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ENZYMATIC CHANGES IN THE MALE REPRODUCTIVE ORGANS BY DELTA-9-TETRAHYDROCANNABINOL

INDIRA CHAKRAVARTY (née SEN)
All India Institute of Hygiene and Public Health, Govt. of India, 110 Chittaranjan Avenue, Calcutta-700 073, India

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Abstract—Like most psychoactive agents, cannabis and its active component delta-9-tetrahydrocannabinol (Δ^9-THC) have been reported to affect the neuroendocrine axis in animals. The effect of Δ^9-THC on some of the functionally important enzymes of the male reproductive organs are reported. The study indicates that Δ^9-THC reduces the activities of the enzymes, β-glucuronidase, α-glucosidase acid phosphatase and fructose-6-phosphatase in a dose related manner in the testis, prostate as well as in the epididymis. It may be concluded that Δ^9-THC may interfere with the normal functioning of the male reproductive organs.

Delta-9-tetrahydrocannabinol or Δ^9-THC is the major active component of cannabis. Cannabis in turn is used to make several kinds of psychoactive drugs like Hashish, Charas, Ganja, Bhang, marihuana etc. Like many of the other psychoactive agents Δ^9-THC has also been reported to cause prominent alterations on the reproductive physiology of both male and female systems, possibly through the neuro-endocrine axis. With regards to male rats it has been documented that the drug causes prominent biochemical changes in most of the reproductive organs [1–5], depresses spermatogenesis [6] causing spermatozoal changes [7, 8] and also depresses circulating testosterone levels [9, 10]. In the present paper we report the effect of Δ^9-THC treatment to adult male rats, with regards to activities of some of the functionally important enzymes of the reproductive organs. The enzymes studied are β-glucuronidase, α-glucosidase, acid phosphatase and fructose-6-phosphatase, all of which are reported to have a prominent action on the maintenance of normal physiological functions of the male reproductive organs.

MATERIALS AND METHODS

Adult male rats of Charles Foster strain weighing about 100–150 g, obtained from the laboratory animal house were used for the study. The rats were maintained at 12 hr light and 12 hr dark with temperature set approximately at 26 ± 2°. Laboratory stock diet and water was given *ad libitum*.

Delta-9-THC obtained from the United Nations Narcotics Laboratory, Geneva, was made into a suspension in saline containing 6% tween-80. The stock solution was kept at 20 mg THC per ml concentration.

Delta-9-THC was injected subcutaneously at doses of 10 mg, 25 mg, and 50 mg per kg body weight at 10 a.m. each morning, for ten consecutive days. The control rats received an equivalent volume (approximately 0.25 to 0.5 ml) of the vehicle each day. Each group had at least six rats and all of them were killed by instant decapitation, 24 hr after the last injection of THC.

The whole of the testis, epididymis and ventral prostate were carefully dissected out, blotted in a filter paper and weighed in an electric balance. The tissues were kept in crushed ice till further treatment.

Enzyme estimations were made, using cell debris-free supernatant, i.e. after cold centrifugation at 3000 *g* in an International Centrifuge. β-Glucuronidase activity was estimated according to the method of Fishman *et al.* [11] using PNP-β-*d*-glucuronide as the substrate. α-Glucosidase activity was measured according to the method of Rao *et al.* [12] using PNP-α-*d*-glucoside as the substrate. The acid-phosphatase activity was assayed by the method of Lowry *et al.* [13] using PNP-phosphate as the substrate at pH 5.0. Fructose-6-phosphatase activity was measured according to the method of Baginski *et al.* [14] and phosphate estimation was done using the assay procedure of Fiske and SubbaRow [15] using fructose-6-phosphate as the substrate. Protein content was measured by the method of Lowry *et al.* [16].

RESULTS

Figure 1 indicates the effect of Δ^9-THC on the β-glucuronidase activities of testis, epididymis and prostate, expressed as μg PNP liberated per mg of protein per hour. It is evident from the figure that THC reduces the activity of the enzyme in all the three reproductive organs in a dose-related manner.

Figure 2 illustrates the role of Δ^9-THC on the enzyme α-glucosidase. In this case also the drug appears to reduce the enzyme activity in prostate, epididymis as well as the testis uniformly with the increase in dose.

Figure 3 indicates the effect of Δ^9-THC on the acid phosphatase activity also signifying a uniformly, dose-dependent reducing effect of the drug on the enzyme action in all three organs.

In the case of fructose-6-phosphatase activity (Fig. 4) a similar decreasing action of the drug on the enzyme activity is evident.

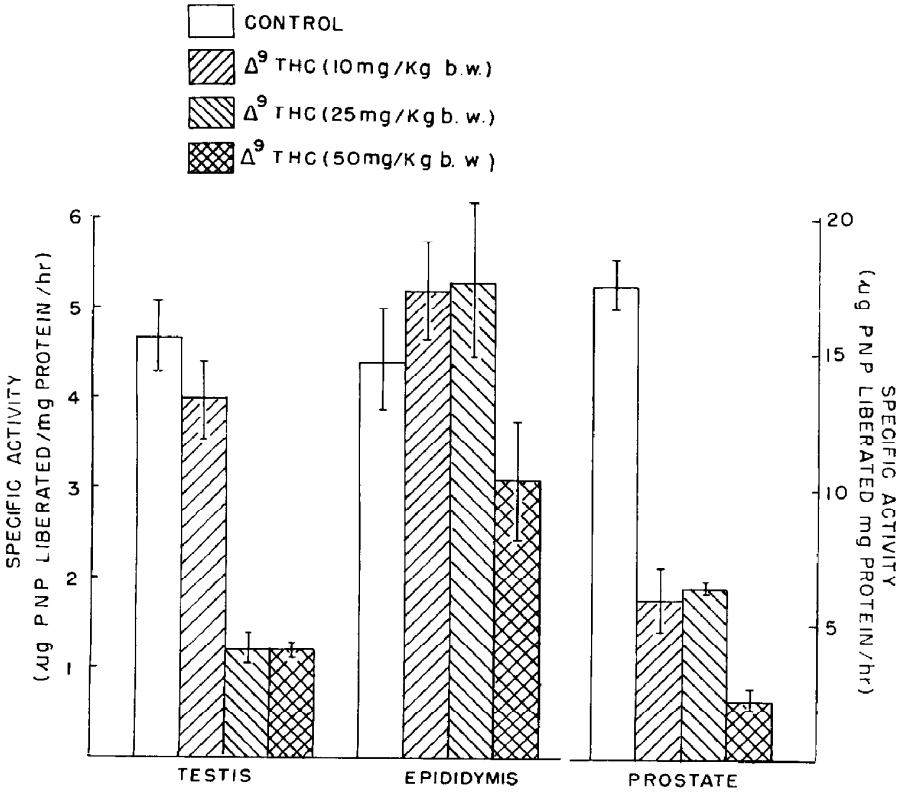

Fig. 1. Delta-9-THC: effect on β-glucuronidase activity in male reproductive organs in adult male rats. Left ordinate indicates activity in the testicular and epididymal tissues; right ordinate that in prostate tissue.

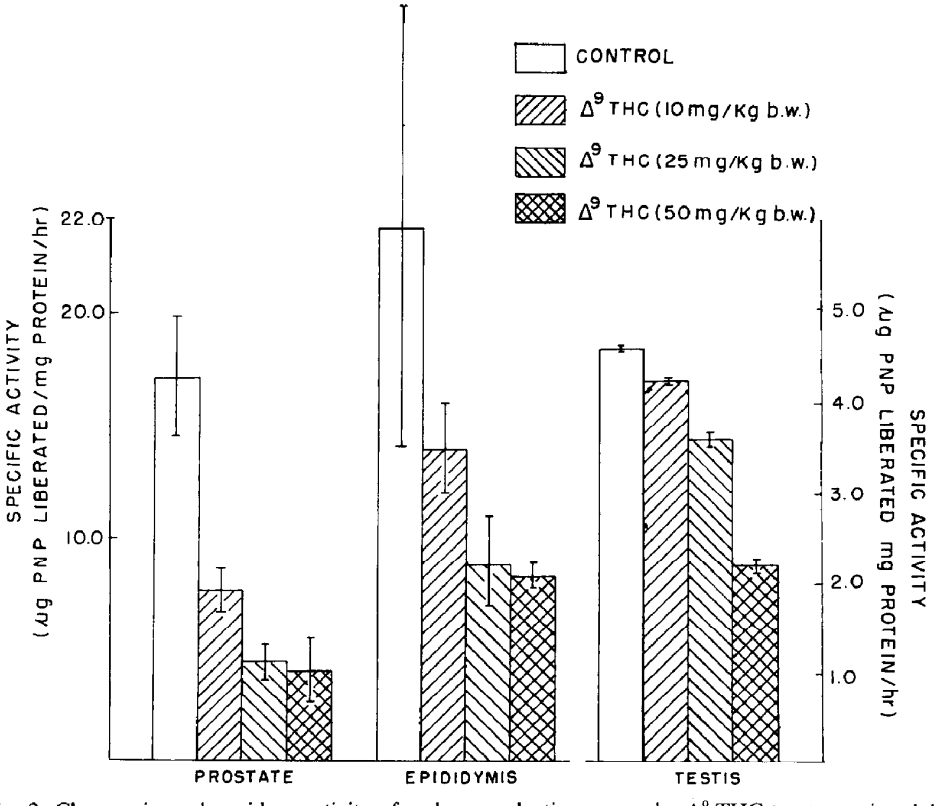

Fig. 2. Changes in α-glucosidase activity of male reproductive organs by Δ^9-THC treatment in adult male rats. Left ordinate indicates activities in the prostate and epididymal tissue; right ordinate indicates that in the testicular tissue.

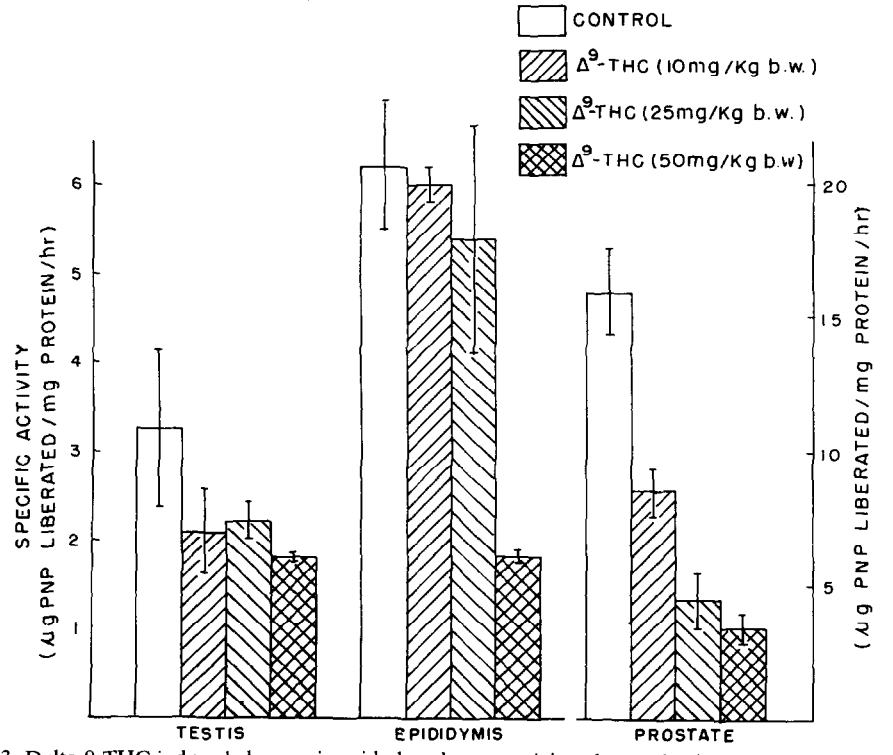

Fig. 3. Delta-9-THC induced changes in acid phosphatase activity of reproductive organs in adult male rats. Left ordinate indicates activities in the testicular and epididymal tissues; right ordinate indicates the activity in the prostate tissue.

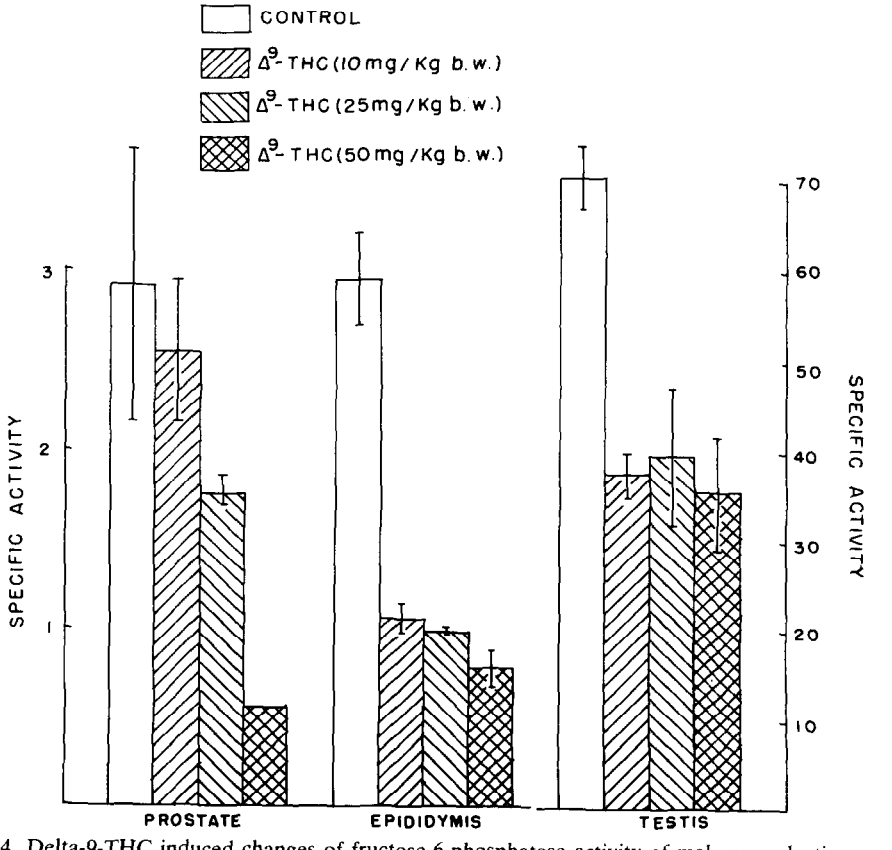

Fig. 4. Delta-9-THC induced changes of fructose-6-phosphatase activity of male reproductive organs in adult male rats. Left ordinate indicates activities in the prostate and epididymal tissues; right ordinate indicates that in the testicular tissue.

Therefore, to sum up, Δ^9-THC appears to reduce the activities of β-glucuronidase, α-glucosidase, acid phosphatase and fructose-6-phosphatase in a dose-related manner, in the prostate, epididymis and testis in adult male rats.

DISCUSSION

Acid mucopolysaccharides and mucoproteins are important constituents of the semen [17], which play an important role with regard to attachment, penetration and fertilization of the egg by sperm [18]. Hyaluronidase is the mucolytic enzyme which brings about the depolymerisation and hydrolysis of hyaluronic acid, an important mucopolysaccharide [19]. Mammalian testis and sperm are the richest animal sources of hyaluronidase which have a prominent role in the process of fertilization [19]. After the action of hyaluronidase the free glucuronic acid is formed due to the action of the enzyme β-glucuronidase. The level of β-glucuronidase has been reported to be decreased by about 50 per cent in the epididymis after castration, and restored to normal after administration of testosterone [20], indicating that the enzyme activity is closely dependent on circulating testosterone levels. Hence Δ^9-THC induced reduction of β-glucuronidase activity in the male reproductive organs (Fig. 1) indicating that the circulating testosterone level may be lowered [9, 10].

Accessory organs of reproduction and their secretions in both rats and mice possess a high level of glycosidase activity [21, 22]. The enzyme activity has been demonstrated to be androgen dependent [23]. Castration reduces the enzyme level which is fully restored to normal after testosterone injections to both immature and mature rats [23]. Hence in this case also Δ^9-THC induced reduction in the α-glucosidase activity indicates an antiandrogenic action of the drug.

Acid phosphatase activity is one of the important male secondary sex characteristics. The level of the enzyme is low in childhood in the accessory organs but increases rapidly at puberty [24]. Administration of androgenic hormone stimulated considerably the phosphatase output from the accessory glands, especially the prostate [25]. Hence reduction in both acid-phosphatase (Fig. 3) as well as fructose-6-phosphatase (Fig. 4) activities further signify that Δ^9-THC has an antagonistic action on the androgen secretion. From the foregoing work it is therefore concluded that Δ^9-THC appears to alter the activity of some of the functionally crucial enzymes of the male reproductive organs, which in turn may interfere with the normal physiological processes of the system.

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