Chapter IV

Effect of lead and cadmium either alone or in combination on hypothalamic-pituitary axis function in non-pregnant rats

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Introduction

Exposure to lead and cadmium is associated with changes in the activity of endocrine system in male and female animals. It was shown that metals can affect the activity of hypothalamus-pituitary-ovarian axis by acting at the hypothalamus (Anderson et al., 1997; Antonio *et al.*, 1999; Das *et al.*, 1993), the pituitary (Lafuente *et al.*, 1999a; Lorenson *et al.*, 1983; Ronis *et al.*, 1998), the ovary (Paksy *et al.*, 1990) and/or the accessory organs (Klinefelter and Hiss, 1998). In earlier chapter it has been shown that estradiol metabolizing enzymes are inhibited by lead and cadmium both in hypothalamus and pituitary. A number of different studies have shown that both these metals modify plasma levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Lafuente *et al.*, 1997, 1999a; Lorenson *et al.*, 1983; Paksy *et al.*, 1989; Zylber-Haran *et al.*, 1982). It is known that catecholamines and indoleamines as well as other neurotransmitters of the central nervous system modulate pituitary hormone secretion (Drouva and Gallo, 1976; Lopez *et al.*, 1989). Thus the metal accumulated at this level after the exposure may change the regulatory mechanism. Various studies have shown that lead exposure can cause changes in catecholaminergic functions (Cooper and Manalis, 1983; Nation *et al.*, 1989; Shih and Hanin, 1978; Winder and Kitchen, 1984). Low-level Cd exposure results in increased catecholamine neurotransmission (Arito *et al.*, 1981; Cooper and Manalis, 1983; Nation *et al.*, 1989; Rastogi *et al.*, 1977; Williams *et al.*, 1978).

Most of the above cited studies were carried out with single metal. Available data on combined exposure of lead and cadmium shows that these metals show additive effect on acetyl choline release at the frog neuromuscular junction (Cooper and Manalis, 1984) whereas Nation *et al.*, 1989 observed lesser effects of combined exposure than that in

isolation. Since hypothalamus is an important tissue, which modulates pituitary hormone secretion it is of further interest to know if these alterations in pituitary hormones are mediated by changes in neurotransmitter content at the hypothalamic level.

Hence, in the present study an attempt has been made to evaluate the effect of lead and cadmium either alone or in combination on the hypothalamic neurotransmitter content and plasma and pituitary levels of LH and FSH. The metal content both in hypothalamus and pituitary was estimated to study whether the changes in hypothalamic-pituitary unit are related to metal accumulation.

Experimental design

There were four groups of 10 animals each in the study: control (sodium acetate), lead acetate, cadmium acetate and lead acetate and cadmium acetate in combination. The animals were treated intraperitonially with 0.05 mg/kg body wt. dose per day for 15 days. The combined treated group was also exposed to same dose by taking half concentration of each metal. The animals were killed by decapitation on the proestrous stage at 10.00 and the decapitation procedure was completed within 30 sec. Blood was collected and serum samples obtained after centrifugation at 2500 ***g*** for 10 min were stored at -20^0C until LH and FSH were measured (RIA method). The hypothalamus and pituitary were immediately removed and processed for further assays. Serotonin, dopamine and norepinephrine were estimated in the hypothalamus samples by the flourimetric method of Shellenberger and Gordon, 1971. The pituitary gland of each rat was homogenized separately in cold 0.9% NaCl (1 ml/10 mg wet weight), centrifuged at 500 ***g*** for 15 min at 4^0C and the supernatant fraction was used for hormone analysis. Hypothalamus and pituitary were analyzed for lead and cadmium levels by GBC 902 Atomic Absorption Spectrophotometer.

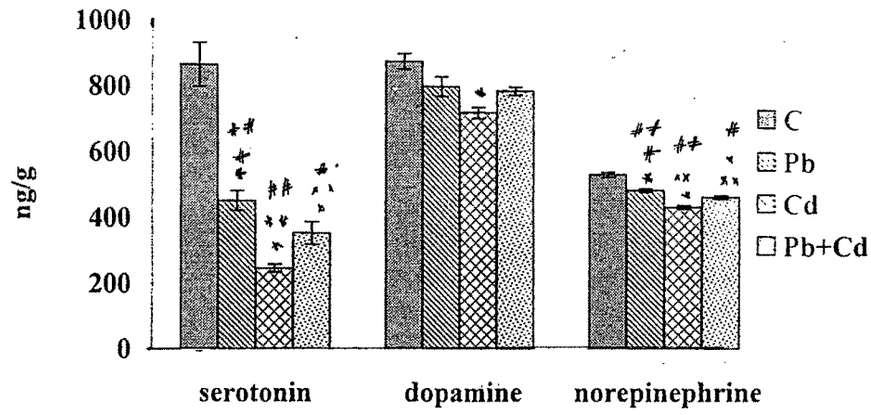
Results

Fig. 1 shows the estimated concentration of hypothalamic serotonin, norepinephrine and dopamine in various groups. The serotonin content was decreased in all metal treated groups with cadmium showing maximum reduction. The combined treatment with lead and cadmium was associated with changes in serotonin relatively similar to those induced by either metal administered alone. The norepinephrine content was decreased in both isolated and combined treatment groups. Among the three groups cadmium exposure was showing maximum reduction in NE content. The dopamine content was decreased in the cadmium exposed animals where as other treatment groups were not showing any significant change in the dopamine level.

The animals in the cadmium and combined groups showed significant decrease in the serum LH (Fig. 2) and FSH (Fig. 3) levels as compared to the control group whereas lead treated group was not showing any significant change in the LH and FSH levels as compared to control. The cadmium exposed animals were showing significant decrease in pituitary LH (Fig. 2) and FSH content (Fig. 3) as compared to control and lead exposed groups. The simultaneous treatment with lead and cadmium induced similar changes in the LH and FSH content as with cadmium alone. The lead exposure failed to cause any change in both LH and FSH level.

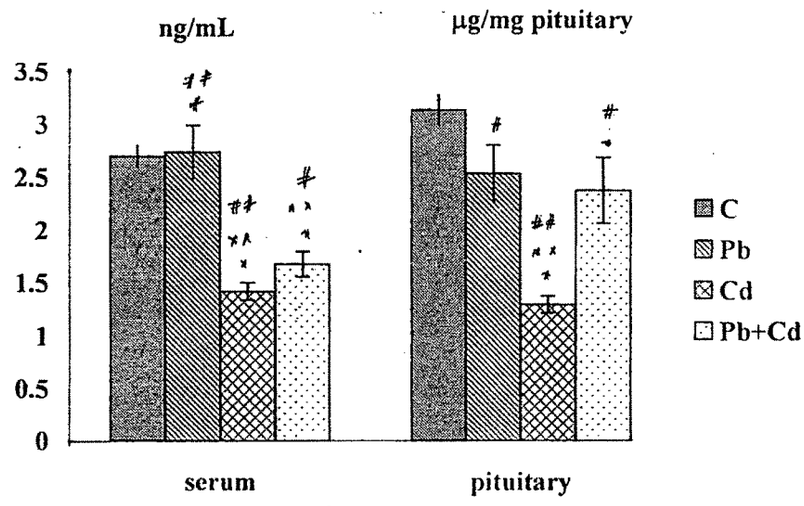
The analysis of metal concentrations showed that the tissues of cadmium and combined metal exposed groups contained greater concentration of cadmium residues than the tissues of control and lead exposed groups (Table 1). Similarly in the case of lead and combined metal exposed groups the lead concentration was significantly higher than that in control and cadmium exposed groups (Table 1).

Fig 1: Effect of lead and cadmium alone and in combination on hypothalamic serotonin, dopamine and norepinephrine content.



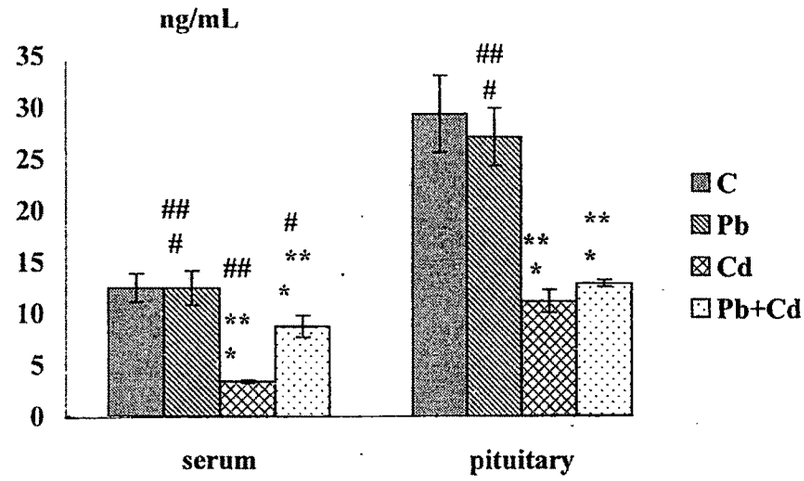
*P<0.05 vs. control; ** *P*< 0.05 *vs.* lead; # *P*< 0.05 vs. cadmium and
P<0.05 vs. lead+cadmium group (n=5)

Fig 2: Effect of lead and cadmium alone and in combination on serum and pituitary LH levels.



*P<0.001 vs. control; ** P<0.001 vs. lead; # P<0.001 vs. cadmium and ## P<0.001 vs. lead+cadmium group (n=5)

Fig 3: Effect of lead and cadmium alone and in combination on serum and pituitary FSH levels.



*P<0.001 vs. control; ** P<0.001 vs. lead; # P<0.001 vs. cadmium and ## P<0.001 vs. lead+cadmium group (n=5)

Table 1. Lead and cadmium levels at the hypothalamus and pituitary of female rats exposed to lead and cadmium alone and in combination for 15 days (0.05 mg/kg body weight).

Group	Hypothalamus		Pituitary	
	Lead	Cadmium	Lead	Cadmium
	(µg/g)		(µg/g)	
Control	2.52±0.095	0.525±0.032	5.05± 0.032	0.745± 0.365
Pb	4.1± 0.27***	0.537± 0.062	9.55± 0.68***	0.82± 0.096
Cd	2.8± 0.021$^{\#}$	1.035± 0.056***$^{\#\#}$	5.7± 0.251$^{\#\#}$	1.47± 0.102*$^{\#}$
Pb+Cd	3.02± 0.125**$^{\#}$	0.84± 0.021***$^{\#}$ $^{\#\#\#}$	6.25± 0.263**$^{\#}$	1.282± 0.375

*P≤0.05; **P≤0.01; ***P≤0.001 vs. control; $^{\#}$P≤0.01; $^{\#\#}$P≤0.001 vs. lead and $^{\#\#\#}$ P≤0.05 vs. cadmium exposed group. (n = 5).

Discussion

The decrease in 5-HT content due to the inhibitory effect of cadmium observed in the present study agrees with the results found by Lafuente *et al.*, 2000 where they have shown the effect in different hypothalamic regions. Das *et al.*, 1993 observed decrease in 5-HT level in the whole hypothalamus after cadmium exposure. Our results indicate that the decrease in serotonin content was more in cadmium exposure group than animals receiving cadmium in combination with lead. Norepinephrine plays an important role in modulating luteinizing hormone releasing hormone (LHRH) neurons that are involved in the regulation of gonadotropin secretion by the anterior pituitary. It has been reported that lead can act at the hypothalamic level to alter LHRH secretion in the rat (Bratton *et al.*, 1994). Lead blocks the NE induced release of PGE_2 resulting in diminished LHRH secretion. This decrease could be due to the altered Ca^{2+} mobilisation, a limiting step in PGE_2 formation and subsequent LHRH release. In the present study we have observed decrease in NE in both isolated and combined metal treated groups. Dopamine content was not affected by lead or simultaneous exposure with lead and cadmium whereas cadmium alone was showing significant decrease in the DA content. In various rodent studies on the subchronic exposure of low level of lead resulted in significant reduction in DA and its metabolites 3,4 dihydroxyphenylaceticacid and homovanillic acid in the nucleus accumbens of rats (Jadhav and Kala, 1994; Kala *et al.*, 1994; Kala and Jadhav, 1995). The discrepancies with other studies could be due to differences in the age of animals, mode of treatment, duration and brain areas analyzed. Lead has been reported to alter calcium homeostasis by affecting both voltage dependent and receptor operated calcium channels (Audesirk, 1993; Bressler and Goldstein, 1991; Oortgiesen *et al.*, 1993) whereas the invitro

studies have shown that lead enhances calcium activated release in brain transmitters (Minnema *et al.*, 1988). Cadmium is shown to inhibit calcium entry and the attendent release of peripheral catecholamines (Hirning *et al.*, 1988). Therefore the changes observed in the cotreatment group might be due to the fact that lead and cadmium compete with calcium at the channel site and compete with each other for entry through terminal membrane channels (Cooper and Manalis, 1984).

The studies on pituitary hormones show that lead and cadmium affect the gonadotropin secretion differently. It is known that serotonin increases both LH and FSH secretion (Ellis *et al.*, 1983; Wuttke *et al.*, 1978). A maximum reduction in both pituitary and serum gonadotropin levels was found in cadmium treated group. Lead is not producing any change in the gonadotropin levels compared to control. Significant changes observed in the combined treatment group can be due to the inhibitory effects produced by cadmium. The hormonal disturbances following combined treatment with lead and cadmium more often paralleled with the effects of cadmium alone than the changes seen in lead alone suggesting antagonism between lead and cadmium. Decreased plasma levels of LH and FSH have been reported after administration of higher doses of cadmium to female rats (Paksy *et al.*, 1989; Varga and Paksy, 1991).

The decrease in LH and FSH levels after cadmium exposure could also be due to the accumulation of cadmium at pituitary causing a direct effect on the hypophyseal level since some literature available indicate this mechanism (Cooper *et al.*, 1987; Lorenson *et al.*, 1983). Several studies have shown that cadmium can compete with calcium at the pituitary level (Milos *et al.*, 1989; Waalkies and Poirier, 1984) which result in altered calcium regulation. Thus it either interferes with calcium influx through membrane channel

(Cooper *et al.*, 1987; Kasprzak and Poirier, 1985; Poirier *et al.*, 1983) or alters intracellular calcium mobilization. The results of our study agree with this hypothesis as both LH and FSH levels were decreased on cadmium accumulation. However, it is interesting to note that the decrease is more observed in case of FSH than LH suggesting additional mechanism for such differential effect. Lafuente et al., 2000 correlated the decease in FSH levels found in postpubertal rats after cadmium exposure to inhibin levels more that to cadmium accumulation at pituitary. Dopamine is a known inhibitor of gonadotropin secretion and this fact can not be correlated with the decreased levels of LH and FSH observed in the present study. Since gonadotropin secretion is under control of multiple regulators it must be the net effect of all these regulators, which has resulted in decreased levels of gonadotropins.

The present investigation found that combined exposure to lead and cadmium showed less concentration of both metals in hypothalamus and pituitary, relative to the metal retention in individual metal treated groups. Nation *et al.*, 1990 found a similar change in blood lead concentration in their studies on the effects of combined exposure of lead and cadmium on behavioral changes. The lesser accumulation of metals after combined exposure could be a reason for the observed antagonistic effects. From the above results it is clear that cadmium exerts more disruptions in the hypothalamic-pituitary axis function compared to lead, and the combined treatment effects are not additive.

Summary

Since hypothalamus is an important tissue which plays an important role in the modulation of pituitary hormone secretion it was of interest to know if the alterations in pituitary hormones by metal exposure are mediated by changes in the neurotransmitter levels at the

hypothalamic level. Therefore the effects of lead and cadmium either alone or in combination on hypothalamic neurotransmitter content and plasma and pituitary levels of Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) were studied. Adult female rats were treated intraperitonially with either lead acetate or cadmium acetate alone and in combination at a dose of 0.05 mg/kg daily for 15 days. Serotonin (5-HT) and norepinephrine (NE) levels decreased in individually and combined metal treated groups whereas dopamine (DA) levels were decreased only in cadmium exposed group. The pituitary levels of LH and FSH were decreased significantly in cadmium and combined treatment groups. In contrast, lead exposure failed to cause any change in serum LH and FSH levels whereas cadmium and combined treatments showed significant decrease in serum LH and FSH levels as compared to control. The accumulation of both metals increased in hypothalamus and pituitary after the treatment. These data suggest that the metal accumulation disrupts the regulatory mechanisms of the hypothalamic-pituitary axis where the effects produced by the combined treatment of metals are not additive.