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SHORT COMMUNICATION

IN VITRO EFFECT OF ALLOXAN ON Na^+–K^+–AT PASE AND SUCCINATE DEHYDROGENASE ACTIVITIES IN BRAIN AND LIVER OF MICE

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Abstract : The present study reports *in vitro* inhibition of the activities of enzymes Na^+–K^+ ATPase and succinate dehydrogenase by alloxan in brain and liver homogenates of Swiss mice. The V_{max} of both the enzymes was reduced in presence of alloxan without any substantial alteration in Km for substrate. Lineweaver Burk's plots showed higher $1/V_{max}$ for alloxan treated samples and convergence of both slopes to intercept –1/Km. The observations pointed to non-competitive type inhibition of the enzymes by alloxan. This may be due to the modification of essential - SH groups present within/adjacent to substrate binding sites by alloxan.

Key words : alloxan Na^+–K^+ ATPase succinate dehydrogenase mice

INTRODUCTION

Alloxan, a β-cytotoxin, chemically called mesoxalylurea, mesoxalylcarbamide, 2, 4, 5, 6-tetra-oxohexahydropyrimidine or pyrimidine tetrone has been extensively used for *in vivo* induction of 'chemical diabetes' in a variety of animal species (1). The chemical acts on its target tissue possibly through Strecker reaction (1) or reaction with SH groups (2) or as a metallic ion chelator (3) or inhibition of membrane bound enzymes (4). Several *in vivo* studies indicated inhibition of membrane bound enzymes leading to metabolic alterations following alloxan diabetes. The activities of the enzyme Na^+–K^+ ATPase (5, 6) and of mitochondrial membrane bound enzyme succinate dehydrogenase (7) decreased following both natural and induced diabetes. These studies, however, did not provide evidence on the direct inhibition of these enzymes by alloxan.

Therefore the present study reports the direct effect of alloxan on some of the kinetic properties of the two enzymes in liver and brain homogenates of Swiss albino mice.

METHODS

Swiss albino mice (15-33 g) of both sexes were maintained at room temperature (30 ±2°C) on a freshly prepared diet (500 g semolina, 50 g milk powder, 20 yeast tablets, NaCl salt 5 g, boiled to make paste to serve 20 animals) and water was provided *ad libitum.*

Tissue processing for enzyme activity : The animals were decapitated with a sharp razor; the brain and the liver were quickly dissected out in precooled Mammalian Ringer (Kreb's Ringer Phosphate) and the adherent tissues were cleaned. The entire brain and a part of the liver were blotted off in Whatman Filter paper No. 1. A 5% homogenate of each tissue was prepared in 0.25 M cold sucrose solution with a homogenizer at a medium speed for one minute. This homogenate was used for assaying the activity of enzymes Na^+–K^+ ATPase and succinate dehydrogenase.

Assay of enzyme activity : (a) Na^+–K^+ ATPase: The assay mixture contained 1 ml of 0.1 M Tris (Fluka, GR) buffer, PH-8.5, 0.5 ml of 50 mM KCl, 0.5 ml of 25 mM ATP (disodium salt, Merck) and 0.5 ml of 5% homogenate. The concentration of Na^+ ions in the

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mixture was the same as K^+ ions. A control tube without the substrate (ATP) was also run simultaneously for correction of endogenous hydrolysis of ATP.

The mixture was incubated for one hour at 37°C. Reaction was terminated by adding 2.5 ml of cold 10% TCA. The whole content was contrifuged in a Remi laboratory centrifuge at 1500 rpm for 15 minutes. The volume of the supernatant was measured and taken for estimation of liberated inorganic phosphorus following standard method (8). The optical densities were measured at 730 nm in a Spectrophotometer (ELICO, Model-CL 27). The enzyme activity was expressed as mg of liberated phosphorus/g tissue/hr.

(b) *Succinate dehydrogenase :* The estimation of succinate dehydrogenase activity was done following the method of Kun and Abood (9). The assay mixture contained 0.5 ml of potassium phosphate buffer (pH 7.4); 0.5 ml of 0.1 M sodium succinate (BDH); 0.5 ml of freshly prepared triphenyl tetrazolium chloride (TTC) and 0.5 ml of the homogenate. A control without the substrate and a blank without the homogenate were also run simultaneously.

The incubation period was one hour at 37°C. The red formozan formed was extracted in 4 ml of acetone (Glaxo) and the optical densities measured at 485 nm in the above Spectrophotometer. The enzyme activity was expressed as µg/formozan/g tissue/hr.

In vitro effects of alloxan on enzyme kinetics : For the studies of kinetics of enzyme activity, two sets of reaction mixtures were prepared. The 1st set consisted of varying concentrations of the substrate with fixed concentration of alloxan (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.5, 4.0, 6.0, 8.0, 10.0 mM ATP for Na^+ $-K^+$–ATPase and 10 mg = 32.43 mM of alloxan and 1.0, 2.0, 3.0, 4.0, 5.0, 10.0, 15.0, 20.0 mM sodium succinate for SDH and 1 mg ≡ 3.123 mM of alloxan). The second set consisted of varying concentrations of alloxan (4, 8, 12, 16 mg for Na^+ $-K^+$ –ATPase and 0.4, 0.8, 1.2, 1.6 and 2 mg for SDH) with fixed concentrations of the substrate (0.25 mM of ATP for Na^+–K^+–ATPase and 0.1 M sodium succinate for SDH). The rest of the respective procedure was the same as described under "Assay of enzyme activity".

The values of Km for both Na^+–K^+–ATPase and succinate dehydrogenase in brain and liver homogenates were calculated from the above series of assays in which the initial velocity of activities were measured at different initial concentrations of substrates with fixed concentrations of the enzyme in presence and absence of alloxan.

RESULTS AND DISCUSSION

In the present study, alloxan reduced the activity of Na^+–K^+–ATPase and succinate dehydrogenase in homogenates of brain and liver. The chemical at a concentration of 3.123 mM reduced the SDH activity by 50% while at a concentration of 32.43 mM reduced the activity of Na^+–K^+–ATPase by 25%. Evidently a higher concentration of the inhibitor was required to inhibit the activity of the latter enzyme.

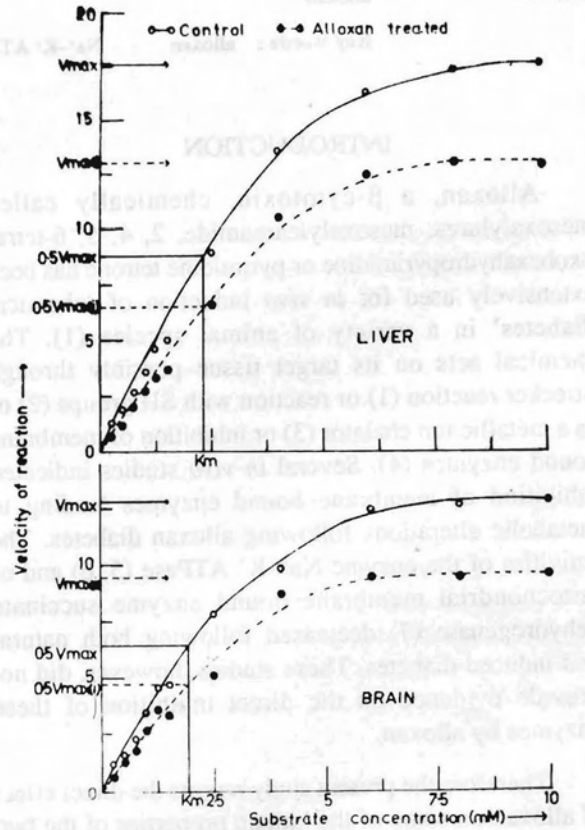

Fig. 1 : *In vitro* effect of alloxan on the activities of enzyme, Na^+ $-K^+$–ATPase (mg Pi released/g tissue wet-weight/hr) with varied substrate concentration (ATP) in brain and liver of Swiss mice (n = 10).

Substrate saturation curves for both the enzymes in tissues of mice (Fig. 1, 2) in presence of alloxan indicate reduced Vmax without changing the Km values at least for Na⁺–K⁺–ATPase. This fact together with proportionate increase in the inhibition by increasing concentrations of alloxan (Fig. 5) probably points to a non-competitive type of inhibition. This view is further corroborated by Lineweaver-Burk's double reciprocal plots where 1/v vs 1/[s] showed higher $1/V_{max}$ values for alloxan treated samples. Both normal and alloxan-treated slopes converged to intercept –1/Km indicating a non-competitive inhibition of both the enzymes by alloxan (Fig. 3, 4). The same is true for SDH activity except a marginal rise in the Km values (Fig. 2). Since an increase in the Km indicates weaker affinity for the substrate at the active sites of the enzyme, one may presume alterations in some of the binding forces by alloxan.

One of the mode of action of alloxan is the binding of –SH groups (2) leading to depletion of tissue glutathione levels (1). Alloxan diabetes caused a decline in –SH groups in kidney homogenates of rats (10). The diminution of glutathione levels may allow oxidative inactivation of critical membrane proteins

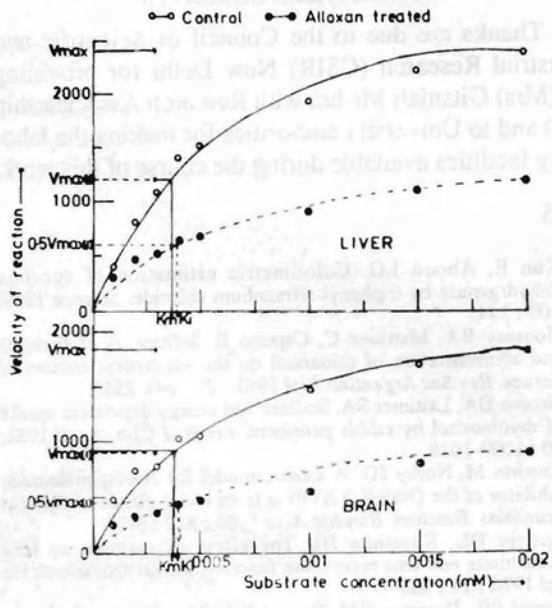

Fig. 2 : *In vitro* effect of alloxan on the activities of enzyme succinic dehydrogenase (μg formazan/g tissue wet-weight/hr) with varied substrate concentration (Na-succinate) in brain and liver of Swiss mice (n = 10).

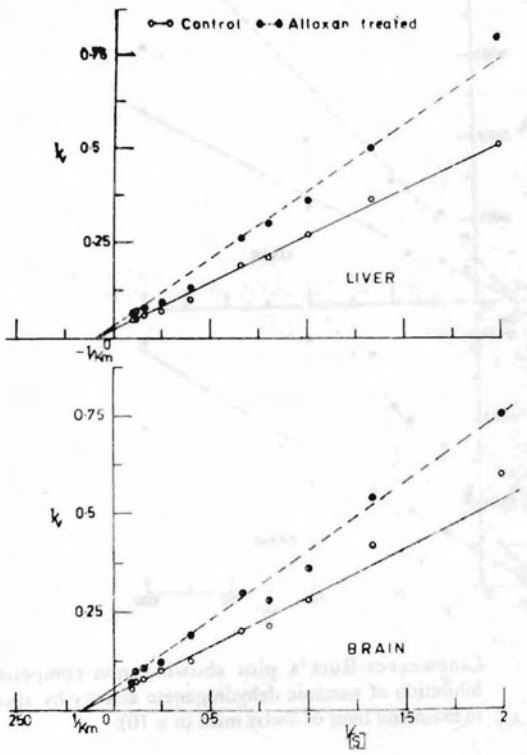

Fig. 3 : Lineweaver-Burk's plot showing non-competitive inhibition of Na⁺–K⁺–ATPase activity by alloxan in brain and liver of Swiss mice (n = 10).

(11) through modification of their –SH groups (12). Oxidation of glutathione in lens has been associated with secondary inhibition of Na⁺–K⁺–ATPase activity speculatively attributed to the direct action of glutathione on formation of disulphide bridges in the enzyme (13). If glutathione availability is responsible for maintaining functionally important sulphydryl groups on vital membrane proteins, then alterations in the levels of GSH should preceed measurable decreases in the activity of these proteins (14). It is therefore reasonable to infer that the reduction in the activity of the enzymes Na⁺–K⁺–ATPase and succinate dehydrogenase by alloxan is either through a direct action of the chemical on functionally important –SH groups present at the active sites or at the surface of the proteins (15) or indirectly through alterations in glutathione levels or both. It appears that binding of alloxan to the sulphydryl groups on the surface of the enzymes changes their conformation and binding on the active sites reduces the number of covalent

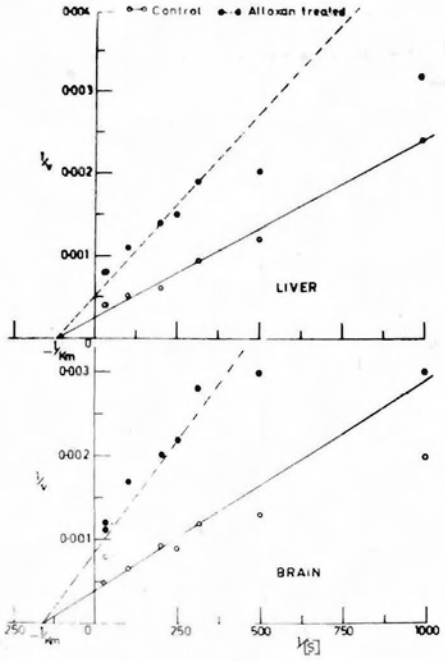

Fig. 4 : Lineweaver-Burk's plot showing non-competitive inhibition of succinic dehydrogenase activity by alloxan in brain and liver of Swiss mice (n = 10).

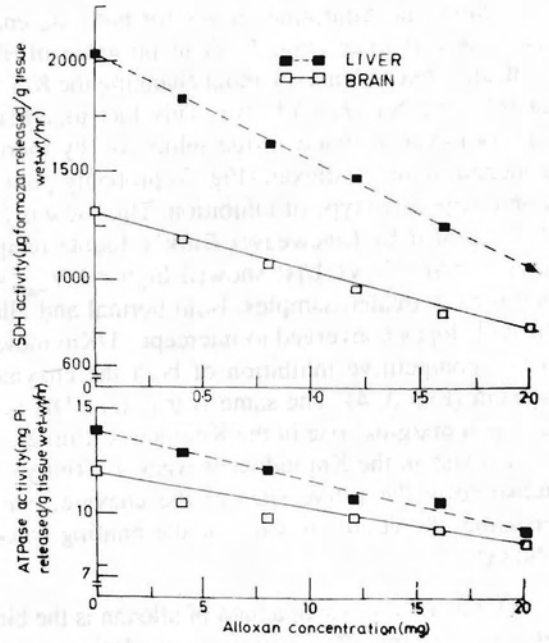

Fig. 5 : *In vitro* inhibition of enzymes, succinic dehydrogenase and Na^+–K^+–ATPase activities by varied alloxan concentrations in brain and liver of Swiss mice (n = 10).

linkages ; in any event leading to poor substrate affinity. It is possible that such conformational changes together with alterations in binding forces at the active sites inhibited the activity of the enzymes, the degree of such inhibition being dependent on the concentration of alloxan.

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