

Chronic Cadmium Administration Preferentially Affects the Responsiveness Mediated by Pre- and Post-synaptic α_2 -Adrenoceptors

Ki-Whan Hong, Byung-Yong Rhim and Uy-Dong Sohn

Department of Pharmacology, College of Medicine, Pusan National University, Pusan 600, Korea

ABSTRACT

Either the contraction of isolated vas deferens or the increase of heart rate in the pithed rats induced by electrical stimulation was significantly augmented by cadmium administration (10 μ mol of cadmium acetate every other day for 2 weeks i.p.). These stimulation-induced responses were diminished by α_2 -adrenoceptor agonist, clonidine, and the inhibition was antagonized by yohimbine. Furthermore, the increase in diastolic blood pressure by clonidine was also significantly reduced after cadmium administration, whereas that by methoxamine was not influenced with this dose range of cadmium. With these results it may be postulated that the long-term cadmium exposure may preferentially affect the responsiveness of the presynaptic as well as the postsynaptic α_2 -adrenoceptors.

Key Words: cadmium, pithed rats, α_2 -adrenoceptor

Abbreviation: Cd; cadmium administration

INTRODUCTION

It has been postulated that cadmium might be a causative agent in human hypertension with the evidences that small doses of cadmium injected or fed to rats for their life times caused hypertension (Schroeder, 1965; Thind *et al.*, 1970; Perry *et al.*, 1976). This hypothesis was supported with the evidences that human subjects exhibiting hypertensive complications showed increased concentration of cadmium in their kidney, compared to the subjects dying of other disease (Schroeder *et al.*, 1966; 1968).

In spite of a controversy in the relationship between cadmium and hypertension (Porter *et al.*, 1974), a positive correlation has been widely reported (Carruthers and Smith, 1979; Templeton and Cherian, 1983). The mechanism by which cadmium raises the arterial blood pressure remains still uncertain. Recently, it was focused on the noradrenergic pathway and prostaglandin metabolism (Caprino *et al.*, 1982; Song and Hong, 1984). Moreover, the plasma dopamine- β -hydroxylase activity has been reported to be increased following sympathetic outflow stimulation after Cd in rats (Fadloun and Leach, 1981).

1. To whom reprint requests should be adressed.

In this investigation, the selective inhibition of α_2 -adrenoceptor regardless of pre- and postsynaptic sites after chronic Cd has been assessed by the experiments using isolated vas deferens and pithed rats.

MATERIALS AND METHODS

In vitro experiments

Male Sprague-Dawley rats weighing 200—250 g were injected intraperitoneally with a dose of 10 μ moles of cadmium acetate every other day for two weeks. In control group, 0.5 ml of normal saline was injected. Rats were sacrificed by decapitation 24 hours after the last injection. The epididymal portion of vas deferens was isolated and mounted in the organ baths containing physiological salt solution maintained at 37°C and continuously gassed with oxygen.

The composition of physiological salt solution was (mM); NaCl, 130; KCl, 4.7; NaH_2PO_4 , 1.18; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.17; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.61; NaHCO_3 , 14.9 and dextrose, 5.5 dissolved in demineralized distilled water and pH was adjusted to 7.4 with 0.1 N HCl. Muscle contraction was recorded using isometric transducer (Myograph F-60) connected to Physiograph (MK IV, Narco Bio-systems) with resting tension of 0.5 g.

Electrical field stimulations were delivered at the pulses of 0.5 msec duration with various frequencies under 40 volts power (Stimulator SI-10, Narco Bio-systems).

In vivo studies

According to the method described by Gillespie and Muir (1967) and Gillespie *et al.* (1970), the rats were pithed. Vagus nerve was sectioned bilaterally in the neck, and carotid artery was cannulated for blood pressure measurement using Statham pressure transducer (Model P 50, Gould). Drugs were administered via jugular vein. Before the experiment all animals received atropine and d-tubocurarine (1 mg/kg i.v. of each).

When the stimulation is necessary, the tip of steel rod was placed between C_7 — T_1 . The parameter of the electrical stimulation of preganglionic nerves to the heart was adjusted (1—2 Hz, 0.5 msec pulse duration at 10 volts) so as to obtain the increase of the heart rates between 90—110 beats per min for 30 sec using electric stimulator (SI-10, Narco Bio-systems).

Drugs used were cadmium acetate (Hayashi Chemical Ind.), methoxamine, kindly donated by Burroughs Wellcome Co. and clonidine (Tokyo Chemical Ind.).

Results of the experiments are expressed as mean \pm S.E. The statistical significance of difference was estimated by Student's t-test and a P value less than 0.05 was considered significant.

RESULTS

Contractile response of rat vas deferens

Fig. 1 displays the frequency-dependent contraction of rat vas deferens. In this experiment the contraction induced by electrical stimulation with the frequency of 40 Hz was 1.06 ± 0.18 g for control and 3.07 ± 0.40 g for Cd group, respectively and these were taken as 100%.

The frequency-response curve of the Cd group markedly shifted to the left (Fig. 1). Moreover, the inhibition of the contraction by clonidine was significantly attenuated in the Cd group ($p < 0.05$), compared to that of control, and the inhibition was antagonized by yohimbine (10^{-6} M) (the data not shown). Methoxamine (10^{-8} — 10^{-7} M) did not show any inhibition on the contraction by electrical stimulation in both groups (Fig. 2).

Cardiac acceleration in pithed rats

Both basal blood pressure and heart rates after pithing were significantly higher in the Cd group than in the control as shown in Table 1.

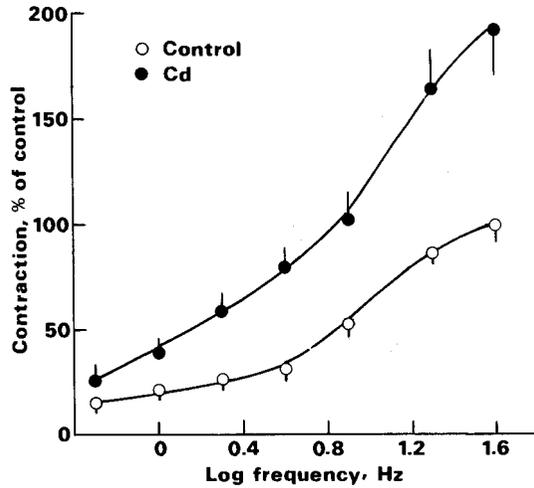


Fig. 1. Frequency-response curves of rat vas deferens of the cadmium administered (●) and control group (○). The contraction amplitude induced by electrical stimulation with 40 Hz frequency was taken as 100%. See others in the text.

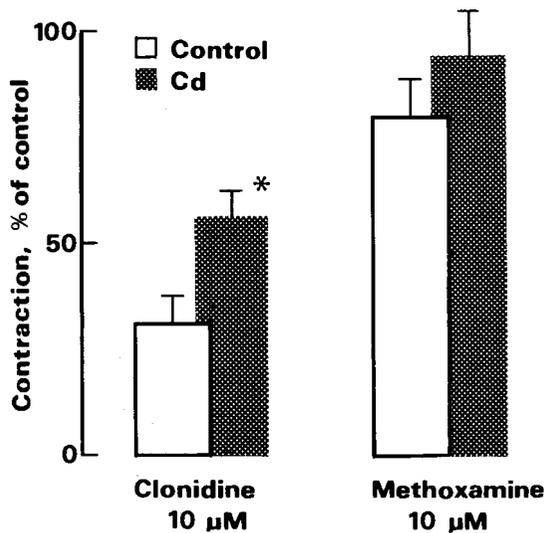


Fig. 2. Comparison of the effects of clonidine with methoxamine on the contraction induced by electrical stimulation delivered at 10 Hz frequency with 0.5 msec pulse duration under 40 volts in both groups.

Table 1. Basal blood pressure and heart rate in the pithed rats

Group	Basal blood pressure (mmHg)	Heart rate (beats/min)
Control	43.2 ± 0.8 (49) ^a	315.7 ± 7.9 (49)
Cd administered	52.9 ± 2.1 (23) ^b	355.0 ± 13.7 (23) ^b

a: Numbers in parentheses are numbers of experiments

b: $p < 0.05$, Significantly different from control value

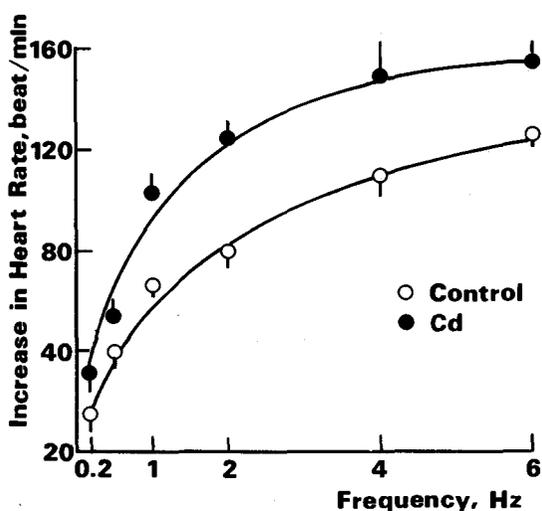


Fig. 3. Frequency-dependent increase in heart rate in the pithed rats. The parameters of the electrical stimulation of preganglionic nerves to the heart was 0.5 msec pulse duration at 10 volts for 30 sec. Control (o); Cadmium administered group (●).

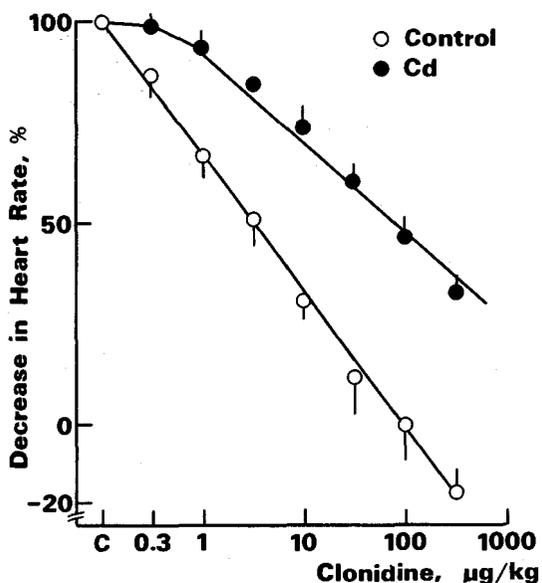


Fig. 4. Dose-dependent inhibition of tachycardia by clonidine in the pithed rats of control (o) and cadmium administered group (●). Others are the same as in Fig. 3.

The heart rate response to cardiac nerve stimulation was augmented to a greater degree in the Cd group. The heart rate evoked by 2 Hz of frequency was 80.9 ± 8.2 beats per min in control and 123.8 ± 10.5 beats per min in Cd group (Fig. 3). Further the reduction of the accelerated heart rate by clonidine was markedly diminished in the Cd group, compared to that of the control (Fig. 4).

Pressor response in pithed rats

Both clonidine and methoxamine (each 1-1,000 µg/kg i.v.) caused a dose-dependent increase in the

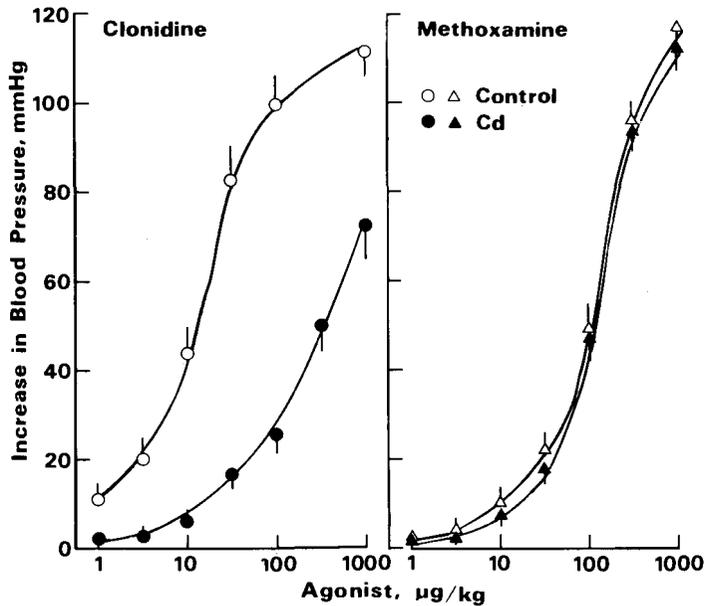


Fig. 5. Dose-dependent responses to clonidine (left) and methoxamine (right) of the increase in diastolic blood pressure in the pithed rats of control (open symbols) and cadmium administered group (closed symbols).

diastolic blood pressure in both groups of pithed rats. The clonidine-induced increase in diastolic blood pressure was significantly reduced in Cd rats, whereas the increase in diastolic blood pressure by methoxamine was not affected after chronic Cd (Fig. 5). The pD_2 value of clonidine (mean dose required to increase in diastolic blood pressure by 50 mmHg) was 13.2 $\mu\text{g}/\text{kg}$ in the control group and around 300.0 $\mu\text{g}/\text{kg}$ in Cd group ($P < 0.01$).

DISCUSSION

α_1 - and α_2 -Adrenoceptors have been postulated to be located post- and presynaptically, respectively (Langer, 1974; Berthelsen and Pettinger, 1977 and Starke, 1977). However, based on the results from the experiments using pithed rats, several investigators have reported the existence of postsynaptic α_2 -adrenoceptor, which can still mediate the agonist-induced increase of arterial pressure (Bentley *et al.*, 1977; Drew and Whiting, 1979; Timmermans *et al.*, 1979), whereas stimulation of presynaptic α_2 -adrenoceptor is known to inhibit stimulation-evoked noradrenaline release from sympathetic nerve ending (Langer, 1981).

The present experiments were designed to elucidate the relationship regarding cadmium and hypertension. So, it was aimed to examine which subtype of α -adrenoceptor is sensitively affected after Cd. The alteration of presynaptic α_2 -adrenoceptor was assessed as the inhibition of the cardioaccelerator response to electrical stimulation and of the contraction of the rat vas deferens by clonidine.

In the present experiment using vas deferens and pithed rats, the frequency-dependent contraction as well as the heart rate increase by the electrical stimulation were augmented to a greater extent in Cd group than in the control. Furthermore, the inhibition of these responses by clonidine was markedly diminished following chronic Cd. From the present results, in agreement with Fadloun and Leach (1981), it might be hypothesized that cadmium primarily affects the presynaptic α_2 -adrenoceptor, the inhibition of which functionally augments the stimulation-evoked noradrenaline release and consequently enforces to enhance the responses.

On the other hand, the effect on the postsynaptic α_2 -adrenoceptor was assessed as the rise in diastolic blood pressure by intravenous injection of α_2 -preferential agonist, clonidine. Cadmium was reported to decrease the responsiveness of the vascular (Hayashi and Toda, 1977; Nasu, 1983) and nonvascular smooth muscle preparations (Osa, 1974; Nasu *et al.*, 1983) to electrical stimulation, potassium ions and noradrenaline. Interestingly, the dose-dependent response to clonidine in raising the diastolic blood pressure in the pithed rats was markedly diminished after Cd, whilst that of methoxamine, α_1 -preferential agonist was not affected. Recently, the hypothesis has been formulated that an influx of extracellular Ca^{++} is necessary for the vasoconstriction initiated by α_2 -adrenoceptor, not by α_1 -adrenoceptor stimulation (Van Zwieten *et al.*, 1983; Shepperson *et al.*, 1981). It was therefore suggested that the decreased hypertensive activity exerted after Cd might be directly related with the inhibitory action of cadmium on the channel regarding the influx of free extracellular Ca^{++} .

However, it is difficult at this stage to correlate the affinity of cadmium for α_2 -adrenoceptor with its inhibitory action. In spite of some arguments on the role of cadmium in the development of human hypertension (Porter *et al.*, 1974; Templeton and Cherian, 1983) the fact that both basal blood pressure and heart rate are higher in the pithed rats with Cd suggests to merit further study.

In conclusion, based on these evidences it may be postulated that long-term cadmium exposure preferentially affect the responsiveness mediated by both pre- and postsynaptic α_2 -adrenoceptors, and the controversy on the development of hypertension due to Cd may be considered to be attributed to these complexities.

REFERENCES

- Bentley SM, Drew GM and Whiting SB: Evidence for two distinct types of postsynaptic α -adrenoceptor. Proc Br J Pharmacol p 116 1977
- Berthelsen S and Pettinger W: A functional basis for classification of α -adrenergic receptors. Life Sci 21:595-606, 1977
- Caprino L, Dolci N, Togna G, Villa P, Bucci R and Carrunchio V: Effects of cadmium on platelet thromboxane and vascular prostacyclin production. Toxicol Appl Pharmacol 65:185-188, 1982
- Carruthers M and Smith B: Evidence of cadmium toxicity in a population living in a zinc-mining area. The Lancet (8121) 1:845-847, 1979
- Drew GM and Whiting SB: Evidence for two distinct types of postsynaptic α -adrenoceptors in vascular smooth muscle in vivo. Br J Pharmacol 67:207-215, 1979
- Fadloun Z and Leach GDH: 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. J Pharm Pharmacol 33:660-664, 1981
- Gillespie JS and Muir TC: A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. Br J Pharmacol 30:78-87, 1967
- Gillespie JS, Maclaren A and Pollack D: A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. Br J Pharmacol 40:257-267, 1970
- Hayashi S and Tda N: Inhibition by Cd, verapamil and papaverine of Ca^{++} -induced contractions in isolated cerebral and peripheral arteries of the dogs. Br J Pharmacol 60:35-43, 1977
- Langer SZ: Presynaptic regulation of catecholamine release. Biochem Pharmacol 23:1793-1800, 1974
- Langer SZ: Presynaptic regulation of the release of catecholamines. Pharmacol Rev 32:337-362, 1981
- Nasu T: Spasmolytic effect of cadmium and cadmium uptake in aorta. Br J Pharmacol 79:751-754, 1983
- Nasu T, Koshiba H, Nase K and Ishida Y: Mechanism of inhibition of contraction by cadmium in guinea-pig taenia coli. J Pharm Pharmacol 35:505-510, 1983
- Osa T: Modification of the mechanical response of the smooth muscles of pregnant mouse myometrium and guinea pig ileum by cadmium and manganese ions. Jap J Physiol 24:107-117, 1974
- Perry HM, Thind GS and Perry EF: The biology of cadmium. Med Clin North Am 60:759-769, 1976

- Porter MC, Miya TS and Bousquet WF: Cadmium: inability to induce hypertension in the rat. *Toxicol Appl Pharmacol* 27:692-695, 1974
- Schroeder HA: Cadmium as a factor in hypertension. *J Chron Dis* 18:647-656, 1965
- Schroeder HA, Kroll SS, Little JW, Livingston PO and Myers MAG: Hypertension in rats from injection of cadmium. *Arch Environ Health* 13:788-789, 1966
- Schroeder HA, Nason AP and Mitchener M: Action of a chelate of zinc on trace metals in hypertensive rats. *Am J Physiol* 214:796-800, 1968
- Shepperson NB, Nicole Duval, Massingham R and Langer SZ: Pre- and postsynaptic alpha adrenoceptor selectivity studies with yohimbine and its two diastereoisomers rauwolscine and corynanthine in the anesthetized dog. *J Pharmacol Exp Ther* 219:540-546, 1981
- Song CS and Hong KW: A study of mechanism involved in cadmium-induced platelet aggregation. *Kor J Pharmacol* 20:41-46, 1984
- Starke K: Regulation of noradrenaline release by presynaptic receptor system. *Rev Physiol Biochem Pharmacol* 77:1-124, 1977
- Templeton DM and Cheria MG: Cadmium and hypertension, *TIPS* 4:501-502, 1983
- Thind GS, Karreman G, Stephan KF and Blakemore WS: Vascular reactivity and mechanical properties of normal and cadmium-hypertensive rabbits. *J Lab Clin Med* 76:560-568, 1970
- Timmermans PBMWM, Kwa HY and Van Zwieten PA: Possible subdivision of postsynaptic α -adrenoceptors mediating pressor responses in the pithed rat. *Naunyn-Schmiedeberg's Arch Pharmacol* 310:189-193, 1979
- Van Zwieten PA, Van Meel JCA and Timmermans PBMWM: Functional interaction between calcium antagonists and the vasoconstriction induced by the stimulation of postsynaptic α -adrenoceptors. *Circ Res* 52(suppl. 1):77-80, 1983

= 국문초록 =

카드미늄의 만성적 투여가 신경접합 전·후 α_2 -아드레날린 수용체에 의한 반응에 미치는 영향

부산대학교 의과대학 약리학교실

홍기환, 임병용, 손의동

전기자극에 의한 적출 정관의 수축 또는 뇌척수제거 흰쥐의 심박동수 증가가 카드뮴 장기투여(매격일 10μ mols, 1회씩 2주간 복강내 주사)로 인하여 대조군에 비하여 현저히 항진되었다.

이러한 전기자극에 의한 반응은 α_2 -효현제인 clonidine에 의하여 억제되었고, 이 억제제는 α_2 -길항제인 yohimbine 투여로 봉쇄되었다. 나아가 methoxamine에 의한 확장혈압의 증가는 카드뮴 투여에 의하여 영향을 받지 아니하였으나 clonidine에 의한 증가는 카드뮴 투여에 의하여 억제되었다.

이러한 결과로 보아 카드뮴 장기 노출에 의하여 시냅스 전 및 후 α_2 -adrenoceptors가 우선적으로 억제되었다고 시사되는 바이다.