

GENERAL PSYCHOPHARMACOLOGY

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Signal Transduction Abnormalities in Schizophrenia: the cAMP System

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ABSTRACT ~ Understanding the neurochemistry of schizophrenia involves the study, not only of neurotransmitters and their receptors, but also of the signal transduction systems that translate their actions into neural activity. Of particular interest is the signal transduction system involving the second messenger cyclic adenosine monophosphate (cAMP), as all dopamine receptors are either positively or negatively coupled to this system. Studies in blood platelets, cerebrospinal fluid, or postmortem brains of patients with schizophrenia demonstrate abnormalities of stimulated cAMP production. Neuroleptic administration in animal models results in altered cAMP metabolism in a pattern opposite to that seen in schizophrenic patients. These studies suggest that abnormal signal transduction may be involved in the pathogenesis of schizophrenia and that the normalization of this defect may be one mechanism of action of neuroleptic drugs. *Psychopharmacology Bulletin*. 2002;36(4): 92-105

Introduction

The search for the neural substrate of schizophrenia has preoccupied psychiatrists for over 100 years. Since the development of neuroleptic drugs and the explosion of knowledge of the chemistry of neurotransmission, work has focused on a search for abnormalities of neurotransmitters and their receptors in the brains of patients with schizophrenia. There is now evidence implicating alterations in the dopaminergic, γ -aminobutyric acidergic and serotonergic neurotransmitter systems in this illness.¹⁻⁵ In addition to this work, a considerable amount of research has investigated the functioning of signal transduction mechanisms in patients with schizophrenia.

Signal transduction refers to the process by which an extracellular signal, such as a neurotransmitter, elicits changes in the intracellular milieu and functioning of cells. In its simplest form, the extracellular signal is detected by a receptor which is also an ion channel. Binding its ligand causes altered conductance and results in altered membrane potential and action-potential firing probability. More common are receptors that produce their intracellular effects through complex multimolec-

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ular signal-transduction pathways. These include the G-protein coupled receptors (GPCRs) which interact with heterotrimeric G-proteins to produce a second messenger which then induces a variety of intracellular effects, including altered phosphorylation of receptors and channels and altered gene expression.⁶ An understanding of any alterations in signal transduction in schizophrenia is especially relevant, given that many of the neurotransmitters implicated in this illness act at GPCRs. For example, all five known dopamine receptors are GPCRs, as are all serotonin receptors with the exception of 5-HT₃.^{7,8} Thus, any alterations in signal transduction function in schizophrenia will be relevant to understanding neuroleptic action, as well as the pathophysiology of the illness. This review will focus on the signal transduction system centered on the second messenger cyclic adenosine monophosphate (cAMP) and review the evidence for dysfunction of this system in schizophrenia and its modulation by neuroleptic drugs.

The cAMP-Mediated Signal Transduction Pathway

The cAMP-mediated signal-transduction pathway has been intensively studied in schizophrenia. This is because all dopamine receptors couple either positively or negatively to cAMP generation, and because alterations in the cAMP signal-transduction pathway affect the in vivo response to neuroleptic medications.^{9,10} The cAMP-mediated signal transduction pathway is a complex system that involves many proteins. The pathway modulates the activity of important substrate proteins. The proteins of the pathway regulate other steps in the cAMP-mediated pathway and furthermore, there is cross talk with other signal transduction pathways at several points. Thus, any definition of the cAMP signal-transduction pathway is inherently arbitrary. Despite this caveat, a core pathway can be appreciated, and, for the purposes of this review, we will consider cAMP-mediated signal transduction to consist of the following steps (Figure). First, the pathway begins with GPCRs that couple to heterotrimeric G-proteins that contain either G_{αs}, G_{αo1f} or G_{αi}. There are many receptors that couple to these G-proteins, including some of known or hypothesized relevance to schizophrenia or the action of neuroleptic medication (Table).¹¹ G_{αs} and G_{αo1f} activate and G_{αi} inhibits adenylyl cyclase, an enzyme that catalyzes the formation of the second messenger cAMP from adenosine triphosphate. cAMP activates protein kinase A (PKA) which is a major effector protein. PKA modulates the function of many different proteins via phosphorylation of specific serine or threonine residues. The actions of PKA may be reversed by serine/threonine phosphatases, most prominently protein phosphatase-1 (PP1), and these enzymes interact in diverse ways. In some systems, phosphorylation by PKA has been shown to activate PP1 by

releasing it from binding proteins that reduce the activity of PP1.¹² In other systems, PKA phosphorylation activates specific PP1 inhibitor proteins (eg, dopamine- and cAMP-regulated phosphoprotein 32 kDa [DARPP-32] and inhibitor-1), enhancing the phosphorylation produced by PKA.^{10,13,14} The dynamic interplay between PKA and PP1 con-

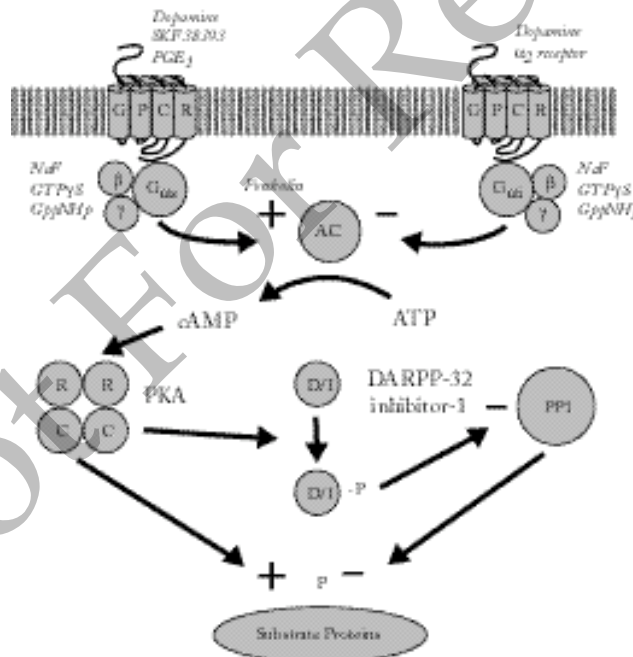
FIGURE

A SCHEMATIC OF THE cAMP-MEDIATED SIGNAL TRANSDUCTION PATHWAY

GPCRs bind agonists and couple through heterotrimeric G-proteins (G_{α} , β , γ) to AC. G-proteins may be positively (G_{α_s}), or negatively (G_{α_i}) coupled to adenylyl cyclase. AC catalyzes the production of cAMP from ATP. cAMP is bound by the R subunits of PKA, which then release the C subunits activating them. These catalytic subunits phosphorylate a variety of substrate proteins altering their activity. This phosphorylation is opposed by specific phosphatases including PP1. In addition, the catalytic subunit of PKA may phosphorylate special inhibitor proteins, including DARPP-32 and inhibitor-1. In their phosphorylated form, these proteins inhibit PP1 activity. Some of the drugs used in the studies reviewed here are shown in italics at their sites of action.

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cAMP=cyclic adenosine monophosphate; GPCR=G-protein coupled receptors; AC=adenylyl cyclase; ATP= adenosine triphosphate; R=regulatory subunit; PKA=protein kinase A; C=catalytic subunit; PP1=protein phosphatase-1; DARPP=dopamine- and cAMP-regulated phosphoprotein; NaF=sodium fluoride; GTP γ S=guanosine-5'- γ -thiotriphosphate; GppNHp=guanosine-5'-[(β γ)-imido] triphosphate.

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trols the phosphorylation level and hence the function of many proteins, including neurotransmitter receptors, ion channels, other signal-transduction proteins, metabolic enzymes, and transcription factors.

The study of signal transduction mechanisms in clinical populations is a difficult undertaking. The ideal of directly studying the organ of interest, the brain, under conditions of controlled drug exposure and rapid and reproducible collection methods is not practical. Therefore, research has been directed in several different ways each with their own advantages and disadvantages. Peripheral tissues, in particular blood platelets, have been collected from patients and control subjects and studied using biochemical or molecular biological methods. This approach has the advantage of ease of procurement and the ability, in some circumstances, to control the recent medication exposure of the patient population and correlate this exposure with the bioassay. The disadvantage is that the relation of signal transduction alterations in peripheral tissues may not directly correspond to defects in the central nervous system.

Other studies have used cerebrospinal fluid (CSF) as a probe of cAMP-mediated signal transduction in the central nervous system. Changes observed in the CSF offer a more direct measure of brain alterations and patient medication exposure can also be acutely controlled. However, CSF is more difficult to obtain than peripheral blood samples and the relationship of an extracellular measurement, that of CSF levels of cAMP, to intracellular events in nerve cells is not established.

Studies of human postmortem material offer the possibility for study of particular brain regions of interest and for directly examining different steps in the pathway, as opposed to measuring one indicator level as in the CSF. The disadvantages of postmortem studies are well known and include the difficulty in obtaining material, the lack of control and/or knowledge of medication exposure, the variability in the agonal state of the patients, and the variability of tissue preservation/preparation. Nonhuman models of schizophrenia have not yet progressed to the point where studies of signal transduction here would be informative of the human illness. However, material from experimental animals treated with neuroleptic medication give useful insights into the effect of neuroleptic drugs in isolation from the underlying illness. The results obtained by these approaches will be reviewed below.

cAMP Signal Transduction Studies in Platelets

Studies of blood platelets have indicated that cAMP-mediated signal transduction may be altered in schizophrenia. In platelets, the cAMP pathway has been studied using the prostaglandin E₁ (PGE₁) receptor, which is coupled to G_{αs},¹⁵ and the norepinephrine α₂ receptor, which couples to G_{αi}.¹⁶ Basal cAMP production in platelets does not differ in

platelets from schizophrenic patients compared to controls,¹⁷⁻²⁰ though one study has reported a decrease in basal cAMP production in platelets from male, but not female, schizophrenics compared to sex-matched controls.²¹ When PGE₁ is used to stimulate cAMP production in platelets, most¹⁷⁻²¹ but not all²² studies show a significant reduction in cAMP production in the platelets of patients compared to normal controls. This decrease in PGE₁-stimulated cAMP production is not due to an alteration of the prostaglandin receptor because direct stimulation of G-proteins with sodium fluoride,^{17,18} or direct stimulation of adenylyl cyclase with forskolin,^{20,23} shows similar reductions in cAMP production in platelets from patients. These results support an alteration in G_s, adenylyl cyclase, or in the coupling that occurs between receptor, G_{αs}, and adenylyl cyclase. This defect in platelet cAMP signaling does not extend to the α₂ receptor, G_{αi}, or their coupling to adenylyl cyclase. There is no difference between patients and control in the ability of norepinephrine to inhibit PGE₁-stimulated cAMP production.^{18,21,23}

The findings of these studies have been remarkably consistent, and have allowed pharmacological manipulation of cAMP levels to better identify the abnormality in patients. However, a critical question remains: Are alterations in peripheral tissues relevant to the brain? This has been addressed by Kanof and colleagues,²⁴ who found that patients with schizophrenia showed a reduced cAMP response to PGE₁, and that this

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TABLE

RECEPTORS THAT COUPLE TO THE cAMP SIGNAL TRANSDUCTION PATHWAY

G _s COUPLED RECEPTORS			G _i COUPLED RECEPTORS
Neurotransmitter	Receptor Family	Receptors	Neurotransmitter
Adenosine	D ₁	A _{2A} , A _{2B}	Glutamate
Dopamine		D ₁ , D ₅	
Serotonin	β	5-HT ₄ , 5-HT _{5A} , 5-HT _{5B} , 5-HT ₆ , 5-HT ₇	Acetylcholine
Adrenoceptors		β ₁ , β ₂ , β ₃	Adenosine
Histamine		H ₂	Dopamine
Calcitonin gene-related peptide		CGRP ₁ , CGRP ₂	Serotonin
Corticotropin-releasing factor		CRF1, CRF2a, CRF2b, CRF2c	Adrenoceptors
Melanocortin		MC1-R, MC2-R, MC3-R, MC4-R, MC5-R	Cannabinoid
Vasoactive intestinal polypeptide		VIP ₁ , VIP ₂ , PACAP	Galanin
Vasopressin	V ₂	Neuropeptide Y	
			Opioid
			Somatostatin

Adapted from: Watling KJ. *The RBI Handbook of Receptor Classification and Signal Transduction*. 3rd ed. Natick, Mass: Research Biochemicals Inc; 1998.

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response was negatively correlated with several rating scales that measure psychosis, including the Clinical Global Impression scale, the Brief Psychiatric Rating Scale subscales for anxiety, depression, and thought disturbance, and the Scale for the Assessment of Thought, Language, and Communication. This provides evidence that the defects in platelets are part of an alteration in signal transduction that includes the brain.

cAMP Signal Transduction Studies in Cerebrospinal Fluid

Some of the earliest investigations into cAMP-mediated signal transduction in schizophrenia were direct measurements of cAMP levels in the CSF of patients. The baseline levels of cAMP do not differ between schizophrenics and controls.^{25,26} To study the production and entry of cAMP into the CSF, without the potential confound of its rate of removal from CSF, probenecid has been used to block the transport of cAMP out of CSF. Probenecid treatment raises the level of cAMP in the CSF.²⁵ In a group of patients free of neuroleptics for 3 weeks, cAMP levels following probenecid were significantly higher in schizophrenics than in patients with depression or other psychoses.²⁷ While the exact source of CSF cAMP is not known, there is reason to believe that it reflects neural processes affected by both the illness and neuroleptics. Within the group of patients with schizophrenia, higher levels of CSF cAMP are correlated with poor prognosis.²⁸ Treatment with neuroleptic medication

G_i COUPLED RECEPTORS

<i>Neurotransmitter</i>	<i>Receptor Family</i>	<i>Receptors</i>
Glutamate	Group II mGluR Group III mGluR	mGluR2, mGluR3 mGluR4, mGluR6, mGluR7, mGluR8
Acetylcholine	muscarinic	m2, m4
Adenosine		A ₁ , A ₃
Dopamine	D ₂	D ₂ , D ₃ , D ₄
Serotonin		5-HT _{1A-F}
Adrenoceptors	α	α _{2A-D}
Cannabinoid		CB ₁ , CB ₂
Galanin		GalR1
Neuropeptide Y		Y ₁ , Y ₂ , Y ₄ , Y ₅ , Y ₆
Opioid		μ, δ ₁ , δ ₂ , κ, ORL1
Somatostatin		sst ₁ , sst ₂ , sst ₃ , sst ₄ , sst ₅

reduces cAMP in the CSF, as measured either with probenecid treatment²⁷ or without it.²⁹ In both studies, those patients who did not respond to medication did not exhibit a fall in CSF cAMP levels.

cAMP Signal Transduction Studies in Postmortem Clinical Tissue

Direct studies of the cAMP signaling pathway in postmortem tissue have also been performed. As reported in platelets of patients, basal cAMP production does not vary between patients and control subjects when examined in the caudate, nucleus accumbens, hippocampus, or cerebellum.^{30,31} In addition, when dopamine was used to stimulate adenylyl cyclase, no difference in cAMP production was seen between patients and control subjects. However, when the selective D₁ dopamine receptor agonist SKF38393 is used, or if the receptor is bypassed and the G-proteins are directly stimulated with GppNHp or sodium fluoride, there is an enhanced stimulation of cAMP production in the caudate and accumbens of patients with schizophrenia.³¹ This suggests that the coupling between G_{αs} and adenylyl cyclase is more efficient in patients with schizophrenia.

The levels of G-proteins have been studied in postmortem material. Using pertussis toxin, which labels G_{αi} and G_{αo}, the levels of G_{αi}/G_{αo} were shown to be reduced in the left putamen and left hippocampus of patients with schizophrenia.^{32,33} The same reports failed to find a difference between patients and controls in the globus pallidus, caudate nucleus, parahippocampal gyrus, amygdala, insular cortex, or lateral temporal cortex. Later, immunoblotting methods, which allow the quantification of individual G_α subunits, were brought to bear on the question of whether the level of G-proteins was altered in schizophrenia. These experiments confirm decreases in the level of G_{αi} and suggest that G_{αs} levels are unchanged in schizophrenia. In the left temporal cortex, both G_{αi} and G_{αo} are decreased on the left, but not the right side.³⁴ There was no difference in G_{αs} levels in either left or right temporal cortex. Another study of the hippocampus, parahippocampus, putamen, caudate, orbitofrontal, and lateral temporal cortex found a decrease in G_{αo} in hippocampus and caudate in the right hemisphere, but no change in G_{αi} levels.³⁵ A study of frontal cortex found no differences in either G_{αi} or G_{αs} between schizophrenics and control subjects, though an increase in G_{αo} in this region was detected in patients.³⁶ Thus, there exists considerable contradiction in the literature, with individual studies finding alterations in restricted regions that do not always overlap from study to study. However, all the studies agree on normal levels of G_{αs} in schizophrenia and some suggest diverse patterns of decrease in G_{αi} levels.

The levels of adenylyl cyclase have been studied using binding of radiolabeled forskolin. At first, forskolin binding was found to be increased in the

left parahippocampal gyrus and the CA1 field of the hippocampus.³⁷ No change was seen in the rest of the hippocampus, including the dentate gyrus. A second group found no change in forskolin binding in the striatum, frontal cortex, hippocampus, or parahippocampus and a decrease in the dentate gyrus.^{38,39} It is not clear whether this conflicting data results from the inherent variance of adenylyl cyclase levels in postmortem material or from binding of forskolin to the glucose transporter in the earlier study.

Other abnormalities seen in the cAMP-mediated signal processing pathway in the brains of schizophrenics include an increase in the level of cAMP response element-binding protein (CREB) in the cerebellum of patients,⁴⁰ and an increase in cAMP binding sites in the left temporal cortex of schizophrenic patients.³⁴ cAMP binding sites represent the regulatory subunits of PKA.⁴¹ PKA exists as a heterotetramer consisting of two regulatory (R) subunits and two catalytic (C) subunits. PKA-R subunits are divided into two major types RI and RII and each of these types exists in two forms, an α and β isoform. The RII subunits are preferentially found in the particulate fraction, while RI subunits are preferentially found in the cytosolic fraction of cells.⁴² Nishino and colleagues³⁴ examined cAMP binding in soluble fractions of temporal cortex consistent with RI containing PKA. However, it remains to be determined which PKA subunits may be involved in schizophrenia.

These data suggest a complex picture of cAMP-mediated signaling in the brains of patients with schizophrenia. Basal activity of adenylyl cyclase is unchanged, but the coupling of G_{α_s} to adenylyl cyclase is enhanced in patients. In addition, the levels of the inhibitory G-protein, G_{α_i} , may be reduced in patients. Adenylyl cyclase appears to be unchanged, while levels of PKA-R subunits and CREB are increased. These alterations would result in a cAMP system primed to respond to stimuli through both enhanced production of cAMP and increased levels of cAMP-regulated effector proteins.

Effects of Neuroleptic Treatment on cAMP Signal Transduction

As alluded to above, an intact cAMP signal transduction pathway is necessary for many neuroleptic actions. When the RII β subunit of PKA is knocked out in mice, they fail to show acute catalepsy or the typical pattern of gene induction following treatment with haloperidol.⁹ Likewise, DARPP-32 knockout mice show reduced effectiveness of raclopride in inducing catalepsy.¹⁰ The effects of neuroleptics on cAMP-mediated signal transduction have been studied in a variety of models. Such studies are critical to understanding the results of studies of patient populations. In particular, data on the effects of neuroleptic medication on signal transduction is needed to differentiate disease effects from medication effects. In addition, if the alterations seen in patient popula-

tions are directly related to the psychotic state, then medication that alleviates psychotic symptoms might be expected to have an effect on cAMP signal transduction, presumably in a direction opposite to that seen in patients. The studies of neuroleptic effects are in the form of examinations of schizophrenic patients on and off medication, of neuroleptic treated patients with other primary diagnoses, or of animals treated chronically with neuroleptic medication.

The effect of neuroleptic medication on cAMP levels in the CSF has been investigated as described above. Neuroleptic treatment reduced cAMP levels, acting to normalize the abnormality seen in patients and this was associated with clinical response. Neuroleptic effects have not been assessed in neuroleptic naive populations, and the time course of effects from prior neuroleptic treatment are not known. Given the possibility of long-lasting side effects, such as tardive dyskinesia, it has not been possible to chronically treat control subjects with neuroleptics for the purpose of studies such as these. Other neuroleptic-treated patient populations exist (eg, bipolar disorder, Alzheimer's disease) but the underlying pathology of these disorders would confound the interpretation of neuroleptic effects. Therefore, the effects of neuroleptic medication on cAMP signaling have been studied in animals treated with neuroleptics.

Most of the work in animals has been done examining the effect of haloperidol on cAMP production in the striatum. Interpretation of these studies is complicated by different lengths of treatment and especially by the different drug-free intervals prior to sacrifice of the animal. Examination of basal cAMP production in striatal homogenates has shown no change after neuroleptic treatment ranging from 7 days to 1 year.⁴³⁻⁴⁹ Neuroleptic withdrawal periods in these studies ranged from 0-8 days. When particulate fractions of the striatum are studied, the findings have been mixed, with increased basal cAMP production found in some studies^{50,51} but not others.^{52,53} Findings of increased basal cAMP production in striatal particulate fractions are associated with longer neuroleptic-free periods prior to sacrifice (8 and 5 days versus 3 and 0 days) and thus may be related to medication withdrawal, rather than treatment.

Dopamine-stimulated cAMP production is not affected by neuroleptic treatment in striatal homogenates,^{43-47,49} though one study showed a decrease in cAMP responsiveness to dopamine after 1 month of treatment but not longer treatments.⁴⁸ In particulate fractions of striatum, studies of dopamine-stimulated cAMP production have given mixed results. Gnegy and colleagues⁵⁴ found no change in dopamine-stimulated cAMP production. Similar results were obtained by Schettini and colleagues,⁵² but when guanosine triphosphate was added a decrease in dopamine-stimulated cAMP production was observed. Hatta and colleagues⁵³ also found that neuroleptic treatment reduced cAMP produc-

tion in the particulate fraction of the striatum. Another study found an increase in cAMP production in striatal particulate fractions.⁵⁰ However, the period of neuroleptic withdrawal prior to sacrifice was particularly long in this study—14 days—compared with 0–3 days in those that found a decrease.^{52,53} Thus, neuroleptic treatment appears to be associated with a decrease in dopamine-stimulated cAMP production in particulate fractions of the striatum.

Changes in dopamine-stimulated cAMP production might well be due to altered receptor levels. To look at the signal transduction pathway isolated from the receptors that access it, G proteins can be directly stimulated with GTP, guanosine-5'-0-(γ -thiotriphosphate) (GTP γ S), guanosine-5'-[(β , γ)-imido] triphosphate (GppNHp), or sodium fluoride. Striatal homogenates from animals treated with haloperidol for 2 weeks with no withdrawal or 3 weeks and an 8-day withdrawal showed no alteration in cAMP production when G proteins were stimulated directly with GTP γ S, sodium fluoride, or GppNHp.^{49,55} Striatal particulate fractions from animals treated with haloperidol for 21 days with a 3-day withdrawal or 365 days with no withdrawal showed reduced cAMP production when stimulated with GTP or GppNHp.^{52,53} Another study found an increase in cAMP production stimulated by GppNHp after 14 days of haloperidol treatment and a 4-day withdrawal.⁵⁶ This effect was not seen when no withdrawal period was used. These results confirm a reduction in cAMP-mediated signal transduction in the striatal particulate fractions and suggest that this is due to an alteration in G-proteins, adenylyl cyclase, or the coupling between them.

The effects of neuroleptic treatment on cAMP signaling do not appear to be due to altered levels of G $_{\alpha s}$ or G $_{\alpha i}$,^{52,55,57-59} although one study found mixed alterations in G-protein levels in different brain regions.⁶⁰ Likewise, forskolin binding to adenylyl cyclase has been reported to be unchanged by neuroleptic treatment,⁶¹ suggesting that the reduction in cAMP production is not attributable to gross reductions in these proteins.

Conclusion

The results reviewed here make a case for altered cAMP-mediated signal transduction as a component of the schizophrenic syndrome. Stimulated cAMP production is increased in the brain and decreased in the platelets of patients, and both of these alterations are correlated with illness severity and prognosis. Neuroleptic drugs reduce stimulated cAMP production in the central nervous system and CSF studies relate this to clinical response. Both the alterations seen in patients and the effects of neuroleptics occur beyond the level of the receptor, perhaps at the coupling of G $_{\alpha s}$ to adenylyl cyclase and this effect may be specific to particulate fractions of adenylyl cyclase.

A number of questions remain to be elucidated. The alterations seen in the brain and platelets are in the opposite direction. This may be attributable in some aspect of the greater cellular complexity of neurons, perhaps to a form of regulation of G_{α_s} -adenylyl cyclase coupling that does not exist in platelets. Studies of the differences in cAMP signal transduction pathways and their regulation between platelets and neurons will suggest candidate proteins and/or genes for involvement in schizophrenia. A second question that begs further study is why the effects of neuroleptics on stimulated cAMP production in animal models are seen in particulate fractions but not homogenates. This finding is particularly intriguing given the wealth of recent evidence that specific localization is critical for the appropriate function of the effector proteins of the cAMP pathway, PKA, and PP1.^{62,63} It will be important to determine the effects of illness and drug treatment on the subcellular localization of cAMP-mediated signal transduction proteins. Third, the studies in patients and drug-treated animals have focused on G-protein function and adenylyl cyclase activity. Further studies will be necessary to determine if abnormalities in the signal transduction pathway extend further to PKA, PP1, etc. Finally, the studies of postmortem tissue and drug-treated animals have focused on the dorsal striatum. This emphasis was natural given the high levels of dopamine, its receptors, and the cAMP signal transduction system in this structure. Further studies⁶⁴⁻⁶⁶ extending such work to the nucleus accumbens and especially the prefrontal cortex will be of great interest, given the evidence for the involvement of these brain regions in the pathology of schizophrenia. ❖

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