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Glycogen phosphorylase levels in the brain of rats treated with psychotomimetic drugs and with tranquilizers

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THE cause of psychosis is still obscure. It is the purpose of this communication to report a finding that appears promising as a possible lead in elucidating such a cause. It was found that a rather close and meaningful relationship exists between the level of glycogen phosphorylase *a* of rat brain and the type of drug that has been administered to the animal. The drugs that depressed the enzyme level belonged, in general, to psychotomimetic drugs, whereas those that enhanced it may be classified as therapeutic in mental illness. This investigation was prompted by a study by one of us* which pointed to a possible, although remote, involvement of the phosphorylase enzyme in psychosis.

Female albino rats of the Wistar strain were used. The animals were sacrificed after a definite period of time following s.c. administration of drugs, and the brains were assayed for phosphorylase activity. The phosphorylase activity determined represents the level found about 3 min after the death of the animal. At this time the activity of *a* is high and is declining very gradually.† Drummond *et al.* have reported that almost fully active *a* was found whether mice were dropped directly into liquid N_2 and the brain removed after freezing, whether anesthetized (pentobarbital) mice were decapitated and the heads frozen, or whether brains were removed from decapitated mice before freezing in liquid N_2.[1] Nevertheless, this high activity might be the result of effects on the "after-death" changes, in view of the report of Breckenridge and Norman that *a*, which is predominantly in an inactive form, is converted within seconds to an active form after death.[2] However, the experiments presented in this note were carried out with very carefully standardized conditions so that any difference observed would be due primarily to difference in treatment and may be significant. Moreover, substances absorbing at 260 mμ were always removed from the homogenate, as well as from glycogen, when deemed necessary, so that the values of *a* would not be influenced by AMP present in the homogenate or glycogen. This procedure was necessary because, without it, the detection of the effect of drugs was impossible.

Large variation in activity was not anticipated, because a slight deviation from homeostasis might be enough to cause mental aberrations. Care was taken, therefore, to enhance the sensitivity for detecting the effects of treatment by improving the technique of assay and by using appropriate experimental designs. The experimental designs were such that the effect of any factor, known or unknown, that might possibly influence the values of determinations would be removed from affecting the error variance by statistical treatment. Statistical analysis, including analysis of variance, was performed on each set of experiments.

The results of three sets of experiments are presented in Table 1. Effects of drugs should be compared with the control of that set of experiments only because conditions of standardization differ slightly from one set to another, resulting in different control values. Comparison of the effects of treatment was made by using the value of phosphorylase determinations without added AMP (active form *a*).

* T. T. Iriye, unpublished.
† Unpublished observation.

TABLE 1. EFFECT OF PSYCHOTOMIMETIC DRUGS, TRANQUILIZERS, AND OTHER DRUGS ON GLYCOGEN PHOSPHORYLASE OF RAT BRAIN *IN VIVO*

Drugs	No. of animals	Dose (per kg.)	Duration of treatment (hr)	−AMP* (phosphorylase units)	Difference from saline	P
Saline	6	5 ml	1	133·0		
LSD-25	6	1 mg	1	124·5	− 8·5	<0·05
BOL-148†	6	1 mg	1	138·8	+5·8	<0·2
Mescaline	6	100 mg	1	125·2	−7·8	<0·05
Bufotenine	6	10 mg	1	127·7	−5·3	<0·2
D-Amphetamine	6	10 mg	1	129·0	−4·0	<0·3
Saline	8	5 ml	4	123·0		
Chlorpromazine	8	10 mg	4	137·4	+14·4	<<0·001
Trifluoperazine	8	10 mg	4	132·0	+9·0	<0·005
Chlorpromazine sulfoxide	8	10 mg	4	124·8	+1·8	<0·5
Saline	8	5 ml	3/4	129·5		
Insulin	8	20 units	3/4	148·3	+18·8	<0·001
Hydrocortisone	8	20 mg	3/4	132·1	+2·6	<0·4
Chlorpromazine	8	20 mg	3/4	133·6	+4·1	<0·2

* Phosphorylase determination without added adenosine-5′-phosphate, a measure of active form *a*.
† 2-Brom-D-lysergic acid diethylamide.

It can be seen from Table 1 that in the first set of experiments LSD-25 and mescaline depressed the enzyme, with less than 5% probability that the difference was due to chance. Bufotenine showed some tendency to depress it. Noteworthy is the finding that BOL-148, which differs from LSD-25 only in having one atom of bromine attached to it, showed no sign of depressing the enzyme level. It is not a psychotogen, although both LSD-25 and BOL-148 have a strong antiserotonin effect on some smooth muscles.[3] Moreover, mescaline required 100 times the dose of LSD-25 to produce a comparable effect. Even wider difference in dosage level of these two drugs is seen in their effect in man.[4]

In the experiment with phenothiazine drugs the effects were observed 4 hr after drug administration. Chlorpromazine and trifluoperazine both showed a distinct enhancement effect, while chlorpromazine sulfoxide, a metabolite of chorpromazine without any therapeutic effect, exhibited no activity. The fact that the effect of chlorpromazine was manifested at 4 hr but not at 45 min (see below) may have significance. This agrees with the reported maximal effect of chlorpromazine at 4 hr in man.[5] However, Breckenridge and Norman reported decrease in effect of chlorpromazine at 2 hr.[2]

In the last set of experiments, insulin alone had a powerful stimulatory effect. Both hydrocortisone and chlorpromazine were without effect at 45 min.

Thus, LSD-25 and mescaline (and possibly bufotenine) constitute one group with a common characteristic of being psychotomimetic, while chlorpromazine, trifluoperazine, and insulin form another group whose common denominator is usefulness as a therapeutic agent in psychosis. Whether these findings are really significant with respect to drug action or merely represent coincidence must await further investigation. If they are significant, then it may be inferred that the phosphorylase enzyme of brain may be involved in some form of mental illness.

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Biochemical Research Laboratory,
Veterans Administration Hospital,
Northport, N.Y., U.S.A.

TOM T. IRIYE
ANDREW KUNA
FRANCIS A. SIMMONDS

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