Drugs, Neurotransmitters, and Schizophrenia

Phenothiazines, amphetamines, and enzymes synthesizing psychotomimetic drugs aid schizophrenia research.

Solomon H. Snyder, Shailesh P. Banerjee,
Henry I. Yamamura, David Greenberg

In searching for biochemical correlates of schizophrenia, the first thing to be determined is whether schizophrenia is a distinct entity or simply a conglomeration of more or less distinctive illnesses. When there are no defined organic pathologic disturbances, the psychiatrist is forced to make diagnoses on the basis of fairly arbitrarily selected symptoms. Accordingly, it is not surprising that from country to country and culture to culture there are great disparities in the criteria for a diagnosis of schizophrenia. Since at least some forms of schizophrenia have powerful genetic determinants (*1*), investigators have been able to discern particular symptoms or histories that correlate with apparent genetic loading. In certain of these studies, it has been found that patients with an acute onset and good premorbid history and prognosis seem to lack a genetic "taint," while genetic factors play a prominent role for those whose disturbance begins more insidiously and progresses to profound deterioration. Whether one or several different forms of the disease are genetically determined is unclear.

For patients with "classic" schizophrenia, about whose diagnosis most psychiatrists would concur, certain psychological characteristics, defined by Bleuler (*2*) as the "fundamental" symptoms of schizophrenia, are fairly constant. These include a peculiar thought disorder; a disturbance of emotional, or affective, responses to the environment; and autism, a withdrawal from interactions with other people. Bleuler felt that hallucinations and delusions, which are certainly among the most dramatic manifestations of schizophrenia, are only secondary symptoms, since they are not constant or essential to the disease. The schizophrenic thought disorder, abnormal affect, and autism are difficult to define and even more difficult to identify reliably and reproducibly in patients. By contrast, secondary symptoms are relatively straightforward and have provided more reliable diagnostic techniques for schizophrenia (*3*). Accordingly, many authors have questioned whether Bleuler's primary symptoms are indeed primary. Still, the notion of focusing upon particular behaviors as reflecting either primary or secondary symptoms of schizophrenia might be helpful in seeking biochemical correlates. For instance, if a particular drug regularly evokes hallucinations but no other symptoms of schizophrenia, we would question its value in explaining the pathophysiology of the disease.

While confusion about diagnosis has been a major stumbling block, one must invoke other explanations for the many false hopes and subsequent disappointments in biochemical studies of schizophrenia. Innumerable "discoveries" of the biochemical abnormality in one or another body fluid of schizophrenics have relentlessly been followed by failures of confirmation in other laboratories. Reported abnormalities in parameters as diverse as carbohydrate, protein, amino acid, and lipid metabolism have been advanced, only to be shown by more careful studies to derive from factors such as drug ingestion, diet, muscular activity, and the effects of chronic hospitalization. Besides these difficulties, the discouraging experiences may also stem from a strategy that is sometimes tantamount to searching for a needle in a haystack. Of the literally millions of chemical systems in the human body, why should nature have chosen to inflict the "schizophrenic abnormality" upon whatever specific chemical the experimentalist happens to be best equipped to measure?

A less direct, but perhaps more heuristic, approach might be to follow up leads suggested by known "biochemical" features of schizophrenia. One aspect of schizophrenia with definite biochemical ramifications is the response of patients to drugs. Drugs can be useful in two ways. Phenothiazine drugs are generally acknowledged to be highly efficacious in alleviating symptoms of schizophrenia. If the actions of these drugs derive from effects on whatever is fundamentally deranged in schizophrenic brains, then understanding the mechanism of action might help elucidate purported abnormal brain functioning in schizophrenia. Another way in which drugs can be useful is in eliciting model psychoses, or intensifying schizophrenic symptoms. Certain drug-induced psychoses may be relatively accurate models of schizophrenic disturbance. In some cases, drugs exacerbate symptoms by increasing the schizophrenic pathology itself, rather than merely superimposing nonschizophrenic symptoms. Knowing the neurochemical bases of such drug action should also help in elucidating the pathophysiology of schizophrenia.

If certain drugs appear to be related to schizophrenic disturbance, then one would be justified in seeking out biochemical systems capable of synthesizing the chemicals predicted by drug action to be relevant to the disease. The most promising leads have involved neurotransmitters, especially the catecholamines and indoleamines, and those drugs with which they interact prominently.

Phenothiazines

More than anything else in the history of psychiatry, the phenothiazines and related drugs have influenced positively the fate of schizophrenic patients. They have enabled many patients, relegated in earlier days to a lifetime in mental institutions, to function nor-

Dr. Snyder is professor of pharmacology and psychiatry, Dr. Banerjee and Dr. Yamamura are research associates, Department of Pharmacology, and Mr. Greenberg is a medical student, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

mally or almost normally in society. In determining the relevance of phenothiazines to brain mechanisms in schizophrenia, we must assess whether their therapeutic action involves something fundamental to the disease or whether these drugs are merely some sort of supersedative. One way would be to compare the clinical efficacy of phenothiazines with that of standard sedatives. In large-scale, well-controlled, multihospital collaborative studies sponsored by the National Institute of Mental Health (NIMH) and the Veterans' Administration (VA), a variety of phenothiazines have been compared to sedatives, especially phenobarbital (*4*). Phenobarbital was no more efficacious than the placebo in any of these studies of schizophrenia, whereas most phenothiazines were significantly more effective than either phenobarbital or placebo. One could conceivably argue that other sedatives, such as diazepam (Valium) or chlordiazepoxide (Librium), which are more powerful antianxiety agents than phenobarbital, might compete better with the phenothiazines in the treatment of schizophrenia. However, most trials of these agents have shown them to be ineffective in the treatment of schizophrenia, despite their accepted efficacy in relieving anxiety. Indeed, since drugs such as diazepam and chlordiazepoxide are more effective than phenothiazines in relieving anxiety, one can conclude that anxiety per se is not a unique and primary feature of schizophrenia. Some authorities have argued that phenothiazines can be used to "quiet down" patients. However, numerous studies have demonstrated that, while phenothiazines do calm hyperactive patients, they also "activate" withdrawn patients (*4*).

The NIMH-VA studies provided another means of judging the extent to which phenothiazines exert a selectively antischizophrenic action. Since a large number of patients was rated for a variety of symptoms, one can analyze the extent to which particular clinical features were affected by the drugs. What Bleuler (*2*) referred to as the fundamental symptoms of schizophrenia tend to show the greatest response to drug treatment. Secondary symptoms, such as delusions and hallucinations, respond somewhat less, and nonschizophrenic symptoms, such as anxiety and depression, fail to show any specific improvement with phenothiazines. By contrast, sedatives relieve agitation with much less influence upon thought disorder or the abnormality of affective response to the environment. From data such as these, one can argue fairly convincingly that phenothiazines exert a unique therapeutic effect on schizophrenic patients. One must be cautious before concluding that the drugs directly reverse whatever is biochemically abnormal in the brains of schizophrenics. Phenothiazines might affect by way of an independent pathway emotional functions that are separately influenced by the site of disturbed activity in the brains of schizophrenics. The fact that phenothiazines, although facilitating remission, do not "cure" schizophrenic patients indicates such relatively indirect action. Indeed, failure to maintain schizophrenic patients on phenothiazines while they are in remission results in a much greater incidence of relapse (*5*).

Stimulants

Amphetamines and related stimulants have two effects on brain mechanisms in schizophrenics. In large doses, amphetamines elicit a psychosis that can be clinically indistinguishable from acute paranoid schizophrenia. In very small doses, the stimulant can selectively exacerbate the symptoms of schizophrenic patients (see box on page 1246).

Many cases of amphetamine psychosis have been misdiagnosed as acute paranoid schizophrenia until the history of drug use was obtained (*6, 7*). Accordingly, Kety (*8*) suggested that amphetamine psychosis might be a heuristic model of schizophrenia. Amphetamine psychosis is most frequently observed in addicts who have consumed enormous amounts of the drug over prolonged periods—for example, 500 to 1000 milligrams of *d*-amphetamine every day for a week or more. Patients develop a paranoid psychosis that usually resolves within a few days after they stop taking the drug. They frequently experience auditory hallucinations much like those typical of schizophrenia, including vague noises and voices and occasionally having conversations with the voices. The visual hallucinations in amphetamine psychotics tend to resemble those observed in very acute schizophrenics (*7*).

One factor that has caused some confusion in relating the symptoms of amphetamine psychosis to those of schizophrenia is the fact that amphetamines can evoke more than one type of psychosis. Amphetamines can give rise to an acute "toxic" psychosis, with delirium, confusion, and disorientation that does not resemble the schizophrenia-like amphetamine psychosis. Toxic amphetamine psychosis usually occurs after only one or two extremely large doses, rather than after prolonged use of the drug. Of the 42 cases of amphetamine psychosis studied by Connell (*7*), visual hallucinations occurred primarily among patients who became acutely psychotic after one or a few large doses, and, hence, were presumably suffering from a toxic psychosis. By contrast, hallucinations were usually auditory in patients whose illness developed gradually, after frequent doses. The toxic amphetamine psychoses probably account for the fact that more visual hallucinations occur in amphetamine intoxication than in schizophrenia. In making comparisons with schizophrenia, one should focus primarily on the "nontoxic" amphetamine psychosis, in which patients retain their orientation to person, place, and time and which often closely resembles clinical schizophrenia.

Some authors have criticized amphetamine psychosis as a model schizophrenia, arguing that it might be related to lack of sleep, overexcitement, or precipitation of psychosis in borderline schizophrenics. However, in controlled studies in which large doses of amphetamine were administered to subjects who had no evidence of preexisting schizophrenia or schizoid tendency, psychosis was uniformly produced within 1 to 4 days (*9, 10*). Thus, amphetamine psychosis is not likely to be simply a precipitation of latent schizophrenia. Since some patients became psychotic in about 24 hours, there could not have been sufficient deprivation of sleep to account for the psychosis. As for the question of overexcitement, after some initial moderate euphoria, most subjects were sullen rather than excited, although it is conceivable that there was "internal" hyperexcitement, which might not be evident to observers.

Although amphetamine addicts who have become psychotic after ingesting large amounts of the drug are often clinically indistinguishable from paranoid schizophrenics, subjects in some experimental studies of amphetamine psychosis apparently lack typically schizophrenic thought disorders or affective disturbances (*9*). However, in other studies, with dosage schedules

more closely mimicking the pattern of ingestion of amphetamine addicts, thought disorder, affective disturbance, and auditory hallucinations are consistently observed (*10*).

One important reservation about treating amphetamine psychosis as a model schizophrenia is that it rarely resembles nonparanoid schizophrenia. It is conceivable that amphetamines possess a "pure" schizophrenia-mimicking action, but that some other effect of the drug transforms the clinical picture into a predominantly paranoid one. Perhaps such paranoid action results from the well-known alerting effects of amphetamines on the central nervous system. One might speculate that the major feature differentiating paranoid schizophrenics from other schizophrenics is a hyperalert striving to turn their bewildering array of psychotic transformations into a coherent and meaningful process.

Amphetamines and related stimulants of the central nervous system can, in small doses, exacerbate symptoms of schizophrenia (*11*) rather than superimpose a distinctive psychosis upon the illness. Patients themselves perceive that their illness is worsening under the influence of the drug. By contrast, when schizophrenics are treated with other psychotomimetic drugs, such as LSD (D-lysergic acid diethylamide), they recognize that the superimposed psychosis differs from their own mental disturbance (*12*). The amphetamine analog methylphenidate (Ritalin) produces a florid exacerbation of schizophrenic symptoms when given in extremely low doses—often as rapidly as 2 minutes after an intravenous injection (*11*). To control for the possibility that amphetamines exacerbate schizophrenic symptoms by a nonspecific stimulation of the central nervous system, Angrist *et al.* (*13*) administered large doses of caffeine to schizophrenic and nonschizophrenic subjects. Although all showed tremor, anxiety, and increased heart rate, none showed an increase of psychotic symptoms.

Yet another item favoring an association between amphetamine-induced mental disturbance and schizophrenia is the fact that phenothiazines and butyrophenones seem to be the best antidotes for amphetamine psychosis and can rapidly reverse amphetamine-induced intensification of schizophrenic symptoms (*10, 14*). By contrast, barbiturate sedatives fail to alleviate amphetamine psychosis and in some cases accentuate the symptoms (*14*).

Psychedelic Drugs

Psychiatric researchers first became interested in LSD primarily as a drug that could elicit model schizophrenia. They were impressed by the fact that LSD reproducibly evoked a psychotic state which differed from toxic drug psychoses in that subjects were always alert and reasonably well oriented to time, place, and person. However, detailed comparisons of the mental states produced by LSD and related psychedelic drugs such as mescaline, dimethyltryptamine (DMT), and psilocybin with the typical functioning of most schizophrenics in mental hospitals revealed many differences (*12*). Psychedelic drugs tend to alter visual perception, with few changes in auditory perception. By contrast, although schizophrenics can experience visual hallucinations, these are much less frequent than auditory hallucinations. The psychedelic drug experience is frequently pleasurable, while for most schizophrenics their psychosis presumably is an unpleasant experience. Whether or not a typically schizophrenic disturbance of thinking and feeling takes place in psychedelic drug–induced psychosis is a matter of controversy. Moreover, unlike the case with amphetamine psychosis, individuals under the influence of drugs such as LSD can be readily distinguished from schizophrenics in mental institutions. Schizophrenics receiving psychedelic drugs report that the drug experience is unlike their endogenous psychosis; it seems like something "different" superimposed upon their fundamental disease (*12*).

However, one should be cautious before rejecting out of hand any possibility of a relationship between psychedelic drug psychosis and schizophrenia. Even if a drug acted by disturbing the same site in the brain that is affected in schizophrenia, one would still not expect the effects of the drug to be identical to those displayed by schizophrenic patients. Patients with schizophrenia have been suffering from their disturbance for many years, probably long before overt symptoms were manifested, whereas the drug experience is acute and short-lived. Moreover, an individual receiving a psychedelic drug knows exactly what is happening to him and can anticipate speedy restitution to normality, while the schizophrenic is afflicted with an unknown and unpredictable long-term process. To test whether a drug truly mimics schizophrenia, one should administer the drug surreptitiously every day for several years, beginning in the subject's early childhood.

This line of reasoning suggests that one should compare drug psychosis to the clinical state displayed by schizophrenic patients during their earliest acute breakdown. There seem to be some striking similarities between the subjective states of some early schizophrenic patients and the effects of psychedelic drugs. Psychedelic drugs elicit feelings of enhanced self-awareness, awe, and ecstasy, with sensations of increased acuity and profundity of all sensory perception. Similarly, in case histories of patients suffering acute schizophrenic breakdowns, Bowers and Freedman (*15*) frequently encountered apparent psychedelic experiences. Perceptual modes were heightened, the patients feeling that they had broken through conventional modes of perceiving, thinking, and feeling to attain a "new creativity." Instead of the flattened affect of chronic schizophrenics, these patients experienced intense joy or dread, which is of interest since with psychedelic drugs one often sees an alternation between extremes of elation and abject terror. Although their thinking was altered, these patients often did not display the typical schizophrenic disturbance of thought or feeling. Moreover, in these acute schizophrenics, changes in visual perception were much more frequent than they were in chronic patients. Snyder and Lamparella (*16*) quantified the presence of various "psychedelic" behaviors in schizophrenia and observed that these were much more frequent in acute patients.

This "psychedelic" phase of schizophrenia seems not to be tolerated for long. Either the acute state subsides and normal mental function is restored, or the bewildering experience is resolved by encapsulation into fixed delusional systems, or restricted modes of interacting, including autistic behavior, altered affect, and a formal thought disorder.

Phenothiazines and Catecholamines

Ascertaining the way in which a drug exerts its therapeutic effects is the pharmacologist's most difficult task. Most drugs elicit a myriad of biological effects, the majority of which are unrelated to the therapeutic action of the drug. Phenothiazines are highly

Relations between Drugs, Catecholamines, and Schizophrenia

Phenothiazines (and related antischizophrenic drugs)

Phenothiazines have true antischizophrenic actions:
They are more effective than sedatives.
They act best on fundamental symptoms.
Blockade of dopamine receptors by phenothiazines is closely related to their clinical efficacy.
The ability of phenothiazines to mimic the preferred catecholamine conformation predicts their therapeutic efficacy.

Amphetamines

In small doses, amphetamines specifically activate schizophrenic symptoms.
Amphetamines can evoke a psychosis that is often indistinguishable from acute paranoid schizophrenia.
Phenothiazines appear to be optimal antidotes for amphetamine psychosis.
Behavioral effects of amphetamines are presumably mediated by catecholamines in the brain.

reactive chemicals capable of pi electron donation or acceptance, hydrophobic binding, and ionic links by way of the side-chain amine; they produce biochemical effects on almost every system that has been examined (*17*). How might one decide which of these effects is most relevant to therapeutic efficacy? Of the large number of phenothiazines that have been employed clinically and that are fairly similar in their chemical structure, some are highly effective in the treatment of schizophrenia, others are somewhat less efficacious clinically; while yet others are definitely ineffective. Biochemical actions that correlate with known clinical actions would be the best candidates to mediate the therapeutic actions of the drugs.

Most of the biochemical effects of the phenothiazines do not correlate with clinical potency. The best correlation is in certain effects upon catecholamines in the brain, especially dopamine; this suggests that the therapeutic action of these drugs is related in some way to a blockade of dopamine receptors in the brain (see box).

The catecholamines, norepinephrine and dopamine, are transmitters in a group of well-defined tracts in the brain. Dopamine is best known as the transmitter of a prominent dopamine tract with cell bodies in the substantia nigra and terminals in the caudate nucleus and putamen of the corpus striatum. The nigrostriatal dopamine pathway degenerates in Parkinson's disease, and the attendant dopamine deficiency appears to account, in large part, for the symptoms of the disease. Thus restoration of the depleted dopamine by treatment with L-dopa, the amino acid precursor of dopamine, has proved to be a veritable "miracle" therapy for Parkinson's disease. There are also prominent dopamine pathways with cell bodies dorsal to the interpeduncular nucleus and terminals in the nucleus accumbens and olfactory tubercle, areas of the limbic forebrain, that have been implicated in emotional behavior (*18*). An extensive network of dopamine neurons has been found in the cerebral cortex (*19*).

Carlsson and Lindqvist (*20*) first suggested that phenothiazine drugs act by blocking dopamine receptors. They observed that chlorpromazine and related antischizophrenic agents elevated concentrations of the methoxylated metabolites of dopamine in the brain, while the antihistaminic phenothiazine, promethazine, which is not effective in treating schizophrenia, did not alter these concentrations. Haloperidol, a butyrophenone drug with antischizophrenic actions similar to, but more potent than, the phenothiazines, was correspondingly more potent in elevating the concentrations of these metabolites. Carlsson and Lindqvist speculated that the phenothiazines block catecholamine receptor sites, whereupon a message is conveyed by means of a neuronal feedback to the cell bodies: "We receptors are not receiving enough transmitter; send us more catecholamines!" Accordingly, the catecholamine neurons proceed to fire more rapidly and, as a corollary, synthesize more catecholamines and release more metabolites. These speculations have been confirmed in studies showing that phenothiazines and butyrophenones do accelerate catecholamine synthesis in proportion to their clinical efficacy (*21*). The influence of these drugs upon dopamine synthesis correlates much better with clinical effects than their actions on norepinephrine synthesis. Indeed, several extremely potent butyrophenone tranquilizers selectively accelerate dopamine turnover with negligible effects on norepinephrine. Kebabian *et al.* (*22*) have shown that a dopamine-sensitive adenylate cyclase in the caudate nucleus is inhibited by low concentrations of phenothiazines and butyrophenones that are clinically effective in treating schizophrenia, but not by phenothiazines that are ineffective in treating schizophrenia. Aghajanian and co-workers (*23*) have demonstrated an inhibitory effect of iontophoresed dopamine on olfactory tubercle cells receiving dopamine terminals. Very low doses of intravenously administered phenothiazines and butyrophenones block this effect of dopamine in proportion to their clinical efficacy in treating schizophrenia.

How do the phenothiazines, which are complex, multiringed structures, interact with the receptor for dopamine, a simple phenethylamine? Chlorpromazine in its preferred conformation, as determined by x-ray crystallography, can be partly superimposed upon the preferred conformation of

Table 1. The relative affinities of phenothiazines and butyrophenones for muscarinic cholinergic receptor binding in the brain correlate inversely with extrapyramidal side effects.

Drug class	Relative affinity for muscarinic receptor*	Frequency of extrapyramidal side effects† (*27, 28*)
Dibenzodiazepine		
Clozapine	385.0	5
Piperidine phenothiazine		
Thioridazine	66.7	4
Alkylamino phenothiazine		
Promazine	15.2	3
Chlorpromazine	10.0	
Trifluopromazine	10.0	
Piperazine phenothiazine		
Acetophenazine	0.91	2
Perphenazine	0.93	
Trifluoperazine	0.91	
Fluphenazine	0.91	
Butyrophenone		
Haloperidol	0.21	1

* Affinity for the muscarinic receptor is defined as the reciprocal $\times 10^{-5}$ of the molarity of the drug that displaces by 50 percent the specific binding of [^{3}H]QNB (1 n*M*) to whole rat brain homogenates (*31*). Effective dose (ED_{50}) values were obtained by log probit plots of the effects of four concentrations of each drug assayed three times. Each experiment was done twice. The same relative affinities were observed in experiments with clozapine, thioridazine, chlorpromazine, and trifluoperazine in rat corpus striatum and in monkey putamen. Similar relative affinities were detected in assays of the effect of thioridazine, chlorpromazine, and trifluoperazine on specific [^{3}H]QNB binding to homogenates of guinea pig ileum. † Rank by class; 1 indicates the most side effects.

dopamine or norepinephrine, providing a molecular mechanism whereby phenothiazines might block dopamine receptors (*24*). In the preferred conformation of chlorpromazine, its side chain tilts away from the midline toward the chlorine-substituted ring (Fig. 1). Presumably the chlorine on ring a is responsible in some way for the "tilt" of the side chain, since if there were no substituent on ring a, both rings a and c would be symmetrical and one would expect the side chain to be fully extended. Accordingly, phenothiazines lacking a substituent on ring a should mimic the conformation of dopamine less efficiently, have less affinity for dopamine receptors, and therefore be less efficacious in the treatment of schizophrenia. Of the numerous phenothiazine tranquilizers that have been widely employed clinically, only two lack a substituent on ring a. Mepazine and promazine, the two phenothiazines lacking a ring a substituent, are significantly less effective as antischizophrenic drugs than the others (*4*). Besides the ring a substituent, another major requirement for therapeutic efficacy is that the side-chain amine of phenothiazines contain three carbons; phenothiazines with two-ringed side chains lack antischizophrenic efficacy. Phenothiazines with two-carbon side chains, such as the antihistamine promethazine and the anti-parkinsonism agent diethazine, are less capable of assuming the dopamine-like conformation than those with three-carbon side chains (*24*).

Besides being associated with antischizophrenic activity, dopamine receptor blockade by phenothiazines and butyrophenones may explain the prominent extrapyramidal side effects of these drugs. By blocking the dopamine receptors in the corpus striatum, these agents produce a functional deficiency of dopamine. One might speculate that, while the parkinsonism-like side effects of the phenothiazine drugs arise by blocking dopamine receptors in the corpus striatum, the antischizophrenic action of the phenothiazines may be related to effects upon dopamine receptors in other areas of the brain, such as the olfactory tubercle, nucleus accumbens, or the dopamine receptor sites in the cerebral cortex.

In most of their biochemical features, dopamine neurons in different parts of the brain and periphery behave quite similarly. Thus the reuptake process of dopamine nerve terminals, which presumably serves to inactivate synaptically released dopamine, appears to be the same in dopamine terminals of the corpus striatum, olfactory tubercle, nucleus accumbens, median eminence, and retina (*25*). Drug responses of dopamine receptors in the olfactory tubercle, corpus striatum, kidney, and superior cervical ganglia are also quite similar (*25, 26*).

Phenothiazines and Acetylcholine Receptors

Since the phenothiazines and butyrophenones act similarly at all dopamine receptors, they should, when given in therapeutic antischizophrenic doses, produce the same incidence of extrapyramidal side effects. Although all antischizophrenic drugs produce extrapyramidal effects, the frequency varies considerably. The piperidine phenothiazine thioridazine produces extrapyramidal side effects less frequently than chlorpromazine (*27*), and the recently introduced antipsychotic agent clozapine elicits few, if any, such side effects (Table 1) (*28*). The piperazine phenothiazines and the butyrophenones evoke a much higher incidence of extrapyramidal side effects than does chlorpromazine. These discrepancies seriously challenge the dopamine hypothesis of schizophrenia.

Recent studies of the muscarinic acetylcholine receptor in the brain may provide a resolution of this dilemma. Acetylcholine actions on smooth muscle, glands, and many sites in the brain involve receptors called "muscarinic" because they are mimicked by the alkaloid muscarine and differ from "nicotinic" acetylcholine effects on skeletal muscle and some spinal cord synapses. For more than a hundred years the belladonna alkaloids, such as atropine, which act as antagonists of muscarinic acetylcholine receptors, have been used to treat Parkinson's disease. Until the advent of L-dopa therapy to replace the dopamine deficiency in the brains of parkinsonian patients, anticholiner-

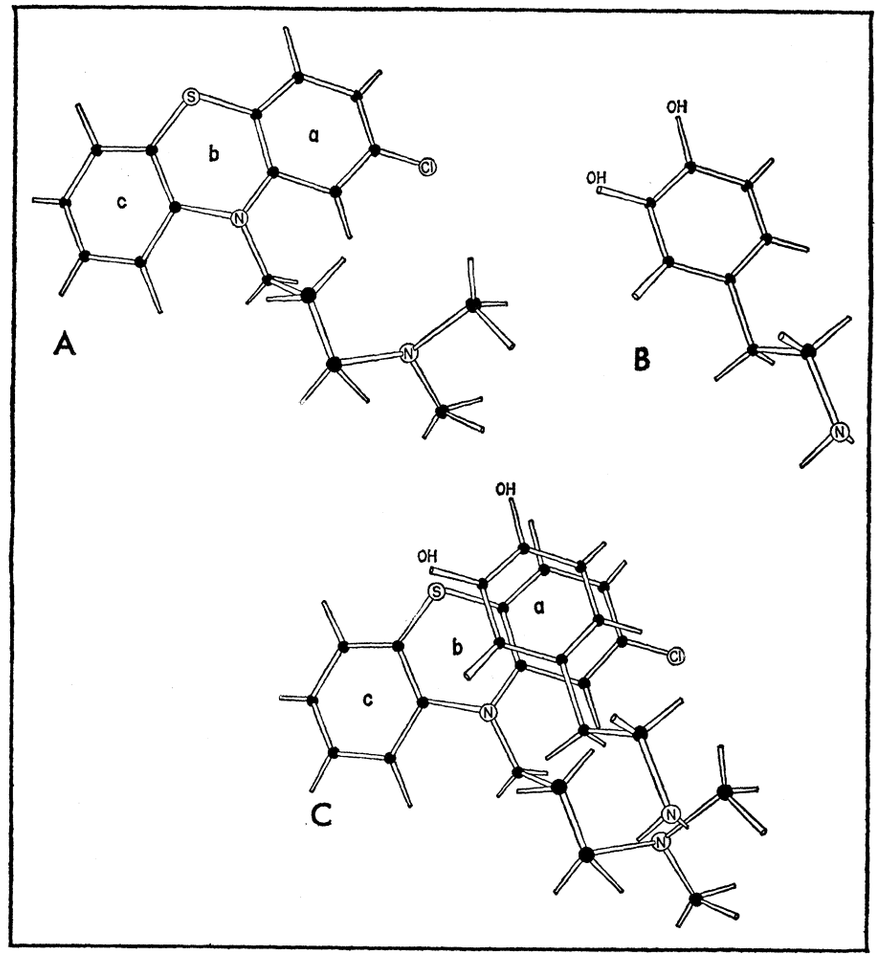

Fig. 1. Conformations of chlorpromazine (A), dopamine (B), and their superimposition (C), determined by x-ray crystallographic analysis. The a, b, and c in (A) and (C) designate rings. [Adapted from Horn and Snyder (*24*)]

gic drugs that act on the central nervous system constituted the major drug therapy for this disease. Anticholinergic drugs have been especially effective in combating the extrapyramidal side effects of phenothiazines. Their therapeutic efficacy apparently reflects a balance in the corpus striatum between dopamine and acetylcholine such that antagonizing acetylcholine effects is equivalent to enhancing those of dopamine, and vice versa.

Phenothiazines often produce muscarinic anticholinergic effects, such as dry mouth and difficulty in urination. Clozapine has few extrapyramidal effects, perhaps because it is a fairly potent antagonist of acetylcholine in smooth muscle (*29*). We speculate that, for all phenothiazines and butyrophenones, extrapyramidal effects vary inversely with anticholinergic potency. Certain phenothiazines may block muscarinic acetylcholine receptors in the corpus striatum, thereby attenuating the extrapyramidal side effects phenothiazines themselves evoke by means of dopamine receptor blockade. The most potent anticholinergics should evoke the fewest extrapyramidal effects; conversely, drugs with the highest incidence of the side effects should be the weakest anticholinergics.

To evaluate this hypothesis, one must be able to quantify the affinity of drugs for muscarinic cholinergic receptors in the brain. Anticholinergic effects on the periphery are readily detected by measuring smooth muscle contractions, but these contractions may not correspond precisely to relative potencies in the brain. Recently, techniques have been developed to identify biochemically the brain's muscarinic cholinergic receptor (*30, 31*). We have measured the reversible binding of 3-quinuclidinylbenzilate (QNB), a potent antagonist of muscarinic cholinergic receptors, to membrane preparations from the central nervous system. The binding of highly radioactive QNB represents an almost exclusive interaction with muscarinic cholinergic receptors (*32*).

With a simple, sensitive, and specific assay for the muscarinic cholinergic receptor in the brain, we evaluated the relative affinities of a variety of antischizophrenic drugs (Table 1 and Fig. 2). Their affinity for the muscarinic receptor in the brain correlates inversely with their tendency to elicit extrapyramidal side effects. Clozapine, which is almost devoid of these side effects, has the greatest potency, similar to that of standard anti-parkinsonism drugs. Thioridazine, which next to clozapine elicits the fewest extrapyramidal symptoms, is second most potent. The alkylamino phenothiazines, whose moderate incidence of extrapyramidal actions is greater than that of thioridazine, have correspondingly less affinity for the acetylcholine receptor. Piperazine phenothiazines and the butyrophenones, whose frequency of extrapyramidal effects is greatest, have the least affinity for the muscarinic receptor. According to this formulation, when given in antischizophrenic doses, all phenothiazines and butyrophenones produce comparable dopamine receptor blockade, thus all have about the same tendency to elicit extrapyramidal side effects. Blockade of acetylcholine receptors by drugs such as clozapine and thioridazine combats these extrapyramidal effects, while, because of their negligible anticholinergic activity, the piperazine phenothiazines and butyrophenones elicit many more extrapyramidal side effects.

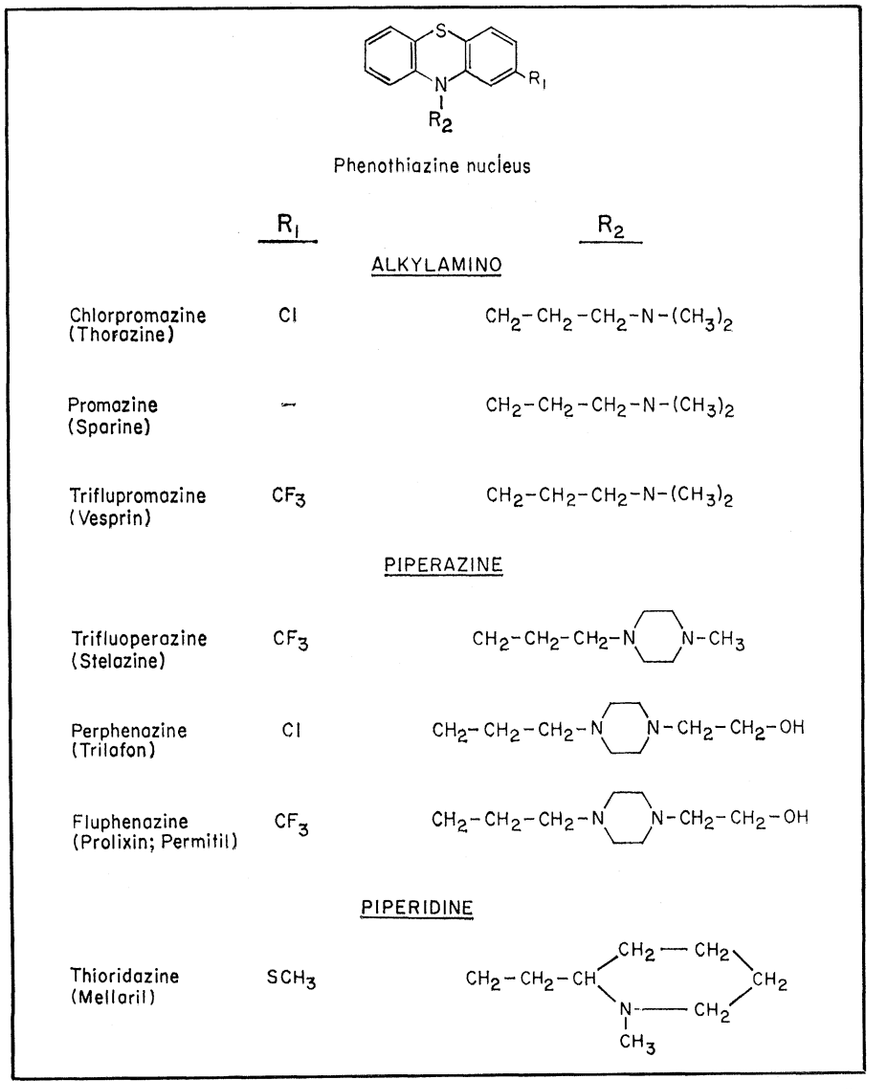

Fig. 2. Structures of different classes of phenothiazines. [Adapted from Snyder (*84*)]

Amphetamines and Catecholamines

While the structural relationship between phenothiazines and the catecholamines was far from obvious, amphetamines, whose chemical structure closely resembles that of the catecholamines, have always been assumed by pharmacologists to act by way of these neurotransmitters. Which of the various dopamine and norepinephrine pathways in the brain mediates particular behavioral effects of amphetamines? In animals, one can make a discrete lesion in individual catecholamine pathways with 6-hydroxydopamine and examine the behavioral consequences. 6-Hydroxydopamine is selectively accumulated into catecholamine neurons,

whereupon it auto-oxidizes and destroys them (*33*). After evaluating the influence of particular lesions on individual behavioral effects of amphetamines, one can make inferences about the tracts mediating the behaviors.

In relatively high doses, amphetamines elicit stereotyped compulsive behavior in animals, the exact pattern varying with different species, but often resembling a searching form of behavior. Rats, whose major means of exploring their environment is olfactory, tend to stay in one portion of the cage, sniff, lick, and especially gnaw (*34*). Cats confined to their cages become involved in repetitive sniffing motions, while less confined cats develop constant, purposeless side-to-side looking movements that may be a response to fear (*35*). Chimpanzees intoxicated with amphetamines display side-to-side looking patterns, as well as self-picking and self-grooming behavior (*36*). These effects of amphetamines closely resemble the stereotyped compulsive behavior frequently observed in addicts who consume large amounts of the drug (*37*). Amphetamine addicts have a compulsion to take objects apart, to sort the parts, and occasionally to put them back together again. Like the monkeys who, under the influence of amphetamines, constantly pick at their skin, amphetamine addicts indulge in "grooming" behavior; this behavior is generally associated with tactile hallucinations that bugs or amphetamine crystals are creeping beneath their skins. Of particular interest is a report that the stereotyped compulsive behavior appears to be an invariable concomitant of psychosis and does not occur in amphetamine addicts who do not develop psychosis (*38*).

An abundance of evidence suggests that stereotyped compulsive behavior of rodents is mediated by way of dopamine pathways in the brain. Thus, making a lesion in the substantia nigra with 6-hydroxydopamine, with complete degeneration of the nigrostriatal dopamine pathway and some loss of dopamine terminals in the nucleus accumbens and olfactory turbercle, abolishes amphetamine-induced stereotyped compulsive behavior, while locomotor stimulation by the drug continues (*39*). Lesions in the corpus striatum can abolish stereotyped behavioral effects of amphetamines (*40*). However, since the nucleus accumbens and the olfactory tubercle are adjacent to the corpus striatum, it is possible that one or the other of these areas is involved in mediating certain components of the stereotyped behavioral syndrome (*41*). Injecting dopamine or apomorphine (which is thought to stimulate dopamine receptors) into the vicinity of the corpus striatum, nucleus accumbens, and olfactory tubercle elicits stereotyped behavior in rats (*42*).

In lower doses, amphetamines stimulate locomotor activity of rodents, an effect that has been thought to mirror the actions of amphetamines in man. Intraventricular administration of norepinephrine produces a similar locomotor activation, but dopamine is much less effective (*43*). Since the naturally occurring (−)−isomer of norepinephrine is much more potent in stimulating locomotor activity after intraventricular administration than is the (+)−isomer, it would seem that locomotor stimulation is dependent on a stimulation of norepinephrine receptors. Postsynaptic dopamine receptors differentiate less well between the isomers of norepinephrine (*44*). Drugs that block the conversion of dopamine to norepinephrine diminish the locomotor stimulant, but not the stereotyped behavioral effects of amphetamines, again suggesting that norepinephrine rather than dopamine is responsible for mediation of this behavior (*45*).

Another dramatic behavioral effect of amphetamines is their ability to greatly facilitate hypothalamic self-stimulation. Animals with electrodes in the lateral hypothalamus will press levers at astronomical rates to obtain electrical stimulation, which suggests that these areas are pleasure centers. This action of amphetamines may be related to the euphoric effects of the drug in man. Several findings indicate that norepinephrine fibers may be responsible for hypothalamic self-stimulation, as Stein suggested (*46*). The "map" for hypothalamic self-stimulation correlates with norepinephrine fiber distribution (*47*). In addition, drugs that block conversion of dopamine to norepinephrine depress hypothalamic self-stimulation (*48*). The depressed self-stimulation rates are restored to normal by intraventricularly administered (−)−norepinephrine, but not by its physiologically inactive (+)−isomer or by dopamine (*48*). There appear to be at least two distinct self-stimulation systems in the brain. Self-stimulation can also be obtained from the area of the substantia nigra, which is rich in dopamine cell bodies (*49*).

Brain lesions, which provide powerful tools for delineating neurochemical mediation of drug effects in animals, can hardly be applied to human beings. Unfortunately, there are no faithful animal models for human conditions such as schizophrenia and amphetamine psychosis. Attempts have been made to draw inferences about the involvement of individual catecholamines in human behavior by using isomers of amphetamines. Such efforts are based on the differences in relative affinities of norepinephrine and amphetamine isomers for dopamine and norepinephrine neuronal uptake systems and for norepinephrine depletion (*25, 50, 51*), although there are discrepant biochemical observations (*52*). In several studies, behavior generally thought to involve primarily norepinephrine is elicited much more efficiently by (+)−amphetamine than by (−)−amphetamine, whereas the two isomers have more similar potencies in enhancing dopamine-mediated behaviors. Thus (+)−amphetamine is seven to ten times as potent as (−)−amphetamine in facilitating hypothalamic self-stimulation, which is generally thought to be norepinephrine-mediated (*46, 53*). By contrast, the two isomers are about equal in facilitating self-stimulation in the dopaminergic substantia nigra (*49*). (+)−Amphetamine is only about twice as potent as (−)−amphetamine in evoking stereotyped behavior in rats (a dopamine-mediated behavior), whereas (+)−amphetamine is ten times as potent as (−)−amphetamine in facilitating locomotor activity (*50*). The rotating behavior of rats following unilateral lesions of the substantia nigra, a motor activity that is determined by dopamine neuronal pathways, is facilitated to an equal extent by (+)− and (−)−isomers of amphetamine (*54*).

Behavioral effects of amphetamine isomers have been explored in man. (+)−Amphetamine is known to be about five times as potent as (−)−amphetamine in its alerting and euphoric effects, which accordingly might be hypothesized to involve predominantly norepinephrine neurons. Amphetamine isomers have been evaluated in studies of amphetamine psychosis (*10*) and schizophrenia (*55*) in human volunteers. In both situations, (+)−amphetamine is less than twice as potent as (−)−amphetamine in the precipitation of amphetamine psychosis in nonschizophrenics and in the florid exacerbation of schizophrenic symp-

Table 2. Characteristics of 5-methyltetrahydrofolic acid (MTHF) mediated amine methylation. Substrates: (i) *N*-methylation utilizes phenethylamines and indoleamines with primary or secondary amine groups (*71, 72*) (tyramine, amphetamine, dopamine, epinine, norepinephrine, normetanephrine, mescaline, tryptamine, *N*-methyltryptamine, 5-methoxytryptamine); and (ii) *O*-methylation utilizes 5-hydroxyindoleamines (*72*) [serotonin, *N,N*-dimethylserotonin (*bufotenin*)]. Affinity for substrates (*72*): MTHF, $K_m = 1 \times 10^{-5} M$; tyramine, $K_m = 1 \times 10^{-3} M$; serotonin, $K_m = 2 \times 10^{-4} M$.

Tissue*	Distribution of *N*- and *O*-methylation of indoleamines in rat		
	O-methylation	*N*-methylation	Ratio of *N*-methylation to *O*-methylation
Brain	97	16	0.16
Heart	145	15	0.10
Intestine	85	37	0.44
Kidney	259	31	0.12
Liver	66	60	0.91
Lung	65	0	0.0
Spleen	52	20	0.38

* Tissues were homogenized in ten volumes of 5 m*M* sodium phosphate buffer (*p*H 7.9). After dialysis, the solutions were centrifuged at 100,000*g* and the supernatants were assayed for enzyme activity. Bufotenin (5 m*M*) and 5-methoxytryptamine (5 m*M*) were substrates for *O*-methylation and *N*-methylation, respectively. [^{14}C]MTHF (20 μM) was the methyl donor. Data are presented as the mean of three experiments. Enzyme activity is expressed as picomoles of methylation per milligram of protein per hour.

toms; this suggests that both phenomena are dopamine-mediated. However, complex and conflicting biochemical data (*51, 52*) preclude firm conclusions. Interestingly, physostigmine, which inhibits acetylcholinesterase and thus increases the effects of acetylcholine, can prevent the psychosis-worsening action of the amphetamine analog methylphenidate (*55*). This suggests a balance between catecholamines and acetylcholine in modulating psychotic symptoms.

There is other evidence pointing to a primary role for dopamine in amphetamine psychosis and amphetamine exacerbation of schizophrenia. The stimulant methylphenidate is more active than amphetamine in exacerbating schizophrenic symptoms (*55*), and in certain biochemical systems it seems to have a more selective effect upon dopamine, as compared to norepinephrine, neuronal disposition (*25, 56*). L-Dopa, whose administration is followed by a considerable formation of dopamine with very little norepinephrine synthesis, exacerbates schizophrenic symptoms in a selective fashion, much like amphetamines (*57*). Although L-dopa does produce psychiatric side effects in parkinsonian patients, there are few reports of anything resembling amphetamine psychosis (*58*). It is conceivable that these results are related to the enormous doses of amphetamine (300 to 500 milligrams) required to elicit a psychosis in nonschizophrenic subjects. To obtain comparable catecholamine-enhancing effects with L-dopa would probably require much higher doses than are generally administered to parkinsonian patients. Cocaine, which facilitates the actions of both norepinephrine and dopamine, produces a psychosis that is virtually identical to amphetamine psychosis (*59*).

Amine-Related Enzymes in Schizophrenics

The relationships among the catecholamines, phenothiazines, amphetamines, and schizophrenia are tantalizing. However, one should be cautious before arguing that these relationships bear upon the hypothetical biochemical lesion in schizophrenia. None of the studies cited presents any direct evidence of a biochemical abnormality in schizophrenic patients. The effects of drugs could well be exerted at a locus extraneous to the fundamental schizophrenic abnormality. What direct evidence is there of abnormal brain chemistry in schizophrenics? Are there biochemical pathways in mammalian brains that could under any circumstances give rise to "psychotomimetic" substances?

Many reports of "abnormal" chemicals, such as adrenochrome and taraxein, in body fluids of schizophrenics have not been confirmed (*8, 60*). The reported selective occurrence of dimethoxyphenylethylamine in urine of the schizophrenics, although confirmed by some workers, has been attributed by others to drugs and diet (*60, 61*).

With reference to biogenic amines, two observations are relevant. Murphy and Wyatt (*62*) observed a highly significant reduction in the activity of monoamine oxidase in platelets of both chronic and acute schizophrenic patients. Concentrations of this enzyme in patients were only about half those in control subjects. Platelet monoamine oxidase concentrations were uninfluenced by drug treatment, and the schizophrenic abnormalities were present in some patients who had never received these drugs. Depressed patients possessed normal enzyme activity, although some bipolar manic-depressive patients displayed monoamine oxidase levels intermediate between those of chronic schizophrenics and controls. Of particular interest is the finding that platelet monoamine oxidase activity was highly correlated in monozygotic twins, only one of whom was schizophrenic (*62*). Thus the reduction in enzyme activity is not a product of the schizophrenic illness; rather, it may provide a genetic marker for vulnerability to schizophrenia. If monoamine oxidase in the brain has the same genetic determinants as the platelet enzyme, then, on the basis of these findings, there should be decreased monoamine activity in the brains of individuals vulnerable to schizophrenia. Preliminary investigations, however, have not revealed abnormalities in monoamine oxidase in the brains of schizophrenics (*63, 64*). The data on amphetamines and phenothiazines are consistent with an excess of dopamine-like activity in the brain, which could conceivably result from reduced monoamine oxidase activity.

Wise and Stein (*64*) have observed lower dopamine β-hydroxylase activity in the brains of schizophrenic patients than in the brains of nonschizophrenic patients. They conducted control experiments to rule out the possibility that their findings resulted from postmortem changes or from the effects of drugs ingested by the patients. As a result of lowered dopamine β-hydroxylase activity, dopamine concentrations might build up in the brains of schizophrenic patients, which again is consistent with what is known of drug actions in schizophrenia. One might also predict, based on observations of Wise and Stein, that net accumulation of norepinephrine would be deficient in the brains of schizophrenics; however, this prediction does not accord with the histochemical data of Olson (*65*), which indicate that norepinephrine fluorescence is the same in the brains of control subjects and schizophrenics.

Amine-Methylating Enzymes that Synthesize Psychotomimetic Drugs

Rather than search for quantitative changes in normal metabolic pathways in schizophrenia, one might search for enzyme systems capable of synthesizing psychotogenic compounds. The known psychedelic drugs are either *O*-methylated (for example, mescaline) or *N*-methylated (for example, dimethyltryptamine). Pollin *et al.* (*66*) observed that, of several amino acids administered to schizophrenic patients, only methionine, and sometimes tryptophan, reliably exacerbated schizophrenic symptoms. As with amphetamines, methionine appeared to worsen the actual schizophrenic symptoms rather than superimpose a toxic psychosis.

Several other investigators have confirmed these findings (*67*). Nonetheless, it is difficult to determine whether the "methionine effect" involved a stimulation of biogenic amine methylation or other quantitatively more prominent pathways of this amino acid. Axelrod (*68*) has described an enzyme in the lungs of rabbits that can transfer the methyl of *S*-adenosylmethionine (AMe) to a variety of phenethylamines and indoleamines. Unfortunately, this enzyme can only be found in rabbit lungs. Relatively feeble enzyme activities have been reported in other mammalian tissues, including the brain, which can *N*-methylate indoleamines and phenethylamines (*69, 70*). Saavedra *et al.* (*70*) observed that serotonin, which might be anticipated to be the naturally occurring substrate, is not methylated.

All these *N*-methylations utilize AMe as a methyl donor, although 5-methyltetrahydrofolic acid (MTHF) can serve as the methyl donor in the methylation of dopamine to epinine (*71*). We have found (*72*) that MTHF can serve as a methyl donor in the methylation of a variety of indoleamines, as well as phenethylamines (*73*) (Table 2 and Fig. 3). With MTHF as the methyl donor, this enzymatic activity is much more vigorous than it is with AMe, suggesting a more important biological role for the MTHF reaction than was evident from earlier studies with AMe. We have found amine-methylating activity in a variety of mammalian tissues, including liver and heart. The enzyme in rabbit lungs differs from that in other tissues, since it is the only one that prefers AMe as methyl donor. Both indoleamines and phenethylamines are methylated by the enzyme. No methylation of serotonin can be demonstrated with AMe, whereas with MTHF, serotonin is the best amine substrate.

Studies with AMe had revealed only *N*-methylation of biogenic amines (*68–70*). Our thin-layer chromatographic analyses in several systems showed that with MTHF, while phenethylamine, tryptamine, and 5-methoxytryptamine are methylated on the amine nitrogen, serotonin is predominantly methylated on the 5-hydroxyl group to form 5-methoxytryptamine. We confirmed the *O*-methylation of indoleamines by showing that bufotenin, in which the amine nitrogen is already fully methylated, is an active substrate and is methylated to form 5-methoxy-*N*,*N*-dimethyltryptamine. This is interesting because 5-methoxy-*N*,*N*-dimethyltryptamine is a very potent psychotomimetic drug (*74*), considerably more active than bufotenin, from which it is formed by *O*-methylation. Indeed, it is questionable that bufotenin is a psychotomimetic drug at all, although its failure to produce psychotomimetic effects may be related to

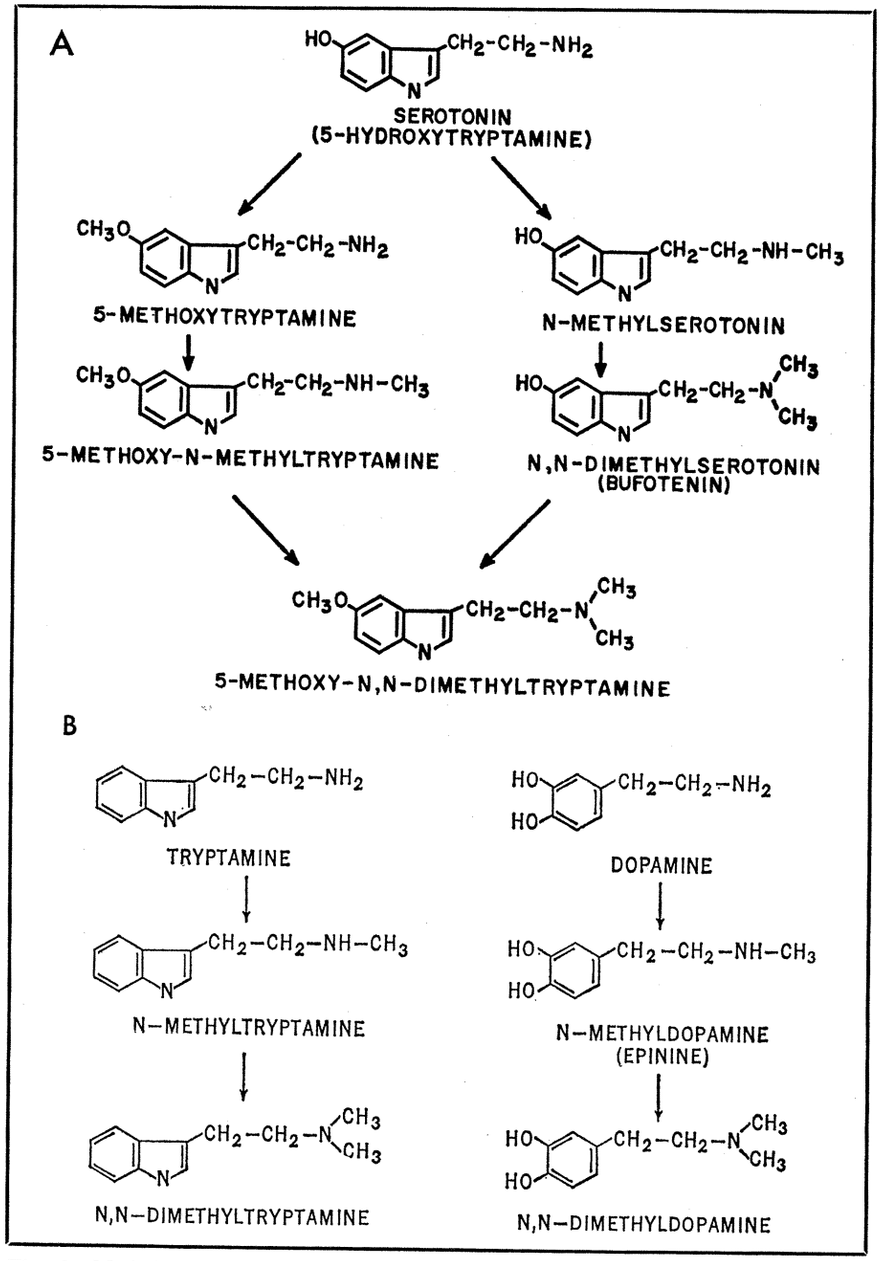

Fig. 3. Methylation reactions of naturally occurring biogenic amines mediated by MTHF: (A) Serotonin can be both *O*-methylated and *N*-methylated; (B) *N*-methylation of dopamine and tryptamine.

difficulty in passing the blood barrier in the brain (*75*).

The inability of serotonin to function as a substrate with AMe may indicate that *O*-methylation requires MTHF, while MTHF and AMe can both serve as donors for *N*-methylation. Arguing for different enzymes are observations that the ratio of methylating activity with AMe to activity with MTHF varies considerably among different tissues, although MTHF is always much more active than AMe in tissues other than the rabbit lung (*72*). Also, partial purification (about 20-fold) of the enzyme from rat brain produced a preparation that methylates vigorously with MTHF, but is completely inactive with AMe (*72*). Although this suggests that one enzyme can use MTHF exclusively in methylating amines, we cannot rule out the possibility that a change in methyl donor properties takes place during purification.

Thus, the amine-methylating enzyme that uses MTHF as methyl donor can transform the neurotransmitter serotonin (5-hydroxytryptamine) into psychotomimetic compounds by *O*-methylation and *N*-methylation (Fig. 3). With the enzyme preparation studied in our laboratory, *O*-methylation of serotonin greatly exceeds *N*-methylation; thus the major first product of enzyme activity is 5-methoxytryptamine. Interestingly, 5-methoxytryptamine has been established as a normal constituent of the brain, with highest concentrations in the hypothalamus (*76*).

Psychotomimetic drugs such as LSD and mescaline are thought to exert their behavioral effects primarily by way of serotonin in the brain (*77*). While serotonin is largely *O*-methylated, tryptamine, which also occurs naturally in the brain (*78*), is *N*-methylated. One of the products of this reaction, *N,N*-dimethyltryptamine (Fig. 3), is a potent psychotomimetic drug.

The fact that serotonin and tryptamine have been definitively shown to be converted to psychedelic-like compounds in the normal brain provokes speculation that variations in the activity of the amine-methylating enzyme play a role in mental disturbances such as schizophrenia. Instead of focusing too narrowly upon possible abnormalities of this enzyme in mental illness, we might do better to consider its potential role in normal mental functioning. The mental state elicited by psychedelic drugs is one of greatly enhanced perception of oneself and one's environment. Similar states occur during mystical and religious introspection and when an individual is profoundly moved by emotions or external events (*79*). Perhaps methoxytryptamine and other methylated amines normally function in modulating people's level of "internal" and "external" perception.

Dopamine, the best neurotransmitter candidate for a role in schizophrenia, can be *N*-methylated to epinine by the amine-methylating enzyme (Fig. 3), as described by Laduron (*71, 73*). He has speculated that schizophrenia arises from a defect in MTHF destruction, resulting in abnormally high MTHF levels and the resulting formation of excess amounts of psychotomimetic, methylated biogenic amines. He cites preliminary evidence that administration of folic acid exacerbates symptoms of schizophrenia, presumably by enhancing the production of MTHF and, thence, psychotomimetic methylated amines.

Whether or not the MTHF-utilizing, amine-methylating enzyme plays a part in schizophrenia or any other psychosis is problematical. The enzyme is not specifically localized in the brain and, in fact, concentrations of it in the brain are lower than those in several other tissues (*72*) (Table 2).

Concluding Remarks

Of various biochemical approaches to the study of schizophrenia, the investigation of brain neurotransmitter interactions with psychotropic drugs has proved most productive in recent years. Analyses of the mechanism of the antischizophrenic activities of the phenothiazines and the ability of amphetamines to worsen schizophrenic symptoms and elicit a schizophrenia-like psychosis have focused attention upon dopamine in the brain. Findings of reduced platelet monoamine oxidase and brain dopamine β-hydroxylase activities in schizophrenics represent enticing but tentative data that would be consistent with a "dopamine hypothesis." The ability of psychedelic drugs to mimic the symptoms of certain early stages of schizophrenia remains a promising lead. An enzymatic activity that utilizes the methyl group of 5-methyltetrahydrofolic acid to *O*-methylate and *N*-methylate phenylethylamines and indoleamines, thereby forming psychotomimetic drugs, is a possible mechanism for the production of such compounds in the mammalian brain. None of these approaches yet affords the definitive "answer" to the riddle of schizophrenia, and roles for other neurotransmitters, such as acetylcholine (*55*) and γ-aminobutyric acid (*80*), are possible.

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51. Norepinephrine and amphetamines are asymmetric phenethylamine structures, which therefore exist in isomeric forms, while dopamine, because of its molecular symmetry, does not possess stereoisomers. The reuptake mechanism in terminals of norepinephrine neurons both in the brain and in most of the peripheral sympathetic nervous system prefers the naturally occurring (—)—norepinephrine isomer (*25*). In all peripheral sympathetic nervous tissues of the rat and in the iris-ciliary body of the rabbit, (+)—amphetamine, the isomer with greater stimulating effects on the central nervous system, blocks norepinephrine uptake more efficiently than (—)—amphetamine. However, in cardiovascular tissues of the rabbit, stereoselectivity is reversed, and uptake is affected more by (—)— than by (+)—amphetamine and more by (+)— than by (—)—norepinephrine (*81*). Studies in our laboratory in synaptosomal (isolated nerve terminal) preparations showed a tenfold greater affinity of (+)— than of (—)—amphetamine for the uptake process of norepinephrine neurons in the brain. Other laboratories have not detected this stereoselectivity (*52*) for reasons that are unclear but that might conceivably relate to difficulties in tissue preparation, since demonstration of stereoselective effects requires careful attention to linearity of uptake with tissue and time and the use of nonsaturating catecholamine concentrations. Dopamine neurons in the brain and retina (*25, 81*) show much less stereoselectivity than norepinephrine neurons in experiments with isomers of norepinephrine, amphetamine, or ephedrine, although distinct stereoselective effects with amphetamines and ephedrines can be demonstrated (*52, 56, 82*). Effects of amphetamines on norepinephrine uptake in vivo are more clear-cut. Inhibition of the initial accumulation of intraventricularly administered [^{3}H]norepinephrine into norepinephrine-rich brain regions is inhibited by (+)— but not by (—)—amphetamine, while the two isomers are equally effective in reducing uptake in the corpus striatum, which is primarily dopaminergic (*50*). Since amphetamine is thought to act as much (or more) by facilitating catecholamine release as by blocking uptake, the effects of amphetamine isomers on processes presumably related mostly to release have been studied (*50, 83*). In rat and mouse brain, (+)—amphetamine is much more potent than (—)—amphetamine in depleting norepinephrine (*50*). Changes in methoxylated metabolites of norepinephrine that may be related to the release process are not affected as differentially by amphetamine isomers (*83*).
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