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Catecholamine Binding to CNS Adrenergic Receptors

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The properties of ^{3}H-catecholamine binding to α- and β-adrenergic receptors in CNS are reviewed. ^{3}H-epinephrine and ^{3}H-norepinephrine label one class of α-receptors throughout the brain, with high affinities for agonists and some antagonists. Agonist affinities at this site are increased in low temperature conditions but are reduced by guanine nucleotides and monovalent cations. Divalent cations reverse both effects. This α-receptor may be coupled to adenylate cyclase by GTP and/or sodium, and uncoupled by divalent cations. ^{3}H-epinephrine labels β_2, but not β_1, receptors in CNS, especially in bovine cerebellum. The same β-receptor does not show agonist-specific GTP-sensitivity, but does exhibit Na^+-sensitivity. This receptor appears to be linked to adenylate cyclase, and sodium rather than GTP may be the coupling agent.

Key words: adrenergic receptor, ^{3}H-catecholamine binding, guanosine triphosphate, clonidine, norepinephrine, dihydroalprenolol

In the study of the biochemistry of neurotransmitter receptors on plasma membranes a valuable tool is the binding of radiolabeled agonist ligands, especially the transmitter itself. Agonist ligand binding permits a more direct assessment of events at the receptor involving changes in agonist affinity and efficacy during desensitization. Direct receptor labeling with hormones and transmitters has been possible with iodinated and tritiated peptide hormones such as insulin [1], glucagon [2], angiotensin [3], and the enkephalins [4], and with biogenic amine neurotransmitters such as dopamine [5] and serotonin [6]. Until recently, identification of adrenergic receptors, primarily in β-receptor tissues, with the endogenous catecholamines ^{3}H-norepinephrine and ^{3}H-epinephrine was virtually unattainable. These earlier studies failed to demonstrate the normal attributes of ligand-receptor binding, such as a high-affinity saturable system with rapid association and such necessary pharmacological properties as drug stereospecificity and a potency rank-order characteristic of adrenergic receptor pharmacology. [For reviews of these data see References 7–9.]

The purpose of this paper is to review some of the data from our laboratory wherein we have demonstrated specific binding of ^{3}H-epinephrine and ^{3}H-norepinephrine to α-adrenergic receptors in membranes from rat and bovine CNS, and β-receptor binding

Abbreviations used: Gpp(NH)p, guanyl-5′-yl imidodiphosphate; DHE, dihydroergokryptine; DHA, dihydroalprenolol.

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of ^{3}H-epinephrine in membranes from bovine cerebellum and rat lung. The physiological significance of these binding sites and their relationship to adenylate cyclase are discussed. In addition, we examine the modulatory influences on catecholamine binding of guanine nucleotides and mono- and divalent metal ions and suggest roles for these phenomena as factors that facilitate or depress coupling of the adrenergic receptor to adenylate cyclase.

CATECHOLAMINE α-RECEPTOR BINDING

Methodology and Characteristics of Binding to Bovine Cortex Membranes

In our laboratory a filtration assay is used to determine receptor binding. A suspension in 50 mM Tris HCl buffer of membranes prepared from CNS tissue, either crude or a lysed P2 fraction, is incubated to equilibrium with radioactive ligand. Nonspecific binding is determined in the presence of an unlabeled drug that has high affinity and specificity for the receptor in question, at a concentration sufficiently high to compete successfully with the ligand and saturate all receptor sites, but that will not inhibit nonspecific ligand binding at the concentration used. Specific binding is the difference between binding determined in the absence and the presence of the unlabeled drug, or "blank." Following incubation, membrane suspensions are filtered under vacuum, and the glass fiber filters are washed with a sufficient volume of buffer maximally to reduce nonspecific binding without dissociating specific binding. In general, radioactive ligands with varying affinities for receptors have similar association constants, and differing dissociation rates account for differences in affinity. With ligands such as ^{3}H-catecholamines, whose dissociation constants (K_D) are in the 10^{-8} M range, the $t_{½}$ for dissociation is about 80 sec [10], and a very rapid filtration and wash procedure is crucial in the prevention of loss of specific binding.

^{3}H-epinephrine binding to bovine cerebral cortex membranes at 37°C is almost entirely nonspecific and shows characteristics similar to earlier ^{3}H-catecholamine binding in myocardium [11], with limited and partial catecholamine stereospecificity; inhibition by the nonreceptor-specific analogue, pyrocatechol; and limited inhibition of binding by the potent, noncatecholamine α-receptor agonist, oxymetazoline [12]. However, the pyrocatechol and oxymetazoline inhibition is additive, and 50% binding of ^{3}H-epinephrine to bovine cortex membranes at 37°C in the presence of 1.0 mM pyrocatechol, 0.1 mM Na_2 EDTA, and 10 μM dithiothreitol displays α-receptor characteristics, with complete stereospecificity for catecholamines, complete nonadditive and potent inhibition by α-agonists and α-antagonists, and a structure -activity relationship for a series of phenylethylamines corresponding to that found at physiological α-receptors (Fig 1) [13]. β-Antagonists such as propranolol and alprenolol are 1,000–10,000-fold weaker than α-antagonists such as phentolamine and ergot alkaloids. The binding of ^{3}H-norepinephrine in the same conditions shows similar α-receptor specificity. In these and all subsequent experiments, pyrocatechol was used to presoak filters and was included in the wash. Pyrocatechol appears to prevent ^{3}H-catecholamine oxidation in the system and selectively to reduce nonspecific binding. Thin-layer chromatography (TLC) studies demonstrates minimal breakdown of ^{3}H-catecholamines in the system. ^{3}H-catecholamine binding to bovine cortical β-adrenergic receptors cannot be detected, for reasons discussed below.

With 0.1 μM oxymetazoline as a blank to define ^{3}H-catecholamine-specific α-receptor binding, both ^{3}H-epinephrine and ^{3}H-norepinephrine binding is saturable with

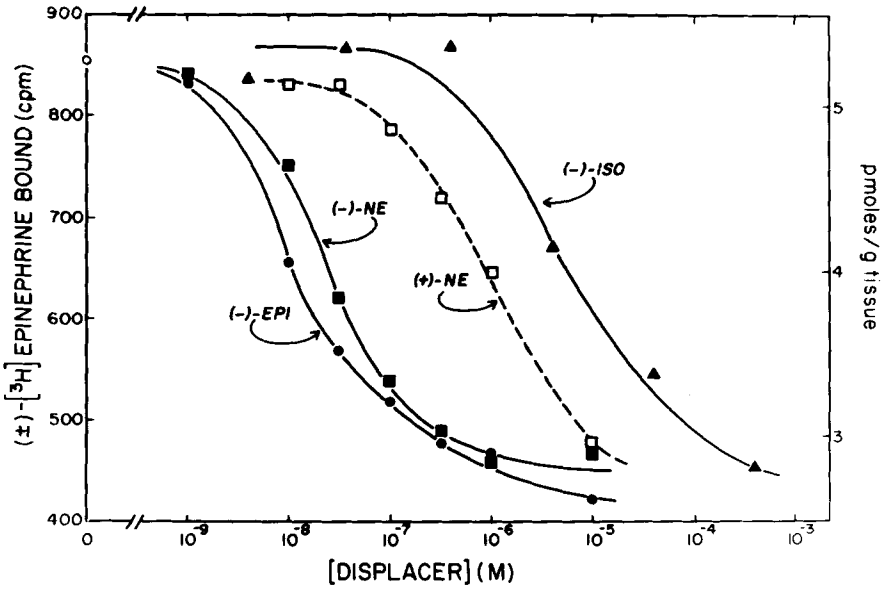

Fig 1. Inhibition of binding of 5nM ^{3}H-epinephrine to calf frontal cortex membranes in the presence of 1.0 mM pyrocatechol, at 37°C [Data from 13].

a single high-affinity component. The K_D values at 37°C are 18 nM and 26 nM for (±)-[^{3}H]epinephrine and (−)-[^{3}H]norepinephrine is 3–4 times more potent than (−)-norepinephrine, which is indicative of α-receptor interactions and is corroborated in inhibition studies with unlabeled catecholamines.

Association of ^{3}H-catecholamine binding to α-receptors at 37°C is rapid; equilibrium binding is reached by 3 min and maintained for 20 min before it decreases slightly [12]. Dissociation at 37°C from α-receptors is rapid, complete, and monophasic, and thus differs from agonist dissociation from liver glucagon and frog erythrocyte β-receptors, which is biphasic and incomplete in the absence of guanine nucleotides. The kinetically-derived K_D values correspond well with equilibrium values.

Multiple CNS α-Receptors

The α-receptor site in bovine CNS labeled by ^{3}H-catecholamines appears identical to that labeled by the noncatecholamine α-agonist, ^{3}H-clonidine. However, the potent benzodioxan ligand ^{3}H-WB-4101 (2-[{2′, 6′- dimethoxy} phenoxyethylamino] methyl benzodioxan) labels with high affinity a CNS site, also with α-receptor characteristics, but with marked affinity differences compared to the ^{3}H-agonist site [12, 14]. Catecholamines and other agonists are much less potent at the ^{3}H-WB-4101 site, whereas some α-blockers, such as WB-4101 itself, phenoxybenzamine, indoramin, prazosin, and phenothiazine neuroleptics, are substantially more potent at the ^{3}H-WB-4101 site. Other antagonists such as ergot alkaloids and phentolamine have equally high affinity for both sites [12, 14]. The possibility that the agonist and antagonist sites are interchangeable states of the same receptor, analogous to the CNS opiate [15] and dopamine [16] receptor systems, has been discounted for several reasons. Agonist and antagonist competitors at either site exhibit noncooperative binding isotherms, suggesting noninter-

TABLE I. α-Receptor Binding Sites in Rat and Calf Cortex

Ligand	B_{max}(pmoles/gm tissue) Rat	Calf
α_1 Receptor		
^{3}H-WB-4101	11	8
α_2 Receptor		
^{3}H-clonidine	14	8
^{3}H-epinephrine	12	9
^{3}H-norepinephrine	10	7
$\alpha_1 + \alpha_2$ Receptors		
^{3}H-dihydroergokryptine	21	16

convertibility [12, 14]. The regional distribution of antagonist and agonist binding sites in bovine CNS shows some marked differences [17]. The strongest evidence for two distinct sites comes from the binding of the ergot ligand ^{3}H-dihydroergokryptine (^{3}H-DHE)[1] to CNS α-receptors. Ergots are equipotent at agonist and antagonist sites, and the inhibition characteristics of ^{3}H-DHE α-receptor binding suggest that it labels both sites [18]. The B_{max} for ^{3}H-DHE binding is equal to the sum of the agonist and antagonist sites in both bovine and rat cortex (Table I). Finally, in bovine cerebellum, where the agonist α-site predominates, ^{3}H-DHE binding is identical to the binding of ^{3}H-catecholamines, whereas in the pons, where the antagonist α-site predominates, ^{3}H-DHE and ^{3}H-WB-4101 binding are very similar [19]. ^{3}H-DHE probably is an antagonist at both sites.

Destruction of presynaptic noradrenergic terminals in the brain with 6-hydroxydopamine leads to a 50% increase in the number of agonist and antagonist α-receptor binding sites, probably by a mechanism akin to denervation supersentitivity [14, 20]. Therefore, neither site appears to correspond to the putative presynaptic noradrenergic "autoreceptor" on nerve terminals. Pharmacologically, however, the α-receptor site labeled by ^{3}H-catecholamines and ^{3}H-clonidine resembles the presynaptic receptor in that α-methylnorepinephrine is more potent than norepinephrine. It has been suggested that α-receptors akin to the autoreceptor may occur postsynaptically in some tissues [21]. Pettinger [21] has designated this receptor the α_2-receptor, as opposed to the classical postsynaptic α_1-receptor, which resembles the ^{3}H-WB-4101 site. Other authors have also suggested the existence of multiple α-receptors [22].

Temperature Effects

An interesting phenomenon is the apparent increase in affinity of ^{3}H-catecholamines for the α-receptor site as the incubation temperature is lowered from 37° to 25° or 4°C (Fig 2). In general, almost all agonists show a 3–4-fold increase in affinity at 25°C, whereas antagonist affinities are somewhat lower than at 37°C. One explanation may be that the receptor-ligand complex is a thermodynamically favored state and becomes more so at lower temperatures. Alternatively, a phase change in the membranes may occur at decreased temperatures to enhance agonist affinity. A third possibility is that, since longer incubation times are needed to attain equilibrium at lower temperatures, the prolonged interaction of the agonist ligand and receptor induces a conformational change to a "desensitized" state of

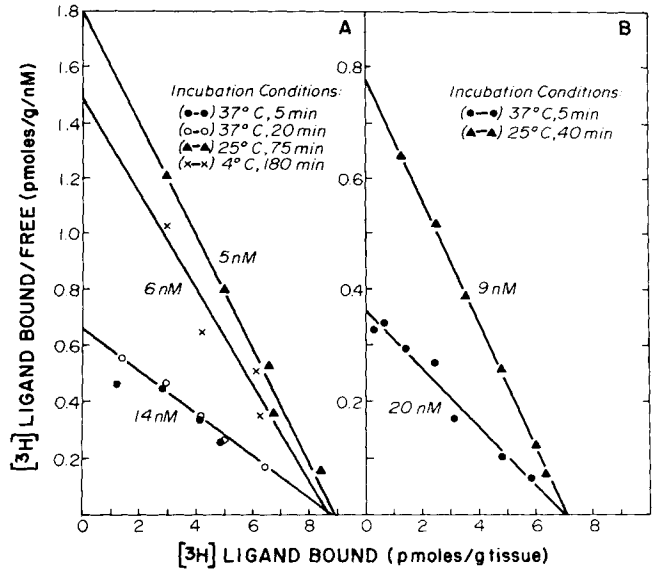

Fig 2. Scatchard plots of the saturation of ^{3}H-catecholamine binding to calf frontal cortex α-receptors in different incubation conditions. K_D values obtained are shown in (A) ^{3}H-epinephrine; (B) ^{3}H-norepinephrine [data from 12].

the receptor, which exhibits higher affinity for agonists. Dissociation of ^{3}H-epinephrine and ^{3}H-norepinephrine is biphasic under low-temperature incubation conditions, although a single population of sites is still apparent, which would be the case if equilibrium existed between the high- and low-affinity forms of the receptor [23]. Since nonspecific binding is unchanged at 25°C, specific binding of 2 nM ^{3}H-epinephrine or ^{3}H-norepinephrine accounts for 75–80% of total binding.

A selective increase in agonist affinities with low-temperature incubation has also been observed in two β-receptor binding systems – agonist inhibition of the antagonist ^{3}H-dihydroalprenolol (^{3}H-DHA) binding to turkey erythrocytes receptors [24], and the bovine cerebellar β-receptor discussed later – both with the agonist ligand, ^{3}H-epinephrine, and agonist competitors of ^{3}H-DHA binding to the same receptors [25].

^{3}H-Catecholamine Binding to Rat CNS α-Receptors

Recently we have demonstrated ^{3}H-epinephrine and ^{3}H-norepinephrine binding to α-receptors in rat cortex membranes, using slight modifications of the procedure developed for bovine CNS α-receptors [26]. The ^{3}H-catecholamine binding site in rat brain is completely analogous to the receptor in rat earlier identified with ^{3}H-clonidine [14]. The rat and bovine ^{3}H-catecholamine binding sites are very similar, except for the finding that the ^{3}H-ligands and all other agonists are severalfold weaker in rat than in calf under identical 25°C incubation conditions. The 25°C K_D values for ^{3}H-epinephrine are 6.5 nM in calf and 17 nM in rat. Thus, for ^{3}H-catecholamines, binding at 25°C in rat is identical to binding at 37°C in calf CNS. Other differences between rat and calf cortical ^{3}H-catecholamine α-receptor sites are that among antagonists, phentolamine and WB-4101 are about 20-fold weaker in rat than in calf. 6-Hydroxydopamine treatment increases the B_{max} of the ^{3}H-epinephrine site in rat cortex, illustrating that the receptor is postsynaptic [20], and

chronic reserpine treatment also enhances ^{3}H-epinephrine binding, although not to the same extent as ^{3}H-WB-4101, or ^{3}H-DHA β-receptor binding [26].

Nucleotide Effects

Daly and co-workers have demonstrated an α-receptor coupled to adenylate cyclase in rat cortical slices, at which clonidine appears to act as an antagonist [27]. They have also found in some brain areas that α-agonists, including clonidine, enhance isoproterenol-stimulated cAMP production, and this enhancement is specifically blocked by α-antagonists [28]. Thus, there may be two CNS α-receptor types, both coupled to adenylate cyclase. α-Receptors in some other tissues are cyclase-coupled, notably in platelets [29] and neuroblastoma/glioma hybrid cell lines [30]. In other tissues, α-receptor interactions may utilize Ca^{2+} as the "second messenger" [31]. In some hormone-receptor systems coupled to adenylate cyclase, the guanine nucleotide, GTP, appears to promote coupling and hormone-stimulated cyclase activity while lowering the affinity of the hormone for the receptor, principally by accelerating the dissociation rate of the hormone. This "modulator" action of GTP has been most completely characterized in the glucagon [32] and β-adrenergic [33] receptor systems. The inference may be drawn that a GTP-sensitive receptor binding site is coupled in physiological conditions to adenylate cyclase. However, Rodbell's group [34] has recently demonstrated the existence of allosteric GTP sites both at the glucagon receptor and at adenylate cyclase. A receptor with an allosteric GTP site could theoretically, therefore, be completely unassociated with adenylate cyclase.

We examined the question of whether agonist binding at either the ^{3}H-catecholamine α-receptor site or the ^{3}H-WB-4101 α-site in bovine cortex showed guanine-nucleotide sensitivity. GDP, GTP, and its phosphohydrolase-resistant analogue, Gpp (NH)p, all potently lower the specific binding of ^{3}H-epinephrine and ^{3}H-norepinephrine, with ED_{50} values in the 1–5 μM range [35] (Fig 3). The corresponding adenine nucleotides are 100–1,000 times weaker. The reduction in ^{3}H-epinephrine binding caused by 10 μM GTP is due to a 6-fold reduction in affinity of ^{3}H-epinephrine for the α-receptor (Fig 4). Unlike the glucagon receptor system [36], no residual high-affinity component of binding is left in the presence of GTP. ^{3}H-epinephrine competition experiments with unlabeled agonists in the presence and absence of 10 μM GTP showed that the affinities of all agonists are lowered 4- to 5-fold by GTP, whereas antagonist affinities at the ^{3}H-epinephrine site are unchanged [35]. The reduction in affinity caused by GTP appears to result from an increase in the rate and extent of ^{3}H-epinephrine dissociation from the receptor (Fig 5). These experiments were conducted at 25°C, so the ^{3}H-epinephrine dissociation rate was biphasic (see above); both the slow and fast phases of dissociation were accelerated by GTP. Similar results were seen with ^{3}H-norepinephrine [35]. 10 μM GTP also produces a modest acceleration of ^{3}H-epinephrine and ^{3}H-norepinephrine association to bovine cortex α-receptors [35] analogous to that seen with glucagon- and β-receptor agonist binding [2, 34, 37], but the dissociation effect of GTP is much greater, and so the net result is the observed reduction in ^{3}H-catecholamine affinity.

At the ^{3}H-WB-4101 α-receptor binding site in bovine cortex, up to 0.1 mM GTP does not affect specific binding and, more significantly, does not lower agonist affinities at that site. ^{3}H-DHE binding in bovine cortex is similarly unaffected (Table II). In several β-receptor binding systems using labeled antagonists, there is a "right shift" of agonist inhibition curves in the presence of GTP [38–40]. One tissue in which this is not the case is the turkey erythrocyte β-receptor, where the receptor and adenylate

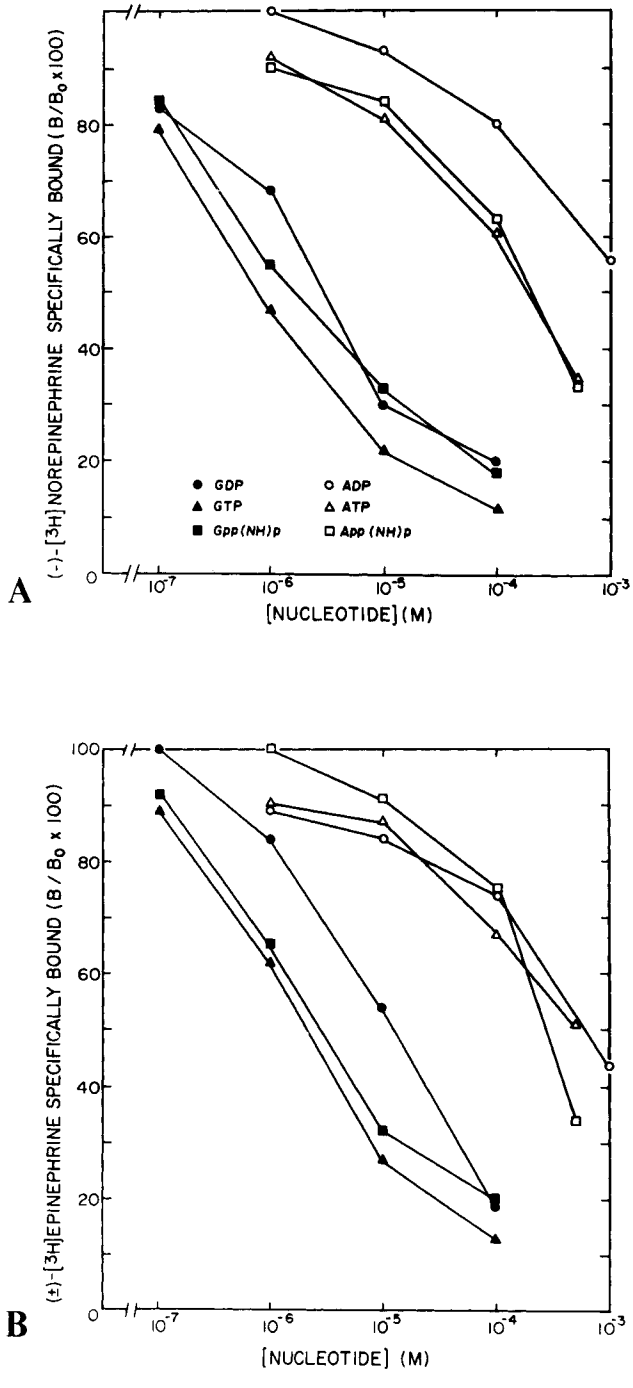

Fig 3. Nucleotide effects on ^{3}H-catecholamine specific binding to calf frontal cortex α-receptors. Values expressed as percent of specific binding in the absence of nucleotides. (A) ^{3}H-norepinephrine; (B) ^{3}H-epinephrine [data from 35].

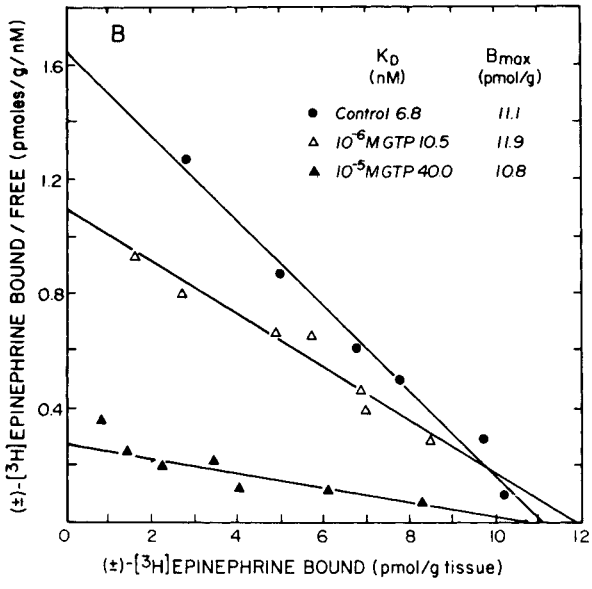

Fig 4. Scatchard plots of the saturation of ^{3}H-epinephrine binding to calf frontal cortex α-receptors at 25°C in the presence and absence of GTP [data from 35].

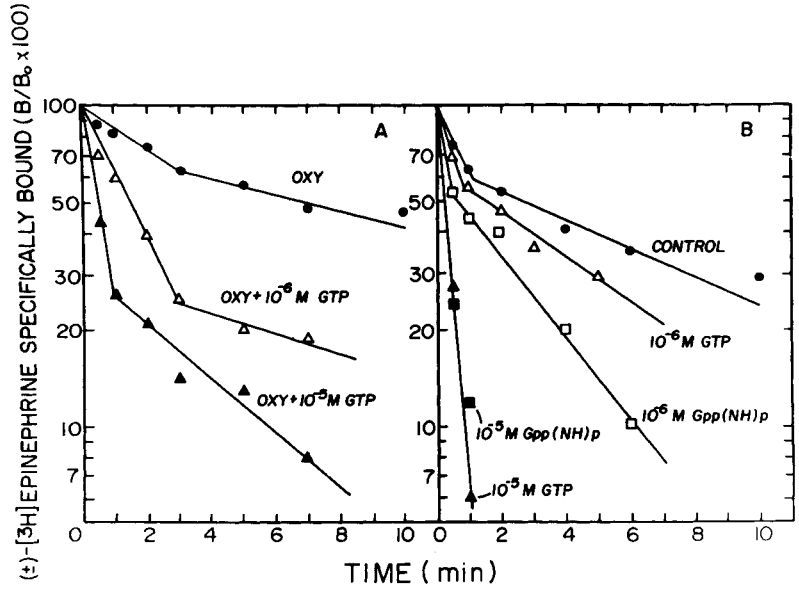

Fig 5. Effect of GTP and Gpp(NH)p on dissociation of ^{3}H-epinephrine α-receptor binding at 25°C. (A) Membranes incubated to equilibrium with ^{3}H-epinephrine alone, then dissociated with 1.0 μM oxymetazoline (Oxy) at zero time, with or without added GTP; (B) membranes incubated to equilibrium with ^{3}H-epinephrine in the presence or absence of nucleotide, then dissociated with 1.0 μM oxymetazoline alone [data from 35].

TABLE II. Effect of 10 μM GTP on Affinities of Competitors for ^{3}H-WB-4101 and ^{3}H-Dihydroergokryptine (^{3}H-DHE) α-Receptor Binding Sites on Calf Frontal Cortex Membranes

	Control	10 μM GTP
Drug	IC_{50} (nM)	
^{3}H-WB-4101		
(−)-epinephrine	1,100	970
Clonidine	600	480
Phentolamine	4.0	2.9
Ergotamine	2.0	1.7
^{3}H-DHE		
(−)-Epinephrine	160	150
Clonidine	65	54

Data from [35].

cyclase may be linked in a rather looser fashion [41]. At the α-receptor labeled by ^{3}H-WB-4101, there is no GTP-induced agonist affinity shift. This receptor, being GTP-insensitive, may not be coupled (or may be only loosely coupled) to adenylate cyclase, whereas the α-receptor labeled by ^{3}H-catecholamines may be cyclase-coupled, since it is GTP-sensitive.

Sodium Effects

An α-receptor in rabbit platelet membranes, labeled by ^{3}H-DHE, shows agonist-specific Na^+-sensitivity, with 100 mM NaCl causing 13–16-fold reduction in the affinity of pure agonists, epinephrine and norepinephrine, at the α-binding site. Clonidine and phenylephrine, which act as partial agonists at best at this receptor, show only a 2–3-fold shift in affinity in the presence of sodium, whereas antagonist affinities are unaltered by sodium [42]. The platelet α-receptor is coupled to adenylate cyclase, and it has been suggested that sodium may have an allosteric "modulator" function similar to that of GTP at β- and glucagon receptor-cyclase systems [42].

In bovine cerebral cortex membranes, sodium decreases the binding of the agonist ligands, ^{3}H-epinephrine, ^{3}H-norepinephrine, and ^{3}H-clonidine, with an ED_{50} of 5–10 mM. Lithium is almost as potent as sodium, and monovalent cations with a larger hydrated radius, eg, potassium, cesium, and rubidium, have a much weaker action [43]. The effect appears to be due to sodium and not the anion, since numerous sodium salts lower agonist binding to the same extent. Saturation and Dixon plot data indicate that sodium lowers the number of agonist binding sites, but this effect may be more apparent than real if sodium converts the majority of sites to a state with such low agonist affinity that specific binding at high ligand concentrations is not readily discernible, and if sodium leaves a residual population of receptors with high agonist affinity. This is the effect of sodium on β-receptor binding (see below). The binding of the antagonist ligands, ^{3}H-WB-4101 and ^{3}H-DHE, is unaffected by monovalent cations, and the affinities of agonist competitors at the antagonist sites are only marginally lowered. It appears, then, that the ^{3}H-WB-4101 α-receptor site does not show Na^+-sensitivity for agonists. Since the ^{3}H-DHE labels both the ^{3}H-WB-4101 site and the ^{3}H-agonist site [18], sodium-induced shifts in agonist affinity, as seen in platelets, may be observed when ^{3}H-DHE binding is

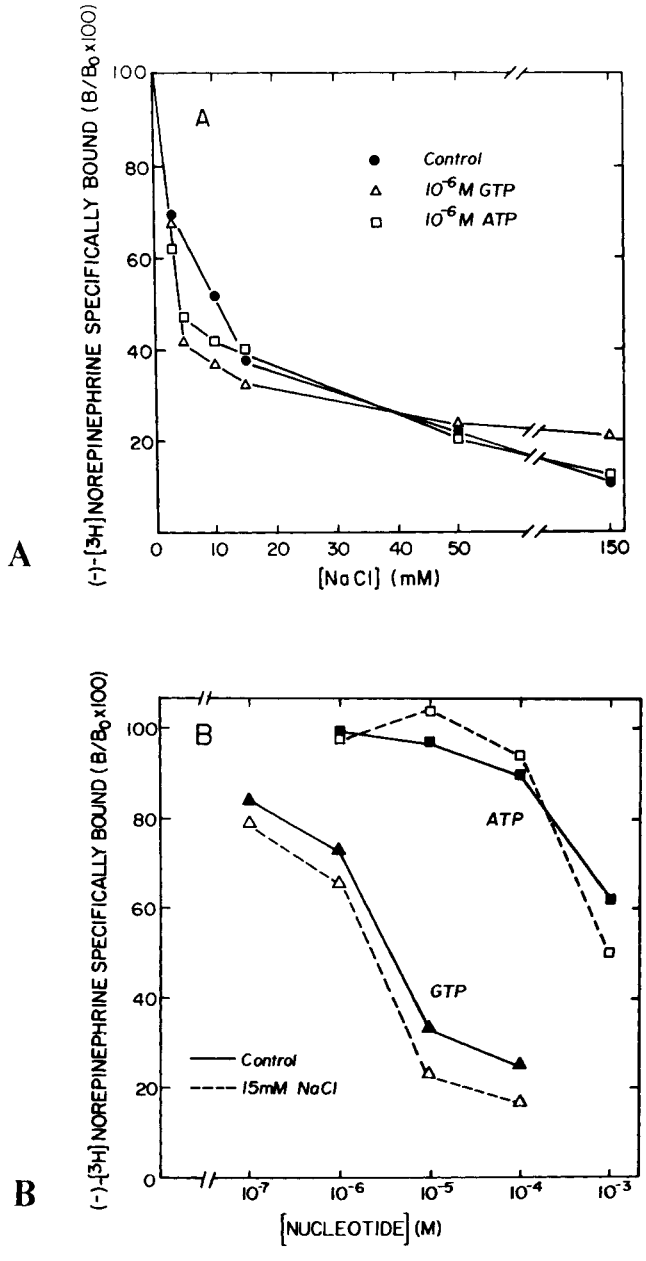

Fig 6. (A) Sodium effect on ^{3}H-norepinephrine α-receptor binding in the presence or absence of GTP or ATP. Values in each instance expressed as percent of specific binding in the absence of sodium. (B) Nucleotide effects on ^{3}H-norepinephrine binding in the presence or absence of sodium. Values in each instance expressed as percent of specific binding in the absence of nucleotide [data from 35].

localized to the ^{3}H-agonist site. Preliminary experiments indicate that this is so [U'Prichard and Snyder, unpublished data].

The α-receptor labeled by ^{3}H-catecholamines is thus both GTP-sensitive and Na^+-sensitive. The effects of GTP and sodium at less than maximal concentrations are additive and not synergistic, as found with opiate receptor agonist binding [Childers, U'Prichard and Snyder, in preparation]. The ED_{50} of sodium in inhibiting ^{3}H-norepinephrine binding is not altered in the presence of 1.0 μM GTP (Fig 6), or 10 μM GTP (data not shown), and conversely the ED_{50} of GTP or ATP is not altered in the presence of 15 mM NaCl (Fig 6).

Divalent Cation Effects

$CaCl_2$ and $MgCl_2$ at 1.0 mM concentrations do not affect the binding of any α-receptor ligand to rat or bovine cortex membranes. $MnCl_2$ by itself at low (0.01–0.1 mM) concentrations causes a 20–30% increase in ^{3}H-clonidine and ^{3}H-epinephrine binding to α-receptors, but at concentrations above 0.1 mM, $MnCl_2$ produces a precipitous drop in ^{3}H-epinephrine specific binding, possibly by oxidizing the ligand. However, Mg^{2+} and Ca^{2+} cause a striking reversal in the GTP-induced lowering of ^{3}H-epinephrine α-receptor binding in both rat and calf cortex (Fig 7), and those ions, and Mn^{2+} in a more effective and potent manner, similarly reverse the GTP effect on ^{3}H-clonidine binding to the same receptors [44]. At bovine cortex α-receptors, where GTP is a potent inhibitor of ^{3}H-catecholamine binding, high concentrations of GTP will still lower binding in the presence of Ca^{2+} and Mg^{2+}, but low GTP concentrations cause an absolute rise in ^{3}H-epinephrine binding with those ions present, with an ED_{50} for this effect of GTP of 0.1–1.0 μM (Fig 7C). At rat cortex ^{3}H-epinephrine α-receptor sites, GTP by itself is a much weaker inhibitor of binding, and in the presence of Ca^{2+} and Mg^{2+}, low concentrations of GTP increase binding, as in the calf, but higher GTP concentrations do not reduce binding again as they do in the calf (Fig 7A).

In contrast to the striking reversal of the GTP effect on ^{3}H-epinephrine binding caused by Ca^{2+} and Mg^{2+}, these ions do not antagonize the Gpp(NH)p reduction in binding to the same extent. The affinity of Gpp(NH)p is lowered somewhat, but there is no true reversal of the nucleotide effect (Fig 7). Thus, whereas GTP and Gpp(NH)p reduce ^{3}H-epinephrine binding to α-receptors in a very similar manner in the absence of Ca^{2+} and Mg^{2+}, their actions are sharply differentiated in the presence of those ions. These findings may be explained on the basis that the M^{2+}-GTP complex has an opposite effect on agonist affinity at the receptor from that of free GTP; the differential interactions with the ions and Gpp(NH)p are not due to a different chelating ability of this nucleotide, but there may be a qualitatively different action of M^{2+}-Gpp(NH)p compared to M^{2+}-GTP. Alternatively, the divalent cations may interact with an entirely different allosteric site at the receptor to counteract the GTP effect. This possibility may be more likely in view of the fact that both ions also counteract the inhibitory effect of Na^+ on ^{3}H-epinephrine binding to bovine cortex α-receptors [44], and Mg^{2+} by itself increases agonist affinity at α-receptor ^{3}H-DHE binding sites in rabbit platelets [42]. Mn^{2+} at very low concentrations can also be shown to counteract the guanine nucleotide effect on ^{3}H-epinephrine binding; the ED_{50} values of the divalent cations are shown in Table III. In general, for both ^{3}H-clonidine and ^{3}H-catecholamine binding, the order of ion potency is $Mn^{2+} > Mg^{2+} > Ca^{2+}$, and the ions are less potent interacting with Gpp(NH)p than with GTP.

Exton and co-workers have found that the α-receptor on hepatocytes is uncoupled from adenylate cyclase in physiological media, and α-agonists stimulate phosphorylase *a* activity via Ca^{2+} as "second messenger" [45, 46]. However in Ca^{2+}-depleted conditions,

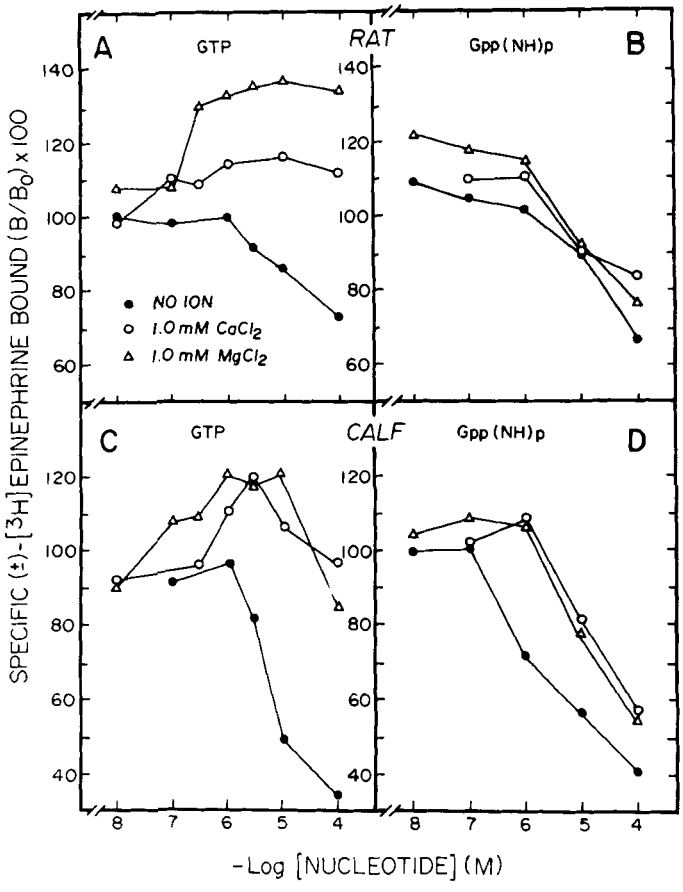

Fig 7. Nucleotide effects on ^{3}H-epinephrine α-receptor binding to rat or calf cortex membranes, in the presence or absence of calcium or magnesium. Values in each instance expressed as percent of no-nucleotide control [data from 44].

the hepatocyte α-receptors are coupled to adenylate cyclase, and α-agonists increase cAMP production [47]. Thus, Ca^{2+} appears in the liver to promote uncoupling of the receptor and adenylate cyclase. An intriguing possibility suggested by the ^{3}H-catecholamine binding studies is that, if GTP and/or Na^+ is the physiological coupling agent, then divalent cations are "uncoupling" agents: by virtue of their ability to antagonize, or severely attenuate, the coupling action of GTP or Na^+. This idea presupposes that the GTP- or Na^+-induced reduction in agonist affinity is an index of coupling, and the reversal of this by M^{2+} is an index of uncoupling. In vascular tissue, Ca^{2+} depletion selectively impairs agonist interaction with α-receptors [48]. and conversely Mg^{2+} increases agonist affinity at platelet α-receptors [42] and is necessary for high-affinity agonist binding to frog erythrocyte β-receptors [49].

CATECHOLAMINE β-RECEPTOR BINDING

Characteristics and Temperature Effects

The α-adrenergic receptor characteristics of ^{3}H-epinephrine binding are uniform in most regions of bovine CNS. However, in the cerebellum ^{3}H-epinephrine binds in the

TABLE III. Divalent Cation ED_{50} Values at ^{3}H-Epinephrine α-Receptor Binding Sites in Rat Cortex

Ion	Reversal of GTP effect (μM)	Reversal of Gpp(NH)p effect (μM)
Mn^{2+}	32	115
Mg^{2+}	140	200
Ca^{2+}	88	170

Data from [44].

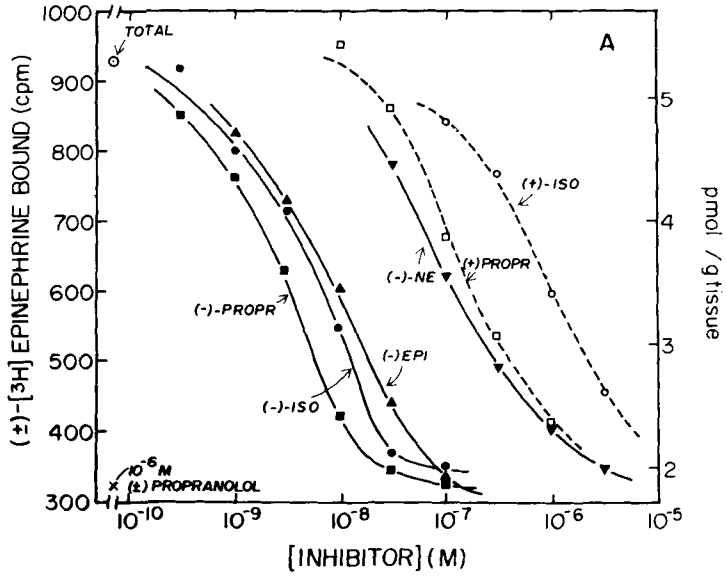

Fig 8. Inhibition of binding of ^{3}H-epinephrine to calf cerebellar membranes in the presence of 1.0 μM phentolamine and 1.0 mM pyrocatechol at 4°C [data from 25].

presence of 1.0 mM pyrocatechol to a mixed population of high-affinity sites. In the presence of 1.0 μM phentolamine, which prevents α-receptor binding, ^{3}H-epinephrine binding is inhibited by propranolol with high affinity, and the majority of binding is to a β-receptor of the β_2 subtype [50] (Fig 8), where in the phenylethylamine series, (−)-isoproterenol is most potent, and (−)-epinephrine is significantly more potent than (−)-norepinephrine. Conversely, in the presence of propranolol, ^{3}H-epinephrine binds exclusively to α-receptors in the cerebellum, and these sites are identical to the cerebral cortex ^{3}H-epinephrine α-receptor site [17, 19]. Using the antagonist β-receptor ligand ^{3}H-dihydroalprenolol (DHA), we found that whereas in most bovine CNS regions the β-receptor is of the β_1 subtype, where (−)-epinephrine and (−)-norepinephrine are equipotent, in the cerebellum the affinity of (−)-epinephrine at the ^{3}H-DHA β-receptor site is increased about 10-fold, the affinity of (−)-norepinephrine binding is observable at β_1 receptors in cortex and other brain areas, where the affinity of epinephrine is too low for detectable specific binding in the usual concentration range. ^{3}H-epinephrine binding to β_2-receptors in rat lung can also be demonstrated, and the rat lung site appears to be

TABLE IV. Inhibition of ^{3}H-Epinephrine (EPI) and ^{3}H-Dihydroalprenolol (DHA) Binding to Calf Cerebellar β-Adrenergic Receptors

Drug	K_iEPI (nM)	K_iDHA (nM)
Agonists		
(−)-Isoproterenol	2.4	36
(−)-Epinephrine	3.2	140
(−)-Norepinephrine	61	2,400
Salbutamol	32	290
Terbutaline	330	2,300
(+)-Isoproterenol	370	1,600
(+)-Norepinephrine	3,000	61,000
Antagonists		
(−)-Propranolol	1.3	0.3
(−)-Alprenolol	1.2	0.4
(+)-Propranolol	130	35
Dichloroisoproterenol	360	180
(−)-Sotalol	160	270
Butoxamine	2,800	960
(−)-Practolol	11,500	4,400

Data from [25].

identical to the cerebellar site. For similar reasons, although ^{3}H-norepinephrine binds to α-receptors in the cerebellum [17], it cannot bind to β-receptors there because its affinity is too low.

The cerebellar and lung ^{3}H-epinephrine β_2 site shows marked stereospecificity for phenylethylamines and β-antagonist enantiomers, and the structure–activity relationships expected at a β-receptor. Nonspecific β-antagonists such as propranolol and alprenolol are very potent inhibitors, with K_i values in the 10^{-9} M range. The sites labeled by the antagonist ligand, ^{3}H-DHA, in both tissues, appear to be identical to the ^{3}H-epinephrine site with respect to antagonist affinities (Table IV) and specificity, but agonists are in general more potent inhibitors of ^{3}H-epinephrine binding.

With 1.0 μM propranolol as a blank to define specific binding, ^{3}H-epinephrine in the presence of phentolamine binds to a single, saturable, high-affinity site in cerebellum and lung [25]. In a similar fashion to ^{3}H-catecholamine α-receptor binding, the affinity of ^{3}H-epinephrine is increased with lower incubation temperatures, from a K_D of 30 nM at 25°C, to 7.5 nM at 4°C, in the cerebellum. Routine assays were performed at 4°C, where specific binding is 70–80% of total in cerebellum, and 50% of total in lung. The K_D for ^{3}H-epinephrine at rat lung β_2-receptors is about 24 nM at 4°C. In both tissues, association of specific binding is quite rapid at 4°C, with equilibrium reached by 20 min. Dissociation is biphasic and incomplete, and is thus similar to dissociation of another β_2-agonist ligand, ^{3}H-hydroxybenzylisoproterenol from frog erythrocyte β_2-receptors [37]. There was a parallel 6- to 10-fold increase in agonist affinities at cerebellar ^{3}H-DHA binding sites when the incubation temperature was lowered from 25° to 4°C, while antagonist affinities were unchanged. Similar findings have been reported with turkey erythrocyte ^{3}H-DHA binding [24]. The K_i values in Table IV are for ^{3}H-epinephrine binding at 4°C and ^{3}H-DHA binding at 25°C. It can be seen that a 6- to 10-fold reduction in agonist K_i values for inhibiting ^{3}H-DHA binding at 4°C would make the ^{3}H-epinephrine and ^{3}H-DHA sites

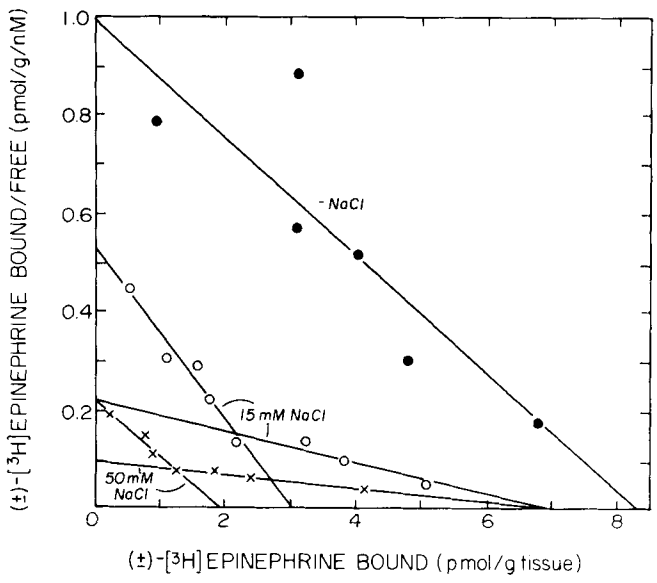

Fig 9. Scatchard plots of the saturation of ^{3}H-epinephrine binding to calf cerebellar β-receptors at 4°C in the presence and absence of sodium [data from 25].

virtually identical with respect to agonists and antagonists. Thus, contrary to the CNS α-receptor binding studies, it appears that ^{3}H-epinephrine and the antagonist ^{3}H-DHA bind to the identical β-receptor site in bovine cerebellum and rat lung.

Nucleotide and Sodium Effects

Guanine nucleotides decrease the affinity of the β-agonist ^{3}H-HB1 at frog erythrocyte β-receptors by increasing the rate and extent of agonist dissociation [37, 49]. Similar effects are observed with agonists at the glucagon receptor [2, 36] and an α-receptor in the CNS (see above). GTP, however, had no observable effect on ^{3}H-epinephrine β-receptor binding in bovine cerebellum. Likewise, GTP did not lower the affinities of agonist inhibitors of ^{3}H-DHA binding in cerebellum, a phenomenon seen also at the turkey erythrocyte β-receptor. In numerous other β-systems, GTP lowers agonist affinities at the ^{3}H-DHA site. Sutherland and co-workers [51] showed that the β-receptor in bovine cerebellum is coupled to adenylate cyclase, but the receptor appears to be GTP-insensitive. Recent experiments in our laboratory with the other β-agonist ligand, ^{3}H-HB1, show the same GTP-insensitivity in the cerebellum in 25°C incubation conditions.

On the other hand, the cerebellar and lung β-receptors, like the ^{3}H-catecholamine α-receptor in CNS, are Na^+-sensitive. Sodium and lithium decrease ^{3}H-epinephrine cerebellar β-receptor binding at low concentrations, with larger ions being much weaker [25]. The effect of sodium is to produce a concentration-dependent reduction in affinity of the majority of ^{3}H-epinephrine sides, leaving a few of the sites in their original high-affinity conformation (Fig 9). Sodium also causes a parallel reduction in agonist affinities as inhibitors of ^{3}H-DHA binding, without affecting antagonist affinities [25]. Thus sodium, instead of GTP, acts as an allosteric "modulator" of agonist affinity at the bovine cerebellar β-receptor and may indeed act as a coupling agent. Although it is

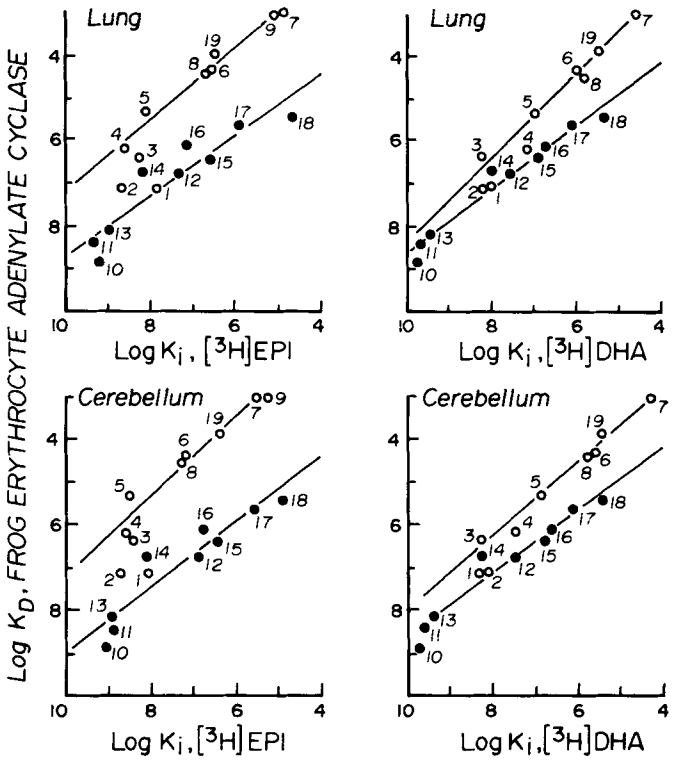

Fig 10. Correlations for β-receptor drugs between affinities in inhibiting ^{3}H-epinephrine (EPI) or ^{3}H-dihydroalprenolol (DHA) binding in rat lung or calf cerebellum, and affinities in stimulating or inhibiting frog erythrocyte adenylate cyclase. Solid symbols are antagonists, open symbols are agonists. (Binding data from [25]; cyclase data from [53, 54].)

not clear if sodium has the same effect as GTP in numerous other β-receptor systems where GTP modulates agonist affinity, it may be the case that adrenergic receptors linked to adenylate cyclase can be divided into three classes characterized as states in which (1) only GTP can couple the receptor and the enzyme; (2) Na^+ is the coupling agent (eg, the cerebellar β-receptor and the rabbit platelet α-receptor); and (3) either GTP or Na^+ can couple the receptor and adenylate cylcase (eg, the neuroblastoma/glioma opiate receptor [52] and the CNS ^{3}H-catecholamine α-receptor). There is no evidence as to the existence of (1) as distinct from (3).

Correlation of ^{3}H-Epinephrine Binding and Adenylate Cyclase Activity

The β_2-receptor-coupled adenylate cyclase of frog erythrocyte has been studied extensively [53, 54]. When affinities of β-agonists and antagonists in this cyclase system are correlated with their affinities at bovine cerebellar or rat lung β-receptors labeled by the agonist ^{3}H-epinephrine at 4°C and the antagonist ^{3}H-DHA at 25°C (Fig 10), it can be seen that for antagonists there is a 1:1 affinity correspondence between the agonist or antagonist binding site and adenylate cyclase inhibition. Agonists are about 10-fold more potent inhibitors of ^{3}H-DHA binding than activators of adenylate cyclase. This difference may be entirely because the ^{3}H-DHA assays were conducted in Na^+-free medium, and NaCl specifically lowers agonist affinities by a factor of about ten.

Agonists are 300 times more potent inhibitors of ^{3}H-epinephrine binding than activators of adenylate cyclase, although their *relative* potencies in binding and cyclase are similar. Two factors that explain this discrepancy are the 4°C incubation conditions, and the Na^+-free medium, both of which selectively increase agonist affinities. The absence of nucleotides in the ^{3}H-epinephrine binding assay, compared to the presence of ATP in the cyclase assay, would not appear to be a factor, since ^{3}H-epinephrine β-receptor binding is GTP-insensitive. However, there may be increased GTP-sensitivity in the presence of NaCl, as seen with opiate agonist binding. The low temperature conditions are clearly not physiological, but increased agonist affinities at the ^{3}H-epinephrine site in the absence of sodium, compared to agonist affinities on the cyclase, may indicate that sodium is a coupling agent for those β_2-receptors. The agonist affinity differential in the ^{3}H-epinephrine binding system due to low-temperature and Na^{2+}-free conditions still does not account entirely for the 300-fold difference in agonist affinities in the binding and cyclase assays. Another constituent or factor may also be involved in selectively increasing agonist affinities at ^{3}H-epinephrine β-receptor sites.

CONCLUSIONS

Endogenous catecholamines can be utilized as radiolabeled probes to identify adrenergic receptors in the CNS. Using ^{3}H-catecholamines and other ligands, we have identified two CNS postsynaptic α-adrenergic receptors, one of which, labeled by ^{3}H-epinephrine, ^{3}H-norepinephrine, ^{3}H-clonidine, and the antagonist ^{3}H-DHE, has high affinity for agonists in the experimental conditions used and has high affinity for some antagonists. This receptor has agonist-specific sensitivity to guanine nucleotides and sodium, which may be agents that induce coupling of the receptor and adenylate cyclase in certain circumstances. The evidence is strong, but inferential, that this α-receptor is indeed linked to adenylate cyclase in the CNS. Divalent cations reverse the actions of GTP and sodium at this receptor and may, by analogy, be considered to be "uncoupling" agents. More direct evidence for an uncoupling role for Ca^{2+} has recently been obtained at α-receptors in the liver [47], and the data here imply that in physiological conditions the receptors are uncoupled from adenylate cyclase and act via Ca^{2+} as a "second messenger." Thus this particular α-receptor in the brain may have the capability of coupling to adenylate cyclase but may not exist in the coupled state normally.

The other CNS α-receptor, labeled by the antagonists ^{3}H-WB-4101 and ^{3}H-DHE, has low affinity for agonists and preferentially high affinity for some antagonists. It seems to be insensitive to sodium and GTP and may not be coupled to adenylate cyclase.

The β-receptors labeled by ^{3}H-epinephrine are necessarily of the β_2 subtype, where epinephrine has greater potency. The same receptors are labeled by the antogonist ^{3}H-DHA, and parallel effects on agonist and antagonist binding can be observed. The bovine cerebellar β_2 receptor is GTP-insensitive, unlike most other β-receptors, but Na^+-sensitive, and Na^+ may be the exclusive coupling agent. There is a strong correlation between receptor binding and cyclase affinities, suggesting that this receptor is indeed linked to adenylate cyclase.

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