INHIBITION OF Ca^{2+}-ATPASE BY BENTHIOCARB IN RAT: AN *IN VITRO* STUDY

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ABSTRACT

In vitro effects of benthiocarb on Ca^{2+}-ATPase were studied in brain and liver of albino rat to elucidate the interaction of benthiocarb with ATPase system. The significant decrease in maximal velocity without appreciable change in apparent Michaelis-Menten constant suggests that the effect of benthiocarb on Ca^{2+}-ATPase is independent of substrate and is of a classical non-competitive type.

INTRODUCTION

BENTHIOCARB is the common name of an organocarbamate herbicide which was commercially introduced in Japan in 1970 under the trade name *Saturn*. It is now popular among rice growers in more than 30 countries. It shows high selectivity in differentiating between rice and barnyard grass. Benthiocarb like other organocarbamates is known to be a neurotoxic compound and it seems to alter mitochondrial enzyme activities such as NAD^+ isocitrate, succinate and malate dehydrogenases[1]. Our unpublished results also show that benthiocarb inhibits ATPase system *in vivo*. Based on this, an attempt was made to elucidate the interaction *in vitro* of benthiocarb with Ca^{2+}-ATPase in rat brain and liver.

MATERIALS AND METHODS

Male albino rats (Wistar strain) in the weight range of 200 ± 25 g maintained under laboratory conditions (temperature 30 ± 2°C; relative humidity 75%; and a light-dark period of 12 h) were used for the study. Technical grade (98%) benthiocarb [S-(4-chlorobenzyl)-N, N-diethyl thiocarbamate] obtained from the Environmental Protection Agency (USA) dissolved in minimum volume of acetone was used. Care was taken to see that acetone concentration is within the limit (5 μl/2 ml reaction mixture) to avoid turbidity which may induce the precipitation of biomolecules.

Preparation of P_2 fraction

Liver and brain were rapidly isolated after decapitation, chilled in ice-cold 0.32 M sucrose buffer and subjected to homogenization using a motor-driven yorko-speed control homogenizer. P_2 fraction of both brain and liver was prepared using the method of Green *et al*[2], the specific activity of Ca^{2+}-ATPase was assayed[3], and the inorganic phosphate (Pi) liberated was estimated[4]. The protein content of the enzyme source was determined[5] using crystalline bovine serum albumin as the standard.

Kinetic analysis

After initial standardization, the specific activity of Ca^{2+}-ATPase was determined. An incubation time of 30 min, an incubation temperature of 37°C, pH of 7.5 and approximately 30–40 μg of protein were selected to ensure maximal velocity. Inhibition of Ca^{2+}-ATPase with different concentrations of benthiocarb (2–16 μM) was studied to determine IC_{50} (the concentration of benthiocarb that inhibits 50% enzyme activity). The IC_{50} values for Ca^{2+}-ATPase of brain and liver were found to be 12 and 15 μM respectively. The effect of benthiocarb was assessed by preincubating the enzyme with IC_{50} of benthiocarb before the reaction was started with the substrate (ATP). Activation of Ca^{2+}-ATPase was assayed by varying ATP concentrations from 1.5 to 10 mM while maintaining other conditions constant. Kinetic parameters, V_{max} and K_m were determined by adopting standard kinetic principles.

RESULTS AND DISCUSSION

The results obtained from the kinetic study of Ca^{2+}-ATPase of rat brain and liver are summarized in table 1. The specific activity of Ca^{2+}-ATPase of both brain and liver was decreased significantly ($P < 0.05$). The decrease in V_{max} was 37.4% and 30.3% for liver and brain Ca^{2+}-ATPase respectively, whereas the K_m of Ca^{2+}-ATPase was not altered significantly in both brain and liver.

Inhibition of Ca^{2+}-ATPase activity by benthiocarb observed (table 1; figure 1A,B) suggests its possible interaction with calcium transport phe-

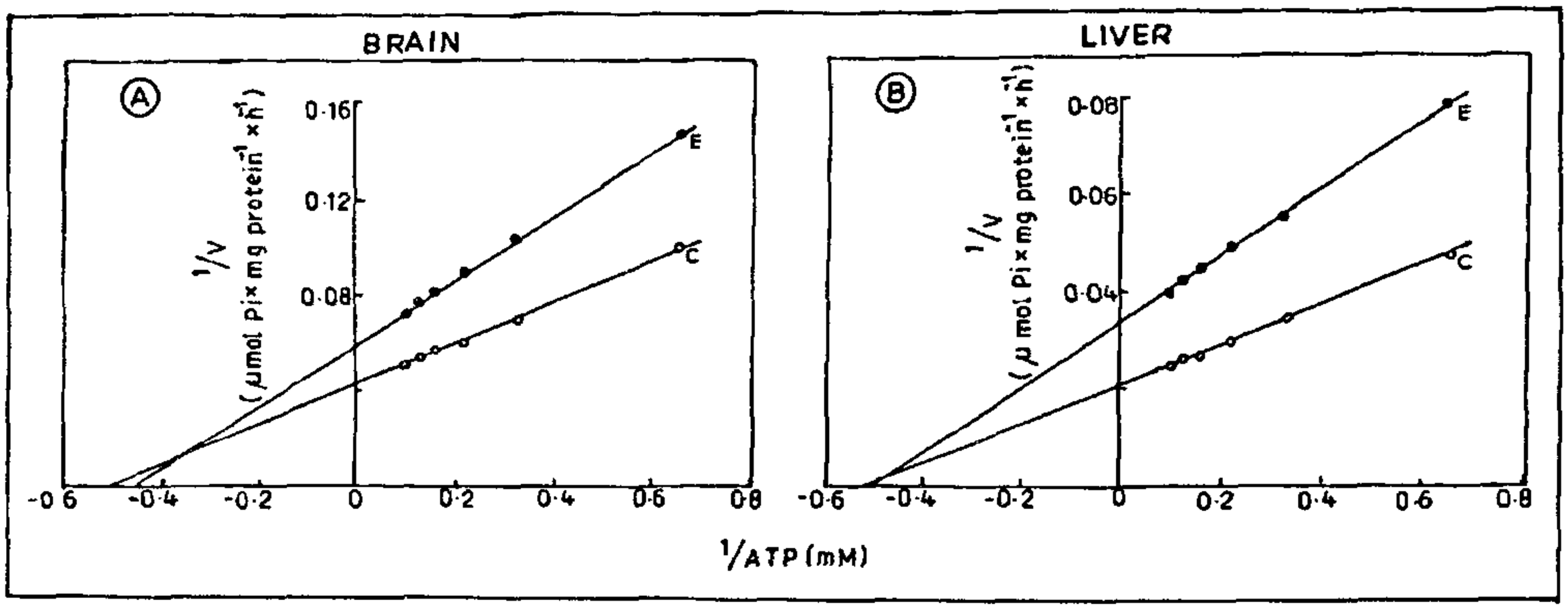

Figure 1. Double reciprocal plots of substrate concentration vs Ca^{2+}-ATPase activity of rat brain and liver with (E) and without (C) IC_{50} of benthiocarb under *in vitro* condition.

Table 1 *In vitro effects of benthiocarb on kinetic constants of Ca^{2+}-ATPase*

	V_{max}[a]		K_m[b]	
Tissue	Control	Experimental	Control	Experimental
Brain	23.8	16.6 (−30.33)[c]	2.04	2.17 (6.37)[c]
Liver	45.5	28.5 (−37.40)[c]	1.82	2.22 (8.80)[c]

[a] Represented in μmol of Pi formed/mg protein/h; [b] Represented in mM of ATP, and [c] % deviations from control.

nomena which might affect calcium-dependent processes in neuronal and hepatic functions. A similar *in vitro* inhibition of Ca^{2+}-ATPase in rat brain synaptosomal function by insecticides including organotin compounds was reported recently[6,7]. Ca^{2+}-ATPase which has a pivotal role in maintaining a constant intracellular calcium concentration has been shown to be activated by calmodulin, a calcium binding protein[8]. The *in vitro* inhibition of Ca^{2+}-ATPase observed is attributed to the possible interactions of benthiocarb with calmodulin resulting in decreased specific activity of enzyme. It was earlier shown that organochlorine and organotin compounds alter the calmodulin content causing alteration in the calmodulin regulated Ca^{2+}-ATPase[9]. Our results therefore confirm the earlier findings.

The significant decrease in V_{max} without an appreciable change in apparent K_m indicates that the effect of benthiocarb on Ca^{2+}-ATPase is independent of substrate (ATP) and is of a classical non-competitive type. The findings of kinetic study also reveal that benthiocarb does not affect the affinity of Ca^{2+}-ATPase for ATP.

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1. Veera Babu, G., *Studies on neuronal and hepatic metabolisms of albino rat (Wistar strain) under induced benthiocarb stress*, Ph.D. thesis, S. V. University, Tirupati, 1987.
2. Green, D. E., Lester, R. L. and Ziegler, D. M., *Biochim. Biophys. Acta*, 1957, **23,** 516.
3. Fritz, F. P. and Hamrick, M. E., *Enzymol. Acta Biocat.*, 1966, **30,** 57.
4. Lowry, O. H. and Lopez, J. A., *J. Biol. Chem.*, 1946, **162,** 421.
5. Lowry, O. H. Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193,** 265.
6. Desaiah, D., Chetty, C. S. and Prasada Rao, K. S., *J. Toxicol. Environ. Health*, 1985, **16,** 189.
7. Prasada Rao, K. S., Chetty, C. S., Trotman, C. H., Ozodinma, J. E. and Desaiah, D., *Cell Biochem. Funct.*, 1985, **3,** 267.
8. Chafouleas, J. D., Dedman, J. R., Munjaal, R. P. and Means, A. R., *J. Biol. Chem.*, 1979, **254,** 10262.
9. Desaiah, D., In: *Biochemical events associated with neurotoxicity of chlordecone*, (ed.) T. Narahashi, Raven Press, New York, 1984.