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The effects of cadmium ions on blood pressure, dopamine-β-hydroxylase activity and on the responsiveness of in vivo preparations to sympathetic nerve stimulation, noradrenaline and tyramine

Z. FADLOUN AND G. D. H. LEACH*

Postgraduate School of Studies in Pharmacology, University of Bradford, BD7 1DP, U.K.

Changes in sympathetic nervous function of the rat caused by acute and chronic treatment with cadmium (Cd^{2+}) have been studied in vivo by measurement of changes in blood pressure and plasma dopamine-β-hydroxylase (DBH) activity. In anaesthetized animals, acute injection of Cd^{2+} (0·1–1 μM) caused an initial fall followed by a rise in both diastolic and systolic blood pressure, plasma DBH activity increased in a dose-dependent manner. Animals subjected to repeated treatment with Cd^{2+} (0·5, 1 μM) daily for 12 days became markedly hypertensive, the increases in the systolic pressure being greater than those seen in the diastolic pressure. In pithed animals the blood pressure responses of the treated animals to electrical stimulation of the lower sympathetic outflow (T_{10}–L_1) and tyramine injection (35, 70, 140 nmol kg^{-1}) were markedly decreased, whilst responses to low doses of noradrenaline (NA) (7, 15, 30 nmol kg^{-1}) were potentiated compared with untreated animals. In addition, plasma DBH activities following sympathetic outflow stimulation and tyramine administration were markedly increased and decreased respectively compared with untreated controls. The data suggest that a correlation exists between changes in sympathetic nervous function and the induction of hypertension caused by Cd^{2+}.

Acute injection of cadmium ions (Cd^{2+}) causes an increase in the blood pressure of the rat (Perry et al 1970). Chronic treatment with Cd^{2+} (5 ppm) added to the drinking water for 6–8 months also causes the development of hypertension in rats (Schroeder & Vinton 1962). The plasma Cd/Zn concentrations have been found to be significantly greater in hypertensive patients and a positive correlation has been shown between the plasma Cd/Zn concentration ratio and the increase in mean arterial blood pressure (Thind & Fisher 1976). It has also been found that chronic feeding of rats with Cd^{2+} (100 ppm), for between 2–10 weeks, significantly increases the heart catecholamine content (Delisle et al 1971). Furthermore, catecholamine release from the isolated superfused bovine medulla is increased after Cd^{2+} administration (Shanbaky et al 1978). To investigate the possible involvement of the sympathetic nervous system in Cd^{2+}-induced hypertension, the effects of Cd^{2+} have been studied on blood pressure and sympathetic function after acute and chronic treatment.

Dopamine-β-hydroxylase (DBH) is responsible for the final step in the biosynthesis of noradrenaline (NA) and occurs together with NA in the vesicles. It is known that DBH is released in a proportional amount to NA after adrenergic nerve stimulation of the hypogastric nerve to the vas deferens in vitro (Weinshilboum et al 1971). In addition, the same authors found that the ratio of NA to DBH discharge is similar to that present in the soluble portion of the synaptic vesicles in the nerve endings. The release of DBH would therefore appear, under certain experimental conditions, to correlate directly with the level of sympathetic nervous activity. Thus, any treatment which interferes with sympathetic function would be expected to alter the level of DBH present in the serum.

Three tests have been used to assess sympathetic function:
(i) electrical stimulation of the lower sympathetic outflow (T_{10}–L_1) using the technique of Gillespie & Muir (1967), (ii) the effect of i.v. injection of NA, (iii) the effect of i.v. injection of tyramine on the systemic blood pressure.

METHODS

Groups of female rats of Sprague-Dawley, CFE strain, 200–250 g were used; group sizes ranged from 4–6. For acute injection a single dose of cadmium (0·1, 0·5 and 1 μM) was intravenously (i.v.) administered in the form of the chloride. The same doses administered intraperitoneally (i.p.) were used for chronic treatment (daily for 12 days). Blood pressure

* Correspondence.

was recorded directly from the common carotid artery in animals anaesthetized with sodium pentobarbitone, 60 mg kg^{-1}. After allowing the blood pressure to stabilize for 10 min, the rat was pithed and artificially respired from a Palmer miniature Ideal pump using a frequency of 60–70 strokes min^{-1} and an inflation volume of 1 ml 100 g^{-1} weight. Electrical stimulation of the lower sympathetic outflow (T_{10}–L_1) was achieved using stimulation parameters of 20 V, pulse width 0·1 ms and 5 s duration according to Gillespie & Muir (1967). Injection of drugs and removal of blood samples were made by means of a cannula inserted into the femoral vein. 300 μl samples of blood were taken for DBH activity measurements.

DBH activity was measured using the method of Kato et al (1974) based on modifications of Algate & Leach (1978). The optimal Cu^{2+} concentration for rendering endogenous inhibitors inactive was found to be 20 μM and the rate of octopamine formation from its tyramine substrate was found to be linear up to 2·5 h: 1 h incubation periods of plasma samples were used throughout the experiments. Doses of Cd^{2+} and drugs are expressed in mol kg^{-1}. In expressing the result of DBH activity the effects after sympathetic outflow stimulation and tyramine injection were compared with the preceding resting value. The significance of the difference between treated and untreated groups was assessed using Student's *t*-test.

Drugs and chemicals used: ascorbic acid (BDH), cadmium chloride (BDH), catalase (Sigma), cupric sulphate (BDH), Dowex-50W, ion exchange resin (Sigma), *N*-ethylmaleimide (Sigma), heparin (Evans), noradrenaline acid tartrate (Sigma), octopamine hydrochloride (Sigma), pargyline hydrochloride (Abbot), sodium fumarate (May and Baker), sodium metabisulphite (Hopkin and Williams), tyramine hydrochloride (Sigma).

RESULTS

Resting systemic blood pressure

The acute intravenous injection of Cd^{2+} (0·1, 0·5 and 1 μM) caused a marked decrease in blood pressure which lasted for less than 1 min and was followed by an increase persisting for several minutes (Fig. 1). Rats pretreated with Cd^{2+} for 12 days appeared healthy but for the exception of the group receiving the highest daily dose of Cd^{2+} (1 μM); these animals showed significant weight loss and were hyperresponsive to handling. The mean body weight of the control group increased by 7% over the 12 day period, whilst the mean body weight of the treated

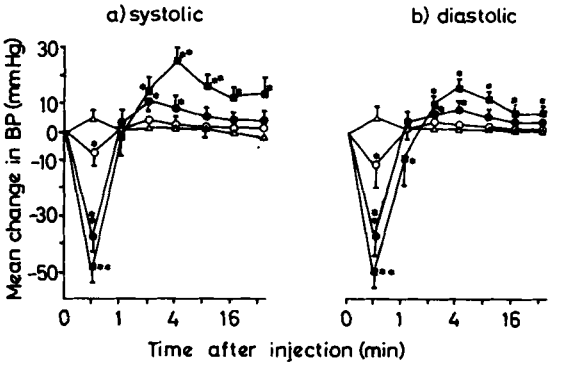

FIG. 1. Blood pressure responses of the pentobarbitone anaesthetized rat to i.v. Cd^{2+} injection. Mean changes ± s.e. in (a) systolic (b) diastolic pressure are shown for 4 treated groups (Δ) saline, (○) 0·1 μM Cd^{2+}, (●) 0·5 μM Cd^{2+}, (■) 1 μM Cd^{2+}, $*P < 0.05$, $**P < 0.01$, n = 4–6.

groups at the end of the treatment period were 99·2, 94·6 and 91·4% of corresponding control group weights for the Cd^{2+} 0·1, 0·5 and 1 μM treatments respectively. The blood pressures of the animals pretreated with 0·5 and 1 μM Cd^{2+} were found, after anaesthetization, to be significantly higher than those of the control animals; the mean systolic blood pressures being 144 and 171 mm Hg respectively (Fig. 2) compared with the pressure in the control group of 124 mm Hg.

The effects of 12 day Cd^{2+} treatment on the blood pressure responses to systemic NA, tyramine and nervous stimulation

The effects of three doses of NA were tested (7, 15, 30 nmol kg^{-1}). No significant differences were seen between the sizes of NA responses in the groups

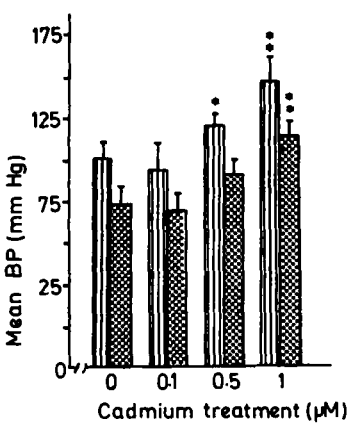

FIG. 2. The effects of a 12 day daily i.p. Cd^{2+} injection on the blood pressure of the pentobarbitone anaesthetised rat measured directly through the carotid artery. The results are shown mean ± s.e., $*P < 0.05$, $**P < 0.01$, ▨ systolic, ▩ diastolic, (n = 6).

treated with Cd^{2+} 0·1 and 0·5 μM and the control group. In the case of the group treated with 1 μM Cd^{2+}, the NA responses were significantly greater than for the controls, especially with the lower dose of NA (Table 1). The responses to tyramine 35, 70 and 140 nmol injected at intervals of 15 min, were significantly reduced in all of the Cd^{2+} treated groups compared with the controls. The extent of the decreases in tyramine responsiveness were seen to be dose-dependent in respect of Cd^{2+} dosage (Table 1).

Electrical stimulation of the lower sympathetic outflow caused blood pressure increases which were frequency-dependent (3–25 Hz) and repeatable for periods up to 3 h. The responses were significantly decreased in a dose-dependent manner in all of the Cd^{2+} treated groups (Table 2).

Dopamine-β-hydroxylase activity

(i) Blood samples removed from animals after acute injection of Cd^{2+}:

Blood samples taken at 0, 5, 10 and 30 min after Cd^{2+} injection showed a significant increase in plasma DBH activity. The activity increased at least twofold after Cd^{2+} (1 μM) (Table 3).

(ii) Blood samples removed from 12 day Cd^{2+} treated rats:

Samples were taken before and 2 min after electrical stimulation of the lower sympathetic outflow (6 Hz) and before and 2 min after tyramine (140 nmol kg^{-1}) injection. The resting level of plasma DBH actively was found to be greater in Cd^{2+} treated rats than in the control animals.

The DBH activity after sympathetic outflow stimulation decreased sharply in the control rats, whilst in the treated groups the activity was significantly increased above the resting values. The increase recorded for the Cd^{2+} (0·5 μM) treated group was the greatest. Tyramine injection did not change the level of plasma DBH activity present in the untreated group. DBH activity was, however, significantly decreased after tyramine injection for the two higher Cd^{2+} treatment groups (0·5 and 1 μM) (Table 4).

Table 1. Mean increases in systolic blood pressure in response to i.v. injection of noradrenaline and tyramine in pithed female rats, doses expressed as dose kg^{-1} weight. The rats were pretreated with Cd^{2+} for 12 days. The results shown are as mean systolic BP (mm Hg) ± s.e. The vehicle for Cd^{2+} administration was 0·2 ml of 0·9% NaCl (i.p.). n = 4–6. Cd^{2+} administered in the form of $CdCl_2$.

Treatment	Initial mean systolic BP (after pithing)	Mean increase in systolic BP: Noradrenaline (nmol kg^{-1}) 7	15	30	Tyramine (nmol kg^{-1}) 35	70	140
Control saline 0·2 ml	57 ± 5	13 ± 6	23 ± 6	45 ± 2	9 ± 2	14 ± 1	22 ± 1
Cd^{2+} 0·1 μM	51 ± 3	14 ± 6	27 ± 9	50 ± 6	8 ± 4	12 ± 4	21 ± 5
Cd^{2+} 0·5 μM	64 ± 2	9 ± 3	21 ± 8	38 ± 5	5 ± 1*	9 ± 1**	14 ± 4**
Cd^{2+} 1 μM	59 ± 6	28 ± 2**	37 ± 3*	47 ± 1	4 ± 1*	8 ± 1**	12 ± 1**

* $P < 0.05$
** $P < 0.02$

Table 2. Systolic mean blood pressure increase following stimulation of the lower sympathetic outflow (T_{10}–L_1) of normal and Cd^{2+} pretreated pithed female rats. Stimulation parameters: pulse width 0·1 ms; duration 5 s; voltage 20 V. The results shown are as mean BP (mm Hg) ± s.e. n = 4–6. The doses of cadmium pretreatment are expressed as μM kg^{-1} weight.

Pretreatment for 12 days (dose in 0·2 ml saline i.p.)	Mean systolic BP (after pithing)	Increase in systolic BP (mm Hg): Stimulation frequency Hz 3	6	12	25
Saline	57 ± 5	10 ± 2	20 ± 4	30 ± 6	43 ± 7
Cd^{2+} 0·1 μM	51 ± 3	7 ± 2	11 ± 4	19 ± 6	26 ± 7
Cd^{2+} 0·5 μM	64 ± 2	11 ± 4	21 ± 6	31 ± 7	43 ± 9
Cd^{2+} 1 μM	59 ± 6	3 ± 1*	5 ± 1*	9 ± 2*	13 ± 3**

* $P < 0.05$
** $P < 0.02$

Table 3. Mean systemic DBH activity measured in plasma samples taken from the femoral vein up to 30 min after acute i.v. injection of Cd^{2+} dissolved in saline. DBH activity is expressed as nmol octopamine ml^{-1} h^{-1}. The results are shown as mean ± s.e. (n = 6).

Treatment injection volume 0·2 ml i.v.	Mean plasma DBH activity (nmol octopamine ml^{-1} h^{-1}) Time after injection (min) 0	5	10	30
Saline	6·4 ± 3·5	6·1 ± 2·9	6·7 ± 3·8	5·4 ± 2
Cd^{2+} 0·1 μM	11·5 ± 2·2	12·4 ± 2·6	15·7 ± 3·3	13·8 ± 2·8
Cd^{2+} 0·5 μM	15·9 ± 0·9	20·4 ± 1·7*	12·7 ± 0·4**	15·7 ± 1·9
Cd^{2+} 1 μM	8·5 ± 4·4	13·9 ± 3·9*	17·0 ± 2·9**	16·9 ± 3·5**

* $P < 0.05$
** $P < 0.01$

Table 4. Mean systemic DBH activity in plasma samples taken at various times and after sympathetic outflow stimulation from Cd^{2+} pretreated female pithed rats. DBH activity is expressed as nmol octopamine ml^{-1} h^{-1}. The results are shown as mean ± s.e. (n = 4–6).

Pretreatment for 12 days (dose = 0·2 ml saline i.p.)	Mean plasma DBH activity (nmol octopamine ml^{-1} h^{-1}) Stimulation 0 min	Stimulation 2 min after	Tyramine 0 min	Tyramine 2 min after
Saline	5·5 ± 2·6	0·2 ± 0·4	8·4 ± 2·1	7·9 ± 1·1
Cd^{2+} 0·1 μM	8·2 ± 1·7	11·8 ± 0·4**	3·8 ± 0·5	3·5 ± 0·8
Cd^{2+} 0·5 μM	7·9 ± 1·8	12·6 ± 2·9**	5·8 ± 1·5	3·3 ± 0·6*
Cd^{2+} 1 μM	7·0 ± 2·1	6·8 ± 1·0	5·5 ± 1·5	2·8 ± 1·89*

* $P < 0.05$
** $P < 0.01$

Although the group results expressed as mean DBH activity values show considerable variations as judged by the extent of their standard errors, each individual animal's results showed a marked change after sympathetic outflow stimulation or tyramine injections when compared with the initial resting value. The wide range of mean group values simply reflects the well established variation reported for resting DBH plasma activity (Algate 1976).

DISCUSSION

Blood pressure

Three methods were used in this study to assess the changes in the sympathetic function following Cd^{2+} pretreatment. Tyramine injection and stimulation of the sympathetic outflow, are both believed to induce the release of NA neurotransmitter but by different mechanisms (Lindmar et al 1967) whilst the third method assessed post-junctional sensitivity in terms of responsiveness to injected NA. Cd^{2+} pretreatment was found to decrease the responsiveness of the cardiovascular system to tyramine and electrical stimulation of the lower sympathetic outflow whilst the responses to NA were either potentiated (small doses) or unaffected.

In previous studies, it has been found that Cd^{2+} in a decreasing order of sensitivity inhibits the responses of vascular (Fadloun & Leach 1980; Hayashi & Toda 1977) and non-vascular isolated smooth muscle preparations (Fadloun & Leach 1979) to electrical stimulation, potassium ions and noradrenaline respectively, NA being the least affected. Increasing extracellular Ca^{2+} reduced the degree of Cd^{2+}-induced inhibition in these isolated preparations. Stimulation of the adrenergic nerves requires extracellular Ca^{2+} (Farnebo 1971) for the release of the transmitter whilst it is believed that tyramine-induced responses are independent of external Ca^{2+} concentrations (Thoenen et al 1969). Since the responses of blood pressure to tyramine and electrical stimulation were inhibited after pretreatment with Cd^{2+}, it is suggested therefore that more than one mechanism is involved in the Cd^{2+}-induced inhibition.

Dopamine-β-hydroxylase

The decrease seen in DBH activity after electrical stimulation in the control group was in accordance with the results presented by Algate (1976). Plasma DBH activity was found to be significantly increased after acute injection of Cd^{2+}, in the 12 day treated group the activity after sympathetic outflow stimulation was also increased. The passage into the systemic circulation of the large DBH molecule is retarded by capillary barriers, so that an undetermined proportion of the enzyme released from nerve endings may enter the circulation through the lymphatic channels. Ngai et al (1974) observed that there was a significant correlation between DBH activity present in the serum and the amounts in the lymph during sympathetic nervous stimulation, the DBH content of the lymph was significantly increased. These findings might help to explain the reduction of DBH activity in plasma following sympathetic stimulation. Another explanation for the reduction could be that sympathetic stimulation causes a release of a high concentration of endogenous DBH inhibitors which may function as intracellular regulators of NA synthesis (Orcutt & Molinoff 1975).

Cd^{2+} is an inhibitor of -SH groups and the endogenous inhibitors of DBH activity present in the tissue are believed to be of a -SH nature. The increase in resting plasma DBH activity seen after Cd^{2+} might therefore be explained by one of the following mechanisms:

(i) increasing the permeability of the lymphatic channel walls, so that more DBH passes more freely into the lumen of the blood vessels, (ii) inhibition of the endogenous tissue inhibitors of DBH activity, (iii) increasing the release of DBH and catecholamines from the adrenal medulla. It has been reported that Cd^{2+} increases the release of catecholamines from isolated superfused bovine medulla (Hart & Borowitz 1974; Shanbaky et al 1978), and this might serve as the reason for the observed changes.

Two major conclusions can therefore be drawn from the present data: (i) it is suggested that the sympathetic nervous system plays a functional role in the hypertensive action of Cd^{2+} although it is difficult at this stage to correlate the changes in the vascular reactivity to the Cd^{2+}-induced hypertension. (ii) Cd^{2+} appears to possess a high affinity for presynaptic mechanisms. This statement is further supported by biochemical studies undertaken in these laboratories.

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