BioMetals (2007) 20:83–91
DOI 10.1007/s10534-006-9017-z
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Effects of acute and chronic cadmium administration on the vascular reactivity of rat aorta

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Received 9 January 2006; accepted 28 April 2006

Key words: Cadmium, Isolated aortic rings, Endothelium, Adult rat, Potassium chloride, Phenylephrine, Acetylcholine

Abstract

The effect of acute and chronic cadmium (Cd) administration on the vascular function of the rat aorta was studied. The rats were randomly divided into four main groups (A: saline controls under chronic administration, B: Cd-treated rats under chronic administration, C: saline controls under acute administration, D: Cd-treated rats under acute administration). After their sacrifice, the aortic rings were divided into rings with endothelium (E+) and without (E−), and suspended in an isolated organ bath with Krebs–Henseleit buffer. Maximal tension (T_{max}, in g) was measured in response to potassium chloride (KCl) and phenylephrine (PE) in all aortic rings. Relaxation response to acetylcholine (ACh) administration was expressed as percent of maximal tension induced by PE. *Chronic administration*: A statistically significant increase of the contraction was observed between groups B (i.m. Cd 0.5 mg/kg for 120 days) and A (i.m. 0.9% NaCl for 120 days) in response to KCl (20–60 mM) and the T_{max} as well (in both the E+ and the E− subgroups). No statistically significant difference was observed in response to PE and ACh exposure. *Acute administration*: A statistically significant increase was observed between group D(E+) (i.m. Cd 2 mg/kg, 8 h before sacrifice) and group C(E+) (i.m. 0.9% NaCl, 8 h before sacrifice) in response to 10–30 mM of KCl, and a significant decrease between D(E−) and C(E−) in response to 10^{-7}–10^{-6} M of PE, though T_{max} was increased between D(E−) and C(E−) with PE exposure. The contractile response levels of the E+ aortic rings to PE and ACh showed no statistically significant difference.

Introduction

Several *in vitro* (cell cultures) and *in vivo* studies have implicated cadmium (Cd) as an environmental toxic factor for the vascular endothelium and the smooth muscle function of the vascular wall. In particular, it has been hypothesized that Cd can be a risk factor of atherosclerosis (under certain conditions) (Kaji *et al.* 1996; Kishimoto *et al.* 1996; Fujiwara *et al.* 1998), elevation of plasma free cholesterol (Bordas & Gabor 1982) and hypertension (Shroeder & Vinton 1962; Perry & Kopp 1983; Balaraman *et al.* 1989a, b; Puri 1999).

In the very comprehensive review of Nomiyama & Nomiyama (2000) (67 references) about the role of Cd in the development (or not) of hypertension, the authors came to the conclusion that

Cd^{2+} tends not to change the blood pressure of normotensive humans or animals, and probably affects only hypertensive Cd-exposed workers. In addition, genetic factors (low urinary kallikrein excretion) (Varoni *et al*. 2003) and the way of Cd^{2+} administration, may influence Cd effects on rat blood pressure. Intravenous (i.v.) and intracerebroventricular (i.c.v.) administration of cadmium chloride ($CdCl_2$: 1 mg/kg and 10 μg/rat, respectively) produced a biphasic response: a transient fall followed by a marked and consistent rise in the rat blood pressure (Lall *et al*. 1997). Prior treatment with centrally administered losartan (an angiotensin II receptor blocker) completely abolished the pressor response to i.c.v. Cd administration, but only partially the i.v. one (Lall *et al*. 1997).

A hypertensive dose- and time-dependent response in Sprague-Dawley rats was observed after i.v. or i.c.v. Cd administration (Puri 1999). Similar results have been also reported after intraperitoneal (i.p.) Cd administration (Wang *et al*. 2002).

Many studies connect Cd-intake and kidney damage (Cd in the kidney accounts for one-third of the total Cd body burden) (Mueller *et al*. 1998; Uriu *et al*. 1998; Asar *et al*. 2004). A possible link between renal tubular damage and dysfunction caused by environmental Cd exposure and increased risk of high blood pressure was recently reported (Choi & Rhee 2003; Baker *et al*. 2005; Satarug *et al*. 2005, 2006).

In isolated organ preparations of rat aortic rings (or of other experimental animals' vessels), it has been observed that Cd-induced contractions were dependent on external calcium (Ca) and were produced by direct stimulation on the cell membrane (Niwa & Suzuki 1982). Cadmium is considered as a Ca-channel antagonist, producing contraction at low concentrations, and relaxation at higher ones (Niwa & Suzuki 1982; Lawson & Cavero 1989; Lawson & Chatelain 1992). Moreover, it has been suggested that Cd produces an endothelial dysfunction by impairing the M_1-type cholinoceptor-mediated response, which seems to be involved in prostanoid release (Bilgen *et al*. 2003).

The present study focuses on the vasoconstricting action of potassium chloride (KCl) and phenylephrine (PE) and the vasodilating action of acetylcholine (ACh) on the aortic rings of male rats exposed to Cd, with a view to explore the functioning of the aorta under both acute and chronic administration.

Materials and methods

Maintenance of the animals

Sixty albino Wistar adult male rats of 6 months were used and were randomly divided into four main groups as shown in Table 1. Each group consisted of two subgroups: the E+ subgroup had its aortic rings tested with their endothelium being intact, while the E− subgroup had its aortic rings tested with their endothelium being denuded. The rats were housed four in a cage, at a constant room temperature (22 ± 1 °C) under a 12-h light: 12-h dark (light 08:00–20:00 h) cycle. Food and water were provided *ad libitum*. Animals were cared for

Table 1. Experimental groups of male Wistar rats (from which aortic rings were excised) that were used in our experiments

Group	Type of treatment	*n*
Chronic i.m. administration (daily for 120 days)		
A(E+)	0.9% NaCl (1 ml) treatment/intact endothelium	7
A(E−)	0.9% NaCl (1 ml) treatment/denuded endothelium	7
B(E+)	Cd (0.5 mg/kg) treatment/intact endothelium	9
B(E−)	Cd (0.5 mg/kg) treatment/denuded endothelium	9
Acute i.m. administration (8 h before sacrifice)		
C(E+)	0.9% NaCl (1 ml) treatment/intact endothelium	7
C(E−)	0.9% NaCl (1 ml) treatment/denuded endothelium	7
D(E+)	Cd (2 mg/kg) treatment/intact endothelium	7
D(E−)	Cd (2 mg/kg) treatment/denuded endothelium	7

Quantities in this table refer to the elements and not to the form (e.g. $3CdSO_4 \cdot 8H_2O$) that these were injected. *n*: number of animals per group; i.m.: intramuscular; Cd: cadmium; E+: intact endothelium; E−: denuded endothelium. For more details, see Materials and Methods.

in accordance with the principles of the "Guide to the Care and Use of Experimental Animals" (Committee on the Care and Use of Laboratory Animals 1985).

Chronic administration

Groups B(E+) and B(E−) received intramuscular (i.m.) injections of Cd (0.5 mg/kg) in 0.5 ml solution, daily for 120 days (4 months). Groups A(E+) and A(E−) acted as controls and received i.m. injections of 0.5 ml 0.9% NaCl solution, for the same period. Cadmium was administered as cadmium sulfate ($3CdSO_4 \cdot 8H_2O$).

Acute administration

Groups D(E+) and D(E−) received i.m. injections of Cd (2 mg/kg) in 0.5 ml solution, 8 h before sacrifice. Groups C(E+) and C(E−) acted as controls and received i.m. injections of 0.5 ml 0.9% NaCl solution, 8 h before sacrifice. Cadmium was administered as cadmium sulfate ($3CdSO_4 \cdot 8H_2O$).

Aortic ring preparations

Thoracic aortas were excised from anaesthetized rats and cut into 3 mm rings. Endothelium was removed by gentle rubbing of the internal surface in all the E− groups (see Table 1). Each ring was suspended in an isolated organ bath filled with Krebs–Henseleit solution, pH = 7.4, (composition in mmol/l: sodium chloride 1.5, potassium chloride 5.6, sodium dihydrogen phosphate 0.5, potassium dihydrogen phosphate 0.5, magnesium chloride hydrate 1, sodium hydrogen carbonate 25, glucose 10, calcium chloride dehydrate 1.8) kept at 37 °C and oxygenated with 95% O_2/5% CO_2 mixture. The rings were connected to force transducers, and isometric tension was recorded (Gould RS 3400). They stretched to a resting tension of 3 g and after an equilibration period, were contracted with KCl 10–60 mM (this stimulates the contractile vascular apparatus independently of receptor mediated vasoconstriction) and phenylephrine hydrochloride (Sigma Chemical Co., St. Louis, MO) 10^{-10}–10^{-5} mol/l. Then, cumulative concentration–response curves to PE and KCl were constructed. Concentration of the drugs was increased when the contractile tension had reached a steady-state response to the prior concentration. At the end of the experiment 10^{-5} mol/l acetylcholine hydrochloride (Sigma Chemical Co., St. Louis, MO) was administered to test for the presence of functioning endothelium on intact rings and for the absence of endothelium on the denuded rings. Maximal tension (T_{max}) in g was measured in response to KCl and PE with intact (E+) and denuded (E−) endothelium respectively. Maximal relaxation (max Relax (%)) after ACh administration was measured and expressed as a percentage of the maximal contraction induced by PE (Pantos *et al.* 2001; Mourouzis *et al.* 2003). For each concentration–contraction relationship, the estimated concentration producing 50% of the maximum response (EC_{50}) to KCl or PE was calculated using the least squares method for curve fitting to the sigmoid model (Karasawa *et al.* 1993). All the experiments were performed at the same time of the day, in order to avoid time-dependent variations (Gorgun *et al.* 1998).

Statistical analysis

All data are expressed as mean values ± SEM. The data were evaluated by variance analysis for repeated measurements. Multiple comparisons were evaluated according to One-Way Between-Groups ANOVA with Bonferroni or Dunnet T3 correction. P values of < 0.05 were considered statistically significant.

Results

A statistically significant increase was observed between group B (chronic Cd administration) and group A (chronic saline control) and their respective subgroups (E+ and E−) regarding the contraction of aortic rings as response to KCl exposure (Figure 1a). This increase for the E+ aortic rings was observed during exposure to 30–60 mM KCl, whereas for the E− aortic rings, during exposure to 20–60 mM KCl. A statistically significant increase was also observed between groups B and A and their respective subgroups (E+ and E−) in T_{max} as a result of KCl exposure (Table 2). No statistically significant difference was observed regarding the response to PE and ACh exposure (Figure 1b, Table 2).

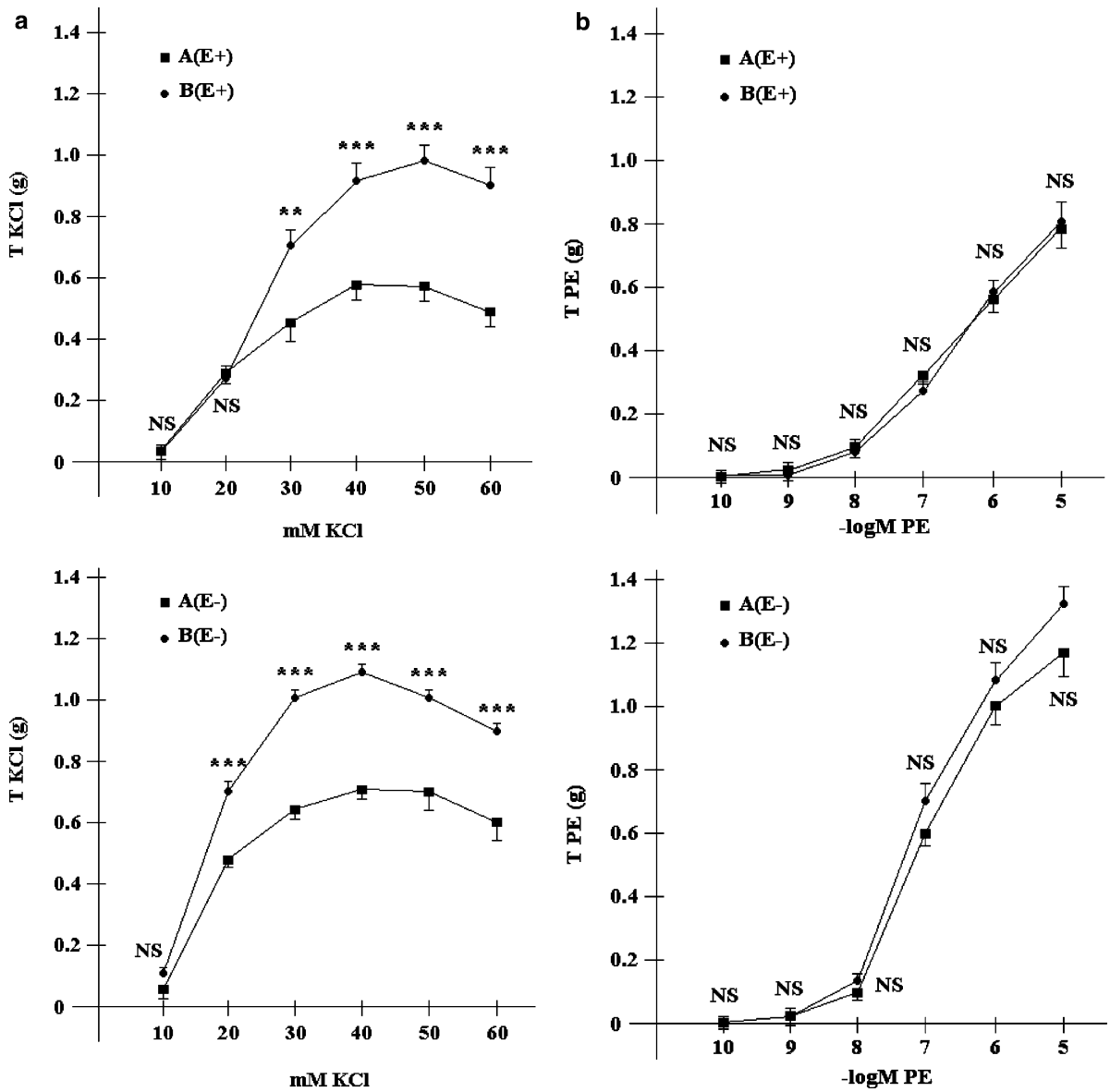

Figure 1. (a) Effect of potassium chloride (KCl) concentration on the steady-state tension (*T*) response of isolated aortic rings with intact (E+) and denuded endothelium (E−), following chronic 0.9% NaCl (■) or Cd (●) i.m. administration (groups A and B respectively). Values for *T* are expressed as mean ± SEM in grams (g). NS = non statistical significance; $^{**}P < 0.01$; $^{***}P < 0.001$ compared to the respective control subgroup A. (b) Effect of phenylephrine (PE) concentration on the steady-state tension (*T*) response of isolated aortic rings with intact (E+) and denuded endothelium (E−), following chronic 0.9% NaCl or Cd i.m. administration (groups A and B respectively). Values for *T* are expressed as mean ± SEM in grams (g). NS = non statistical significance compared to the respective control subgroup A.

As concerns the acute administration, a statistically significant increase was observed between group D (acute Cd administration) and group C (acute saline control) regarding the contraction of the E+ aortic rings only, as response to exposure to 10–30 mM KCl (Figure 2a). There was no statistically significant difference in T_{max} during KCl exposure among the above groups (Table 2). There was a statistically significant decrease between groups D and C regarding the E− aortic rings only, as response to exposure to 10^{-7}–10^{-6} M PE (Figure 2b). A statistically significant increase in T_{max} was also observed between groups D(E−) and C(E−) compared to their respective controls,

Table 2. Maximal recorded tension (T_{max}) in response to potassium chloride (KCl) and phenylephrine (PE), tension (T) in response to acetylcholine (ACh) (concentration 5 $-\log M$) and maximal relaxation (max Relax) after ACh administration (measured and expressed as a percentage of the maximal contraction induced by PE) of aortic rings with intact (E+) or denuded (E−) endothelium (following chronic or acute 0.9% NaCl or Cd administration)

Group	T_{max} KCl (g)	T_{max} PE (g)	T ACh (g)	ACh max Relax (%)
A(E+)	0.59 ± 0.03^{a}	0.78 ± 0.06^{a}	-0.35 ± 0.05	44.87
B(E+)	1.00 ± 0.04^{b}	0.81 ± 0.07^{b}	-0.35 ± 0.03	43.21
A(E−)	0.72 ± 0.02^{c}	1.18 ± 0.06^{c}	-0.21 ± 0.02	17.80
B(E−)	1.09 ± 0.03^{d}	1.34 ± 0.05^{d}	-0.26 ± 0.02	19.40
C(E+)	0.58 ± 0.04^{e}	0.77 ± 0.05^{e}	-0.38 ± 0.03	49.35
D(E+)	0.63 ± 0.04^{f}	0.78 ± 0.04^{f}	-0.38 ± 0.02	48.72
C(E−)	0.63 ± 0.03^{g}	1.15 ± 0.06^{g}	-0.25 ± 0.04	21.74
D(E−)	0.68 ± 0.02^{h}	1.04 ± 0.05^{h}	-0.23 ± 0.03	22.12

For more details, see Materials and Methods. Values for T_{max} and T are expressed as mean ± SEM in grams (g). Cd: cadmium; i.m.: intramuscular; s.s.: statistical significance; NS: non statistical significance; Tmax KCl: b/d, e/f, g/h, e/g, f/h = NS; a/c = $P < 0.01$; a/b, c/d: $P < 0.001$; T_{max} PE: a/b, c/d, e/f, g/h = NS; a/c, b/d, e/g, f/h = $P < 0.001$. No significance was noted as concerns the T ACh and the ACh max Relax values.

as a result of PE exposure (Table 2). The contractile responsiveness of the E+ aortic rings to PE and ACh showed no statistically significant difference.

Discussion

We examined the responses in KCl, PE and ACh of E+ and E− aortic rings of rats chronically or acutely exposed to i.m. Cd administration. The overall analysis of our data revealed an increase in vasoconstriction of the aortic rings under chronic Cd administration, in response to KCl. The results are in accordance with those of Niwa *et al.* (1981) in *vas deferens* of guinea pigs, and those of Sakurada & Wakabayashi (1999) in Cd-incubated rat aortas, while had some differences with those of Ozdem & Ogutman (1999). However, the latter investigators administered Cd in different doses, way and time-period.

Increased vasoconstriction was observed in the acute administration of Cd (2 mg/kg) only in the E+ aortic rings and with low KCl concentrations. A tendency for reduced response to KCl was observed in the E− aortic rings. A similar observation was refereed by Niwa & Suzuki (1982). Significant vasoconstriction was also observed in isolated ventral E− aortic rings of the shark *Squalus acanthias* (homologous of mammal aorta) after Cd administration (Evans & Weingarten 1990).

Potassium chloride contracts smooth muscle cells independently of a_1-receptors. It is probable that these results are not due to an up-regulation or increased sensitivity of a_1-receptors, since no corresponding increase in response to PE was recorded.

We could speculate some probable mechanisms for the aortic ring responses to KCl of our experiment. Firstly, an increase of intracellular Cd^{2+} ion concentration might occur. According to Sakurada & Wakabayashi (1999), Cd accumulation increases rat aorta vasocontractility, in part because of the facilitation of phosphoinositide hydrolysis. It is likely that Cd^{2+} creates equal or stronger bonds with calmodulin than Ca^{2+}, since calmodulin does not have the intrinsic ability to discriminate between Ca^{2+} and Cd^{2+} (Sutoo *et al.* 1990), maybe due to its similar ion size (Cd^{2+}: 0.97 Å, while Ca^{2+}: 0.99 Å) (Atkins & Beran 1992), resulting in increased dopamine synthesis that inhibits sympathetic nerve activity and reduces blood pressure (as observed in spontaneously hypertensive rats by Sutto & Akiyama 2000). This mechanism could explain the increased response to KCl in the acute Cd^{2+} administration, taking under consideration that Ca-channel antagonists such as nifedipine and verapamil can block Cd^{2+} effects (Balaraman *et al.* 1989a, b; Madeddu *et al.* 1993). In particular, the preventive effect of Ca-channel antagonists on acute pressor response to i.c.v. Cd administration in conscious normotensive rats suggests that the latter can interfere with Ca^{2+} transmembrane influx in the brain, increase intraneuronal Ca^{2+} concentration and thus, influence the central control of blood pressure.

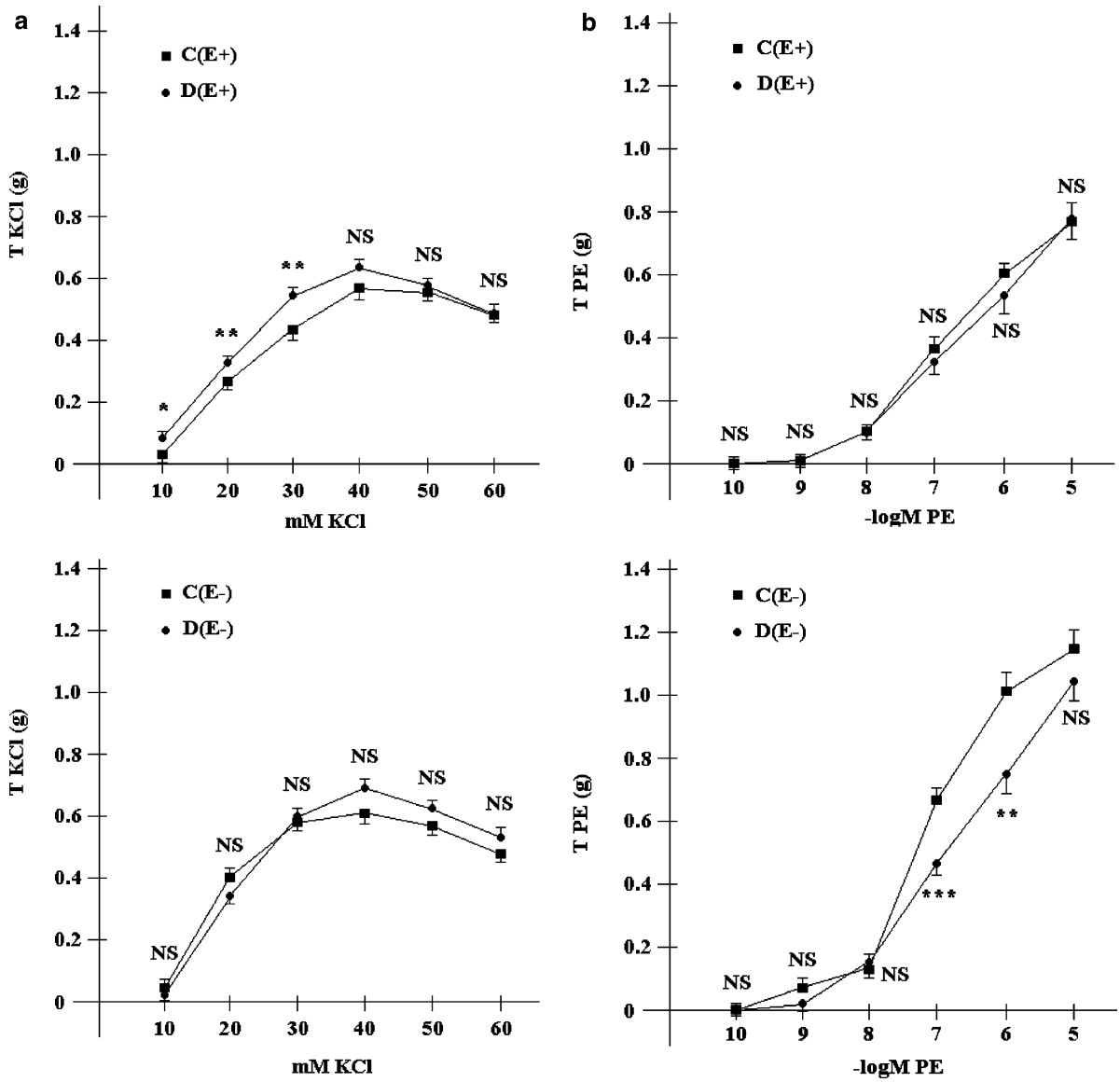

Figure 2. (a) Effect of potassium chloride (KCl) concentration on the steady-state tension (*T*) response of isolated aortic rings with intact (E+) and denuded endothelium (E−), following acute 0.9% NaCl (■) or Cd (●) i.m. administration (groups C and D respectively). Values for *T* are expressed as mean ± SEM in grams (g). NS = non statistical significance; $*P < 0.05$; $**P < 0.01$ compared to the respective control subgroup C. (b) Effect of phenylephrine (PE) concentration on the steady-state tension (*T*) response of isolated aortic rings with intact (E+) and denuded endothelium (E−), following acute 0.9% NaCl or Cd i.m. administration (groups C and D respectively). Values for *T* are expressed as mean ± SEM in grams (g). NS = non statistical significance; $**P < 0.01$; $***P < 0.001$ compared to the respective control subgroup C.

Another possibility that could explain the increased response of the aortic rings (both E+ and E−) to KCl is the proliferation of smooth muscle cells during the chronic Cd^{2+} administration through intracellular Ca-dependent signaling (Fujiwara *et al.* 1998). Furthermore, as the latter observed, Cd increased intracellular radioactive Ca^{2+} concentration in bovine aortic smooth muscle cells. Potassium chloride acts on smooth muscle cells via high voltage K^+-sensitive Ca^{2+}-channels or via slow K^+-channels or via ATP-sensitive K^+-channels. This channels' activation induces a K^+ inward current to the cell, which provokes membrane hyperpolarization and vaso-

dilatation. In our study, because of high external K^+ concentration in the medium, K-channels are closing, the intracellular Ca^{2+} concentration increases, membrane depolarization occurs, and vasoconstriction follows.

An alternative mechanism that could support our findings concerning the increased response to KCl in E+ aortic rings of acute Cd^{2+} administration, could be based on the observation that Cd^{2+} provokes intracellular oxidative stress (Carageorgiou *et al*. 2005), resulting in less NO formation (Buzard & Kasprzak 2000) and less vasodilation consequently. This could also be supported by the findings of Ramasamy & Delong (2000) (where Cd administration in bovine aortic endothelial cells caused an increase of NO synthase (NOS) mRNA and protein content, but not of endothelial NOS (eNOS) activity) and the study of Demontis *et al*. (1998). In this study, the pressure responses to i.c.v. Cd-administration were reduced by L-arginine i.c.v. pretreatment. Since L-arginine increases the availability of substrate for NO biosynthesis, it is possible that the pressor effect of i.c.v. Cd-administration is due, at least in part, to a reduced NO formation, subsequent to an inhibition of brain NOS. Moreover, the above study revealed that the hypertensive effect of i.c.v. Cd-administration was also prevented by calcium chloride ($CaCl_2$), a co-factor of the brain Ca/calmodulin-dependent neuronal NOS (nNOS) (Demontis *et al*. 1998). Similar data by Weaver *et al*. (2004) further confirmed that Cd does not activate nNOS. In addition, reduced NO in the serum of rats treated with Cd was observed by Martynowicz *et al*. (2004) and Skoczynska & Martynowicz (2005).

As concerns PE, no statistically significant differences were observed after chronic Cd administration on either E− or E+ aortic rings. It appears that Cd did not influence a_1-adrenergic receptors. This is in accordance with the Niwa & Suzuki (1982) data, where phentolamine (an a-blocking agent) administration did not inhibit the (low Cd concentration-induced) rat isolated aorta preparation contraction. These contractions were Ca-dependent, and were abolished in Ca-free mediums. Repeated Cd administration induces tachyphylaxis and full suppression of the contractions, while tachyphylaxis did not affect noradrenaline-induced contractions. Obviously, Cd does not act via a_1-adrenergic receptors.

On the contrary, a significant reduction of the contraction by PE was observed in E− aortic rings of acute high-dose Cd treated rats. This could be explained by the high Cd concentration (2 mg/kg), provoking enhanced Ca-channel antagonism and thus, resulting in the reduction of PE contraction. A similar observation was recorded by Lawson & Chatelain (1992) and by Niwa & Suzuki (1982).

However, according to the Nomiyama & Nomiyama (2000) review conclusions, prolonged low Cd administration may elevate blood pressure slightly, whereas a higher dose may depress it (but only in hypertensive humans or animals). In our experiment, the rat strain used was a normotensive Wistar one and showed reduction of the PE contraction after acute high-dose Cd administration in the E− aortic rings. However, a question arises: why this does not happen in the E+ aortic rings in response to PE in the acute Cd administration? A transient fall in blood pressure followed by persistent rise, was observed by Puri (1999) after*in vivo* (i.v. or i.c.v.) Cd administration in male rats. Cadmium-induced hypertension in rats was prevented by Ca-antagonists (Balaraman *et al*. 1989a, b; Madeddu *et al*. 1993). In the Ozdem & Ogutman (1999) study, a reduction in noradrenaline and other vasoconstrictive agents was observed in the E+ aortic rings of the Cd-treated rats. However, the authors did not examine E− aortic rings.

The lack of statistically significant impairment in response to ACh of aortic rings' dilatation between Cd- and saline-treated rats, reveals that Cd did not damage the endothelium (at least under our experimental conditions). However, Kishimoto *et al*. (1994) observed a dose-dependent endothelium damage in cultured human umbilical arterial endothelial cells and an inhibition of EDRF (endothelium-derived relaxing factor). Vascular smooth-muscle cells, as well as endothelial cells, may be a critical target of Cd toxicity (Kaji *et al*. 1996).

From the above analysis of our findings and the available literature, it is obvious that many factors (such as genetic background, experimental animal strain, as well as way, duration and dose of administration) influence the effect of Cd on the arterial wall and the development (or not) of hypertension (Nomiyama & Nomiyama 2000; Varoni *et al*. 2003; Satarug *et al*. 2005, 2006). Since the presence of Cd in the ecosystem is gradually increasing (being accumulated into our

body and exerting a wide variety of adverse effects – including oxidative stress) (Carageorgiou *et al.* 2005), people with hereditary sensitivities (atherosclerosis, kidney or liver function deficiencies) must be informed and prudent in order to avoid high cholesterol food intake and smoking (Carageorgiou *et al.* 2004).

Acknowledgement

This work was funded by the University of Athens.

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