Bull. Environ. Contam. Toxicol. (1996) 56:165-170

Bulletin of Environmental Contamination and Toxicology

Age-Related Change in Rat Testicular ATPase Activities in Response to HCH Treatment

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Received: 20 January 1995/Accepted: 20 June 1995

[1]HCH (Y-hexachlorocyclohexane), an organochlorine insecticide is used widely for agricultural and hygienic purposes in most of the developing countries including India. Like other organochlorine, HCH can also enter into the body by various means such as through food chain, through inhalation and through dermal contact. It has been reported that HCH preferably accumulates in adipose tissues and in membrane lipid layer (Zhu et al. 1986; Lopez-Aparicio et al.1 988). Besides exhibiting neurological (Woolley et al. 1985; Tusell et al. 1987) and hepatological (Junqueira et al.1988) toxicities, HCH is also reported to produce adverse effect on male reproductive systems of rats. HCH-induced toxicity in male reproductive system includes degeneration of seminiferous tubules (Dikshith et al.1978 ; Srinivasan et a1.1988) and inhibition of stimulatory activity of adrenergic agonist upon cAMP accumulation in prostatic epithelial cells (Carrero et al. 1990). Mechanism of action of HCH on male reproductive system particularly in the testis is poorly understood. Mitochondrial fraction from rat testis has been shown to metaboiize ATP at a faster rate than that from liver or kidney (Hollinger 1971). Although testis from immature and mature rats catabolized ATP at a very high rate (Hollinger 1971), by means of Ca^{+2} and Mg^{+2} ATPase, there is a marked change in the enzyme activity during maturation (Delhumeau-Ongay et al.1973). It is understood that pesticide-induced testicular damage is more pronounced in immature rats in comparison to adult rats (Sjoberg et al.1985 Jinna et al.1989). Keeping this in view, an effort has been made in the present study to examine the effect of chronic exposure of HCH on testicular Ca^{+2} and Mg^{+2}- ATPase and Mg^{+2} ATPase in immature (30 days old) and adult (90 days old) rats since no information on this subject is yet available.

MATERIALS AND METHODS

Male wistar rats of 30 day old (weighing 60-80 g.) and 90 day old (weighing 300-350 g) were purchased from National Institute of Nutrition, Hyderabad, India. They were acclimated to laboratory conditions for about 10 days before any experimentation. Rats were maintained with artificial illumination for 12 h followed by a dark period of 12 h. They were fed gram (Cicer arietinum) and freshly prepared diet containing flour and vitaminized powered milk. Tap water was supplied *ad libitum.*

Rats of each age group were divided into three groups containing 5 animals per group. The second and third group were fed 10 mg and 20 mg HCH per kg. body weight respectively by oral intubation for 7, 15 and 30 days. The first group which served as control, was fed only olive oil in the similar manner. The technical grade HCH, obtained

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from Southern Pesticide Corporation Ltd., India, was suspended in olive oil. Volume of oil or pesticide suspended in oil, fed to animal was 0.1 ml. The animals treated for different durations were sacrificed by decapitation after 24 h of the last treatment and testis was immediately dissected out and processed for ATPase assay.

A 10% (w/v) homogenate of decapsulated testis was prepared in ice-cold homogenizing buffer (Tris-HCI, 50 mM; sucrose, 0.32 M; pH, 7.5) with the help of a glass-teflon motor driven homogenizer with 7-8 up and down strokes. The homogenate was centrifuged at 14000 x g for 10 min to get the mitochondrial fraction. The precipitate was washed twice and finally suspended in homogenizing buffer (200 mg tissue/ ml). Testicular ATPase activity was determined calorimetrically by estimating inorganic phosphate liberated from ATP hydrolysis (Chen et al.1956). For Ca^{+2}- and Mg^{+2}- ATPase, 1 ml reaction mixture contained 50 mM Tris buffer (pH 7.5), 0.5 mM EGTA, 1.5 mM $CaCl_2$, 3mM ATP (disodium salt) and 50-70 µg protein. For Mg^{+2}-ATPase, 1 ml reaction mixture contained 50 mM Tris-HCI buffer (pH 7.5), 0.5 mM EGTA, 5 mM $MgCl_2$ 3 mM ATP and 50-70 µg of protein. Reaction mixtures were pre-incubated for 10 min at 37°C in a shaking water bath and reaction was started after adding ATP to the mixture and was incubated for 30 min at 37°C. The reaction was terminated by adding 0.1 ml of 50% TCA. The mixture was centrifuged at 5000 rpm for 10 min and liberated inorganic phosphate was estimated in the supernatant. Enzyme activity was expressed as µmole phosphate liberated per mg protein per hour. Protein content was determined using the method of Lowry et al (1951).

Data for each group were subjected to analysis of variance (ANOVA) followed by Duncan's new Multiple range test. The data are expressed as mean ± standard deviation of five animals. The level of statistical significance employed in all cases was $P < 0.05$.

RESULTS AND DISCUSSION

The influence of HCH on the activity pattern of testicular Ca^{2+}- Mg^{2+}- ATPase and Mg^{2+}-ATPase in immature rats (30-days old) is evidently similar. Fig. 1 and Fig.2 clearly indicate that the activities of both the enzymes get enhanced after 7 and 15 days of HCH treatment (both 10 mg and 20 mg HCH/kg body wt./day) while no significant variation in their activities could be seen after 30 days of pesticide treatment in comparison to respective control groups.

In mature rats (90-days old) both the doses of HCH (10 mg and 20 mg HCH/kg body wt./day) significantly reduced the activities of Ca^{2+}- Mg^{2+}-ATPase and Mg^{2+}- ATPase after 7 days of treatment as compared to control. Subsequently a remarkable increase in the activity of both the enzymes was observed after 15 and 30 days of pesticide treatment and the trend being more or less similar in both the doses.

In developing rat testis, an active proliferation of germ cells and differentiation is a high energy requiring process. At this stage, in immature rats, the variation in the values of 15-days treated control group to that of 7 and 30 days treated control group may be attributed to the physiological status of rat.

The present study indicates that response of testicular ATPase not only depends upon the dose and duration of the pesticide treatment but also depends upon the age of the animal. The age related variation in the testicular ATPases in response to HCH could be due to efficiency of delivery of pesticide in the testis and also susceptibility of testicular ATPases to the pesticide. The difference in absorption, distribution, metabolism and excretion of the pesticide between 30 and 90 day-old rats may also contribute to age related variation in testicular ATPase. It has been reported that higher proportion of intestinal tissue in relation to body weight (Younoszai and Ranshaw 1974) and the relative higher blood flow through

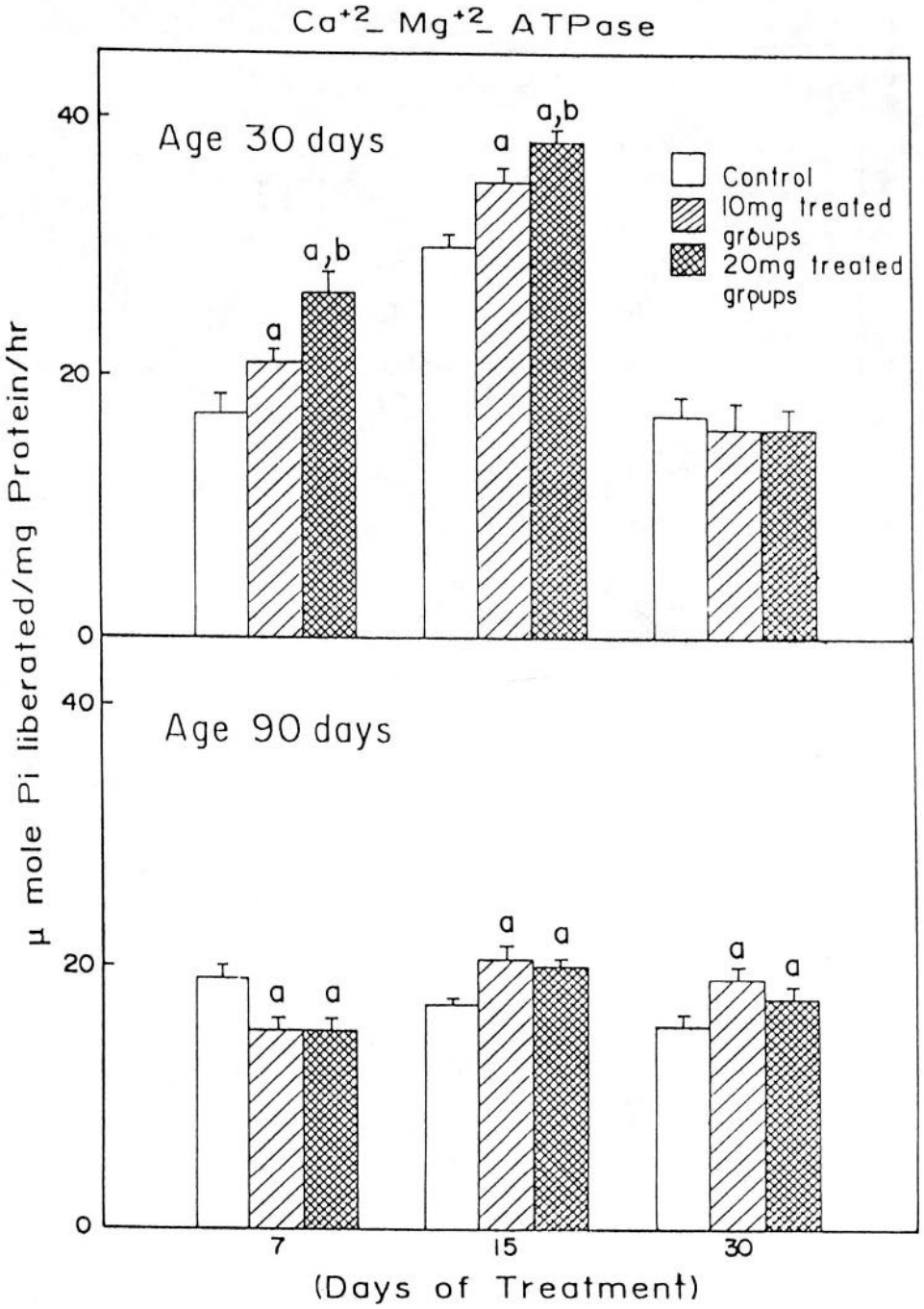

Figure 1. Ca^{+2}- Mg^{+2}-ATPase activity in 30-day (immature) and 90-day(adult) rats after exposure of 10 and 20 mg of HCH/ kg. body wt./day for a period of 7.15 and 30 days (Each value is represented as mean (±) SD. of five individual animals of that group)

a: Statistically significant (P< 0.05) compared to control

b: Slatistically significant compared to 10 mg HCH treated group

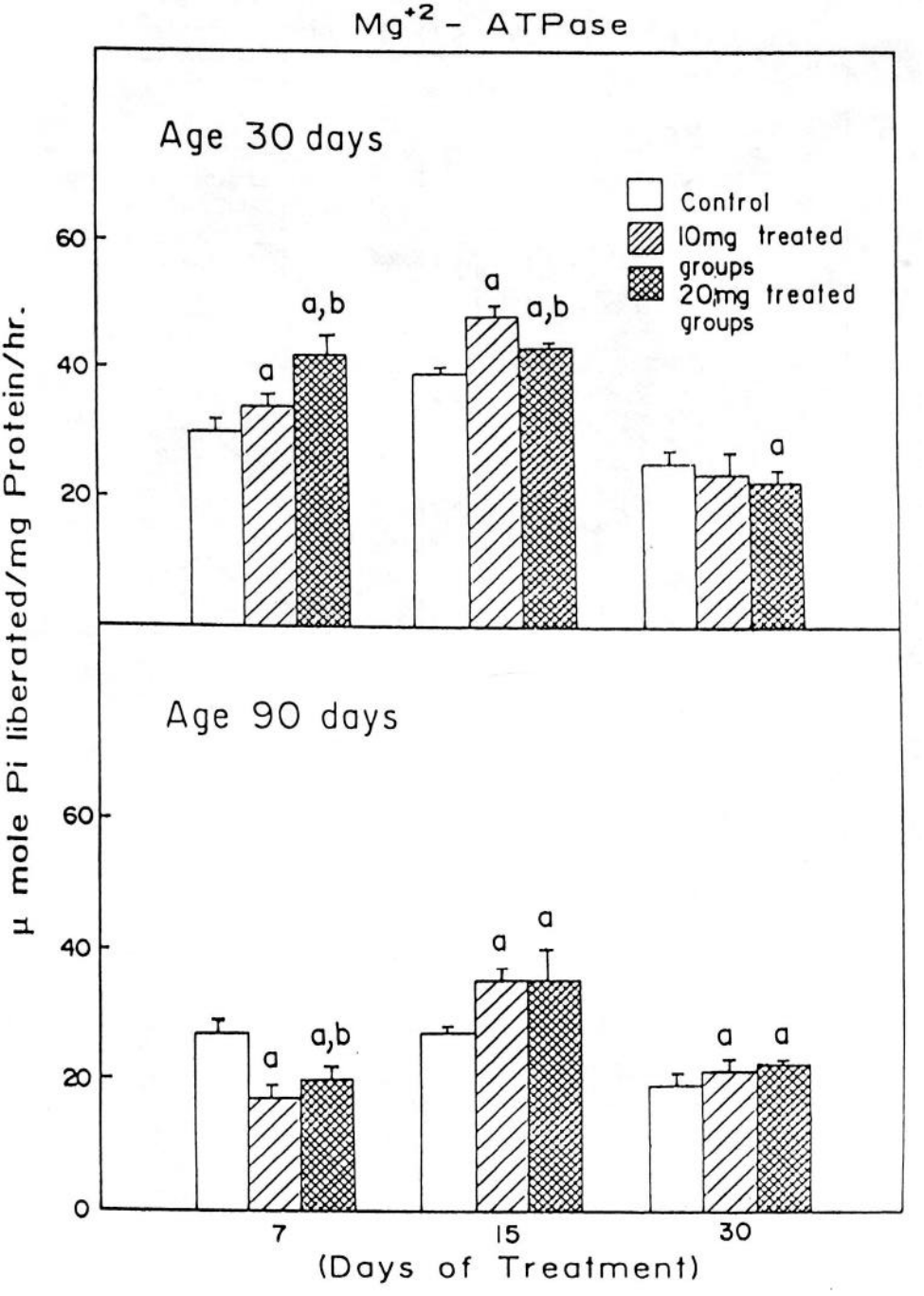

Figure 2. Mg^{+2}- ATPase activity in 30-day (immature) and 90-day (adult) rats after exposure of 10 and 20 mg of HCH/ kg. body wt./day for a period of 7, 15 and 30 days (Each value is represented as mean (±) S.D. of five individual animals of that group)

a: Statistically significant (P< 0.05) compared to control

b: Statistically significant compared to 10 mg HCH treated group

gastro-intestinal tract (Varga and Csaky 1976) may be the factors causing increased absorption of the pesticides in young rats. Testicular damage in response to DHEP has been reported to vary with the age of the animal (Sjoberg et al. 1982).

ATP is known to play a key role in storage and utilization of metabolic energy in most of mammalian tissues including that of testis. It has been shown that mitochondrial fraction of the rat testis catabolize ATP at a faster rate and to more complete extent than mitochondria from liver and kidney (Hollinger 1971). Further it was observed that catabolism of ATP by testicular mitochondrial fraction occurred mainly due to Ca^{+2}- Mg^{+2}-ATPase activity which changed during maturation (Delhumeau-Ongay et al. 1973). It was also noticed by them that the activity of this enzyme in the testis was increased when germinal cell regression in the testis was induced either chemically or surgically which pointed out the intratubular distribution of the enzyme. Therefore, increase in the enzyme activity in the testis of the rat in response to HCH may be due to degeneration of germ cells. Delhumeau-Ongay et al.(1973) interpretated that increase in Ca^{+2}- Mg^{+2}-ATPase following regression of germ cells is due to proliferation and differentiation of primitive germ cells after destruction of existing germ ceils and/or due to phagocytosis of degenerating cells by Sertoli cells. This may be correlated with an HCH induced increase in Ca^{+2}- Mg^{+2}-ATPase activity of the testis. HCH has been reported to cause testicular hypertrophy and cellular damage when fed orally to mice (Nigam et al. 1979). Intra-testicular injection of HCH to rats resulted in degeneration of seminiferous tubules and total or partial arrest of spermatogenesis (Dikshith and Datta 1972). Formation of giant germ cells and atrophy of seminiferous tubules along with change in levels of many testicular enzymes was observed when adult male rats were fed HCH orally (Dikshith et al. 1978).

The elevation of mitochondrial Mg^{+2}-ATPase in rat testis by HCH is note worthy. Several metabolic processes in various tissues including testis need ATP as substrate and 70% of this ATP is being produced in mitochondria through oxidative phosphorylation (Van Rossum 1971). It is now well established that Mg^{+2}- ATPase catalyses ATP synthesis in the mitochondria through oxidative phosphorylation (Boyer et al.1977). Therefore increase in this enzyme activity may result in the increased production of ATP and thus, the availability of ATP to Ca^{+2}- Mg^{+2}- ATPase gets enhanced as a results of degeneration of germinal epithelium.

Acknowledgments. We thank Prof. D.R Naik for his keen interest in the work and Dr. S.P. Bhunya, Head, Department of Zoology for providing necessary facilities.

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