

Effects of Cadmium as a Possible Endocrine Disruptor upon the Serum Level of Sex Steroids and the Secretion of Gonadotropins from Pituitary in Adult Rats

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Objective: The objective of this study is to assess the effect of cadmium (Cd) upon the female reproductive system by investigating the serum levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) as well as sex steroids such as estradiol (E₂) and progesterone (P).

Materials and Methods: Sprague-Dawley (SD) female rats were daily injected subcutaneously with CdCl₂ for 7 or 14 days at a dose of 0, 0.5 and 1.0 mg /Kg body-weight (BW) and the serum levels of FSH, LH, E₂ and P were measured by radioimmunoassay. Also, the levels of FSH and LH in the presence or absence of extraneously added gonadotropin-releasing hormone (GnRH) were determined in the similarly treated rats.

Results: Although the FSH and LH levels were not changed by CdCl₂ administration, the serum concentration of E₂ was significantly decreased by the injection with both high and low doses of CdCl₂. In some conditions the serum P level was also decreased. To examine the reactivity of pituitary cells to GnRH in CdCl₂-treated rats, we measured the serum levels of FSH and LH in the presence or absence of extraneously added GnRH and then found that although the secretion of FSH and LH was increased more or less by GnRH administration in any combinations, the increase in the serum LH level of CdCl₂-treated rats was significantly lower than that of control rats.

Conclusion: Cd may decrease the reactivity of LH cells to GnRH, resulting in lower serum levels of E₂ and P through the disorder of ovarian cycles.

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Introduction

Cd is a toxicant that has a long biological half-life and a wide-ranging destructive effects on many organs and systems. However, only a little has been known about the mechanism of Cd to induce the reproductive disorder.

In fact, during these several decades the toxicity of Cd for the reproductive system has aroused public concerns. Shiva et al. (1982) reported that Cd caused scatter hemorrhages in mice and inhibited the ovulation, resulting in temporary sterility in rabbit^[1]. Zhang et al. (1999) found that Cd may delay the estrous cycle and increase the proportion of atretic follicles^[2]. Ding et al. (1992) also found that Cd inhibits the ovulation in rats, but the ovulation can be recovered by the injection of supplementary LHRH^[3]. Since it is well known that the estrous cycles, tightly connected with ovulation, are highly dependent upon the hypothalamus-pituitary-gonad axis, the information on the serum levels of gonadotropins and sex steroids would be essential to understand the reproductive toxicity of Cd for females.

In the present study, we measured the serum concentrations of FSH, LH, E₂ and P by radioimmunoassay in the female rats, which were administered with CdCl₂. Moreover, to assess the effect of CdCl₂ upon the reactivity of pituitary to hypothalamic stimulus, the serum level of FSH and LH was also examined in the CdCl₂-treated rats after the exposure to a GnRH analogue.

Materials and Methods

Animals

Adult female Sprague-Dawley (SD) rats weighing

180-250g (n=78) were purchased from the Center of Experiment Animal (Shanghai, China) and used in the present study.

Chemicals

Cadmium chloride (CdCl_2) was purchased from Tingxin Chemical Plant (Shanghai, China). Alarelin, a synthetic nonapeptide analogue of GnRH, was from Maanshang Biochemical & Pharmaceutical Factory (Anhui, China). Radioimmunoassay kits for FSH, LH, E_2 and P were from North Institution of Biological Production (Beijing, China).

Experimental procedures

Experiment I: The effect of Cd upon the serum levels of FSH, LH, E_2 and P

Forty-eight rats were weighed and randomly assigned to three groups. The rats of experimental groups, low dose (n=16) and high dose (n=16), were subcutaneously injected daily with a dose of 0.5 and 1.0 mg/Kg BW of CdCl_2 (as a Cd amount), dissolved in saline, for 7 days, respectively. The control group (n=16) was injected daily with saline alone. Then, 8 rats from each group were selected randomly and the blood was drawn via eyeball rid for the preparation of serum. The remaining 8 rats of each group continuously received the daily injection of CdCl_2 solution for more 7 days, and then the blood was collected similarly. The collected sera were stored at -20°C till to be used for radioimmunoassays.

Experiment II: The effect of extraneous GnRH analogue upon the serum level of FSH and LH

Thirty rats were weighed and randomly assigned to three groups; control, low dose and high dose. Each group consisting of 10 rats received daily injections with saline alone, 0.25 mg/Kg BW or 1.0 mg/Kg BW, respectively, for 30 days. At the end of the protocol, rats in estrous phase determined by vaginal smear were injected with $3.0 \mu\text{g/Kg}$ BW of Alarelin. Before and 40 min after the injection, the blood was collected via eyeball rid and the concentrations of FSH and LH were measured.

Assay for the serum concentration of various hormones

Serum FSH, LH, E_2 and P were determined by radioimmunoassays. All procedures were carried out according to the instruction accompanying the kit.

Statistical analysis

All data were expressed as mean \pm standard deviation (SD). Differences between different groups were analyzed by a computer based Statistical Package for the Social Science (SPSS version 10.0) for statistical significance using. One-way analysis of variance with Student-Newman-Keuls (SNK) test. A P value less than 0.05 was considered statistically significant.

Results

Generally, the rats injected with CdCl_2 presented various degrees of spiritual cachexia and anorexia appetite. Also, around the site of injection, a tubercle of soybean size and in some cases an abscess was formed especially in the high dose group.

First of all, we examined the effects of CdCl_2 upon the serum levels of various hormones related to the female reproductive activity. As shown in Table 1, when rats were treated with CdCl_2 for 7 days, the levels of E_2 and P were significantly decreased at the low or the high dose of CdCl_2 respectively, compared to that of the control group. On the other hand, no changes in the serum levels of FSH and LH were found.

Table 1. Serum levels of various hormones in the rats administered with CdCl_2 for 7 days

Group	FSH (mIU/ml)	LH (mIU/ml)	E_2 (pg/ml)	P (ng/ml)
Control	5.127 \pm 1.651	10.561 \pm 1.845	285.72 \pm 123.66	6.907 \pm 3.631
Low dose	4.567 \pm 1.885	11.392 \pm 1.521	168.68 \pm 33.04	1.321 \pm 1.475*
High dose	4.362 \pm 1.372	10.130 \pm 1.889	148.42 \pm 53.18*	3.497 \pm 2.310

* significantly different from the control, $P < 0.05$ (SNK)

When rats were administered with CdCl_2 for 14 days, the serum level of E_2 was declined to 50-55% of the control level by injections with CdCl_2 , and that of P was also markedly decreased to about 20% of the control, as shown in Table 2. Again, however, the levels of FSH and LH were not significantly affected by CdCl_2 injections.

Table 2. Serum levels of various hormones in the rats administered with CdCl₂ for 14 days

Group	FSH (mIU/ml)	LH (mIU/ml)	E ₂ (pg/ml)	P (ng/ml)
Control	3.344±0.575	10.486±1.148	230.64±83.07	5.513±4.348
Low dose	2.768±0.698	11.382±1.228	117.61±35.14*	5.144±6.394
High dose	3.944±1.510	9.959±1.567	128.97±36.21*	1.204±0.687*

* significantly different from the control, P<0.05 (SNK)

As a next step, to gain some insights into the decrease in the production of ovarian steroids in CdCl₂-treated rats, we investigated the reactivity of the release of pituitary gonadotropins to GnRH. When the rats were injected extraneously with Alarelin, a synthetic GnRH analogue, the serum levels of FSH and LH were increased more or less compared to that before the injection. However, in the CdCl₂-treated rats, the increase in the serum level of LH was significantly reduced, as shown in Table 3. These results indicate that the reactivity of pituitary LH cells to GnRH was decreased by CdCl₂ administration.

Table 3. Serum levels of FSH and LH in the rats exposed to CdCl₂ before and after the injection of GnRH

Group	FSH (mIU/ml)		LH (mIU/ml)			
	before injection (n)	after injection (n)	before injection (n)	after injection (n)	before injection (n)	after injection (n)
Control	5.345±0.347 (8)	6.909±1.139** (9)	2.944±0.986 (9)	6.161±1.298** (9)	2.944±0.986 (9)	6.161±1.298** (9)
Low dose	5.551±1.618 (8)	7.016±1.148* (9)	1.757±0.810 (8)	4.576±1.292** Δ (8)	1.757±0.810 (8)	4.576±1.292** Δ (8)
High dose	5.675±1.266 (8)	7.806±1.669* (9)	2.969±1.405 (8)	4.699±1.355** Δ (8)	2.969±1.405 (8)	4.699±1.355** Δ (8)

* significantly different from that before injection, P<0.05 (SNK)

** significantly different from that before injection, P<0.01 (SNK)

Δ significantly different from that of the control, P<0.05 (SNK)

n the number of rats in each group

Discussion

In the present study, we have examined the effects of Cd upon the serum levels of various hormones, which are involved in the female reproductive function, and have found that the levels of E₂ and P are significantly decreased by Cd administration. Moreover, we have also found that the reactivity of pituitary LH cells to extraneously added GnRH is markedly reduced by Cd, indicating that Cd may exert its deteriorative effects on pituitary as well as ovary itself in rats. Cd has been considered as an important environmental endocrine disruptor (EED)^[6-9], since it has a distinct

female reproductive toxicity^[8], such as abnormal menstruation in women^[10]. In fact, both ovary and uterus are known to accumulate Cd to a great extent. Also, it is known that in the experimental animals exposed to Cd, the delay in the estrous cycle^[2], the retardation of follicular growth and development^[2], and the inhibition of ovulation^[1,3] occurred, leading to temporary sterility[1]. However, the mechanism underlying the toxic effects of Cd on the female reproductive system was largely unknown. Therefore, our present results seem to be significant in the understanding of Cd action to mimic the endocrine system constituting hypothalamus-pituitary-ovary axis.

Ding et al. (1992) previously reported that the serum level of LH was greatly decreased in the rats administered subcutaneously with a single dose of 5-10 mg/Kg BW of Cd. However, our results revealed that the serum levels of FSH and LH were not significantly different between control and Cd-treated groups even in 7-days or 14-days exposure. Although there is no appropriate explanation for the discrepancy in these results at present, the difference in the experimental protocol to give Cd to rats may affect the final level of LH.

In the present study, the serum levels of E₂ in the high dose group with 7-days Cd exposure or in the high and low dose groups with 14-days Cd exposure were significantly lower than that of the corresponding control group (P<0.05). Moreover, we also found that the serum P level in the low dose group with 7-days Cd exposure and in the high dose group with 14-days Cd exposure were significantly lower than that of the control group (P<0.05). In accord with these results, Paksy et al. (1992) reported that Cd suppressed the steroid synthesis in cultured granulosa and luteal cells^[11], indicating the direct action of Cd on ovarian cells. On the other hand, we found that the concentrations of serum LH and FSH were significantly increased after the injection of a GnRH analogue, compared to that before the injection, as was in agreement with the report of Varga et al. (1991)^[4]. However, after the injection of GnRH the serum LH level in the rats exposed to Cd was significantly lower than that of the control group. These results indicate that the reactivity of pituitary LH cells, not FSH cells, to GnRH was decreased by the Cd exposure, possibly resulting in a loss of LH surge, which is essential to induce ovulation. Therefore, the lower serum levels of E₂ and P in the Cd exposed female rats may be explained by the retardation of ovarian cycles as well as the direct inhibition of steroid synthesis in ovarian cells.

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