J.Amer.med.Ass. 184, 283-284 (1963). LSD 1150/BOL 153

184, No 4

Phosphorylase Enzyme of Brain in Mental Illness

Tom T. Iriye, PhD, and Francis A. Simmonds, BS, Northport, N.Y.

THE PHOSPHORYLASE ENZYME which catalyzes the conversion of glycogen to glucose-1-phosphate has become implicated recently in some important biochemical and physiological functions of the organism, eg, contraction of the heart,[1, 2] production of the corticoids by the adrenal cortex,[3, 4] etc. These and some other biochemical and pharmacological phenomena have suggested possible involvement of the phosphorylase enzyme of the brain in the synthesis of norepinephrine.[5] If such is the case, the effect on the phosphorylase enzyme would affect the transmission of nerve impulses, and, hence, mental phenomena.

Investigation was begun, therefore, to search for substances which affected the enzyme level of the brain in vivo. The work which has been done so far is a preliminary study in an attempt to substantiate the hypothesis. This communication is not intended to offer proof of the hypothesis. Nevertheless, results obtained suggested a correlation between the phosphorylase enzyme and mental illness.

This investigation consists of two phases of experiments, differing in the method of phosphorylase determination. In both phases of work albino rats of Wistar strain were used, being fed or fasted in the first phase and fasted in the second phase. The fasted rats were deprived of food for 24 hr. About 45 to 75 min. after injection subcutaneously with test drugs, rats were killed with an overdose of chloroform. The brain was removed as quickly as possible and put in deep freeze. After complete freezing the brain was processed for phosphorylase determination.

The phosphorylase enzyme exists in two forms, the active form *a* and the inactive form *b*, in dynamic equilibrium in vivo. The phosphorylase assay in the absence and presence of AMP (adenosine-5′ phosphate) is a measure of the active form *a* and the total enzyme, respectively. The activity level of the enzyme is expressed in absolute values of the *a* and the total enzyme as well as in per cent ratio of *a* to total enzyme.

First Phase.—The phosphorylase determination of the first phase was made with a modification of the method used by Hess and Haugaard.[1] The results of the first phase are shown in Table 1.

Of the drugs tested, insulin was the only drug which increased the enzyme activity as compared to a saline solution which served as a control. Even this was achieved with a rather drastic treatment, ie, administration of subconvulsive doses to rats starved 24 hr.

The two substances found to depress the activity were dextro amphetamine sulfate and epinephrine. Of these two, the amphetamine was more powerful. Other substances tested, ie, corticotropin, cortisone, hydrocortisone, and chlorpromazine had no effect.

According to the results obtained, reduction of phosphorylase activity is associated with two anxiety-producing drugs, amphetamine and epinephrine, which are closely related structurally to the psychotogens, mescaline and adrenochrome. On the other hand, increase of phosphorylase activity is related to insulin, which is used in the treatment of mental illness. The fact that these three drugs, which are closely associated with mental illness, affect the phosphorylase level indicates that there may be a relationship between the phosphorylase enzyme and mental illness.

The above tentative conclusion was strengthened by results of the second phase of the investigation.

Second Phase.—The phosphorylase determination of the second phase was conducted with a method which was perfected in our laboratory. By this method, the stability of the homogenate is assured and hence practically devoid of artifacts.

The experiment was conducted in such a manner that statistical evaluation of the results can be made by pairing each observation of a treatment group with the corresponding observation of the control group. This was done as follows: Since there were four groups including control, four rats, each belonging to different groups, were treated and processed in one day and assayed all in one day, the next day, or a few days later. Stability of frozen samples was assured by previous studies. The order of treatment, processing, and assay was altered deliberately in order to cancel any variation. The drugs tested were d-lysergic acid diethylamide (LSD-25), 2-brom-d-lysergic acid diethylamide (BOL-148), and mescaline. The control group received a saline solution. The dosage range of LSD-25 and BOL-148 was 1 to 2 mg/kg. That of mescaline was 50 to 100 mg/kg. All drugs were administered in a volume equivalent to 0.5 ml/100 gm body weight.

LSD-25 and mescaline are known psychotogenic substances while BOL-148, which differs from LSD-25 only in having one atom of bromine attached to

Drs. Iriye and Mr. Simmonds are Research Biochemists at the Veterans Administration Hospital.

Read before the Panel on Psychopharmacology, Role Perceptions, Injections, and Granulomata at the 111th Annual Meeting of the American Medical Association, Chicago, June 27, 1962.

Table 1.—Effect of Various Agents on Phosphorylase Activity of Rat Brain in Vivo

Test Substances	Animals, No.	Dosage Range per Kg	Fed (F) or Starved (S)	— AMP (A)* (Phosphorylase Units)	+ AMP (B)† (Phosphorylase Units)	Ratio $\frac{A}{B} \times 100$
Saline solution (control)	31	5 ml	Mixed	127	218	58.2
Insulin	7	16-32 units	S	142‡	213	66.7§
d-Amphetamine	6	16-32 units	F or Partly S	135	223	60.4
Epinephrine	8	5-10 mg	S	103§	210	49.1§
Corticotropin	6	2.5-5 mg	S	111§	220	50.5§
Corticotropin	6	10-20 units	F	123	221	55.7
Hydrocortisone	6	10-20 units	S	124	214	58.1
	5	20 mg	S	120	208	57.4

*Phosphorylase determination without added AMP.
†Phosphorylase determination with added AMP.
‡Significant (t test).
§Highly significant (t test).

Table 2.—Effect of Hallucinogenic Drugs on Phosphorylase Activity of Rat Brain in Vivo

Test Substances	Animals, No.	Dosage Range per Kg	— AMP (A) (Phosphorylase Units)	+ AMP (B) (Phosphorylase Units)	Ratio $\frac{A}{B} \times 100$
Saline solution (control)	12	5 ml	125	181	69.0
LSD-25	12	1-2 mg	112†	168‡	67.0
BOL-148*	12	1-2 mg	128	183	69.7
Mescaline	12	50-100 mg	120	182	65.8†

*Included to show contrast with LSD-25.
†Significant at 1% level.
‡Significant (statistical analysis by pairing observations and using t test).

its nucleus, is not a psychotogen although both LSD-25 and BOL-148 have a strong antiserotonin effect on some smooth muscles. If it can be demonstrated that LSD-25 in a very small dose and mescaline in a somewhat larger dose inhibit the phosphorylase activity while BOL-148 does not, it is a very strong indication that abnormal mental phenomenon may be causally related to depressed phosphorylase enzyme. This is precisely what happened. Both LSD-25 and mescaline depressed the phosphorylase enzyme while BOL-148 had no effect whatsoever. Moreover LSD-25 was effective in a dosage range of 1 to 2 mg/kg, a fact which reflects the extremely small dose required to produce a hallucinogenic effect by LSD-25. Lower limit of the effective LSD-25 dosage was not pursued.

Table 2 shows the effect of LSD-25, BOL-148, mescaline, and saline solution on the phosphorylase activity level of the rat brain. Of the drugs tested, LSD-25-treated rats had the lowest level of phosphorylase activity on the basis of absolute values. The activity of both the active form *a* and the total enzyme decreased. Compared to the saline-solution-treated group which served as a control, the depression was highly significant. However, the per cent ratio was not significant because both the active form *a* and the total enzyme decreased together. In the mescaline-treated group only the active form *a* dropped and consequently the per cent ratio decreased. The decrease was significant at 1% level.

The inhibitory effect of LSD-25 and mescaline on the phosphorylase enzyme of the brain and the absence of such effect in BOL-148 strongly suggest that the inhibition of the phosphorylase activity is somehow involved in the hallucinogenic properties of the LSD-25 and mescaline.

Conclusion

The investigation showed that known hallucinogenic drugs, mescaline and LSD-25, and certain anxiety-producing drugs, amphetamine and epinephrine, caused depression of the phosphorylase enzyme, while elevation of its activity was associated with insulin, a drug used in the treatment of mental illness. BOL-148, which is an analogue of LSD-25, without any psychotogenic property, had no effect whatsoever on the enzyme. Moreover, LSD-25 was effective in a very small dose. From consideration of all these facts, it was concluded that the phosphorylase enzyme may be implicated in mental illness.

Veterans Administration Hospital, Northport, Long Island, N.Y. (Dr. Iriye).

Sandoz Pharmaceuticals Division, Sandoz Chemical Works, Inc., Hanover, N.J., supplied the LSD-25 and BOL-148 used in this investigation.

Generic and Trade Names of Drugs

Dextro amphetamine sulfate—*d-Amfetasul, Dexedrine Sulfate, Dextro Amphetamine Sulfate.*
Corticotropin—*Acth, Achtar, Corticotropin.*
Cortisone acetate—*Cortisone Acetate, Cortogen Acetate, Cortone Acetate.*
Hydrocortisone—*Cortef, Cortifan, Cortril, Hycortole, Hydrocortone.*
Chlorpromazine—*Thorazine.*

References

1. Hess, M. E., and Haugaard, N.: Effect of Epinephrine and Aminophylline on Phosphorylase Activity of Perfused Contracting Heart Muscle, *J Pharmacol Exp Ther* 122:169-175. (Feb) 1958.
2. Kukovetz, W. R., et al: Action of Sympathomimetic Amines on Isometric Contraction and Phosphorylase Activity of Isolated Rat Heart, *J Pharmacol Exp Ther* 127:122-127 (Oct) 1959.
3. Haynes, R. C., Jr., and Berthet, L.: Studies on Mechanism of Action of Adrenocorticotropic Hormone, *J Biol Chem* 225:115-124 (March) 1957.
4. Haynes, R. C., Jr.: Activation of Adrenal Phosphorylase by Adrenocorticotropic Hormone, *J Biol Chem* 233: 1220-1222 (Nov) 1958.
5. Iriye, T. T.: Unpublished study.