AMINES IN SCHIZOPHRENIA

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THIS essay is not confined to catecholamines in schizophrenia, because to do so would convey the impression that other amines are irrelevant to the disease. Some drug actions suggest a role for dopamine; others implicate the indoleamines. The 'dopamine story' derives from the influences of antischizophrenic phenothiazines and butyrophenones upon the synaptic activities of dopamine and studies of amphetamine psychosis as a model schizophrenia mediated via brain dopamine. Aspects of indoleamines relevant to schizophrenia are: (a) the psychotomimetic actions of indoleamine-related psychedelic drugs, and (b) the existence of enzymes capable of methylating indoleamines to form psychedelic drugs in the human body.

DOPAMINE

Phenothiazine–catecholamine interactions

Effects of phenothiazines upon brain catecholamines would be relevant to schizophrenia only if the phenothiazine drugs exerted a selective antischizophrenic action so that their primary site of action might be assumed to play a prominent role in brain dysfunction in schizophrenia. This is a very strong assertion which can only be proposed tentatively. However, several lines of investigation do favour the notion that phenothiazines exert a unique antischizophrenic therapeutic action. In very extensive and well controlled studies comparing phenothiazines with sedatives such as barbiturates and the antianxiety agents, a greater efficacy for the phenothiazines has consistently been demonstrated. Moreover, nonsedating phenothiazine drugs are just as effective in treating schizophrenics as the 'sedating' ones. In addition, phenothiazines exert just as much therapeutic benefit in withdrawn as in hyperactive patients. Careful analysis of various symptoms of schizophrenic patients shows that the so called 'fundamental' symptoms of schizophrenia respond selectively to phenothiazine drugs, while accessory and non-schizophrenic symptoms are not as dramatically affected (KLEIN and DAVIS, 1969). These sort of data suggest strongly that the phenothiazine and butyrophenone drugs do exert a very specific therapeutic action in schizophrenia. Of course this action need not necessarily be at 'the schizophrenia receptor' in the brain.

How do the phenothiazines exert their antischizophrenic action? Because they are highly reactive chemicals, phenothiazine effects have been demonstrated upon almost every biochemical system studied. However, most of these actions do not correlate at all with clinical efficacy. Certain phenothiazines, such as the antihistamine promethazine are essentially devoid of antischizophrenic activity, yet are equally active as chlorpromazine in mediating many biochemical effects. The best correlation with clinical efficacy has emerged from studies of the actions of these drugs upon dopamine and norepinephrine disposition in the brain. CARLSSON and LINDQVIST (1963) first advanced

the concept that phenothiazines block catecholamine receptors and that a feedback mechanism causes the catecholamine neurons to increase their firing rate. An increased firing rate should be reflected in enhanced turnover of the catecholamines, which has been well documented (ANDÉN, CARLSSON and HÄGGENDAL, 1969). The acceleration of catecholamine turnover after phenothiazine or butyrophenone drug treatment is more striking for dopamine than norepinephrine, and changes in turnover rate of dopamine correlate better with clinical efficacy than do changes in norepinephrine turnover. Thus, if phenothiazine action involves catecholamine receptor blockade, dopamine is the best candidate. Dopamine receptor blockade in the caudate by phenothiazines has been directly demonstrated by measurements of caudate adenylate cyclase (KEBABIAN, PETZOLD and GREENGARD, 1972) and more recently by direct recordings from olfactory tubercle cells which receive dopamine terminals and upon which the inhibitory effect of iontophoresed dopamine is blocked by phenothiazine or butyrophenone administration (AGHAJANIAN, BUNNEY and KUHAR, 1973).

A molecular mechanism whereby phenothiazines can fit into dopamine receptors is suggested by the close similarity between the optimal conformation of chlorpromazine determined by X-ray crystallography and the optimal conformation of catecholamines (Fig. 1). Indeed the ability of phenothiazines to assume the conformation which mimics dopamine very closely parallels their clinical efficacy. For instance, compounds lacking A ring substituents would be less capable of mimicking the dopamine conformation and, in the case of mepazine and promazine, have been shown to be less effective than other phenothiazines in alleviating schizophrenic symptoms (HORN and SNYDER, 1972). Moreover phenothiazines with a shorter side chain than chlorpromazine, e.g. promethazine, would also mimic the dopamine conformation less well and are less effective in the treatment of schizophrenia.

Amphetamines and catecholamines

Amphetamines link catecholamines and schizophrenia in a fashion opposite to the phenothiazines. While the phenothiazines antagonise schizophrenic symptoms and block dopamine receptors, amphetamines exascerbate schizophrenic symptoms and can elicit a schizophrenia-like psychosis in non-schizophrenic individuals, while enhancing the synaptic actions of dopamine. The amphetamine psychosis produced by large doses of the drug is an acute paranoid psychosis which is frequently mistaken by experienced psychiatrists for acute paranoid schizophrenia (CONNELL, 1958; BELL, 1965). Of all drug psychoses, amphetamine psychosis best mimics schizophrenia, since it is the only drug psychosis which frequently deceives skilled clinicians into a diagnosis of schizophrenia.

Some psychiatrists have speculated that amphetamine psychosis is merely a drug precipitation of latent schizophrenia, an effect of sleep deprivation or of overstimulation. However experimental studies in human subjects with no demonstrable schizoid tendencies have shown that amphetamine can reproducibly elicit psychosis in almost all subjects (GRIFFITH, CAVANAUGH, HELD and OATES, 1972). Since some subjects become psychotic within twenty four hours and since after the first 12 hr most subjects are not overtly stimulated, it is unlikely that the psychosis is related to sleep deprivation or hyperstimulation.

It has been argued that the psychosis does not faithfully mimic schizophrenia, because a typically schizophrenic thought disorder and disturbance of affect cannot be

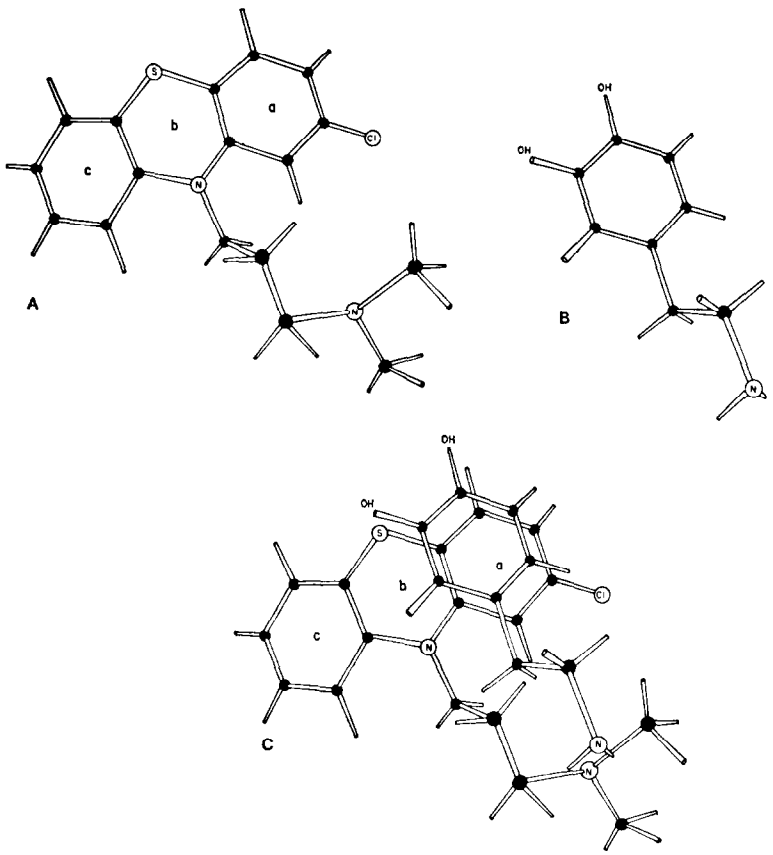

FIG. 1

proven in these individuals. However, in some experimental studies of amphetamine psychosis, the subjects did show thought disorder and affect disturbance (ANGRIST and GERSHON, 1970, 1971).

It is possible that a 'contaminating' alerting action of amphetamine transforms a schizophrenia-mimicking effect of the drug into a paranoid picture (SNYDER, 1972, 1973). In an acute paranoid process, clear-cut thought and affective disorders would not be as readily observed as in a more slowly developing schizophrenia. In addition the concept of a dual action of amphetamine, namely an alerting effect transforming a purer schizophrenia-like state into a paranoid process would explain another objection to the concept of amphetamine psychosis as a model schizophrenia, namely its failure to mimic undifferentiated schizophrenia. Thus, because amphetamine psychosis is almost always manifested as a paranoid psychosis, it is too restricted to be a 'model schizophrenia', unless one postulates extraneous pharmacological actions of the drug transforming the 'schizophreniform psychosis' into a paranoid psychosis.

Another link between amphetamine and schizophrenia is the ability of very small doses of amphetamine or related agents such as methylphenidate, to produce a florid exascerbation of schizophrenic symptoms (JANOWSKY, EL-YOUSEL, DAVIS and SEKERKE, 1973). These drugs do not exascerbate depressive or manic psychosis.

Moreover the psychotic symptoms represent an increase in the patient's schizophrenic psychosis, rather than superimposition of unrelated psychotic symptoms as occurs when schizophrenics are treated with psychedelic drugs.

Most pharmacologists assume that amphetamines act via catecholamines. It is difficult to determine whether amphetamine psychosis or amphetamine exascerbation of schizophrenic symptoms involve dopamine or norepinephrine, since there are no suitable animal models of schizophrenia. Studies with amphetamine isomers have made possible limited neurochemical inferences in humans. *d*-Amphetamine is much more potent than *l*-amphetamine in enhancing behaviours presumably mediated by norepinephrine, while the two isomers are fairly similar in enhancing dopamine mediated behaviours (TAYLOR and SNYDER, 1970, 1971; SNYDER, 1972, 1973). Accordingly it is of interest that *d*- and *l*-amphetamine are similar in their potency in eliciting amphetamine psychosis (ANGRIST, SHOPSIN and GERSHON, 1971) and in exascerbating schizophrenic symptoms (DAVIS and JANOWSKY, 1973). This suggests that dopamine mediates amphetamine psychosis as well as the exacerbation of schizophrenic symptoms by amphetamine.

INDOLEAMINES

Initial evidence suggesting a role of indoleamines in schizophrenia derived from studies of the psychotomimetic effects of psychedelic drugs such as LSD, psilocybin and dimethyltryptamine (DMT) which have been interpreted as resembling the symptoms of schizophrenia. Although one can readily differentiate psychedelic drug psychoses from schizophrenia (HOLLISTER, 1962) there is some evidence that, at least in its early stages, schizophrenia can present a remarkably 'psychedelic' picture (BOWERS and FREEDMAN, 1966). Early in their disease many schizophrenic patients describe perceptual distortions with remarkable visual changes, awesome feelings of enhanced self-awareness and unity with the universe, all of which seem very much like the symptoms evoked by psychedelic drugs.

The existence of psychedelic drug psychosis and the fact that drugs such as mescaline and dimethyltryptamine resemble catecholamines and indoleamines respectively prompted the speculation that the brains of schizophrenics might methylate normally occurring amines to form psychotomimetic agents which account for schizophrenic symptoms. *O*-dimethylation of catecholamines might form dimethoxydopamine, which resembles mescaline, but is not itself psychotomimetic (FRIEDHOFF and VAN WINKLE, 1967). Alternatively one might postulate the *N*-methylation of indoleamines (POLLIN, CARDON and KETY, 1961; MANDELL and SPOONER, 1968). Enzymes which can *N*-methylate a variety of biogenic amines have been reported in rabbit lung (AXELROD, 1962), chick and human brain (MORGAN and MANDELL, 1969; MANDELL and MORGAN, 1971) and in several mammalian tissues (SAAVEDRA and AXELROD, 1972; SAAVEDRA, COYLE and AXELROD, 1973). Unfortunately, these are all relatively feeble enzymatic activities. While all of these *N*-methylations utilise *S*-adenosylmethionine (AMe) as a methyl donor, recently LADURON (1972) showed that methyltetrahydrofolic acid (MTHF) can serve as the methyl donor in the methylation of dopamine to epinine. We have found that MTHF can serve as a methyl donor in the methylation of a variety of indoleamines as well as phenethylamines (BANERJEE and SNYDER, 1973). With MTHF as methyl donor, this enzymatic activity is much more vigourous than with AMe suggesting a more important biological role for this reaction than was evident from earlier studies with AMe.

We found amine methylating activity in a variety of mammalian tissues. The enzyme from rabbit lung (AXELROD, 1962) was quite different from that in other tissues, since it was the only one which preferred AMe as methyl donor. In all of the tissues MTHF was considerably more active than AMe as methyl donor (Table 1). Both indoleamines and phenothylamines were methylated. Strikingly, no methylation of serotonin could be demonstrated with AMe, while with MTHF serotonin was the best amine substrate.

TABLE 1. SPECIES AND TISSUE DISTRIBUTION OF METHYLTRANSFERASE ACTIVITY

Tissues were homogenised in 10 volumes of 5 μM Na-phosphate buffer, pH 7·9, and enzyme activity was assayed in the 100,000 *g* supernatant fraction after dialysis with serotonin (5 mM) or tyramine (5 mM) as substrates and *S*-adenosylmethionine (AMe) (1 μM) or 5-methyltetrahydrofolic acid (MTHF) (1 μM) as methyl donors. Data are the mean of 3 experiments whose results varied less than 20 per cent).

Tissue	Methyltransferase activity*						Ratio of enzyme activity with AMe to activity with MTHF	
	AMe as methyl donor			MTHF as methyl donor				
	Serotonin	Tyramine	Ratio Serotonin/ Tyramine	Serotonin	Tyramine	Ratio Serotonin/ Tyramine	Serotonin	Tyramine
Rabbit lung	32·0	24·0	1·33	8·0	4·0	2·0	4·0	6·0
Brain	0	0·05	0	2·6	1·4	1·9	0	0·04
Liver	0	0·2	0	2·1	1·2	1·7	0	
Rat brain	0	0·4	0	3·0	1·3	2·3	0	0·31
Liver	0	1·2	0	4·3	1·4	3·1	0	0·90
Lung	0	0·35	0	4·0	1·3	3·1	0	0·27
Heart	0	0·4	0	8·0	3·0	2·7	0	0·14
Chick brain	0	1·0	0	6·0	4·0	1·5	0	0·25
Heart	0	2·0	0	26·0	11·0	2·3	0	0·18

* nmoles/mg protein/hr.

Purification of the enzyme 20-fold from rat brain by ammonium sulphate fractionation and negative adsorption on alumina-C-γ gel (BANERJEE and SNYDER, 1973) produced a preparation which methylated vigourously with MTHF but was completely inactive with AMe, suggesting the existence of an enzyme which uses MTHF exclusively in methylating amines, although the possibility of a change in methyl donor properties during purification cannot be ruled out. The purified enzyme as well as the crude supernatant fraction was most active towards serotonin (Table 2). The K_m of the partially purified enzyme for MTHF was 1 μM and its K_m values for serotonin and tyramine were 0·1 mM and 1 mM respectively.

Thin layer chromatographic analysis in several systems showed that, while phenethylamines, tryptamine, and 5-methoxytryptamine are methylated on the amine nitrogen, serotonin is predominantly methylated on the 5-hydroxyl group to form 5-methoxytryptamine. Confirmation of the *O*-methylation of indoleamines was obtained by showing that bufotenin, in which the amine nitrogen is already dimethylated, is vigourously methylated to form the potent hallucinogen, 5-methoxy-*N*,*N*-dimethyltryptamine. The relative methylation of bufotenin and 5-methoxytryptamine is constant in 4 different ammonium sulphate fractions of the enzyme purified from rat liver, suggesting that the same enzyme mediates *O*- and *N*-methylation. The failure of serotonin to function as a substrate with AMe may indicate that *O*-methylation requires MTHF, while both MTHF and AMe can serve as donors for *N*-methylation.

In considering what role this enzyme might play in the patho-physiology of schizophrenia or other psychoses, it is of interest that serotonin is its best substrate.

TABLE 2. SUBSTRATE SPECIFICITY OF PARTIALLY PURIFIED RAT BRAIN METHYLTRANSFERASE WITH MTHF AS METHYL DONOR

The enzyme preparation used was the supernatant fraction after 75% ammonium sulphate precepitation Enzymatic activity is expressed as nmoles/mg protein/hr. The following specific extraction procedures were used for the optimal isolation of a given product: (1) extracted with a mixture of toluene and isoamyl alcohol (3:2) and dried overnight at 80°C in a chromatography oven: (2) extracted with a toluene isoamyl alcohol mixture (97:3) and dried in oven; (3) extracted with isomyl alcohol and dried in oven; (4) extracted as in (2) but counted with no previous drying procedure because of the volatility of the product.)

Substrate	Specific activity	Extraction procedure
Tyramine	4·4	1
Tryptamine	4·3	2
Serotonin	8·8	3
β-phenylethylamine	2·0	4
Octopamine	2·7	1
N-methyltryptamine	3·4	2
N-methylserotonin	7·2	3
Desmethylimipramine	0·0	3

However, it is important to bear in mind that brain was one of the least active of tissues examined.

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