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Adrenergic Ligand Liposolubility in Membranes

Direct Assessment in a *Beta*-Adrenergic Binding System

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SUMMARY

The binding of [^{3}H]dihydroalprenolol to adipocyte membranes may be displaced not only from *beta*-adrenergic receptors but also nonstereospecifically from other membrane compartments by *beta*-adrenergic and other adrenergic agents. The EC_{50} of this nonstereospecific displaceable binding is directly related to the liposolubility (*n*-octanol : water partition coefficient) of the displacing ligand.

The tritiated *beta*-adrenergic antagonist [^{3}H]DHA[1] has been used in many mammalian tissues to characterize and to quantitate *beta*-adrenergic receptors (1). In rat adipocyte membranes the binding results are confusing. Isoproterenol displaces [^{3}H]DHA from the cell surface *beta*-adrenergic receptor in a stereospecific manner. The *beta*-adrenergic antagonist propranolol displaces [^{3}H]DHA from the *beta*-adrenergic receptor (stereospecific binding) but also nonstereospecifically from another membrane compartment. In kidney, lung, brain, and liver (2–5) it has also been shown that propranolol is capable of displacing more [^{3}H]DHA from tissue membranes than is isoproterenol. It has been suggested that the difference in [^{3}H]DHA displacement properties of propranolol and isoproterenol is due to their differing liposolubility (i.e., their ability to partition in a lipid medium) (6, 7). This hypothesis has not been tested directly. We determined the liposolubility of a number of adrenergic ligands by measuring their partition coefficients in an *n*-octanol : water system. This was compared with the concentration of the adrenergic ligand which displaced 50% of the [^{3}H]DHA from adipocyte membranes in the presence of 10^{-4} M isoproterenol (i.e., 50% displacement of nonstereospecific binding). A direct correlation was shown between the liposolubility and the non-*beta*-adrenergic membrane affinity of these adrenergic ligands. The implications of these results are wider than their application to *beta*-adrenergic binding studies. Adrenergic agents apparently distribute in other compartments of mammalian cell membranes according to their liposolubility. Furthermore, a number of cellular functions (not involving the *beta*-adrenergic-receptor adenylate cyclase system) have been related to the liposolubility of adrenergic agents [reviewed by Mendel and Almon (6) and Deacon *et al.* (8)]. While the present study may provide a clue to some problems in the literature in explaining sensitivities of adrenergic agents in various mammalian tissues (9), it may also provide a novel measure in adrenergic pharmacology not mediated through the *beta*-adrenergic receptor (8).

In fat cell membranes, [^{3}H]DHA displacement characteristics of isoproterenol and propranolol differed. In Fig. 1 the difference in [^{3}H]DHA displacement by 10^{-4} M isoproterenol from that displaced by 10^{-4} M propranolol illustrates the maximal difference between the displacement by the two agents in fat cell membranes at 10 nM [^{3}H]DHA. The binding displaced by isoproterenol has been shown, in a separate study, to be *beta*-adrenergic binding. It is the [^{3}H]DHA further displaced from the membrane by propranolol and other agents (Fig. 1B) which we have related to the displacing adrenergic agent's partition coefficient or relative liposolubility. We have termed this binding nonstereospecific displaceable binding.

It has been shown that isoproterenol (almost lipid-insoluble) and propranolol represent *beta*-adrenergic agents with extremes in *n*-octanol:water partition coefficients (relative liposolubility) (6, 10). Therefore, the nonstereospecific membrane displacement of [^{3}H]DHA that we are relating to liposolubility should not be displaced in the presence of high concentrations of isoproterenol but totally displaced in the presence of high concentrations of propranolol (Fig. 1). As far as possible we attempted to examine the nonstereospecific displaceable

[1] The abbreviation used is: DHA, (−)-dihydroalprenolol.

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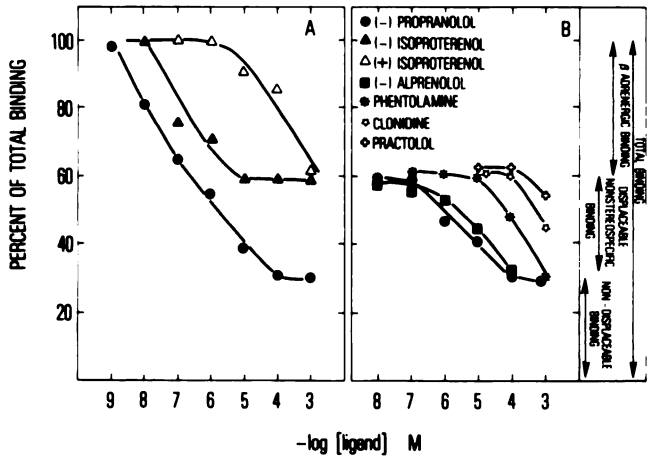

FIG. 1. *Displacement of 10 nM [^{3}H]DHA from rat adipocyte membranes by adrenergic ligands in the absence (A) and presence of 10^{-4} M isoproterenol (B)*

Epididymal fat pads were minced, and isolated adipocytes were prepared by collagenase digestion [5 mg of collagenase per gram (wet weight) of adipose tissue]. Membranes were prepared by homogenization of the adipocytes in 0.25 M sucrose with 0.01 M Tris-HCl and 0.001 M EDTA at pH 7.4, centrifugation at 12,000 × *g*, and two successive washes of the pellet in assay buffer (0.05 M Tris-HCl with 0.01 M $MgCl_2$ at pH 7.4). Incubation of the membranes (150–250 μg of protein) with [^{3}H]DHA was carried out at 30° for 10 min. Separation of membrane-bound from "free" [^{3}H]DHA was accomplished by vacuum filtration of the incubate over glass-fiber filters (GF/C Whatman) and washing with a total of 20 ml of ice-cold assay buffer. In A, binding displaced by isoproterenol was shown to fulfill all of the necessary characteristics of *beta*-adrenergic binding, including stereospecificity, whereas propranolol displaced both the *beta*-adrenergic and nonstereospecific components of [^{3}H]DHA binding. In B, [^{3}H]DHA displacement is shown by various adrenergic ligands in the presence of 10^{-4} M isoproterenol which was used to occupy the *beta*-adrenergic binding sites. In the experiment shown in B, 10 nM [^{3}H]DHA was used but the nonstereospecific displacement was observed with [^{3}H]DHA concentrations as low as 2 nM. With increasing [^{3}H]DHA concentrations the nonstereospecific displaceable binding progressively increased and represented approximately 80% of the displaceable binding at 100 nM [^{3}H]DHA.

binding without the influence of the specific *beta*-adrenergic binding. Therefore, 10^{-4} M isoproterenol was included to occupy the surface *beta*-adrenergic binding sites while 10^{-4} M propranolol displacement was used to displace totally the nonspecific displaceable binding (Fig. 1A).

In Fig. 1B is shown the ability of various adrenergic agents in the presence of 10^{-4} M isoproterenol to displace [^{3}H]DHA from membranes. The displacing ability of these agents ranked in the same order as their octanol:water partition coefficients at pH 7.5 (Table 1). The displacement by these agents displayed no stereospecificity, which is an essential characteristic of *beta*-adrenergic binding. Furthermore, there was a highly significant correlation ($r = 0.9609$, $p < 0.001$) between the partition coefficients and concentrations of these adrenergic agents causing half-maximal [^{3}H]DHA displacement in the presence of 10^{-4} M isoproterenol (Fig. 2).

These results do not distinguish whether the displacing adrenergic ligand competitively displaces [^{3}H]DHA from a membrane compartment or whether it changes the

TABLE 1

Doses of adrenergic ligands displacing 50% of 10 nM [^{3}H]DHA bound (EC_{50}) in the presence of 10^{-4} M (−)-isoproterenol in adipocyte membranes and their n-octanol:water partition coefficients (P)

Experiments were performed at pH 7.5. These results are representative of three separate experiments showing similar results.

Ligand	EC_{50}	P^a
	M	
(+)-Propranolol	9×10^{-7}	12.97
(−)-Propranolol	3×10^{-6}	10.83
(−)-Alprenolol	8×10^{-6}	5.72
(+)-Alprenolol	1.2×10^{-5}	7.05
Phentolamine	3×10^{-5}	2.06
Hydroxybenzylpindolol	6×10^{-5}	1.24
Clonidine	1.2×10^{-4}	0.14
Phenylephrine	2×10^{-3}[b]	0.03
Practolol	1.2×10^{-2}[b]	0.022
(−)-, (+)-Isoproterenol, Epinephrine, norepinephrine	$>10^{-2}$	<0.002

[a] Methodology as in Fig. 2.
[b] Curve extended to achieve this value.

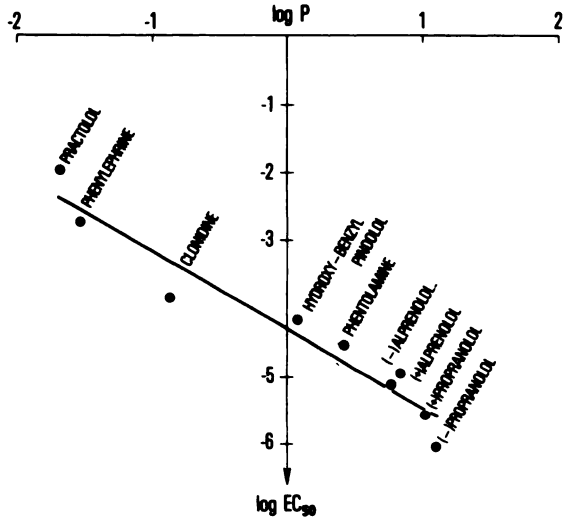

FIG. 2. *Correlation between the logarithm of partition coefficients (P) and the logarithm of doses of the adrenergic ligands causing 50% displacement of nonstereospecific displaceable binding*

Experimental conditions for binding were as described in Fig. 1. Partition coefficients in an *n*-octanol:water system were measured at pH 7.5 using methods similar to those described previously (13). Tris-HCl buffer (0.05 M) with 0.01 M $MgCl_2$ at pH 7.5 was used as the "water" phase in order to use the same buffer as the binding system. The concentrations of the ligands in the buffer and the *n*-octanol:water ratio used were as follows: (−)- and (+)-propranolol, 10^{-3} M, 1:4; (−)- and (+)-alprenolol, 10^{-3} M, 1:1; phentolamine, 10^{-4} M, 10:1; hydroxybenzylpindolol and clonidine, 5×10^{-4} M, 5:1; practolol, 10^{-4} M, 10:1; and isoproterenol, epinephrine, and norepinephrine 2×10^{-4} M, 10:1. Absorption maxima of the adrenergic agents in the water phase lay between 243 and 290 λ.

membrane distribution of [^{3}H]DHA by altering the [^{3}H]DHA partitioning (6). They do demonstrate directly that there are interactions between adrenergic agents (including alprenolol) in membranes which are not pertinent to their interaction with the *beta*-adrenergic receptor. The results also show that, under the standard conditions used in many *beta*-adrenergic binding assays employing

[^{3}H]DHA, the [^{3}H]DHA partitions in the membrane. The partitioning reactions are then included, at least in part, as *beta*-adrenergic binding.

To exclude these nonstereospecific interactions from *beta*-adrenergic receptor assays it would be possible to use isoproterenol or the natural agonists to displace [^{3}H] DHA from receptors at the cell surface. On the other hand, studies using [^{125}I]hydroxybenzylpindolol or the more recently developed [^{125}I]pindolol radioligands (11) (with lower partition coefficients) may circumvent the inclusion of the non-*beta*-adrenergic binding. The routine use of phentolamine in [^{3}H]DHA binding assays—as has been widely employed—eliminates a large portion of the [^{3}H]DHA partitioning component and therefore the inadvertent inclusion of nonstereospecific binding.

It is possible that this nonstereospecific binding may have physiological and pharmacological significance. The metabolism of adrenergic agents relates to their liposolubility (8), as does their ability to cross the blood-brain barrier (10). It has been shown that the partition coefficient of *beta*-adrenergic agents relates directly to their local anaesthetic properties (12). Adrenergic agents have been shown to act on sodium-potassium ATPases, although not necessarily through the adenylate cyclase-dependent *beta*-adrenergic receptor mechanism (13). Our observations may relate to and possibly provide a measure of the *beta*-adrenergic agent's ability to interact with these non-adenylate cyclase-dependent mechanisms. Finally, it is stressed that our observations concerning nonstereospecific [^{3}H]DHA displacement made in adipocyte membranes seem to apply to other mammalian tissues (2–5). Therefore, the non-*beta*-adrenergic interactions of the adrenergic agents demonstrated here may have considerable pharmacological importance.

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