EFFECTS OF CADMIUM ON THE TENSION OF ISOLATED RAT AORTA (A POSSIBLE MECHANISM FOR CADMIUM-INDUCED HYPERTENSION)

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Abstract……A possible mechanism for the pressor effect of cadmium was investigated in isolated rat thoracic aorta. Cadmium produced contractions at low concentrations, but relaxations at high concentrations. Phentolamine, a sympathetic alpha blocking agent did not inhibit the cadmium-induced contractions. These contractions were reduced in accordance with the decrease in Ca content in medium and were abolished in Ca-free medium, rather inducing a small degree of relaxation. When low concentrations of cadmium were applied repeatedly for a short period of time, the contractions were remarkably reduced and finally abolished. Noradrenaline-induced contractions were not affected after the completion of cadmium-tachyphylaxis.

Low concentrations of cadmium potentiated K-, Ba- and noradrenaline-induced contractions, while high concentrations suppressed them.

These results suggest that cadmium-induced contractions are dependent on external Ca and that they are produced by direct stimulation on the cell membrane. In addition, low concentrations of cadmium accelerate Ca availability, while high concentrations inhibit it.

Key words: Cadmium, hypertension, contraction, aorta, Ca, K, Ba, noradrenaline

INTRODUCTION

It is recognized that excessive exposure to cadmium results in renal damage (Schroeder 1964; Thind et al. 1975; Perry et al. 1977a), infertility (Schroeder et al. 1966; Dixit et al. 1975; Suzuki et al. 1978) and cardiovascular disease (Perry et al. 1974a; Kopp et al. 1978). In recent years, cadmium has particularly attracted interest as a potential etiologic agent in human hypertension.

In the past, the association of hypertension with cadmium exposure has been
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examined in epidemiologic studies (Hickey et al. 1966; Perry et al. 1974b) in order to establish a cause-effect relationship, with experimental hypertension having been reported following parenteral (Perry et al. 1965, 1967b, 1970, 1973; Schroeder et al. 1966) and oral (Perry et al. 1973, 1974a, 1977a, 1977b) administration. There have also been several hypotheses that may relate to a possible hypertensive mechanism, including sodium retention (Perry et al. 1973, 1974a, 1977a, 1977b), elevated circulating renin activity (Perry et al. 1973, 1974a) and increased cardiac output (Perry et al. 1967b, 1970, 1974a, 1977a, 1977b).

On the other hand, cadmium has been reported to have produced relaxation of isolated blood vessels (Toda 1973; Porter et al. 1975; Cooper et al. 1977; Hayashi et al. 1977) and some smooth muscle organs (Triggle et al. 1975; Suzuki et al. 1977, 1978). It has been also demonstrated that cadmium perfusion through the hindquarters of the rat produced vasoconstriction (Perry et al. 1967b, 1970). It has not, however, been easy to explain these conflicting results or the hypertensive mechanism for cadmium.

The present study, in order to clarify cadmium hypertension, was conducted using isolated rat aortas.

MATERIALS AND METHODS

Mature rats were anesthetized with ether and thoracic aortas were removed. Thereafter the preparations were cut spirally and mounted in an organ bath containing Locke's solution. This solution was maintained at 30°C and continuously bubbled with a gas mixture of 95% O₂ and 5% CO₂ at pH 7.3. The thoracic aorta strips were subjected to a 2.0 g resting tension with changes in tonus being recorded isometrically.

Each strip was exposed to 10⁻⁰⁻¹⁰⁻⁴ g/ml concentrations of Cd and a full, single dose-response curve of Cd was obtained. In our low Ca media experiment, Cd 10⁻⁷ g/ml was employed. After a control contraction was obtained, the Ca concentration of the medium was altered and the strips were incubated for 30 min in the altered medium. The sequence of Ca concentrations in mM was 2.2, 1.0, 0.4, 0.2 and 0.1. The procedure for Cd 10⁻⁴ g/ml in a Ca-free medium was studied for 30 min after exchange to the Ca-free medium, with Cd repeatedly being applied at intervals of 20 min.

In an experiment concerning the effects of Cd on the responses to vasoconstrictors, cumulative dose-response curves were obtained by stepwise increases in the concentration of vasoconstrictor agents. When the change in tension by each dose of Cd tested was stabilized, the vasoconstrictor agents were applied in the same fashion. A second dose-response curve was compared with its control curve.

The drugs used were as follows: CdCl₂ (Sigma), KCl (Wako), BaCl₂ (Wako), noradrenaline (Nad) hydrochloride (Sankyo), phen tolamine mesylate (Chiba-Geigy), tyramine hydrochloride (Sigma).

RESULTS

1. Effect of cadmium on tonus.

While low concentrations of cadmium (Cd) produced contractions, high con-
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centrations resulted in relaxations. The minimal concentration required for contractions was $5 \times 10^{-4}$ to $10^{-3}$ g/ml, while the maximal contraction was obtained at $10^{-7}$ g/ml.

In concentrations above $10^{-7}$ g/ml, contractions were gradually reduced and $10^{-4}$ g/ml or more produced only relaxation. The tensions evoked by Cd of $10^{-8}$, $10^{-7}$, $10^{-6}$ and $10^{-5}$ g/ml were 33.2, 49.4, 37.8 and 35.4 mg, respectively (Table 1). At the maximal contraction, the value of Cd ($10^{-7}$ g/ml) was equivalent to 61.8% of Nad ($10^{-7}$ g/ml).

<table>
<thead>
<tr>
<th>Concentrations of cadmium</th>
<th>Contraction and relaxation</th>
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<tbody>
<tr>
<td>$10^{-8}$ (g/ml)</td>
<td>33.2±16.0 (mg)</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>49.4±11.4</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>37.8±6.2</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>35.4±11.1</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>-117.3±41.8</td>
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Contraction and relaxation (−) were measured at isometric tension in normal medium.

2. Effect of the sympathetic alpha-blocking agent, phentolamine.

The contractile responses to Cd, Nad or tyramine were examined in the presence of a sympathetic alpha-blocking agent, phentolamine. $10^{-6}$ g/ml phentolamine completely inhibited the contractions by $10^{-6}$ g/ml Nad and $10^{-4}$ g/ml tyramine, but had no effect on contractions evoked by $10^{-7}$ g/ml Cd (Fig. 1).

![Fig. 1. Effects of the alpha-blocking agent phentolamine on contractions by noradrenaline, tyramine and cadmium.](image)

3. Cadmium-induced tachyphylaxis.

When $10^{-7}$ g/ml Cd was applied repeatedly at intervals of 30 min, Cd-induced contractions were gradually reduced and resolved. Administration at intervals of 60 min or more, however, did not induce such tachyphylaxis. $10^{-7}$ g/ml Nad-induced contraction was not affected by Cd-tachyphylaxis, as shown in Fig. 2.

4. Effect of low Ca media on cadmium-induced contractions.

Cd $10^{-7}$ g/ml-induced contractions were reduced in low Ca media. The reduction
corresponded with the degree of Ca decrease in the medium. That is, the reductions were, respectively, about 35% in 1.0 mM Ca medium, 44% in 0.4 mM Ca medium and 85% in 0.2 mM Ca medium. Contractions were not elicited in 0.1 mM Ca medium and a small degree of relaxation was produced in Ca-free medium (Fig. 4).

In contrast, an extremely high concentration of Cd $10^{-4}$ g/ml produced contractions in Ca-free medium. These contractions were not affected by repeated applications (Fig. 5).

![Fig. 2. Cadmium-induced tachyphylaxis.](image)

![Fig. 3. Contractions by cadmium in low Ca media.](image)

![Fig. 4. Responses to cadmium in normal and Ca-free media.](image)
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Fig. 5. Contractions by cadmium in Ca-free medium.

Fig. 6. Effects of cadmium on the dose-response curve of KCl. Each point represents the mean ± S.E.
5. Effect of cadmium on K-, Ba- and Nad-induced contractions.
   a) Effect of cadmium on K-induced contractions.

   Application of low concentrations of Cd (10^{-8} - 10^{-7} g/ml) enhanced K-induced contractions, while high concentrations of Cd (10^{-6} g/ml or more) inhibited them (Fig. 6). This inhibition was particularly striking at 10^{-4} g/ml Cd.

   b) Effect of cadmium on Nad-induced contractions.

   Cd of 10^{-8} g/ml enhanced Nad-induced contractions, while 10^{-7} g/ml had no significant effect. High concentrations (10^{-6} g/ml or more) inhibited these contractions, with 10^{-4} g/ml Cd completely blocking them (Fig. 7).

   c) Effect of cadmium on Ba-induced contractions.

   Low concentrations of Cd (10^{-8} - 10^{-7} g/ml) increased Ba-induced contractions, but high concentrations (10^{-5} g/ml or more) inhibited them. Ba-induced contractions were greatly inhibited with 10^{-5} g/ml Cd (Fig. 8).

Fig. 7. Effects of cadmium on the dose-response curve of noradrenaline. Each point represents the mean value ± S. E.
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Fig. 8. Effects of cadmium on the dose-response curve of BaCl₂. Each point represents the mean ± S.E.

DISCUSSION

Cadmium (Cd) produced contractions at low concentrations and relaxations at high concentrations. The minimal effective concentration for contractions produced by Cd ranged from \(5 \times 10^{-6}\) to \(10^{-4}\) g/ml. Maximal contractions were obtained at \(10^{-7}\) g/ml Cd, with a contractile value which was about 60% of those produced by Nad. These facts suggest that Cd generates strong constriction of the blood vessels in low doses. These findings may be supported by the report that Cd perfusion through rat hindquarters produced vasoconstriction at \(1.6 \times 10^{-6}\) M (1.8 \(\times 10^{-6}\) g/ml) (Perry et al. 1967b, 1970).

The sympathetic alpha-blocking agent phentolamine did not inhibit the Cd-induced contractions, suggesting that Cd did not act directly or indirectly on alpha adrenergic receptor.

It has been reported that Cd inhibited the activities of monoamine oxidase and catechol-O-methyltransferase (Glausser et al. 1976; Revis 1977; Williams et al. 1978), and that Cd caused the release of catecholamine from the adrenal medulla (Hart et al. 1974), resulting in elevated blood pressure. This suggests that Cd may release catecholamine from sympathetic nerve ending thus leading to vasoconstriction. The fact, however, that the concentrations of phentolamine which blocked the effects of tyramine or Nad did not affect the Cd-induced contractions may refute these possibilities.
It is generally accepted that responses to contractile agents are dependent on Ca release, Ca influx and the direct effect on contractile proteins. This study showed that Cd-induced contractions were reduced with decreasing Ca in medium and were abolished in Ca-free medium, thus suggesting that such contractions were highly sensitive to external Ca content.

When low concentrations of Cd were applied repeatedly during a short period of time, contractions remarkably diminished and were finally resolved. In addition, Nad-induced contractions were unaffected following the completion of Cd-tachyphylaxis. Nad-induced contractions have been postulated to be associated with Ca release (Yanagawa et al. 1979). These findings suggest thatCd-tachyphylaxis may not be due to a disturbance of Ca release and/or Ca carrier proteins, but rather to interference on the cell membrane.

$10^{-4}$ g/ml and higher concentrations of Cd produced relaxation. In previous experiments, high concentrations of Cd produced relaxation in isolated blood vessels (Perry et al. 1967a; Toda 1973; Porter et al. 1975). The present findings indicate, likewise, that high concentrations of Cd produced only relaxation. Thus, the dual action of Cd most likely depends upon the dose of the metal. It may be possible that high concentrations induce a predominantly stabilizing action on membrane and/or disorder in Ca carrier proteins, producing relaxation. In addition, generic differences (Perry et al. 1965, 1967b; Toda 1973) may be one reason for conflicting results.

Extremely high concentrations of Cd produced contractions in Ca-free medium, with no exhibition of tachyphylaxis. In the preparations in which Nad- and K-induced contractions were abolished, the same contractions remained. There is a possibility that very high concentrations of Cd bind to contractile proteins like divalent cations such as Ca or Sr, producing contractions.

Low concentrations of Cd potentiated contractions produced by K, Ba and Nad, while high concentrations suppressed contractions by these agonists. A previous report revealed that the responses to these agonists were produced by Ca influx and Ca release (Yanagawa et al. 1979). It seems therefore likely that low concentrations of Cd accelerate Ca release and Ca influx, but that high concentrations inhibit them.

It has been indicated that Cd increased the pressor response to norepinephrine (Revis 1977; Nechay et al. 1978; Williams et al. 1978). The present results revealing that low concentrations of Cd potentiated not only Nad-induced contractions, but also those induced by K and Ba, seem to show a nonspecific action for Cd. In the case of inhibitory effects for Cd, it has also been reported on the malfunction of contractile proteins in high concentrations of Cd (Blum 1962; Suzuki et al. 1977).

In summary, our work suggests that Cd produces contractions or relaxations by direct stimulation on the cell membrane, depending upon the concentration of the metal.

REFERENCES

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