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Research Article

HISTOCHEMICAL SHIFTS IN THE PROFILE OF OVARIAN DEHYDROGENASES OF SEXUALLY MATURE CYCLING FEMALES OF SWISS ALBINO MICE DUE TO SODIUM FLUORIDE INGESTION

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ABSTRACT
Distribution of lactate dehydrogenase (LDH) and succinic dehydrogenase (SDH) in the ovary of mice treated with two chronic doses of sodium fluoride (NaF), for five days, in drinking water. LDH histochemical reactivity was observed following Hess et al., (1958) technique and SDH histochemical reactivity was obtained using the method of Nachlas et al., (1957). A decreasing trend of enzyme reactivity was observed after 5 mg/kg BW dose of NaF. A further decrease in both LDH and SDH enzyme reactivity was evident after 10 mg/kg BW. Functional relationship of these enzymes in reference to NaF damage in ovary is discussed.

***Key Words**: Fluoride, Toxicity, Ovary, Dehydrogenises, Albino Mice.*

INTRODUCTION
Dehydrogenases play a crucial role in supplicating energy needed for various metabolic functions in somatic and germ cells. They form a group of enzymes of mitochondrial and cytoplasmic origin which facilitate many oxidoreduction reactions responsible for generating ATP. Lactate and succinate dehydragenaese are important oxido reductases which are linked not only with the events of spermatogenesis and androgenesis but also with oxagenesis and steroidogenesis. Steroid dehydrogenases subserve a fundamental function of catalysing the synthesis and or inter conversion of vast array of steroid hormones in specialised cells designated as steroidogenic cells. Mammalian ovary serves as an ideal model to determine the repertoire of not only enzymes of TCA-cycle but also of the steroidogenic pathways. The latter function is restricted only to the interstitial, granulosa and leutine cells (Aitken and Roman, 2009).

Lactate and Succinate dehydrogenases(LDH and SDH) are the key enzymes. The former is concerned with the conversion of Lactate - Pyruvate, while the latter has been shown to be the histochemical ‘marker for the loci of mitochondria in cells. Both enzymes have been localised in the ovary of rodents, large number of mammalian sp. e.g. lagomorphs, ungulates, primates and in some spp. of bats (LALL, 1986).

Histochemical and biochemical data indicated that cells possess endogenous mechanism to switch on the glycolytic cycle under oxygen deficit conditions or resort to TCA cycle under oxygen rich conditions (Jaroli, 1980; *Trivedi, 1991*).

The purpose of the present study was to determine the histochemical site and pattern of distribution of Succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH) in the ovarian cells/layers of Swiss albino mice and to determine the shifts in profiles of these enzymes after treatment with increasing dose of NaF (5 mg and 10 mg/kg BW) for five days. The aforesaid enzymes were chosen mainly because they play important roles in carbohydrate metabolism and would serve as indicators of the energy requirement for folliculogenesis. The activity of these enzymes may vary in the process of follicle maturation (Guraya, 1985).Chiropterans are enigmatic animals as they are not only the true flying mammals, but also have diverse reproductive patterns (Wimsatt, 1979). Rhinopoma breeds once each year and possesses ovaries which function alternately in each cycle (Lall, 1986; Trivedi 1991). SDH and LDH have been localised in the ovaries of rodents, lagomorphs, ungulates, primates and some species of bats (species not mentioned in the text of the book) (Guraya, 1985).

MATERIALS AND METHODS

Sexually mature females of Swiss albino mice were bred in our laboratory for the experiment. Animals were divided into control and experimental groups A and B. Experimental animals of group 'A' were given 5 mg/kg BW NaF in drinking water and in 'B' 10 mg/kg BW. Control animals were given deionised water. Ovaries were dissected out and washed with DW. Fresh cryosections (7microns-10microns) were cut on cryostat. Serial sections were incubated in different substrate media for the localization of enzyme Spp. described as under:

Succinic dehydoogenase (SDH)

SDH was demonstrated by the method of Nachlas *et al*.,(1957) using nitro blue tetrazolium (Nitro BT) as an electron acceptor. Sections were incubated in the substrate medium containing tetrazolium for 15 mins at 37 degree centigrade. Sections were post fixed in 10% neutral formalin after rinsing in distilled water. Blue diformazon granule deposits were taken as indicative of enzyme activity.

Lactic dehydrogenase (LDH)

LDH was histochemically detected according to the technique of Hess *et al*., (1958) as described by Pearse, (1968). The sections were incubated in the appropriate substrate solution containing nitroblue tetrazolium (Nitro BT) and nicotinamide adenine dinucleotide (NAD) for 1 5 mins at 37 degree centigrade and post fixed in 10% neutral formalin. Violet blue diformazon deposits were taken as manifestations of LDH activity.

Control reaction

Suitable controls for SDH and LDH were run simultaneously. Sections were incubated in medium where substrate is replaced with 1 ml of distilled water or they were boiled at 80 degree centigrade prior to incubation. The enzyme activities in the ovarian constituents were visually appraised and graded.

RESULTS AND DISCUSSION

I. Succinic dehydrogenase (SDH)

(i) Control

SDH activity was very intense in the granulosa cells of the primary, secondary, pre-antral and antral follicles. In atretic follicles, a well intense staining for SDH was evident. Interestingly stroma tissue also demonstrated intense SDH reactivity

(ii) SDH activity after treatment with 5 mg/kg dose of NaF for 5 days

Medium to low SDH enzyme reactivity was visualized in the granulosa cells investing primary, secondary, pre-antral and antral follicles. The atretic follicles as well demonstrate weak SDH reaction. The growing follicles displayed very little reaction as compared to Oocytes and refrectory follicles. Corpora lutea displayed heterogeneous distribution of diformazon granules. Interestingly, stromal tissue demonstrate intense enzyme reaction.

(iii) SDH activity after treatment with 10 mg/kg dose of NaF for 5 days

A decremental trend in SDH intensity was quite evident in the ovarian tissue after 10 mg/kg BW treatment of NaF from control level. Weak enzyme reactivity was displayed by primary, secondary, pre-antral and atretic follicles. Stroma tissue also demonstrates intense enzyme reaction.

II. Lactic dehydrogenase (LDH)

(i) Control

LDH reactivity was very intense in the ovarian tissue, primary, secondary pre-antral and antral follicles show strong reaction. Stroma tissue also shows reaction similar to SDH. Granulosa cells demonstrate intense to moderate LDH reactivity.

(ii) LDH activity after treatment with 5 mg/kg dose of NaF for 5 days.

Cells of the primary, secondary, pre-antral and antral follicles displayed a very weak staining reaction. A weak LDH activity was observed in the atretic follicles. LDH activity was relatively low in stroma cells and in the interstitial tissue. Degenerating follicles also displayed very weak enzymatic activity. Weak

reaction was observed in the lutein cells of corpus luteum. Non growing follicles displayed base level of enzyme reaction vis-a-vis. growing and atretic follicle. Difference in enzyme activity was observed in the ovarian follicle types, stroma and interstitial cells.

(iii) LDH activity after treatment with 10 mg/kg dose of NaF for 5 days .

Interestingly after 10 mg\ kg BW treatment of NaF a decreasing trend of LDH reactivity is well, established. Primary and secondary follicles demonstrate negligible reaction. Atretic and graffian follicles demonstrate weak LDH staining. Interestingly no reaction was observed in the stroma tissue. However, these layers show weak enzyme reactivity.

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