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CADMIUM INDUCTION OF RENAL AND HEPATIC ORNITHINE DECARBOXYLASE ACTIVITY IN THE RAT

EFFECTS OF SEX HORMONES AND INVOLVEMENT OF THE RENIN–ANGIOTENSIN SYSTEM

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Abstract—We investigated the effect of sex hormones on the sex-dependent response of rat kidney ornithine decarboyxlase (ODC) activity to cadmium (Cd) administration and the involvement of the renin–angiotensin system in mediating stimulation of the liver enzyme by the metal. The response of renal ODC to Cd, which occurs in intact adult males but not in females, is also detectable in prepubertal and castrated males. Upon treatment with 17β-estradiol, the basal levels of enzyme activity in intact or castrated adult males were enhanced and Cd administration failed to increase them further. In adult females the kidney enzyme became responsive after ovariectomy. Also, in prepubertal females renal ODC was induced by Cd, and this was prevented by treatment with 17β-estradiol. Under the same conditions, changes in the levels of Cd accumulation within the kidney, that might account for variations in the response of ODC activity, did not occur. Cd caused an increase in renin activity starting minutes after its injection. Captopril, which specifically inhibits the conversion of angiotensin I to angiotensin II, prevented completely the induction of liver ODC by this metal; stimulation of the enzyme by Co was not affected by the drug. A similar inhibitory effect was exerted by propranolol. Adrenalectomy had no influence on the response of hepatic ODC to Cd; the decarboxylase was unaffected by aldosterone administration. It is suggested that Cd may induce liver ODC through the increase in angiotensin II following stimulation of renin by the metal.

Ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17; ODC) catalyses the first step in the biosynthetic pathway of the aliphatic polyamines in animal tissues and plays a crucial role in regulating the whole process. This enzyme activity is induced in various biological systems by diverse stimuli, especially but not exclusively [1, 2] by those affecting normal or pathological cell growth [3, 4]. In spite of the long list of agents capable of inducing ODC in mammals, very little is known about the pathways through which they affect this enzyme. Similarly, in spite of the effects of increasing polyamine concentrations described in a plethora of *in vitro* systems, it is poorly understood which cellular processes are specifically affected *in vivo*.

The ability to respond to such a variety of factors suggests that ODC induction, with the consequent increase in polyamine levels, is a general response as part of the organ adaptation to stress [1, 2]. This implies a multifunctional role of these endogenous polycations.

We have shown recently that cadmium (Cd), cobalt (Co) and other heavy metals are able to induce hepatic ODC and not tyrosine aminotransferase in the goldfish *Carassius auratus* [5]. Stimulation of ODC in this teleost, however, was not a constant outcome of Cd administration since it occurred only at certain times of the year depending on the water temperature and, probably, on seasonal hormonal fluctuations [6]. Also in the rat, the hormone endowment appears to affect the response of ODC to Cd or Co treatment. In fact, it has been reported [7] that the renal decarboxylase responds to treatment with either metal in males but not in females, while the activity of the liver enzyme was enhanced in both sexes.

Because of the increasing levels of metal pollution in our surroundings, there is interest in understanding how Cd may interfere with cell metabolism. In the present paper we investigated how sex hormones may affect induction of rat kidney ODC by Cd and addressed the question of identifying possible neuroendocrine pathways involved in the Cd effect on this enzyme activity.

MATERIALS AND METHODS

Animals. Male and female Wistar rats from a commercial source were housed in a temperature- and light-controlled room. Animals, fasted overnight, were injected i.p. with $CdCl_2$ (5.0 mg/kg body wt) or s.c. with $CoCl_2$ (60 mg/kg body wt) in 0.9% NaCl and killed after 4 or 5 hr respectively. Propranolol was given i.p. at a dose of 10 mg/kg body wt 30 min before metal administration. Estrogen-treated rats were injected s.c. with 17β-estradiol (0.2 mg/kg body wt) in ethanol 40% for 6 consecutive days before metal injection. Captopril (Capoten, Squibb, Anagni, Italy) was administered orally as an aqueous suspension (10 mg/kg body wt) 60 min before metal

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administration. In all treatments control animals received the appropriate vehicle. Animals undergoing adrenalectomy were operated on 10 days before the experiments and then given saline to drink. All the operations were performed under ether anesthesia. The organs were quickly removed and frozen in liquid nitrogen.

ODC assay. The tissues were homogenized in ice-cold 10 mM Tris–HCl buffer (pH 7.4) containing 0.25 M sucrose. The supernatant was obtained by centrifuging at 20,000 *g* for 30 min. ODC was determined in the supernatant by quantitating the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine (sp. act. 57 mCi/mmol, Amersham International, Amersham, U.K.). The incubation mixture contained in a final volume of 0.5 mL: 50 mM Tris–HCl pH 7.5, 6.0 μM EDTA, 40 μM pyridoxal-5′-phosphate, 0.5 μCi of DL-[1-^{14}C]-ornithine and approx. 1.0 mg of protein. The incubation was carried out at 37° for 60 min and stopped by addition of 10% trichloroacetic acid [8]. Cd concentrations in the range of those found in the tissue extracts did not affect the enzyme activity (not shown).

Cd *determination.* Cd was measured by atomic absorption spectrophotometry (Instrumentation Laboratories, model IL II) as described previously by Davalli *et al.* [5].

Determination of protein. Protein concentrations were determined according to Lowry *et al.* [9], with bovine serum albumin as a standard.

Statistical analysis. Student's *t*-test was applied to determine the level of significance. $P < 0.05$ was considered statistically significant.

RESULTS

After confirming [7] that renal ODC activity is induced by injection of Cd into male (Table 1) and not female rats (Table 2), we determined whether this depends on high levels of circulating androgens exerting a permissive effect on ODC stimulation by the metal in males, or on high levels of female sex hormones preventing this Cd effect in females. Thus, we tested the effect of the metal on the kidney enzyme of male and female rats with low levels of circulating sex hormones.

In castrated adult or prepubertal males (Table 1) the response of the renal enzyme was not lowered as compared with that of intact adults (it was in fact amplified), showing that physiological levels of circulating androgens are not required for the renal enzyme to be induced by the metal.

In sexually mature females (Table 2) the renal decarboxylase was not significantly induced by Cd, but ovariectomy of 80-day-old females, performed 16 days before killing, caused the enzyme to respond to the metal. The same result was obtained when ovariectomy was performed at prepubertal age (60 days before killing, not shown). The renal ODC of prepubertal females was similarly stimulated by Cd treatment (Table 2).

Administration of 0.2 mg/kg body wt of 17β-estradiol for 6 days to immature females caused an increase in the basal level of the enzyme activity and prevented any further stimulation by Cd; the enzyme

Table 1. ODC activity and Cd concentration in male rat tissues 4 hr after i.p. injection of $CdCl_2$

$CdCl_2$ (mg/kg body wt)	Intact adult		Castrated adult		Prepubertal		Castrated adult + 17β-estradiol		Intact adult + 17β-estradiol	
	0	5.0	0	5.0	0	5.0	0	5.0	0	5.0
Kidney										
ODC activity	217 ± 40	658 ± 125*	123 ± 19	851 ± 140†	290 ± 58	2171 ± 302†	751 ± 103	591 ± 91	4814 ± 532	3299 ± 401
Cd	ND	5.15 ± 0.20	ND	5.26 ± 0.58	ND	4.84 ± 0.61	ND	8.39 ± 0.80‡	ND	7.16 ± 1.08‡
Liver										
ODC activity	11 ± 3	99 ± 17†	8 ± 1	100 ± 21†	7 ± 1	95 ± 16†	7 ± 1	98 ± 17†	13 ± 2	100 ± 14†
Cd	ND	44.03 ± 1.64	ND	49.23 ± 1.86	ND	42.03 ± 2.00	ND	51.14 ± 1.28	ND	43.64 ± 2.91

The enzyme activity is expressed as pmol $^{14}CO_2$/hr/mg protein and Cd concentrations are μg/g wet tissue; in both cases the values are means ± SD (N = 5–10).
For ODC activity, values significantly different from the respective untreated controls are * $P < 0.01$, † $P < 0.001$; for Cd concentration: ‡ $P < 0.05$.
ND, not detectable.

Table 2. ODC activity and Cd concentration in female rat tissues 4 hr after i.p. injection of $CdCl_2$

$CdCl_2$ (mg/kg body wt)	Intact adult 0	Intact adult 5.0	Castrated adult 0	Castrated adult 5.0	Prepubertal 0	Prepubertal 5.0	Prepubertal +17β-estradiol 0	Prepubertal +17β-estradiol 5.0
Kidney								
ODC activity	220 ± 40	256 ± 48	156 ± 25	332 ± 65*	309 ± 53	1400 ± 256†	747 ± 159	344 ± 60*
Cd	ND	7.30 ± 0.72	ND	7.98 ± 0.74	ND	6.81 ± 0.31	ND	7.00 ± 0.91
Liver								
ODC activity	12 ± 3	117 ± 25†	5 ± 1	76 ± 12†	24 ± 4	376 ± 70†	17 ± 3	163 ± 35†
Cd	ND	42.13 ± 3.2	ND	73.32 ± 4.48	ND	48.86 ± 4.51	ND	42.96 ± 3.84

See legend to Table 1 for all details.
Cd concentration in the liver of castrated adults is significantly higher than in that of intact adults ($P < 0.01$).

Table 3. ODC activity and Cd concentration in rat tissues 4 hr after i.p. injection of $CdCl_2$

$CdCl_2$ (mg/kg body wt)	Adult males 0	Adult males 5.0	Adult females 0	Adult females 5.0
Heart				
ODC activity	25 ± 3	44 ± 6*	53 ± 9	113 ± 18*
Cd	ND	1.00 ± 0.12	ND	2.23 ± 0.19‡
Adrenals				
ODC activity	48 ± 7	197 ± 30†	12 ± 1	121 ± 21†
Cd	ND	4.09 ± 0.51	ND	7.3 ± 0.7§

Details as in legend to Table 1, except * $P < 0.05$.
Cd concentration in females was significantly higher than in males: ‡ $P < 0.05$, § $P < 0.01$.

activity in fact decreased upon treatment with the metal (Table 2).

The effect of the estrogen on renal ODC induction by Cd was confirmed in experiments with male rats (Table 1). In fact, similarly to females, treatment of intact or castrated males with 17β-estradiol increased the basal level of kidney ODC activity (especially in intact rats) and prevented completely the stimulating of the enzyme by Cd.

In the liver the response of ODC to Cd was not substantially affected by changes in the levels of endogenous sex hormones or by treatment with 17β-estradiol in either males (Table 1) or females (Table 2).

Besides in the liver and kidney, ODC activity was significantly stimulated by Cd in the heart and the adrenals of both males and females (Table 3). However, in prepubertal rats of both sexes the heart enzyme was not stimulated (not shown).

In order to check whether the negative effect of endogenous or exogenous female steroids on ODC induction by Cd occurred through a possible effect of the latter hormones on the levels of Cd accumulation within the tissues, we measured the actual tissue concentrations of the metal by means of atomic absorption spectrophotometry. The amount of Cd accumulated in various organs of rats with different levels of sex hormones, 4 hr after injection of 5.0 mg/kg body wt of $CdCl_2$, are shown in Tables 1, 2 and 3. The extent of accumulation among the organs studied differed dramatically. The liver exhibited the highest concentrations of the metal and in this organ variations in the levels of circulating sex hormones did not affect significantly the Cd concentration except in adult females upon ovariectomy, where a significant increase occurred (Table 2). In both the heart and adrenals the Cd concentration was higher in females than in males (Table 3). With regard to the kidney, the Cd concentration increased in males treated with estradiol (Table 1), while no significant changes occurred in females when changing the levels of sex hormones (Table 2).

In other experiments, not reported here, we tested the effect of lower doses of $CdCl_2$ on ODC activity and metal accumulation within the same organs. Liver and male kidney ODC was induced even with a dose of $CdCl_2$ as low as 0.615 mg/kg body wt. When injecting 1.25 and 5.0 mg/kg body wt $CdCl_2$, in the adrenals only were the levels of Cd accumulation proportional to the amount administered; in the liver, kidney and heart Cd accumulation with the lower dose was 35–50% of that attained with the higher dose. With a dose of 1.25 mg/kg

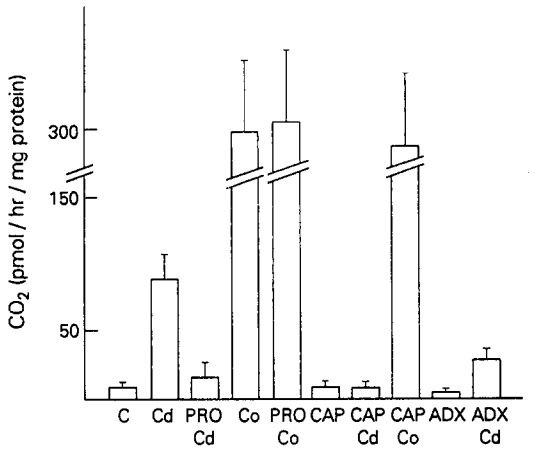

Fig. 1. Effect of propranolol (10 mg/kg), captopril (10 mg/kg) and adrenalectomy on induction of male rat liver ODC activity by $CdCl_2$ (5.0 mg/kg body wt, i.p.) or $CoCl_2$ (60 mg/kg body wt, s.c.). The animals were killed 4 hr after Cd and 5 hr after Co injection. PRO and CAP were administered 30 and 60 min before the metals, respectively. Each point represents the mean ± SD for eight rats. C, control; PRO, propranolol; CAP, captopril; ADX, adrenalectomized.

body wt renal ODC was stimulated in castrated females and not intact ones (as with 5 mg/kg body wt, Table 2); however, with the low dose, Cd accumulation within the kidney decreased by about 25% upon castration.

It has been reported [10–12] that Cd administration increases plasma renin activity. Under our conditions a several-fold increase in plasma renin activity was detectable (by radioimmunoassay) starting as soon as 15 min after Cd injection (data not shown). Thus, the neuroendocrine mechanism(s) possibly involved in hepatic ODC stimulation by Cd, was investigated by studying the effect of inhibitors of the renin–angiotensin system. Figure 1 shows that the β-adrenergic blocker, propranolol (10 mg/kg body wt), known to inhibit the release of renin [13], almost totally prevented hepatic ODC induction (metal accumulation within the tissue was not affected by this treatment, not shown). The same β-adrenergic antagonist did not prevent the induction of liver ODC caused by another divalent cation, Co. Similarly, captopril (10 mg/kg body wt), a potent inhibitor of the converting enzyme, catalysing the conversion of angiotensin I to angiotensin II [14], totally inhibited the induction of ODC by Cd, but not by Co. On the contrary, mineralcorticoid hormone depletion by adrenal ablation had no effect on induction of the decarboxylase, and administration of up to 60 μg/kg body wt of aldosterone did not exert any stimulatory effect on the enzyme (not shown).

DISCUSSION

Interest in understanding the mechanisms of metal ion toxicity has grown recently in connection with the increasing metal pollution of our surroundings. Due to its wide distribution in the environment in industrial countries, Cd represents a typical agent of metal pollution. It has been suggested that human exposure to Cd may cause various pathological processes such as cancer, hypertension, growth inhibition etc. [15].

In the present paper the response of rat kidney and liver ODC to Cd was studied after acute treatment of the animals with a high dose (5.0 mg/kg body wt) of $CdCl_2$ [7]. It is important to note, however, that qualitatively the same results were obtained by us with 0.615 and 1.25 mg/kg body wt of $CdCl_2$, while sex dependence of renal ODC stimulation was found also with 0.305 mg/kg body wt [7]. Although the present results cannot be directly extended to humans chronically exposed to Cd, they may represent clues for understanding the mechanisms by which metabolic processes are affected by this metal. Under the same conditions, ornithine aminotransferase did not respond to Cd, while tyrosine aminotransferase was affected very slightly (Davalli, unpublished observations).

It appears from our data that Cd induction of male rat kidney ODC also occurs when the levels of circulating androgens are low, as in castrated and prepubertal animals. Thus, the lack of response in females can hardly be ascribed to lack of androgens. It seems rather that the physiological concentrations of circulating sex hormones present in adult females are the factor which prevents the response of renal ODC to Cd, since in both castrated and prepubertal females the renal enzyme is induced by the metal. Estrogens may be the female steroids, directly or indirectly, involved in nullifying the stimulatory action of Cd on renal ODC, as treatment of the animals with 17β-estradiol prevented completely ODC stimulation by Cd in both females and males. Interpretation of the latter results, however, is complicated by the stimulatory effect of the hormone *per se* on basal enzyme activity [16].

Kidney ODC remained unaffected by Cd during the various phases of the oestral cycle, when the levels of sex hormones undergo extensive physiological changes (Davalli, unpublished observations).

It has been reported that, under different conditions, estrogens cause increases in Cd uptake in rat renal tissue [17, 18]. In our experiments, Cd accumulation increased in males upon treatment with estradiol (Table 1), but there was no significant difference in Cd accumulation in castrated or prepubertal females treated or not with estradiol with respect to intact adults, in spite of the changes occurring in the response of renal ODC to Cd (Table 2). Thus, the mechanism by which estrogens affect renal ODC induction by Cd under our experimental conditions does not seem to be attributable to variations in the extent of metal accumulation.

The existence of conflicting data on the mechanism of Cd-induced acute pressor hypertension [19–25] prompted us to investigate the possible role of the renin–angiotensin system in hepatic ODC induction. After confirming the finding in the literature [12] that Cd is able to induce renin activation (not shown), we found that captopril, administered 60 min

before Cd, abolished completely the induction of hepatic ODC by the metal (Fig. 1), suggesting that interruption of the pathway renin–angiotensin–aldosterone at the level of angiotensin activation prevents the signal for hepatic ODC induction from being effective. Also, the administration of propranolol or nadolol (not shown) prior to metal injection abolished the increase in hepatic ODC (Fig. 1) without changing the Cd concentration within the liver (not shown). This is in agreement with other reports suggesting that Cd may cause the release of catecholamines [25], which, by interacting with the juxta-glomerular cells, in turn cause the release of renin. Thus, the inhibitory action of the β-blocker strengthens the hypothesis of an involvement of the renin–angiotensin system in hepatic ODC induction by Cd.

Depletion of mineralcorticoids by adrenalectomy did not affect liver ODC induction by Cd (Fig. 1) and aldosterone administered at doses of up to 60 μg/kg body wt had no stimulatory effect on the enzyme (not shown). Therefore, aldosterone does not seem to play a role in this mechanism; rather, angiotensin II may be the factor that conveys to the liver the signal for induction of the decarboxylase. In effect, angiotensin II activates in the liver the production of angiotensinogen [26]; the increase in ODC activity, which is usually associated with increases in protein synthesis, might be a part of this mechanism.

Involvement of the renin–angiotensin system in the stimulation of hepatic ODC by Cd does not appear to be a general pathway for the induction of this hepatic enzyme by heavy metals. In fact, the potent stimulatory action of Co was not affected by previous administration of either propranolol or captopril (Fig. 1). Thus, the two heavy metals apparently stimulate hepatic ODC via completely different pathways.

In conclusion, we show here that in the adult female rat kidney the physiological levels of circulating estrogens prevent the response of ODC to Cd without affecting substantially the levels of Cd accumulation, while in the rat liver stimulation of this enzyme by Cd occurs through formation of active angiotensin. These results suggest the existence of a new pathway through which the metabolism of hepatic polyamines may be controlled *in vivo* and show that the Cd effect on certain metabolic events occurs through the specific interaction of Cd with the endocrine system. At present we do not have a convincing interpretation of the possible functional meaning of these responses.

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