Early Inhibition of Succinic Dehydrogenase by Manganese in Rat Gonads

Raushan Husain, Prahlad K. Seth, and Satya V. Chandra
Industrial Toxicology Research Centre, Post Box No. 80
Lucknow-226001, India

Although brain is considered to be the chief site of injury in manganese poisoning (CANAVAN et al. 1934), sterility in male rabbits has been observed to occur even earlier than neurological disturbances (CHANDRA, 1972; CHANDRA et al. 1973). This testicular dysfunction was found to be the result of temporally correlated reduction in the activity of succinic dehydrogenase (SDH) and adenosine triphosphatase (ATPase) and degeneration of seminiferous tubules after a single intratracheal administration of manganese dioxide (SETH et al. 1973). Sexual impotency after chronic manganese exposure has also been reported in humans (MEENA et al. 1967). Therefore, it appeared of interest to investigate the early alterations in SDH activity of gonads after a single exposure to manganese. Present communication describes the effect of manganese on SDH activity of rat gonads at different time intervals between 1-90 days after a single intraperitoneal injection of manganese sulphate. Morphological studies were also conducted at the time of maximum inhibition of SDH activity.

EXPERIMENTAL

Industrial Toxicology Research Centre bred adult male (150-200 g) and prepuberal female albino rats (4 to 6 weeks old with vaginal openings closed; 30-40 g) were used. Males and females were divided into two groups each. Animals of group I of each sex were given 5 mg/kg of $MnSo_4.4H_2O$) in 0.5 ml of normal saline intraperitoneally and the animals of group II received the same volume of saline to serve as controls. The animals were later killed by decapitation at predetermined time intervals and the organs were collected for preparation of tissue homogenates and histopathological studies.

Activity of succinic dehydrogenase [SDH; succinate (acceptor) oxidoreductase, E.C. 1.3.99] was studied in 10% (W/V) homogenates of testis and ovary prepared in cold isotonic sucrose with the help of a Potter-Elvehjem type homogenizer by the method of SLATER and BONNER (1952) as described earlier (SETH and HUSAIN 1974). Estimation of protein was according to LOWRY (1951) using serum bovine albumin as standard.

Gonads were immediately fixed in 10% neutral formalin. Serial paraffin sections cut at 6μ were stained with haematoxylin, eosin and PAS for histological observations.

Bulletin of Environmental Contamination & Toxicology,
Vol. 16, No. 1 © 1976 by Springer-Verlag New York Inc.

RESULTS AND DISCUSSION

The results in Table 1 indicate that Mn treatment significantly decreased SDH activity in gonads in both sexes at various time intervals. In ovary the decrease in the enzyme activity reached maximum 57.5% on day 8. Later, on day 16 it showed a tendency to recover but the enzyme level still remained significantly below than that observed at day 1 after manganese injection.

TABLE 1

Effect of manganese on the SDH activity of rat gonads

Days after manganese administration	nmoles of $K_3Fe(CN)_6$ reduced/min/mg protein	
	Testis	Ovary
0	6.60±0.001	13.2±0.011
1	5.30±0.003 (19.6)*	12.2±0.018 (7.5)
2	5.20±0.003 (21.2)	11.7±0.002 (11.3)
4	3.59±0.002 (45.6)	11.30±0.004 (14.4)
8	3.81±0.010 (42.2)	5.60±0.017 (57.5)
16	3.66±0.001 (44.5)	8.10±0.004 (38.6)
30	3.81±0.001 (42.2)	-
90	3.94±0.016 (40.3)	-

*Values in parentheses indicate percent inhibition of the enzyme activity.

Analytical data are expressed as mean ± S.E. from five animals in case of testis and of three values each based on pooled ovaries from two animals.

In testis the maximum decrease (45.6%) in the enzyme activity was observed on day 4 which remained approximately at the same level throughout the period of observation.

SDH is a mitochondrial enzyme and is involved in the oxidative metabolism for energy production. Localization of manganese in mitochondria (MAYNARD and COTZIAS 1955) and inhibition of SDH and lactic dehydrogenase in brain, liver and testis in rats (SINGH et al. 1974) and of SDH and ATPase in testis of rabbits (CHANDRA et

al. 1973; SETH et al. 1973) observed previously suggests that this metal exerts its toxic effect by altering the energy metabolism of the cell. Early inhibition of succinoxidase by cadmium in rat gonads has been reported by DAS et al. (1961). Cadmium, mercury and lead have been shown to inhibit the succinoxidase of pigeon breast muscle preparations (BARRON and KALNITSKY 1947) and other steps of energy generating process (VALLEE and ULMER 1972). Such results suggest that interference with energy generating process could be one of the modes by which metal ions affect cellular physiology.

The histological studies conducted on day 4 in testis and on day 8 in ovary did not reveal any significant difference between control and treated rats.

Although according to our two previous studies (SETH et al. 1973; SINGH et al. 1974) a correlation between inhibition of SDH activity and degeneration of seminiferous tubules was observed, it was, however, not possible from these studies to indicate the initial time course of the two events. The significant inhibition of SDH activity from day 1 onward and absence of any histopathological change even at the time of maximum inhibition of SDH, as observed in the present study, suggest that disturbance of energy metabolism is the early manifestation of manganese poisoning which may presumably lead to histological changes later on. In this connection observation of SINGH et al. (1974) on brain is worth recalling. These authors also observed marked biochemical changes in the brain unaccompanied by any histopathological change in manganese treated rats.

ACKNOWLEDGMENTS

The authors are grateful to Dr. S. H. Zaidi, Director, Industrial Toxicology Research Centre, for his support of this work and to Dr. J. D. Kohli for his helpful suggestions and revision of the manuscript. Technical assistance of Mr. Kanhaiya Lal and photographic help to Mr. Musleh Ahmed is gratefully acknowledged.

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