TECHNICAL STRATEGIES FOR THE STUDY OF CATECHOLAMINES IN MAN

DENNIS L. MURPHY
Laboratory of Clinical Science, NIMH, Bethesda, Maryland, U.S.A.

BECAUSE of necessary limitations in obtaining tissue and in administering certain drugs and isotopes, studies of catecholamine metabolism in man often depend on indirect procedures or utilise specialised methods not required in animal investigations. Most human studies have been directed towards the identification of alterations in catecholamine metabolism produced by drugs or by physiologic changes (e.g. exercise) or pathologic states (e.g. cardiovascular, neurologic and psychiatric disorders). The major tactics utilised in the study of biogenic amines in man are outlined in Table 1. Only a small amount of material subsumed under this title can be mentioned here, but some major strategies for catecholamine studies in man (e.g., the probenecid approach to CSF amine metabolite formation) are reviewed in detail elsewhere in this symposium, and some reviews directed toward the study of amine synthesis, turnover and metabolic pathways of biogenic amines in man are listed in the bibliography (SCHILDKRAUT and KETY, 1967; MANDELL and SPOONER, 1968; FRANZEN and EYSELL, 1969; KOPIN, 1972; BARCHAS and USDIN, 1973). This paper will more narrowly focus on attempts to study catecholamines, catecholamine-related enzymes and catecholamine transport, storage and release mechanisms at the cellular level in man. Examples from current studies in this laboratory will be used for illustration.

STUDY OF BIOGENIC AMINE-RELATED ENZYMES IN MAN

Increasing attention is being devoted to the study of enzymes from biogenic amine pathways found in plasma, blood cells and cultured fibroblasts. Many genetically-based disorders of metabolism in other fields have been identified on the basis of enzyme alterations in leukocytes (HSIA, 1972), and in erythrocytes, platelets and plasma (HARRIS, 1971).

Monoamine oxidase (*EC* 1.4.3.4, *MAO*)

MAO has been studied in human platelets and plasma. The platelet enzyme is located in mitochondria, and has many substrate and inhibitor characteristics in common with mitochondrial MAO's of the B type found in other tissues (PAASONEN and SOLATUNTURI, 1965; ROBINSON, LOVENBERG, KEISER and SJOERDMA, 1968; COLLINS and SANDLER, 1971; NEFF and GORIDIS, 1972, MURPHY and WEISS, 1972). The purified platelet enzyme is electrophoretically homogenous, with a molecular weight of 235,000 (COLLINS and SANDLER, 1971). The soluble plasma enzyme is a pyridoxal-containing enzyme which has different substrate specificities compared to platelet and other tissue MAO's and is inhibited by carbonyl reagents but is relatively insensitive to tissue MAO inhibitors such as pargyline, tranylcypromine and iproniazid (ROBINSON *et al.*, 1968; MCEWEN, 1972).

Treatment with MAO inhibiting drugs (MAOI) under ordinary clinical conditions leads to marked inhibition of the human platelet (ROBINSON *et al.*, 1968) and brain

TABLE 1. METHODOLOGIC APPROACHES TO THE STUDY OF CATECHOLAMINES IN MAN

I.	Direct measurement of amines, amine metabolites and amine-related enzymes in blood, urine, cerebrospinal fluid and biochemical or histofluoresence analysis in tissues (e.g. blood cells, surgically-obtained specimens and autopsy material).
II.	Use of radioactively-labeled amine precursors and amines to study pathways of amine synthesis and metabolism.
III.	Use of metabolic loads to stress pathways of amine synthesis and degradation.
IV.	Use of drugs with relatively specfic effects to alter amine synthesis, degradation, turnover, transport, storage and release, with measurement of effects on amines and amine metabolites as above.

enzymes (GANROT, ROSENGREN and GOTTFRIES, 1962), with MAO activity reduced 70–90 per cent after several days of treatment. Inhibition persists for up to 2–3 weeks after discontinuation of the MAOI. Platelet MAO activity appears little affected by drugs other than MAOI or by environmental changes and menstrual cycle changes, although MAO activity in platelets appears to increase with age, particularly over age 50 (ROBINSON, DAVIS J. M., NIES, COLBURN, DAVIS J. N., BOURNE, BUNNEY, SHAW and COPPEN, 1972; MURPHY D. L., unpublished); females tend to have 10–20 per cent higher MAO levels, a difference which again is especially evident over age 50 and also prior to puberty (ROBINSON *et al.*, 1972; MURPHY, unpublished).

Studies comparing MAO activity in normal and psychiatrically-ill monozygotic and dizygotic twins, sibling pairs and random unrelated pairs suggest a high order of genetic influence on platelet MAO activity (Table 2). Moderately reduced platelet MAO activity has been described in bipolar manic–depressive patients and in individuals with acute schizophreniform psychoses, while chronic schizophrenic individuals have more markedly reduced levels (MURPHY and WEISS, 1972; MURPHY and WYATT, 1972). In contrast, slightly elevated (MURPHY and WEISS, 1972) or moderately elevated (NIES, ROBINSON, RAVARIS and DAVIS, 1971) levels are found in other depressed patients. In monozygotic twins discordant for schizophrenia, the twins with and without schizophrenia have MAO values which are reduced in comparison to normal controls, suggesting that the reduction in schizophrenic individuals does not represent an effect of the disorder or its treatment, but rather may provide a genetically-related measure reflecting vulnerability to schizophrenia (WYATT, MURPHY, BELMAKER, COHEN, DONNELLY and POLLIN, 1973).

TABLE 2. INTRACLASS CORRELATION COEFFICIENTS FOR PLATELET MONOAMINE OXIDASE ACTIVITY MEASURED IN NORMAL, SCHIZOPHRENIC AND BIPOLAR TWINS AND IN SIB AND RANDOM PAIRS

	Intraclass correlation
Normals	
Monozygotic twins ($N = 9$)	0·88
Dizygotic twins ($N = 10$)	0·45
Sib pairs ($N = 37$)	0·28
Random pairs matched for age and sex ($N = 37$)	0·12
Monozygotic twins discordant for schizophrenia ($N = 13$)	0·65
Monozygotic twins concordant for bipolar manic-depressive illness ($N = 3$)	0·83

Catechol-O-methyl transferase (EC 2.1.16, COMT)

This enzyme has been identified in human erythrocytes (AXELROD and COHN, 1971) and has similar properties and substrate specificities as COMT in other animal tissues (COHN, 1970). The enzyme appears to be a stable characteristic of the individual and is not affected by tricyclic antidepressants, phenothiazines, lithium carbonate, α-methyl-*para*-tyrosine or *para*-chlorophenylalanine (COHN, 1970; DUNNER, 1971), although chronic L-dopa administration has been reported to increase red cell COMT activity in Parkinsonian patients (WEISS, COHN and CHASE, 1971). Age and sex are said not to influence activity, although reduced COMT levels were observed in women but not in men hospitalised for depression (COHN, 1970). Females with unipolar depression had a significantly greater reduction in COMT activity than did female bipolar patients, while schizophrenic females and men with antisocial personalities were no different from normal controls. Recovery from depression or the development of mania was not associated with any change in enzyme activity (DUNNER, 1971).

Dopamine β-hydroxylase (DBH) and other catecholamine-related enzymes

Plasma DBH also appears to be a relatively stable characteristic of the individual (WEINSHILBOUM and AXELROD, 1971), with twin studies suggesting a marked genetic influence on measured activity (LAMPRECHT *et al.*, 1973). The characteristics of this enzyme in man and alterations found in some clinical populations, including such genetically-based disorders as torsion dystonia (WOOTEN, ELDRIDGE, AXELROD and STERN, 1973) and dysautonomia (WEINSHILBOUM and AXELROD, 1971), are reviewed elsewhere in this symposium. Other catecholamine-related enzymes including tyrosine hydroxylase, *l*-amino acid decarboxylase and phenylethanolamine *N*-methyl transferase have been little studied in man.

STUDY OF BIOGENIC AMINE CELLULAR TRANSPORT AND STORAGE IN MAN

The platelet model

Platelets accumulate biogenic amines and store them in vesicles, reaching concentration gradients over 100:1 for serotonin and dopamine and 5–13:1 for norepinephrine and epinephrine (PLETSCHER, 1968; ABRAMS and SOLOMON, 1969; BORN and SMITH, 1970; BOULLIN and O'BRIEN, 1970; PAASONEN, AHTEE and SOLATUNTURI, 1971; MURPHY and KOPIN, 1972). The transport K_m for serotonin in platelets is 3×10^{-7} M, which is very similar to K_m for serotonin uptake in brain slices (SNYDER, KUHAR, GREEN, COYLE and SHASKAN, 1970). Although dopamine, norepinephrine and epinephrine competitively inhibit serotonin uptake in platelets, a saturable uptake mechanism for catecholamines has only been demonstrated for dopamine (BOULLIN, 1970). Since isolated platelet vesicles also bind catecholamines less avidly than serotonin (DAPRADA and PLETSCHER, 1969), it would appear that the platelet provides a better model system for serotonergic than catecholaminergic neurons. However, the similarity of effects of many drugs such as reserpine, MAO inhibitors and tricyclic antidepressants on both catecholaminergic and serotonergic cell processes permits utilisation of the platelet model system in monitoring drug effects in individuals (ROBINSON *et al.*, 1968; MURPHY, COLBURN, DAVIS and BUNNEY, 1970).

However, this apparent specificity as a serotonergic cell provides an opportunity to investigate the accumulation and fate of other amines not ordinarily found in serotonergic cells; for example, the accumulation of dopamine or a dopamine metabolite during treatment with L-dopa (BOULLIN, 1970; MURPHY *et al.*, 1970), and the accumulation of octopamine during monoamine oxidase inhibitor treatment (CAHAN, MURPHY and MOLINOFF, 1973). *In vitro*, serotonin stores appear to be displaced by dopamine, norepinephrine, epinephrine, tyramine and octopamine. *In vivo*, L-dopa administration leads to a reduction in platelet serotonin content (MURPHY, *et al.*, 1970). Whether this alteration results from competition by catecholamines for uptake of serotonin at the cell membrane level or from serotonin displacement from vesicles, this evidence suggests the possibility that 'false-transmitter' type effects demonstrated in animals may occur in man. Furthermore, the demonstration of several-fold higher levels of octopamine in platelets isolated from individuals receiving monoamine-oxidase inhibiting drugs compared to normal controls strengthens the suggestions made on the basis of animal studies that octopamine accumulation in cells might contribute to some effects of MAO-inhibiting drugs in man such as hypotension (KOPIN, FISCHER, MUSACCHIO and HORST, 1964).

Other cell systems

While human erythrocytes do not contain amine storage vesicles and do not achieve concentration gradients for biogenic amines greater than 2–3:1, some studies of amine distribution across the erythrocyte cell membrane and of catecholamine metabolism by these cells have been accomplished (MURPHY and KOPIN, 1972; DANON and SAPIRA, 1973). Some biochemical (BOURNE, BUNNEY, COLBURN, DAVIS J. M., DAVIS J. N., SHAW and COPPEN, 1968; BEVAN-JONES, PARE, NICHOLSON, PRICE and STACEY, 1972) and histofluorescence studies (DE LA TORRE, 1972) of catecholamines in human brain tissue have been made. Axonal transport of dopamine β-hydroxylase has been studied in biopsy specimens of human sural nerves (BRIMIJOIN and DYCK, in press).

STUDY OF BIOGENIC AMINE RECEPTOR FUNCTIONS IN MAN

Platelets and leukocytes possess an adenyl cyclase system which is responsive to biogenic amines and biogenic amine-affecting drugs (MOSKOWITZ, HARWOOD, REID and KRISHNA, 1971). Platelets can be stimulated by prostaglandin E_1 to produce a 10-fold increase in cell cyclic AMP content; in intact cells, norepinephrine reduces this cyclic AMP formation elicited by prostaglandin E_1, while the norepinephrine effects are antagonised by phentolamine but not by propranolol. This apparent adrenergic α-receptor function in platelets is affected by some psychoactive drugs *in vitro* and by at least one such drug, lithium carbonate, *in vivo* in man (MURPHY, DONNELLY and MOSKOWITZ, in press). Lithium has similar antagonistic effects on catecholamine and other hormone-induced adenyl cyclase activity in other tissues. Norepinephrine and epinephrine also produce platelet aggregation, a cell response which can be prevented by phentolamine and phenoxybenzamine, while propranolol is much less potent than the α-receptor antagonists (MUSTARD and PACKHAM, 1970).

CONCLUSION

These investigations of biogenic amine enzymes, transport and metabolic functions, and receptor effects of amines in human cells suggest a potential for the use of such

approaches in confirming amine metabolic changes studied by other techniques, and in determining individual differences in response to amine-affecting drugs. While cells such as platelets do not synthesise biogenic amines, their enzymes and their transport and storage functions appear to be affected by various drugs in ways similar to those observed in nerve tissue and brain. The possibility of studying genetically-based differences in these membrane-based processes is suggested by the evidence that genetic factors are major determinants of the activity of such enzymes as monoamine oxidase in these cells.

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