GONADOTROPHIN, TESTOSTERONE AND PROLACTIN INTERRELATIONSHIPS IN CADMIUM-TREATED RATS

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SUMMARY

We have investigated the long-term effect of a single subcutaneous injection of cadmium chloride on plasma testosterone and gonadotrophin levels and the prolactin response to the dopaminergic antagonist metoclopramide in the rat. Twelve days after treatment with cadmium there was testicular necrosis, associated with a decrease in testosterone concentration and atrophy of the accessory sexual glands. By 185 days, partial recovery of the accessory sexual glands indicated by Leydig cell regeneration and a slight rise in testosterone levels had occurred. There was, however, persistent damage to the germinal epithelium. Concentrations of LH increased eightfold above controls by day 12, remained raised until 60 days and then decreased to threefold above controls at 280 days. In contrast, FSH levels reached a maximum between 60 and 130 days and remained persistently raised.

The peak prolactin response to metoclopramide in cadmium-treated rats was depressed 12 days after cadmium administration and levels remained low at 19 and 75 days. Normal prolactin responses to metoclopramide were obtained 130 days after cadmium treatment using 1·0 mg metoclopramide/kg or 280 days after treatment using 0·25 mg/kg. When control and cadmium-treated rats were castrated at 280 days and then given metoclopramide 10 days later, the prolactin response was significantly reduced. It is concluded that the impaired prolactin response to metoclopramide in cadmium-treated rats is reversible. Prolactin returns to normal in parallel with regeneration of the Leydig cells, partial restoration of the accessory sex organ weight, slight increase in plasma testosterone and decrease in LH levels. These results suggest that testosterone is not solely responsible for the maintenance of normal prolactin secretion in the male rat.

INTRODUCTION

Parenteral administration of a single small dose of cadmium salts results in testicular necrosis and subsequent atrophy of the accessory sexual glands (Gunn & Gould, 1970). After some time, recovery of the latter can be observed consequent to Leydig cell regeneration. However, the germinal epithelium remains persistently damaged (Gunn & Gould, 1970).

There is some slight similarity in morphology between seminiferous tubule failure in man and the late stages of cadmium-induced azoospermia in the rat. Azoospermic or oligospermic patients with high gonadotrophins, normal levels of testosterone and intact secondary sex characteristics have an exaggerated prolactin response to thyrotrophin releasing hormone (TRH) (Spitz, Zylber, Cohen, Almaliach & LeRoith, 1979). Patients orchidectomized because of prostatic carcinoma, have normal prolactin responses (LeRoith, Liel, Caine & Spitz, 1981). However, there is a decreased prolactin response to both TRH and metoclopramide in the castrated rat (Zylber, Gershman & Spitz, 1979). Administration of
testosterone propionate, in a dose sufficient to maintain the weight of the accessory sex organs, only partially restored prolactin secretion in the castrated rat (Zylber-Haran, Gersham & Spitz, 1981a).

The aim of the present study was to assess the interrelationships between testicular morphology and circulating plasma levels of testosterone, gonadotrophins and prolactin in cadmium-treated rats during a period of 280 days after cadmium administration.

MATERIALS AND METHODS

Animals

Male rats (180–220 g) of the Charles River strain were used in all studies. They were housed under a schedule of 14 h light : 10 h darkness, and water and rat chow were available ad libitum. Forty rats were treated with a single subcutaneous injection of 0.02 mmol cadmium chloride (BDH Chemicals Ltd, Poole, Dorset)/kg in 0.2 ml distilled water. This dose damages the seminiferous tubules and interstitial tissue, but recovery of the latter is observed within 6–8 weeks (Gunn & Gould, 1970). Control animals were injected with distilled water. In some rats, castration was performed under ether anaesthesia through a single mid-scrotal incision.

Blood sampling and pituitary gland collection

At 12, 19, 60, 75, 130 and 280 days after cadmium administration, groups of six cadmium-tREATED and six control rats were subjected to a metoclopramide test, as described previously (Zylber et al. 1979). In brief, rats were anaesthetized with ketamine (Ketalar; Parke Davis, Michigan, U.S.A.; 100 mg/kg) and a catheter was inserted into the jugular vein. After a 30-min waiting period a basal sample was taken, metoclopramide (Pramin; Rafa, Jerusalem, Israel; 0.25 or 1.0 mg/kg) was administered subcutaneously and blood samples were taken at 10, 20, 30 and 45 min. Twelve additional rats not given cadmium chloride were subjected to the metoclopramide test (1.0 mg/kg) 12 and 280 days after castration. Two groups of control and cadmium-treated rats were castrated at 280 days and given metoclopramide (1.0 mg/kg) 10 days later. At 19, 75 and 185 days after cadmium treatment the pituitary glands of six cadmium-treated and six control rats were dissected out, weighed and kept at −20 °C. Before assay, the gland of each rat was homogenized separately in cold 0.9% NaCl (1 ml/10 mg wet weight), centrifuged (500 g for 15 min at 4 °C) and the supernatant fraction used for analysis.

Histology

The testes of three cadmium-treated and three control rats at 19, 75, 130 and 175 days after cadmium treatment were fixed in Bouin's solution. Sections embedded in paraffin wax were stained using haematoxylin and eosin, periodic acid-Schiff or the Von Kossa technique (Krajian, 1963).

Radioimmunoassay

Plasma and pituitary prolactin, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured (Zylber-Haran et al. 1981a, b) by double-antibody radioimmunoassays using reagents kindly supplied by the National Pituitary Agency, NIAMDD, Maryland, U.S.A. Results are expressed in ng/ml NIAMDD rat PRL-RP-1, LH-RP-1 and FSH-RP-1 standards supplied with the kit. Samples from a complete experiment were determined in the same assay. Interassay coefficients of variation for prolactin, LH and FSH assays were 15.5, 16.0 and 10.5% respectively. Corresponding intra-assay coefficients of variation were 7.9, 9.2 and 6.9%. For the testosterone assay, plasma samples were extracted with diethyl ether. The supernatant fractions were dried under nitrogen at 37 °C and the dried material was resuspended in 0.05 M-Tris-HCl buffer (pH 8.0) containing 0.15 M-NaCl, 0.1% gelatin and
0.02% sodium azide. Fractions of the resuspended ether extracts were incubated for 18 h at 4°C with 5000 counts [1,2,6,7,16,17-3H(N)]testosterone/min (New England Nuclear, Boston, U.S.A.) and goat anti-testosterone-7α-bovine serum albumin serum (Miles Yeda, Rehovot, Israel) at a final dilution of 1:100. The cross-reaction of the anti-testosterone serum with dihydrotestosterone was 23.8%. Bound and free testosterone were separated by the dextran-coated charcoal technique. To avoid interassay variation, all samples were measured within a single assay. One-way analysis of variance was used to determine mean statistical differences between the various groups. Student’s t-test was used to compare treated rats with their corresponding controls.

RESULTS

Testes and accessory sex organs

Nineteen days after administration of cadmium chloride, there was massive coagulative necrosis of the seminiferous tubules and interstitial tissue although occasional Leydig cells were spared. After 75 days, an apparent reduction in size of the seminiferous tubules was observed and their basement membrane was thickened and irregular. Many of the tubules were completely fibrosed and calcified. An apparent increase in number of interstitial cells was noted beneath the albuginea. After 130 and 175 days similar changes were observed, but in addition, nodules of hyperplastic proliferating Leydig cells were found (Plate). The testes of the control rats remained normal throughout.

By 12 days weights of seminal vesicles (100.8 ± 19.9 (s.d.) mg/100g body weight) and ventral prostate (23.8 ± 4.6 mg/100g body weight) in cadmium-treated rats were both significantly (P < 0.001) decreased as compared with controls (451.1 ± 54.2 and 102.7 ± 14.9 mg/100g body weight respectively). However, after 185 days of treatment, a partial recovery of the accessory sexual glands was observed (seminal vesicle 244.7 ± 90.9 mg/100g body weight and ventral prostate 58.6 ± 28.9 mg/100 body weight). Nevertheless the accessory sexual gland weight was still less than in the controls (P < 0.001).

Testosterone

Plasma levels of testosterone in control rats changed very little over a period of 280 days (Text-fig. 1). Twelve days after cadmium administration there was a significant (P < 0.001) reduction in the plasma testosterone concentration, but by 60 days levels began to increase, reaching a maximum value between 130 and 280 days. These values were significantly higher (P < 0.001) than at 12 days but still lower (P < 0.001) than the control value.

Gonadotrophins

An analysis of variance indicated that plasma levels of LH and FSH in control rats did not change significantly throughout the experimental period of 280 days (Text-fig. 1). Twelve days after administration of cadmium, mean ± S.D. plasma LH levels increased to 626.7 ± 189.9 ng/ml (P < 0.001), reaching the highest value after 60 days. Thereafter, levels decreased and by 280 days were 253.8 ± 116.4 ng/ml which was significantly less (P < 0.001) than the level at 12 days, but still greater (P < 0.001) than control levels. Plasma FSH levels increased significantly (P < 0.001) from 445.0 ± 95.8 ng/ml in untreated rats to 2047.1 ± 405.3 ng/ml 75 days after cadmium treatment. By 280 days, levels were similar to those at 12 days. Pituitary LH content increased at 19 days (P < 0.001) and remained raised at 75 and 185 days. Likewise, pituitary FSH content increased 19 days after cadmium treatment (P < 0.001) and remained high (Table 1).

Prolactin

Basal plasma prolactin levels of cadmium-treated rats were 4.59 ± 1.22 ng/ml and similar to those of control rats (4.84 ± 1.22 ng/ml). In both groups levels remained constant over 280
Table 1. Effect of cadmium treatment of male rats on pituitary LH, FSH and prolactin content (% of control values). Values are mean ± S.D.; number of rats shown in parentheses.

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>LH</th>
<th>FSH</th>
<th>Prolactin</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>217.2 ± 50.6 (5)</td>
<td>168.2 ± 44.9 (6)</td>
<td>78.3 ± 16.6 (6)</td>
</tr>
<tr>
<td>75</td>
<td>231.4 ± 31.2 (4)</td>
<td>220.6 ± 57.8 (5)</td>
<td>81.9 ± 18.8 (6)</td>
</tr>
<tr>
<td>185</td>
<td>242.5 ± 85.6 (5)</td>
<td>182.1 ± 29.6 (5)</td>
<td>94.5 ± 12.0 (5)</td>
</tr>
</tbody>
</table>

Twelve days after cadmium administration, pituitary prolactin content decreased ($P<0.01$) and remained low until 75 days but returned to normal at 185 days (Table 1).

Prolactin response to metoclopramide

With both doses of metoclopramide (0.25 and 1.0 mg/kg) and at all time intervals, the prolactin response of control rats did not change significantly over 280 days. The prolactin response to metoclopramide (0.25 mg/kg) in cadmium-treated rats was significantly less than in controls ($P<0.001$) at 12, 19, 60, 75 and 130 days (Text-fig. 2a). However, by 280 days, the peak prolactin response had increased and was not significantly different from that of controls (Text-fig. 2a).

When control and cadmium-treated rats received metoclopramide 1.0 mg/kg (Text-fig. 2b), the prolactin response was similar, but was reduced only at 12, 19, 60 and 75 days after cadmium treatment.
Text-fig. 2. Plasma prolactin responses to metoclopramide at doses of (a) 0·25 mg/kg in control (solid lines) and cadmium-treated (broken lines) rats and (b) 1·0 mg/kg in control, cadmium-treated and castrated (---) rats as a function of time over 280 days. Values are means ± S.E.M. of six animals.

cadmium treatment ($P < 0.001$). By 130 and 280 days, the peak prolactin response had increased to the control levels.

The prolactin response to metoclopramide (1·0 mg/kg) in control and cadmium-treated rats was compared with that of castrated rats at 12 and 280 days (Text-fig. 2b). After 12
Text-fig. 3. Plasma prolactin response to metoclopramide (1·0 mg/kg) in control (— — ) and cadmium-treated (−−−) rats at 280 days. (These are the same data as those shown in Text-fig. 2b except that the prolactin value at 45 min is included.) At this time castration was carried out and control (−−−) and cadmium-treated (−−−) rats were rechallenged with metoclopramide (1·0 mg/kg) 10 days later (i.e. 290 days). Values are means ± S.E.M. of six animals.

days, the prolactin response in castrated rats was not significantly different from cadmium-treated rats. By 280 days, the prolactin response of cadmium-treated rats had returned to normal, although that of castrated rats remained diminished.

Control and cadmium-treated rats were castrated at 280 days and 10 days later given metoclopramide (1·0 mg/kg). Castration of control and cadmium-treated rats significantly ($P < 0·01$) reduced the prolactin release compared with the non-castrated groups (Text-fig. 3).

**DISCUSSION**

A variety of experimental procedures have been used to damage the seminiferous tubules selectively without impairing Leydig cell function. These include radiation (Hopkins, Dulsch, Gauss, Hilscher & Hirschhauser, 1978), induction of cryptorchidism (Kerr, Rich & De Kretser, 1979), vitamin A-deficient diet (Rich & De Kretser, 1977), administration of hydroxyurea (Rich & De Kretser, 1977) and busulfan (Debeljuk, Arimura, & Schally, 1973). Cadmium is highly toxic to the testes of a number of animals. Parizek & Zahor (1956) reported complete necrosis of the rat testis after a single injection of cadmium in a dose of 0·02 mmol/kg body weight. After the initial necrotic phase, Leydig cell regeneration occurs without any tubular regeneration (Allanson & Deanesly, 1962; Roe, Dukes, Cameron, Pugh & Mitchley, 1964).

In the initial phase characterized by coagulative necrosis of tubular and interstitial cell elements, there was an increase in plasma levels of LH and FSH and a decrease in plasma testosterone with atrophy of the accessory sexual glands. This is compatible with earlier reports (Schenck, Senge & Graf, 1975; Chandler, Timms, Morton & Groom, 1976).

The second stage of cadmium damage was characterized by interstitial cell proliferation and hyperplasia, a slight increase in testosterone levels with a partial restoration of ventral prostate and seminal vesicle weight and slight decrease in LH levels. However, none of these parameters had fully returned to normal. Levels of FSH remained markedly raised and histologically there was no regeneration of the seminiferous tubules which were fibrosed and calcified. This is compatible with observations by Favino, Baillie & Griffiths (1966) who showed that testosterone synthesis increased progressively with interstitial tissue regeneration.

Prolactin secretion is controlled predominantly by an inhibitory dopaminergic mechanism (Clemens & Meites, 1977). Metoclopramide increases prolactin secretion by induction of
Prolactin secretion in cadmium-treated rats

dopamine blockade (Carlson, Briggs & McCallum, 1977). Our previous studies have shown that a single subcutaneous injection of metoclopramide elicited a dose-dependent increase in plasma prolactin levels in both control and castrated male rats, higher doses producing greater increases. Control rats consistently responded with a greater plasma prolactin increase than castrated rats (Zylber-Haran, Gershman & Spitz, 1981b). This was reconfirmed in this study, in which the diminished prolactin response of castrated rats persisted for 280 days. Similarly, in cadmium-treated rats, a reduction in prolactin release was observed at an early stage in parallel with testicular necrosis. The impaired prolactin response to metoclopramide was reversible; restoration of the prolactin response was observed at 130 days with metoclopramide (1·0 mg/kg) and at 280 days with a lower dose. Prolactin secretion returned to normal in parallel with regeneration of the Leydig cells, partial increase in plasma testosterone levels and accessory sex organ weight, and decrease in plasma LH levels.

The mechanism for the alteration in prolactin dynamics in the cadmium-treated rats is unknown. However, there is evidence that castrated rats have an increase in dopaminergic tone. Kizer, Palkovits, Zivin, Brownstein, Saavedra & Kopin (1974) reported a significant rise in the tyrosine hydroxylase activity in the median eminence after castration and this increased activity was partially reversed by treatment of gonadectomized animals with testosterone. Gudelsky, Annunziato & Moore (1977) showed that 5 days after ovariectomy there was a marked increase in the dopamine concentration of the median eminence, and daily administration of oestradiol benzoate reversed the increase in dopamine induced by ovariectomy. Our results can be explained by an alteration in dopaminergic tone in the cadmium-treated rats with reduction in the prolactin response to metoclopramide. This returned to normal in parallel with regeneration of Leydig cells and partial increase in testosterone concentration.

Testosterone is not the sole product responsible for the restoration to normal of prolactin secretion in cadmium-treated rats. Administration of testosterone propionate to castrated rats in a dose sufficient to maintain the weight of the accessory sex organs only partially restores prolactin secretion (Zylber-Haran et al 1981a). In contrast, in the present study, the prolactin response returned to normal without full recovery of the accessory sexual organ weight and plasma testosterone levels. Also, in patients with primary testicular failure and testosterone levels in the low normal range, an exaggerated prolactin response to TRH was observed (Spitz et al. 1979). In addition to testosterone, oestradiol also increases prolactin release in man and animals (Kalra, Fawcett, Krulich & McCann, 1973; Yen, Ebara & Siler, 1974). Labrie, Drouin, Ferland, Lagace, Beaulieu, De Lean, Kelly, Caron & Raymond (1978) reported that preincubation of anterior pituitary cells in culture with oestradiol-17β led to an almost complete reversal of the inhibitory effect of dopamine on both basal and TRH-induced prolactin release. This clearly indicates an important interaction of oestradiol with dopamine receptors and oestradiol could be involved in the restoration of the prolactin response.

It is concluded that Leydig cell secretory products play an important role in mediating prolactin secretion in the male rat. Evidence for this is the fact that the prolactin response to metoclopramide returned to normal in parallel with regeneration of the Leydig cell, partial increase in testosterone levels and accessory sex organ weight, and decrease in LH levels. In contrast, rats castrated for 280 days demonstrated an impaired prolactin response to metoclopramide. The normal response of the 280-day cadmium-treated rat was reduced by castration.

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REFERENCES


DESCRIPTION OF PLATE

(Haematoxylin and eosin: × 200)

Pathology 175 days after treatment of rat with cadmium showing calcified atrophic seminiferous tubules and nodular Leydig cell (NLC) hyperplasia adjacent to the albuginea.