Cyclic Nucleotides in the Nervous System

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Preface

Research on cyclic nucleotides in nerve tissue continues relentlessly, as it has for almost two decades. An immense amount of information has accumulated on the biochemical and regulatory aspects of cyclic nucleotide metabolism, but efforts to relate this knowledge to physiological processes have met with very limited success. The overwhelming preoccupation, of course, is whether the cyclic nucleotides play a role in synaptic transmission and, if so, to define such an action in electrophysiological and molecular terms. Much indirect and circumstantial evidence indeed implicates both cyclic AMP and cyclic GMP in synaptic events, but it is still not possible to assign an unequivocal physiological role to either cyclic nucleotide in the vertebrate nervous system. This is due, in part, to the anatomical and functional complexities of the central nervous system and to a lack of understanding of its basic electrophysiology. Recent advances, especially from studies on cyclic nucleotides in less complex nervous systems, appear likely to change this disappointing state of affairs. If cyclic nucleotides are involved in neuronal information processing, especially as messengers or as modulators of neurotransmitter action, it is accepted they would do so by mediating phosphorylation-dephosphorylation reactions, probably at both pre- and postsynaptic sites. It is in this area that some of the most exciting evidence is being provided that cyclic nucleotides can indeed alter the electrical and permeability properties of neuronal membranes in a manner compatible with neurotransmitter action.

This volume deals with biochemical events and processes, focusing mainly on synaptic transmission. It examines the evidence that cyclic nucleotides can modulate neurotransmitter action at synapses and discusses whether altered cyclic nucleotide metabolism can account in any way for the actions of a variety of pharmacological agents and chemicals that affect neuronal excitability. The volume concentrates on studies that have appeared since 1979, incorporating earlier studies only when necessary for clarity and for providing essential background information. Otherwise the reader is referred to several comprehensive reviews that appeared during the years 1975 to 1979 (refs. 44, 130, 166, 308, 448, 494); several restricted reviews (refs. 24, 717) have appeared more recently. This volume will be of interest to all neuroscientists.

George I. Drummond

CHAPTER 1

Adenylate Cyclase

The fact that receptors for neurotransmitters are coupled to adenylate cyclase in nerve tissue in a manner analogous to hormone-receptor-enzyme coupling in peripheral tissues has provided compelling, albeit indirect, evidence for the involvement of cAMP in nerve transmission. Impressive advances have been made in understanding the mechanisms by which hormones regulate adenylate cyclase in nonneuronal tissues, and this knowledge will undoubtedly be the basis for understanding neurotransmitter-coupled systems in nerve. It is now established that hormone-sensitive adenylate cyclase comprises at least three distinct and separable entities: the hormone receptor, a guanine nucleotide-binding regulatory protein (N), and the catalytic unit. A distinct receptor exists on the cell surface for each hormone interacting with the system. The regulatory protein, in addition to binding GTP, appears to be associated with GTPase activity and has a crucial role in the coupling process by which the hormone-bound receptor conveys activation upon the catalytic unit, which in turn forms cAMP from ATP. The most notable success in resolving and purifying the components of the adenylate cyclase system has come from the work of Lefkowitz and his colleagues, who have purified the β-adrenergic receptor to homogeneity from frog erythrocytes (604,605) and from Gilman and his associates, who have solubilized and purified the regulatory protein to homogeneity from liver plasma membranes (460,626) and turkey erythrocyte membranes (257). Success with the regulatory protein was due, in part, to the availability of a mutant or variant of the S49 murine lymphoma cell line, cyc^-, which lacks the regulatory protein but contains the catalytic unit. The assay for regulatory protein depended on the capacity of fractions containing this component to reconstitute guanine nucleotide, fluoride, and hormonal sensitivity when added to membranes derived from cyc^- cells (257,460,626). The catalytic unit of hormone-sensitive adenylate cyclase has not yielded to extensive purification from any source and only recently has been successfully resolved from the regulatory protein by physical means following solubilization (544). Several comprehensive reviews are available on the general properties of the system (545), on the role of guanine nucleotides (621), and on the molecular mechanisms of hormone-receptor coupling (18, 377,388,542,623).

Some progress has been made in elucidating the components of the brain adenylate cyclase system. Strittmatter and Neer (635) have solubilized the enzyme system from bovine cerebral cortex using sodium cholate and have partially resolved the catalytic component from the regulatory protein. The resolved catalytic activity was not responsive to guanylyl-imidodiphosphate (p[NH]ppG), a stable analog of GTP, or to sodium fluoride and was much less active when assayed with MgATP as substrate than with MnATP, properties that would be expected of the free catalytic unit. The separated regulatory protein reconstituted p[NH]ppG and fluoride sensitivity when added to the catalytic unit (450,635). The solubilized rat brain enzyme has been partially purified (628); the activity was not sensitive to p[NH]ppG, indicating that the regulatory protein had been removed. These facts and the fact that the brain enzyme responds to several neurotransmitters in a guanine-nucleotide-dependent manner strongly indicate that the primary components of the neuronal adenylate cyclase system and the mechanisms of receptor coupling are basically analogous to those from peripheral tissues.

Some hormones in vertebrate tissues have an inhibitory, rather than a stimulatory, action on adenylate cyclase. Since inhibition is a receptor-mediated process, receptors for such hormones appear to be *negatively* coupled to adenylate cyclase. In fact, receptors for several putative neurotransmitters in the CNS also appear to be negatively coupled to the enzyme. These include dopaminergic D_2, adenosine A_1, and opiate receptors and will be dealt with later. From studies in peripheral tissues, particularly adipose tissue, it has been revealed that inhibition requires GTP, and a regulatory protein that seems to be distinct from the one mediating hormonal activation of the enzyme has been identified. Thus there appear to be two regulatory proteins in the plasma membrane of cells through which hormones may be coupled to exert regulatory actions on adenylate cyclase—one mediating stimulation of the enzyme termed N_s and a second mediating inhibitory influences termed N_i (103,286,377,401,542). At present much more is known about N_s than about N_i.

A. Actions of Neurotransmitters

As mentioned above, receptors for several neurotransmitters that exist on the surface of neurons in specific areas of the brain appear to be coupled to adenylate cyclase (130,308,448). This, in fact, has constituted one of the most compelling factors in the concept of cAMP serving as a messenger of neurotransmitter action. As in peripheral tissues, neurohormone receptors in nerve frequently exist as multiple species or subtypes, for example, H_1 and H_2 histamine receptors, α_1- and α_2-adrenergic receptors, and 5-HT_1 and 5-HT_2 serotonin receptors (618,619). In the past, receptor subtypes have been identified largely on the basis of pharmacological considerations; more recently they can be identified by specific binding techniques employing, in most instances, a variety of radioactively labeled agonists and antagonists (618,619).

Hormone and neurohormone receptors that are linked to adenylate cyclase are frequently sensitive to guanine nucleotides. Guanine nucleotides selectively decrease the affinities of agonists but not of antagonists for receptor sites (388, 618,623). This effect has been interpreted as guanine nucleotide (GTP) involvement in the coupling between the hormone receptor, the regulatory component, and the catalytic unit. Guanine nucleotide sensitivity has been considered by some investigators to be a property of only those receptors linked to adenylate cyclase (618) and is sometimes used as evidence for association of a binding site with the enzyme. As mentioned above, neurotransmitter receptors may be coupled to adenylate cyclase through the inhibitory regulatory protein N_i so as to exert inhibition on the enzyme. In such cases, it appears that receptor subtypes involved are distinct from those that are coupled through the stimulatory regulatory protein N_s. Since receptors for several putative neurotransmitters are heterogenous, the possibility for dual control by a single neurohormone exists. That is, a given neurohormone may interact with a receptor subtype through N_s to stimulate adenylate cyclase, and with a different receptor subtype through N_i to affect inhibitory control (103,401). In the brain the two systems may exist in different regions, in different cell types in a given region, or possibly in a single cell type. It should be realized also that different receptor subtypes for a single neurohormone may be coupled to the stimulatory system; for example, both β_1 and β_2 receptors activate adenylate cyclase. Finally, neurohormone receptors, or one or more of their subtypes, may not be coupled to adenylate cyclase. In the succeeding sections the nature of receptors for several neurotransmitters in the CNS and the possible significance of their association with adenylate cyclase will be examined.

1. Dopamine

Dopamine (DA) is found in large amounts in the basal ganglia (especially the caudate nucleus), the nucleus accumbens, the olfactory tubercle, the central nucleus of the amygdala, the median eminence, and some areas of the frontal cortex. There are dopaminergic neurons within the ventral hypothalamus that innervate the median eminence and intermediate lobe of the pituitary, hypothalamic neurons that connect the dorsal and posterior hypothalamus with the lateral septal nuclei, and long projections between DA-containing nuclei in the substantia nigra and their targets in the striatum, the limbic zones of the cerebral cortex and other areas of the limbic system (see 442). Application of DA onto neurons or spontaneously firing cells may block action potentials, cause excitation, or both. On stimulation of nigral neurons, the caudate neurons respond within milliseconds with increased firing, then become inhibited. The inhibition may be due to the fact that DA elicits a prolonged postsynaptic potential. Involvement of the dopaminergic nigrostriatal system in extrapyramidal motor function is well known, and Parkinsonism may result from either a deficiency of DA in the basal ganglia or a blockade of DA receptors. Neuroleptic drugs

block certain behaviors (particularly rotation and locomotion) elicited by DA agonists. Thus these agents cause catalepsy in animals and Parkinson-like symptoms in patients. These and other facts, as well as the observation that there is an apparent increase in DA receptors in brains of schizophrenics (358,359,411), have been the basis of the DA hypothesis of schizophrenia, which suggests that this disorder results from overactivity of certain dopaminergic pathways in the brain (see 587).

In each region of the brain where DA synapses exist there is a DA-sensitive adenylate cyclase. DA stimulation of the enzyme is inhibited by antipsychotic agents such as the phenothiazines. When this became known, intense interest arose in the possibility that DA-sensitive adenylate cyclase was related to the postsynaptic DA receptor and could be involved in the molecular action of the antipsychotic drugs, an idea developed by Greengard and his associates. The DA-stimulated adenylate cyclase, especially in the caudate nucleus and mesolimbic system (olfactory tubercle and nucleus accumbens), was extensively investigated using a variety of agonists, antagonists, and neuroleptic agents in an effort to correlate clinical antipsychotic efficacy and potency with ability to block DA receptors and to inhibit DA-stimulated adenylate cyclase, and also to determine if blockade of the DA-sensitive enzyme was involved in production of extrapyramidal side effects of antipsychotic drugs. These studies, which took place in the early 1970s, have already been reviewed (108,130,308,587,642) and will be summarized only briefly here. The main findings were that within a particular series of compounds, for example, the phenothiazines, a correlation existed between clinical potency and ability to inhibit DA-sensitive adenylate cyclase. However, discrepancies became apparent if one compared the potency of neuroleptics belonging to different chemical groupings. The main inconsistency was that the butyrophenones, relative to the phenothiazines, were very weak inhibitors of DA-sensitive adenylate cyclase but were highly potent antagonists of DA-mediated effects *in vivo* and were highly potent antipsychotics. Other discrepancies were also revealed; for example, clozapine, which is a weak *in vivo* agent and does not produce extrapyramidal effects, is a potent inhibitor of DA-sensitive adenylate cyclase in the caudate nucleus; metoclopramide produces extrapyramidal reactions and blocks DA mechanisms in animals but does not inhibit striatal DA-sensitive adenylate cyclase; sulpiride has antipsychotic activity but does not block DA stimulation of the enzyme in either striatal or mesolimbic areas; and in addition, molindone, an effective antipsychotic similar to the phenothiazines, is a very weak inhibitor of the enzyme. Moreover, some potent antidepressants are powerful inhibitors of DA-stimulated adenylate cyclase but lack antipsychotic action (642). Seeman (587) draws attention to another discrepancy, namely, that even with respect to the phenothiazines the neuroleptic concentrations required to produce 50% inhibition of DA-stimulated enzyme activity would be in the range 100 to 10,000 nM, concentrations that would not exist clinically. In other words, even though a correlation exists with respect to clinical potency and inhibition of DA-sensitive adenylate cyclase

within the phenothiazine series, the concentrations required to inhibit the enzyme are much higher than those detected in plasma during clinical treatment.

a) Characterization and Classification of DA Receptors

A conclusion from all these studies was that DA-sensitive adenylate cyclase is most likely not the DA receptor through which antipsychotics mediate their action in schizophrenia or through which DA antagonists block dopaminergic effects on motor stimulation. The possibility arose that not all DA receptors were linked to adenylate cyclase in the caudate nucleus or limbic system. This stimulated a search for a "neuroleptic" receptor and to determine if it was indeed a DA receptor. The result has been an avalanche of binding studies using an array of radioactively labeled ligands, including neuroleptics of all classes, DA agonists and antagonists, and ergot alkaloids. The interpretation of these binding studies is complex, partly as a result of DA receptor heterogeneity and the confusion that has resulted from different systems being used for their classification, but mainly from the use of such a diversity of radioligands, many of which have overlapping specificity for several receptor subtypes. A detailed coverage is beyond the scope of this review; a number of reviews are available (102,108,116,119,309) including an exhaustive review by Seeman (587) (which cites 1,278 studies). From all this activity has come the understanding that in addition to there being multiple DA receptor subtypes in the brain, adenylate cyclase is not linked to each receptor subtype and that a DA receptor that is apparently not linked to adenylate cyclase is involved in neuroleptic action. A major advance occurred when binding sites for the butyrophenone [^{3}H]haloperidol were discovered in the striatum (see 587). The regional distribution of these sites was similar to both that of DA-containing nerve terminals and to sites responding to iontophoretically applied DA. The IC_{50} of haloperidol in displacing [^{3}H]haloperidol was 2 nM, in good agreement with the concentration of this antipsychotic agent found in the serum of patients receiving the drug clinically (587) and, much more importantly, the IC_{50} values of a wide range of neuroleptics in displacing [^{3}H]haloperidol correlated beautifully with their clinical antipsychotic potencies [117,431,588,587 (especially Fig. 2)] as well as with their behavioral effects as DA antagonists in animals. Thus the idea became quite well established that the antipsychotics interact primarily with a DA receptor in the striatum, which can be quantitated by binding of [^{3}H]haloperidol and which is not linked to adenylate cyclase. Incidentally, chronic administration of neuroleptics (chlorpromazine and thioridazine) to rats gives rise to increased DA receptors in the brain. The effect is specific for DA receptors and persists for several months after withdrawal of the drug (98,99,646). This may be related to DA receptor supersensitivity, which is thought to produce tardive dyskinesia in patients on antipsychotic therapy.

Kebabian and Calne (309) were the first to classify DA receptors. Their classification was based on two facts: (a) in the bovine parathyroid a DA receptor

that stimulates adenylate cyclase is present and (b) in the anterior and intermediate lobes of the pituitary there exists a DA receptor that functions to inhibit prolactin and α-melanocyte-stimulating hormone (α-MSH) release and that does not mediate increases in cAMP. They proposed that DA receptors in these two tissues were distinct and designated them D_1 and D_2 dopamine receptors; thus the prototype of the D_1 subtype linked to adenylate cyclase became the parathyroid, and the prototype of the D_2 subtype not linked to the enzyme, the anterior pituitary. By this time (1979) it was possible to identify DA receptors at five sites within the nigrostriatal system—dopaminergic neurons contain autoreceptors that regulate either electrical firing of these cells in the substantia nigra (site 1) or tyrosine hydroxylase activity on the terminals (site 2), cortical neurons that project to the striatum and possess [^{3}H]haloperidol binding sites (site 3), neurons that are intrinsic to the caudate and that contain DA-sensitive adenylate cyclase (site 4), and terminals of striatal neurons that project to the substantia nigra and also contain the DA-sensitive enzyme (site 5). According to Kebabian and Calne (309; see also 116), DA receptors at sites 1 to 3, since they appear not to be associated with adenylate cyclase, would be classified as D_2 and those at sites 4 and 5 as D_1. Incidentally, the D_2 receptors in the pituitary can be labeled with [^{3}H]haloperidol and appear similar to the majority of these sites identified in the striatum. In addition to the receptor displaying high affinity for [^{3}H]haloperidol, Seeman and his associates (391,647) obtained evidence for a second receptor in the striatum that was primarily labeled with [^{3}H]DA, that is, DA displaced [^{3}H]DA with high potency (IC_{50}, 1–7 nM), whereas the IC_{50} for DA displacing [^{3}H]haloperidol was 10 μM. They concluded that these two [^{3}H] ligands label different populations of receptors. Seeman (587) classifies the receptor that preferentially binds [^{3}H]DA as D_3. Seeman (587) has designated still another DA receptor subtype, D_4. This latter designation was based on work of Meunier and Labrie (432), which suggested the presence of a DA-inhibited adenylate cyclase in intermediate lobe pituitary cells. To summarize, Seeman has designated four DA receptor subtypes: D_1 refers to the site for DA-stimulated adenylate cyclase and is characterized by being stimulated by micromolar concentrations of DA and antagonized by micromolar concentrations of neuroleptics. D_2 refers to the dopaminergic site that is labeled with [^{3}H]haloperidol, is sensitive to nanomolar concentrations of neuroleptics but micromolar concentrations of DA. D_3 is sensitive to nanomolar concentrations of DA but micromolar concentrations of neuroleptics; D_4 is sensitive to nanomolar concentrations of both DA and neuroleptics. A suitable ligand for the D_1 site is [^{3}H]*cis*-flupenthixol (283,284) for which there is an excellent correlation between IC_{50} values for neuroleptics on DA-sensitive adenylate cyclase and competition for binding sites for this radioligand. D_2 receptors can be labeled with [^{3}H]haloperidol as well as many other neuroleptics, such as [^{3}H]spiperone, [^{3}H]pimozide, [^{3}H]domperidone, and [^{3}H]sulpiride; D_3 sites may be labeled with [^{3}H]DA, [^{3}H]LSD, or [^{3}H]apomorphine; and [^{3}H]bromocryptine, [^{3}H]pergolide, [^{3}H]lisuride, or [^{3}H]LSD may be used for D_4 sites (587).

There is some confusion regarding D_2 and D_4 receptors. Kebabian and Calne (309) originally used the term D_2 because butyrophenones were potent antagonists of the nanomolar efficacy of DA in inhibiting prolactin and α-MSH release in the pituitary; DA did not stimulate cAMP production in this tissue. Several recent studies have demonstrated that in the anterior and intermediate pituitary, DA inhibits both vasoactive intestinal peptide (VIP)- and β-adrenergic-stimulated adenylate cyclase activities which stimulate prolactin and α-MSH release (109,215,466). In the study of Onali and associates (466), DA did not alter basal enzyme activity but did inhibit the stimulation of adenylate cyclase elicited by VIP. Apomorphine and 2-bromo-α-ergocryptine mimicked the effect of DA, whereas (−)-sulpiride and classic neuroleptics antagonized it. It was reasonable to conclude that D_2 receptors affect pituitary secretion by inhibiting the activation of anterior pituitary adenylate cyclase by VIP. Meunier and Labrie (432) have examined the potency of DA and a number of DA agonists (lisuride, pergoline, dihydroergocryptine, apomorphine) to inhibit cAMP accumulation in intermediate lobe pituitary cells in culture. These agents inhibited both basal and isoproterenol-stimulated cAMP formation with nanomolar potency (EC_{50} values varied from 0.05 nM for lisuride to 5 nM for DA). Inhibition was reversed by a number of neuroleptics also with nanomolar potency [IC_{50} values varied from 0.02 nM for spiroperidol to 0.9 nM for (+)-butaclamol]. It seems clear then that the D_2 receptors in these cells are actually *negatively* coupled to adenylate cyclase [supposedly by involvement of the inhibitory regulatory protein N_i (103,401)]. Others (606,625) have also identified DA receptors in the pituitary; in the intermediate lobe guanine nucleotides decrease agonist ([^{3}H]*N*-*n*-propylnorapomorphine) binding, but not antagonist ([^{3}H]spiroperidol) binding, observations in harmony with the binding site being related to adenylate cyclase. This is the receptor that Seeman designated D_4 (587). The anomolous situation now exists in which a D_2 subtype receptor is present in the striatum which has nanomolar affinity for neuroleptics and micromolar affinity for DA and is not linked to adenylate cyclase and a D_2 receptor is present in the anterior pituitary which has nanomolar potency for both neuroleptics and DA and is negatively coupled to adenylate cyclase. Meunier and Labrie (432) have made an interesting suggestion that DA receptors coupled positively to adenylate cyclase could be designated DA_+, those coupled negatively DA_-, and those not coupled to the enzyme DA_0. This would still not be entirely satisfactory for a tissue such as the striatum, where two receptors exist (D_2 and D_3), neither of which is likely to be associated with the enzyme.

b) Interrelationships of DA Receptor Subtypes

The relationship between D_2 and D_3 receptors in the striatum is unclear. Phenoxybenzamine, a classical α-adrenergic blocking agent, also irreversibly inactivates the binding of [^{3}H]spiroperidol, a D_2-selective ligand in bovine caudate membranes (252). Doses completely eliminating the binding of this radio-

ligand leave the binding of [^{3}H]DA to D_3 sites unaffected. The binding sites for these two ligands thus seem indeed to represent distinct DA receptor subtypes. [^{3}H]Apomorphine binding can be resolved into a phenoxybenzamine-labile population of sites, which resemble D_2 receptors, and a phenoxybenzamine-resistant population of sites, which have an affinity for [^{3}H]DA comparable to the D_3 receptor; [^{3}H]apomorphine therefore seems to label a portion of D_2 receptors in addition to D_3. Lew and Goldstein (378) have suggested that D_2 and D_3 receptors can be distinguished simply by heating striatal homogenates at 53°C; under these conditions [^{3}H]DA binding was destroyed but [^{3}H]spiroperidol binding remained intact. However, Hamblin and Creese (253) have found that [^{3}H]apomorphine binding is decreased with a time course and temperature dependence indistinguishable from those of [^{3}H]DA binding. Since apomorphine binds to both D_2 and D_3 receptors (252), it was expected that [^{3}H]apomorphine binding should have been partially heat-resistant. In a series of interesting experiments they have demonstrated that the effects of heat treatment actually resemble those produced by guanine nucleotide on other hormone-receptor interactions, that is, heat treatment greatly lowered the affinity of agonists at both D_2 and D_3 receptors while leaving antagonist affinities unchanged. Heat treatment and GTP caused identical decreases in the affinity of agonists in the displacement of [^{3}H]spiroperidol (253); the effects of heat treatment and GTP were not additive. These investigators suggest that heat treatment inactivates not the receptor itself, but a guanine nucleotide regulatory protein that regulates both D_2 and D_3 receptor binding affinities and is essential for formation of high-affinity agonist-receptor complexes. This, of course, implies involvement of a guanine nucleotide regulatory protein with receptors not linked to adenylate cyclase (542). Divalent metal cations have been demonstrated to enhance the binding of agonists ([^{3}H]apomorphine) to D_3 receptor sites (122) and to increase the potency of agonists at D_2 sites labeled with [^{3}H]spiroperidol (670). Hamblin and Creese (254) have reported that incubation of striatal membranes with Mg^{2+} and inclusion of this cation (as well as other divalent cations) in the binding assay allow high-affinity binding of [^{3}H]DA to D_2 receptors. [DA is reputed to have low (μM) affinity at these sites.] These ions also increase the maximum binding of D_3-specific [^{3}H]DA binding. Guanine nucleotides abolish all D_2-specific [^{3}H]DA binding, whereas only the Mg^{2+}-dependent portion of D_3 sites appears to be GTP-sensitive (254). It has been suggested that the striatal D_2 receptor exists in two agonist affinity states whose interconversion is affected by guanine nucleotides and divalent metal ions. The GTP-sensitive Mg^{2+}-dependent nature of [^{3}H]DA binding to D_3 sites led to the postulate (254) that these sites may actually represent a high agonist affinity state of the D_1 receptor subtype, which, of course, is coupled to adenylate cyclase.

The actions of guanine nucleotides have had a significant bearing on the classification of DA receptors. Guanine nucleotides selectively reduce agonist binding to DA receptors in brain membranes (118,121,123) and decrease agonist displacement of antagonist binding to DA receptors (743). GTP inhibits

[^{3}H]apomorphine binding to DA receptors and reduces agonist potency in inhibiting [^{3}H]spiroperidol binding, suggesting that both these ligands may, in part, label receptors linked to adenylate cyclase (123). Kainic acid lesions, however, abolish the sensitivity of DA receptor binding to guanine nucleotides (123), that is, GTP no longer affects the potency of DA as an inhibitor of [^{3}H]spiroperidol binding. Also, after kainic acid lesions, GTP no longer reduces [^{3}H]apomorphine binding. Thus [^{3}H]spiroperidol and [^{3}H]apomorphine binding involves two DA receptors, only one of which seems affected by guanine nucleotides.

The properties of the benzamide sulpiride are also of interest with respect to DA receptors. This antipsychotic exhibits many of the actions of classic DA antagonists but is completely ineffective in inhibiting DA-sensitive adenylate cyclase. Because of this it has been suggested (645) that D_1 receptors are sulpiride-insensitive and only D_2 receptors interact with this agent. [^{3}H]Sulpiride binding sites have been identified in striatal preparations by several investigators (203, 204,645,732); guanine nucleotides decrease the affinity of DA and other DA agonists for the binding sites as measured by their ability to displace [^{3}H]sulpiride (202,203) but do not affect [^{3}H]sulpiride binding. Such findings might be considered contrary to the idea that sulpiride binds only to DA receptors not linked to adenylate cyclase. In this regard Freedman et al. (203) have suggested that guanine nucleotides might influence [^{3}H]sulpiride binding by interacting with an inhibitory regulatory protein.

Stoof and Kebabian (634) have described a series of experiments that provide evidence of the presence in the neostriatum of a DA receptor that is inhibitory to adenylate cyclase. In their study they used efflux of cAMP from striatal slices as a measure of DA stimulation, the inference being made that efflux is a valid measure of cAMP formation. Their evidence is based on the following: SKF 3839, a D_1 receptor agonist, stimulates efflux, which is antagonized by fluphenazine but not by (−)-sulpiride (a pituitary D_2 antagonist); efflux of cAMP therefore involves activation of D_1 receptors. LY-141865, an agonist on pituitary D_2 receptors, but with no effect on D_1, does not affect efflux either in the presence or absence of (−)-sulpiride. DA (which interacts with D_1 and D_2) increases efflux and (−)-sulpiride markedly potentiates the DA effect; in addition apomorphine enhances efflux only in the presence of (−)-sulpiride. LY-141865 reduces SKF 3839-stimulated efflux by a maximum of 58% and this inhibitory effect is reversed by (−)-sulpiride. The data, they believe, can be explained by the presence of a DA receptor that *inhibits* cAMP formation occurring as a consequence of D_1 receptor stimulation. DA would interact with both receptors, simultaneously stimulating and inhibiting the formation of cAMP. (−)-Sulpiride potentiates the DA-stimulating effect by blocking the second receptor, removing the inhibitory constraint, and thereby stimulating cAMP formation. Similarly, apomorphine stimulates both receptors, but its stimulatory action is manifest only when the second (inhibitory) receptor is blocked by (−)-sulpiride. LY-141865 would antagonize the stimulatory effects of SKF 3839 by activating

the inhibitory receptor and this is reversed by (−)-sulpiride by virtue of its capacity to block this receptor. If this is true, and insofar as the effects of (−)-sulpiride are concerned it agrees with the suggestion of Freedman et al. (203), it means that a pituitary-like D_2 receptor inhibitory to adenylate cyclase exists in the striatum. Whether it is coupled to adenylate cyclase directly or whether it antagonizes the effects of D_1 receptor stimulation indirectly is not known.

The neuroleptic D_2 receptor has been solubilized from human (140), dog (228,412), and calf striatum (95,96) using digitonin as detergent. In the preparation from human brain (140), the receptors were labeled with [^{3}H]spiperone; neuroleptics had inhibitory potencies on the solubilized receptor similar to those at the membrane-bound site. These preparations may be useful in further clarification of the binding properties and other characteristics of the neuroleptic receptor, especially its relationship to other DA receptor subtypes.

c) DA-Sensitive Adenylate Cyclase

Only very few studies have been carried out on DA-sensitive adenylate cyclase in brain during the past several years. Enjalbert et al. (175,176) found that DA-sensitive adenylate cyclase in rat brain was inhibited by several classic serotonergic antagonists (e.g., methiothepin, cyproheptadine, cinanserin, and metergoline). In fact these agents were more potent antagonists of the DA-stimulated enzyme than of the serotonin (5-HT)-sensitive species. It seemed that the two receptors exist in agonist and antagonist forms and the antagonist forms may be closely related. Ca^{2+}-calmodulin also seems to interact with DA-sensitive adenylate cyclase (505,506). GTP is required for DA stimulation of the striatal enzyme (428). Like many hormones, DA stimulates striatal adenylate cyclase by decreasing the K_a for free Mg^{2+} and by increasing V_{max} without changing K_m for substrate (428). The enzyme is present primarily in synaptic membrane fractions of caudate nucleus (560). The K_D for [^{3}H]DA binding (1.5 μM) was almost identical to the K_a for DA activation of the enzyme. In this study (560), which used very low concentrations of [^{3}H]DA in the binding assay, a high-affinity binding site for DA (K_D, 12 nM) was also identified. Whether or not this site is the same as the D_3 subtype was not made clear. The enzyme was solubilized by means of the nonionic detergent Lubrol 12A9, and the solubilized enzyme retained DA sensitivity. The [^{3}H]DA binding component was separated from the catalytic activity by gel exclusion chromatography (559). The resolved catalytic activity was no longer responsive to DA, but DA sensitivity was restored by adding back the [^{3}H]DA binding component. Possibly the DA binding component also contained the regulatory protein. Hoffman (273,274) has solubilized the DA-sensitive enzyme in caudate nucleus using cholate as the detergent. The cholate solubilized basal activity was resolved from the component that conferred DA sensitivity by means of gel filtration. The components of the system could also be solubilized by the nonionic detergents Brij 56 or

digitonin, but the sensitivity to DA was lost. Removal of the nonionic detergent by replacement with cholate and phospholipid, followed by removal of cholate, restored DA sensitivity. This is one of the very few instances where hormone sensitivity has been reconstituted in adenylate cyclase preparations from a mammalian tissue following solubilization. The study also points to the importance of phospholipid in DA receptor-cyclase coupling, a fact that is also indicated in experiments in which DA stimulation of adenylate cyclase in rat striata was destroyed by phospholipase A_2 (10) and restored by adding back phosphatidylserine. These solubilized preparations may be exploited in efforts to elucidate more clearly the regulation of the enzyme by DA.

It is obvious that many questions remain to be answered regarding the nature of DA receptors in the brain, of their possible interrelationships, and of their association with adenylate cyclase. It seems well accepted that the D_2 neuroleptic receptor is not linked to adenylate cyclase, but how it relates to the pharmacological action of neuroleptic drugs or to the pathology of schizophrenia is largely unknown. Nothing is known about the physiological significance of the D_3 receptor subtype or for that matter of the D_1 subtype linked to adenylate cyclase. Whether this receptor, together with cAMP formed from adenylate cyclase stimulation, has anything to do with the inhibitory action of DA on synaptic potentials is largely hypothetical. If a DA receptor subtype that is inhibitory to cAMP formation exists in the striatum, its functional role will also require elucidation.

2. α- and β-Adrenergic Agents

Several noradrenergic pathways in the brain are well delineated. Norepinephrine (NE)-containing neurons of the pontine nuclei and locus ceruleus extend axons to cortical regions including the cerebral, cerebellar, and limbic cortices and through different pathways to the hypothalamus. In the cerebellar cortex the NE input from the locus ceruleus is to the Purkinje cells; in the hippocampus and dentate gyrus, the recipient cells are the pyramidal neurons. The capacity of catecholamines, especially NE, to stimulate cAMP formation in several brain regions and to activate adenylate cyclase in brain membrane preparations has been known for almost 2 decades (see 44,130,448,642). Moreover, stimulation of the locus ceruleus increases cAMP levels in the cortex, hippocampus, striatum, and hypothalamus by mechanisms involving NE liberation (44,337).

a) Nature and Distribution of Adrenergic Receptors

In brain, as in other tissues, β-adrenergic and α-adrenergic receptors are heterogeneous; for example, both β_1 and β_2 subtypes are present. It is possible to measure β-adrenergic receptors directly by the specific binding of the high-affinity antagonists [^{3}H]dihydroalprenolol and [^{125}I]iodohydroxybenzylpindolol and the agonist [^{3}H]hydroxybenzylisoproterenol (721). NE is a selective β_1-ad-

renergic agonist; β_1-selective antagonists are practolol, atenolol, metoprolol, and ρ-oxprenolol, β_2-selective agonists are isoproterenol, zinterol (MJ 1999), terbutaline, and salbutamol (438,619). Both α_1 and α_2 subtypes of α-adrenergic receptors (619,624,718) are present in the mammalian CNS. Prazosin, indoramin, and WB-4101 are selective antagonists at α_1-adrenergic sites, piperoxan and yohimbine are relatively selective antagonists at α_2-adrenergic receptors, and clonidine and other imidazolines are selective agonists (619); [^{3}H]clonidine has been a particularly useful selective radioligand. Both α_1- and α_2-adrenergic receptors are blocked by phentolamine and the ergot alkaloids; [^{3}H]dihydroergocryptine is an effective radioligand for quantitating α adrenergic receptors (721). Both α_1- and α_2-adrenergic receptors in brain appear to be located postsynaptically (664,668,669) and seem to represent distinct noninterconverting species (487). GTP decreases binding of [^{3}H]agonists to α_2-adrenergic receptors but not to the α_1 subtype (666) and Na^+ decreases selective binding to α_2 receptors but has no effect on α_1 noradrenergic receptors (235). Similarly, binding of [^{3}H]clonidine is decreased by guanine nucleotides (669); divalent cations (Ca^{2+}, Mg^{2+}, or Mn^{2+}) reverse the inhibition by GTP and antagonize the Na^+-induced inhibition of α_2 agonist binding in calf cortex membranes. Depending on the assumptions made, these selective effects of cations and guanine nucleotides could be used as evidence that only the α_2 receptor is associated with adenylate cyclase. The situation is likely more complex because [^{3}H]clonidine has been shown to bind to two distinct receptor populations in calf cortex membranes, a high-affinity site (K_D, 1.1 nM) and a low-affinity site (K_D, 5.4 nM) (55). The binding specificities of the two sites differ with agonists being more potent at the high-affinity site and antagonists more potent at the low-affinity site. The regional distribution of the two sites also differs. Guanine nucleotides selectively decrease binding to the high-affinity site without affecting the low-affinity site (549,550,663,675). This could imply that α_2-adrenergic receptors exist in adenylate cyclase-coupled and adenylate cyclase-independent forms. The neuroblastoma × glioma hybrid NG108–15 contains only receptors of the α_2 subtype (16,249,298), which may also occur in two or more affinity states (298) and are coupled to adenylate cyclase in an *inhibitory* manner (298,556,557). These sites, incidentally, are very similar to those high-affinity sites in bovine brain labeled with [^{3}H]clonidine and [^{3}H]epinephrine (549,666). Recently Leibowitz and associates (360) examined the distribution of α receptors in numerous brain areas, including several hypothalamic areas, using [^{3}H]ρ-aminoclonidine and [^{3}H]WB-4101 [(2′,6′-dimethoxyethylamino)-methylbenzodioxan]. Regional variations in [^{3}H]WB-4101 binding (representing α_1 receptors) were relatively small, whereas binding of [^{3}H]ρ-aminoclonidine (representing α_2 receptors) showed a wider range in binding. The ratio of [^{3}H]ρ-aminoclonidine to [^{3}H]WB-4101 binding differed significantly in different hypothalamic areas (range, 1.5:1–4:1); the median eminence was exceptional in that it contained appreciable [^{3}H]ρ-aminoclonidine binding but virtually no [^{3}H]WB-4101 binding sites. Since these authors have also shown (360, see refs. 16–19 therein) that injection of NE