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SHORT COMMUNICATIONS

The effect of thiamine on monoamine oxidase

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IN ADDITION to the more widely studied inhibitors of monoamine oxidase (MAO), a number of quaternary nitrogen compounds inhibit this enzyme *in vitro*.[1] The structure of these compounds is very diverse, including long chain N-alkyl derivatives of pyridine, such as pyridine aldoxime dodecyl-iodide,[2] and ammonium salts such as hexamethonium and decamethonium.[1] In a recent publication,[3] Gal and Drewes demonstrated that in thiamine-deficient rats, the MAO-activity of the brain and intestine is increased, as compared to the activity of rats fed *ad libitum* with a normal laboratory diet. These workers suggested that the increase might be related to the stress theory of Selye. It is the purpose of this communication to propose that, *in vivo*, thiamine, which has a quaternary nitrogen in its thiazole moiety, is either itself an inhibitor of MAO, or more likely, is metabolized via a pathway which produces another quaternary nitrogen compound which is an inhibitor of MAO. Thus, the increased MAO-activity in the thiamine-deficient animals might be accounted for by a diminution in the amount of this thiamine-like inhibitor. The MAO-inhibition caused by this thiamine-like compound also might explain the hypotension and ganglionic blockade which thiamine produces in man and experimental animals; [4, 5] Gertner has recently shown that MAO-inhibitors block ganglionic transmission in cats and dogs,[6] while hypotension in man has been noted with the administration of inhibitors of MAO.[7]

Experiments *in vitro* show that thiamine and thiamine pyrophosphate are weak inhibitors of MAO: At 10^{-2} M, thiamine inhibits the MAO of rat liver mitochondria 70 per cent, as determined by the method of Weissbach *et al.*,[8] a result of little significance with respect to the proposed mechanism *in vivo*. Furthermore, rats given 70 mg of thiamine per kg subcutaneously, and sacrificed after 2 hr, when thiamine pyrosphosphate levels in liver are elevated,[9] showed no inhibition of the MAO-activity of liver, brain, and intestine, as compared with untreated controls. However, this does not constitute a refutation of the proposed theory, if the rate of formation of the postulated thiamine metabolite proceeds maximally with the amount of thiamine provided by a normal diet.

Substantial indirect evidence can be provided for the theory. Although in higher animals most of the thiamine which is absorbed is excreted unchanged, the fate of the thiamine which is degraded is largely unknown.[10] Molluscs, carp, and some other lower species possess an enzyme, thiaminase,[11] which catalyses the reaction of thiamine with a variety of basic compounds with loss of the 4-methyl-5-β-hydroxyethylthiazole portion, and the formation of other quaternary compounds derived from the pyrimidine portion.[10] No similar enzyme has been found in mammalian tissue, but only small amounts may be present. Such a reaction could produce a potent quaternary inhibitor of MAO. It is significant that von Muralt, using labeled thiamine, has shown that, on excitation of a peripheral nerve, metabolites of thiamine appear, which are as yet unidentified.[12] One of these might be the postulated inhibitor; if so, this would provide a mechanism for preventing the oxidation of any neurohumor which is a substrate for MAO.

The hypotension and ganglionic blockade produced both by thiamine and by known inhibitors of MAO is suggestive that these compounds are related. This action of thiamine is unrelated to its properties as a coenzyme.[4] The blockade is also different in kind from that produced by hexamethonium,[5] as is that caused by the inhibitors of monoamine oxidase.[6]

A final argument which may be offered is that in a thiamine-deficiency state in man (beri-beri), the initial symptoms are emotional—depression, apathy, and disinterest,[13, 14] a syndrome a physician might now treat with an inhibitor of MAO, which indeed would be only replacement therapy, if thiamine administration to thiamine-deficient organisms does in fact produce an MAO-inhibitor.

It is interesting that thiamine-deficient rats also show increased cholinesterase activity[15] and

diamine oxidase activity,[16] while thiamine is also a weak inhibitor of these enzymes, *in vitro*. It has been proposed that *in vivo* thiamine inhibits both of these enzymes.[15, 16] Thus, there is both precedent for the theory presented and a wider scope for a mechanism which would delay the catabolism of neurohumors such as acetylcholine, histamine, serotonin, and the catecholamines.

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Inhibition of dopamine β-oxidase by imipramine

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PREVIOUSLY we have reported that phenylethylamines and phenylpropylamines inhibit the conversion *in vitro* of dopamine to norepinephrine by dopamine β oxidase. The conversion of dopamine to norepinephrine may be the rate-limiting step in the biosynthesis of norepinephrine and, consequently, inhibitors of dopamine hydroxylation may be important therapeutic agents in lowering norepinephrine levels or increasing dopamine levels *in vivo*. The present report shows that imipramine (N-(γ-dimethylaminopropyl)-iminodibenzyl), a known anti-depressant agent, inhibits the conversion *in vitro* of dopamine to norepinephrine.

The enzyme dopamine β-oxidase was prepared by the method of E. Y. Levine *et al.*,[1] and the incubation was carried out by the procedure described in the previous paper.[2] At the end of the period of incubation, the solution was analyzed for the enzymically formed norepinephrine by two different experimental procedures. In the first procedure, dopamine-C^{14} was used as a substrate, and after acetylation of the amines the enzymically formed norepinephrine-C^{14} was separated from dopamine-C^{14} by paper chromatography.[3] The amount of dopamine-C^{14} which disappeared from the incubation mixture, as well as the amount of norepinephrine-C^{14} formed in the incubation mixture, was calculated from the radioactivity of each of these compounds. In the second series, non-radioactive dopamine was used as a substrate and the enzymatically formed norepinephrine was determined by a modification of the fluoremetric method of von Euler and Floding.[4] The degree of inhibition rate of dopamine β oxidase was determined in both procedures by comparing the amount of norepinephrine formed in an incubation mixture which contained only the substrate and in an incubation mixture which contained imipramine and the substrate. Table 1 shows the effects of imipramine on the conversion of dopamine to norepinephrine. It is evident that when the concentration of inhibitor to substrate is 2:1 approximately 50 per cent inhibition of norepinephrine synthesis is observed.