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Acute effects of cadmium on the renin angiotensin system in rats

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Abstract—The effect of cadmium acetate (1 mg/kg i.p.) on the renin angiotensin system was examined in male Sprague–Dawley rats. Blood pressure, plasma renin activity (PRA) and serum angiotensin converting enzyme (ACE) levels were determined in the rats. Cadmium acetate produced a hypertensive response which was not associated with elevated PRA. However, ACE levels in the serum were significantly decreased in the cadmium-treated group as compared with normal controls ($P < 0.01$).

Several studies have confirmed the hypertensive nature of cadmium in rats [1–4]. The role of cadmium in relation to the renin angiotensin system (RAS*) is still not well understood [4]. Eakin *et al.* [5] have reported that oral feeding of cadmium failed to alter the plasma renin activity (PRA), while Perry and Erlanger [6] have reported raised renin levels after cadmium exposure in rats. Thind *et al.* [7] have reported that cadmium produced an increase in the levels of renin activity in rabbits. There has not been any report published on the serum converting enzyme levels after cadmium treatment in rodents. Therefore, in this study the effect of acute cadmium administration was studied on blood pressure, PRA and angiotensin converting enzyme (ACE) activity in the serum of Sprague–Dawley rats.

Materials and Methods

Male Sprague–Dawley rats (200–230 g), bred in the CDRI animal house, were used. In this study rats were housed in polyethylene cages with free access to water and food in a temperature-controlled room (26 ± 2°). Rats were anaesthetized with urethane (25% solution 0.6 mL/100 g BW i.p.) and systemic blood pressure was recorded by placing a polyethylene catheter (PE 60) in the common carotid artery. Arterial pressure was measured with a Statham pressure transducer connected to a Grass Model VII polygraph. The jugular vein was cannulated for infusion of cadmium and normal saline. Blood sampling was carried out via a carotid cannula (1 mL) and 1 mL of normal saline was pushed very slowly. Blood was sampled during the control period followed by cadmium treatment when the peak hypertensive effect was observed. Blood was collected in EDTA-containing test-tubes and centrifuged immediately (5000 *g* for 15 min). The plasma was separated and PRA determined by radio-immunoassay (RIA kit supplied by Bhabha Atomic Research Centre, Bombay). The intra- and interassay coefficients of variance were 2% and 14% (N = 5), respectively. Serum ACE was estimated by using a tripeptide hippuryl-L-histidyl-L-leucine as described by Leberman [8]. Rats were treated with cadmium acetate (1 mg/kg i.p.) or normal saline (control animals) and were killed 60 min after the injection. Blood was collected in sterilized test-tubes and the serum was separated by centrifugation (5000 *g* for 15 min). Serum ACE activity was measured on the same or the next day. Results are expressed as means ± SEM. Data was analysed using the paired Student's *t*-test. A value of $P < 0.05$ was considered to be statistically significant.

Results and Discussion

The results are summarized in Fig. 1. Cadmium (1 mg/kg i.v.) produced a hypertensive response in urethane-anaesthetized rats. Blood pressure during the control period was 75 ± 6.9 mmHg and was increased by cadmium to 111 ± 7.9 mmHg (N = 6). The difference is highly significant ($P < 0.01$). The PRA during the control period, 0.89 ± 0.22 ng/mL/hr, was not significantly altered by cadmium treatment (0.85 ± 0.23 ng/mL/hr).

Serum ACE activity in the control rats was 19.33 ± 0.90 U/mL and was reduced by cadmium treatment (1 mg/kg i.p.) to 0.79 ± 0.21 U/mL. The difference between the two values is statistically highly significant ($P < 0.01$). The present study has confirmed our previous findings that cadmium produces a hypertensive response in rats [1, 2]. The mechanism of cadmium-induced hypertension is not well understood. In the present study, it was observed that

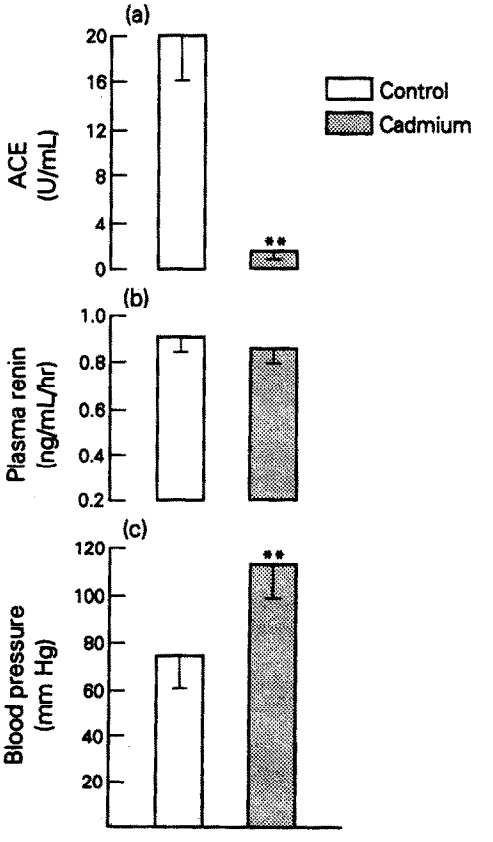

Fig. 1. Effect of cadmium (shaded bars) and saline control (open bars) on (a) ACE activity, (b) PRA and (c) blood pressure of male Sprague–Dawley rats. Values are means ± SE. **$P < 0.01$.

* Abbreviations: RAS, renin angiotensin system; PRA, plasma renin activity; ACE, angiotensin converting enzyme.

cadmium strongly inhibited the coverting enzyme which converts the active decapeptide angiotensin I to active pressure peptide angiotensin II. This leads to an increase in feedback of PRA. However, the PRA did not change suggesting that feedback upregulation is not invariable associated with an increase in PRA. Takemore *et al.* [9] and Benctas *et al.* [10] using different ACE inhibitors in rats was well as in essential hypertensive patients did not observe a significant increase in PRA.

Cadmium-induced hypertensive response could be due to the activation of the aminergic system in rats. We have reported previously that cadmium treatment of rats produced increased serum levels of catecholamines [3]. The present observation is supported by Fadloun and Leach [4] who reported the induction of dopamine-β-hydroxylase activity by cadmium in rats. Dazu and Pratt [11] reported that angiotensin II activates the target organs resulting in the release of norepinephrine. Cadmium-induced increase in the blood pressure of rats is independent of the PRA. However, the possible effects of vasopressin or some specific peptide produced by cadmium need to be elucidated. In conclusion, cadmium produced a hypertensive response and significantly inhibited serum ACE activity without effecting the PRA.

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Heparin: does it act as an antioxidant *in vivo*?

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Abstract—Previous studies have shown that heparin antagonizes oxygen radical-mediated injury to endothelial cells, suggesting an antioxidant role of the drug. In the present investigation, the hypothesis that heparin exerts direct antioxidant effects was tested in several experimental models. We have found that 1, 3, 5, 10, 20, 40 and 80 U/mL of heparin do not scavenge superoxide anion, hydrogen peroxide, hydroxyl radical or the stable free radical 1,1-diphenyl-2-pycrylhydrazyl. Moreover, the drug is ineffective towards iron-driven linolenic acid peroxidation, autooxidation of brain homogenate and linolenic acid peroxidation mediated by human internal mammary artery homogenate. Specific studies on the potential iron-binding–inactivating capacity of heparin prove the drug to be totally ineffective. Finally, the loss of protein sulphydryls from human plasma induced by hypoxanthine–xanthine oxidase-generated oxygen radicals is not prevented by heparin. In conclusion, heparin, even at concentrations far higher than those usually used therapeutically, has no direct antioxidant properties. Thus, other mechanisms not strictly antioxidant-type must be involved in heparin-mediated cell protection against toxic oxygen metabolites.

Heparin, a glycosoaminoglycan, is employed clinically as an antithrombotic for both preventive and therapeutic purposes, and its use as an antiatherogenic agent has also been stressed [1, 2]. Recent studies have shown that heparin pre-incubation protects cultured endothelial cells from damage by toxic oxygen metabolites, suggesting that the drug has antioxidant properties [3]. Moreover, Hladovec [4] demonstrated a decrease in circulating endothelial cells