with the almost total inhibition of adrenaline-induced responses, such as increased rates of glycogenolysis and respiration. Although previous studies with either hepatocytes (Aggerbeck *et al.*, 1980; Blackmore *et al.*, 1978, 1981) or the perfused liver (Reinhart *et al.*, 1981, 1982b) have shown that numerous pharmacological agents can inhibit hormone-induced responses, these effects were correlated only with binding data obtained by using isolated membrane fragments enriched in plasma membranes, and therefore may not be relevant to conditions *in situ* (Taylor *et al.*, 1983).

A further feature revealed by these studies *in vivo* is that the binding and internalization of [^3H]adrenaline occurs independently of Ca^{2+} redistributions. The depletion of the hormone-sensitive Ca^{2+} pool had no effect on the rate of [^3H]adrenaline uptake, whereas all other α -adrenergic agonist-induced responses are inhibited by this treatment (Reinhart *et al.*, 1982b, 1984). Hence the interaction of adrenaline with α -adrenergic receptors may give rise to both the uptake and metabolism of the α -agonist (McKillop & Powis, 1976), as well as to the generation of some messenger that gives rise to the redistribution of cellular Ca^{2+} . The finding that adrenaline linked to Sepharose can still induce physiological responses (Dehaye *et al.*, 1980) makes it unlikely that [^3H]adrenaline uptake in itself plays a role in the generation of some intracellular messenger.

The observed inhibitory effects of prazosin and yohimbine on the rate of [^3H]adrenaline uptake and hormone-induced responses indicates that tissue-related factors play a significant role in the interactions between these antagonists, adrenaline and α -receptors. Binding assays carried out *in vitro* have shown that hepatic α -receptors are predominantly of the α_1 subtype and that prazosin is more potent than yohimbine in preventing hormone-induced responses in isolated hepatocytes (Hoffman *et al.*, 1979, 1980a; Aggerbeck *et al.*, 1980; El-Refai & Exton, 1980).

In the intact tissue a different pattern of responses is observed, since relatively high concentrations of prazosin are required to inhibit either [^3H]adrenaline uptake or hormone-induced responses, and the effects of yohimbine are complex, occurring at two concentration ranges of the antagonist. These results may in part be due to the rapid uptake and metabolism of prazosin by the intact liver (Rubin & Reid, 1983), resulting in a decreased concentration of this antagonist at the α -receptor site. Since this effect appears to be less

apparent in hepatocytes (Aggerbeck *et al.*, 1980; Hoffman *et al.*, 1980a), non-parenchymal cells may then contribute to the uptake of prazosin. In the intact liver yohimbine maximally inhibits adrenaline-induced responses at concentrations that inhibit only a portion of the total prazosin-sensitive rate of [3 H]adrenaline uptake. If prazosin-sensitive hormone uptake involves the binding of adrenaline to α -adrenergic receptors, then the data indicate that only a proportion of the receptor pool bound by adrenaline is coupled to the intracellular signal-transducing mechanism. Such a view is consistent with previous proposals that α -receptor subtypes may exist in more than one form (El-Refai & Exton, 1979, 1980; Hoffman *et al.*, 1980a,b; Geynet *et al.*, 1981), but our data do not allow us to differentiate between binding to high- and low-affinity forms of α_2 -receptors (Hoffman *et al.*, 1980a,b) or precursor forms of α_1 -adrenergic binding sites (Geynet *et al.*, 1981). However, these data highlight the importance of examining tissue-specific effects when considering α -adrenergic-agonist-antagonist interactions *in vivo*, particularly in liver, the organ involved in the uptake and metabolism of most pharmacological agents. Thus our study has revealed that many pharmacological agents can inhibit the uptake of [3 H]adrenaline, and hence presumably adrenaline-receptor interactions. However, it is important to differentiate between inhibition at coupled receptors and inhibition at receptors not involved in mediating physiological responses. Many of the agents examined in the present study appeared to interact at these latter sites, whereas trifluoperazine and verapamil inhibited hormone uptake at both coupled and presumably uncoupled receptors. The development of pharmacological agents with a high affinity for coupled α -adrenergic receptors should reveal much information about receptor binding *in vivo*.

We conclude that the interaction of adrenaline with hepatic α -adrenergic receptors is far more dynamic than indicated by receptor-binding assays *in vitro*, and that an assessment of hormone-receptor interactions *in situ*, as described in this work, may represent a powerful approach to understanding the action of many drugs used in the treatment of hypertension and cardiac failure (Rubin & Reid, 1983), or in examining the effects of starvation, thyroidectomy and cholestasis on adrenaline-induced responses (El-Refai & Chan, 1982; Preiksaitis *et al.*, 1982; Aggerbeck *et al.*, 1983).

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