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ORIGINAL PAPER

**Yıldırım Bayazıt · Peyman Uçar Ertuğ · Meral Ürünsak
Cemil Göçmen · İ. Atilla Arıdoğan · Tahsin Turunç
Ergin Şingirik**

Effects of chronic cadmium exposure on contractility of the rat detrusor

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Abstract The chronic effects of Cd^{2+} on the myogenic contractions induced by acetylcholine (ACh), and the neurogenic contractions induced by electrical field stimulation (EFS) of the rat detrusor were investigated. Wistar Kyoto rats weighing 150–250 g were randomly divided into four groups each containing ten animals. Three groups received intraperitoneal Cd^{2+} (0.25, 0.5 and 1 mg/kg, respectively) dissolved in saline twice a week for 3 months. The control group received only saline (0.3 ml). At the end of 3 months, the urinary bladders were surgically removed and a strip of detrusor was prepared from each bladder. An atomic absorption device and the standard addition method were used to determine blood levels of Cd^{2+} and the Cd^{2+} levels of the remaining parts of each bladder. The responses of the detrusor strips were studied in organ chambers. The tissues were first treated with ACh and then with EFS. The responses were recorded by isotonic transducers. The tissue Cd^{2+} levels were significantly increased in the Cd^{2+} treated rats in a dose-dependent manner except in the 0.25 mg/kg Cd^{2+} treated group. ACh-induced contractions were significantly attenuated only in the 1 mg/kg Cd^{2+} treated rats. The contractions induced by EFS were significantly decreased in all of the Cd^{2+}-treated groups, but there were no significant differences between the groups. This study showed that Cd^{2+} exposure for 3 months impairs neurogenic and myogenic contractile activity in the rat detrusor muscle. This action seems to be at least partly due to an inhibition of the cholinergic muscarinic system. This may have clinical implications for people who are exposed to Cd^{2+}.

Keywords Cadmium · Contraction · Bladder · Electric stimulation · Acetylcholine

Y. Bayazıt (✉) · İ. A. Arıdoğan · T. Turunç
Department of Urology, Faculty of Medicine,
Çukurova University, Balcalı, 01330, Adana, Turkey
E-mail: bayazit@mail.cu.edu.tr
Tel.: +90-322-3386305
Fax: +90-322-3387087

P. Uçar Ertuğ · C. Göçmen · E. Şingirik
Department of Pharmacology, Faculty of Medicine,
Çukurova University, Balcalı, 01330, Adana, Turkey

M. Ürünsak
Department of Microbiology, Faculty of Medicine,
Çukurova University, Balcalı, 01330, Adana, Turkey

Introduction

Cadmium (Cd^{2+}) is a very toxic metal which has a long biological half-life in the human body [6]. In the general population, Cd^{2+} exposure can be mainly due to diet and smoking. Recent studies have suggested that Cd^{2+} may play a role in the pathogenesis of kidney diseases, hypertension and sexual dysfunction [1, 3, 17]. The main sites of Cd^{2+} deposition are the kidneys and the liver [12]. Animal studies have shown that Cd^{2+} can also be a potent neurotoxic agent for the peripheral nervous system [25, 26]. In a recent study it was suggested that Cd^{2+} intake may impair the neurogenic responses of the mouse corpus cavernosum to electrical field stimulation (EFS) [4].

Transmural EFS was shown to induce frequency-dependent neurogenic contractions which could be inhibited by tetrodotoxin (TTX) in detrusor muscles obtained from human and rats [15, 19]. Experimental studies have shown that this neurogenic contraction elicited by EFS has atropine-sensitive (cholinergic) and atropine-resistant (non-cholinergic) components [11, 22]. It was suggested that in the normal human bladder the contraction induced by EFS is cholinergic in nature, but in the functionally disturbed bladder part of the contraction is atropine resistant, a finding that may have clinical implications [11].

The role of neurogenic and myogenic activity of the detrusor muscle on bladder function has been well established. To our knowledge, however, there has been no attempt to investigate the effects of chronic exposure to Cd^{2+} on rat detrusor contractility. The purpose of this study was to investigate whether chronic Cd^{2+} treatment impairs neurogenic contractions induced by

EFS and myogenic contractions induced by acetylcholine (ACh) in the rat detrusor muscle.

Materials and methods

In vivo experiments

Wistar Kyoto rats weighing 150–250 g were housed separately according to their experimental groups in a room with a 12 h light–12 h dark cycle and controlled temperature (19–21°C) and humidity (45–60%). Rats were randomly divided into four groups. Each group was composed of ten animals receiving food and tap water ad libitum throughout the experiments. The first group acted as age-matched controls in which saline was intraperitoneally injected (0.3 ml) twice a week for 3 months. The other three groups received intraperitoneal Cd^{2+} (0.25, 0.5 and 1 mg/kg, respectively) dissolved in saline similarly to the control group. At the end of the 3 months the rats were anesthetised with ketamine (25 mg/kg, intraperitoneally) and bled to death after the collection of blood samples for determining Cd^{2+} levels. The urinary bladders were surgically removed and then placed in a Petri dish containing Krebs solution (NaCl mM 119, KCl 4.6 mM, $CaCl_2$ 1.5 mM, $MgCl_2$ 1.2 mM, $NaHCO_3$ 15 mM, $NaHPO_4$ 1.2 mM, glucose 11 mM). One detrusor strip (2×10 mm) was prepared from each bladder. Atomic absorption spectroscopy (Perkin Elmer, 2380, standard addition method) was used to determine the blood levels of Cd^{2+} and Cd^{2+} levels of the remaining parts of each bladder [5, 18, 24, 27].

In vitro experiments

The preparations were mounted under 0.5 g tension in 5-ml organ baths containing Krebs solution maintained at 37°C and aerated with 95% O_2 and 5% CO_2. Tissues were allowed to equilibrate for 1 h. The changes in the muscle length were recorded on polygraph paper (Ugo Basile, Gemini 7070) via isotonic transducers (Ugo Basile, 7006). After an equilibrium period, the tissues were first treated with ACh and then with EFS. ACh was cumulatively re-added to the organ bath in concentrations of 10^{-9}–10^{-3}. EFS (0.5–32 Hz, 30 V, 0.1 ms with square waves) was applied for 30 s at 2-min intervals to confirm the elicited contractions of the tissue. This was delivered by a Grass S88 stimulator via two parallel platinum electrodes embedded in Perspex. After the frequency-response curves of EFS had been recorded, the tissue was incubated with atropine (0.2 μM) for 30 min and the procedure was repeated with only 16 Hz in atropine. In the preliminary experiments, we tested the effect of muscarinic receptor blockage with atropine (0.2 μM) on the frequency-response curves of EFS, but in each experiment, we applied only 16 Hz EFS because atropine suppresses only high frequency responses and has little effect at low frequencies of EFS [2]. Following each frequency of EFS, recording was stopped and the tissue was washed with fresh Krebs solution and thus relaxed back to the baseline level. At the end of the resting period, after ACh and EFS applications were completed, the tissues were contracted with 50 mM KCl solution and the experiments were terminated.

Drugs and solutions

Stock solutions of atropine sulfate and ACh were dissolved in distilled water. Cadmium chloride was dissolved in saline solution. All of the drugs were purchased from Sigma.

Statistical considerations

The contractions were calculated as the percentage of peak contraction elicited by 50 mM KCl solution. The mean values (±SE) for each group were calculated separately. All data were evaluated with the Bonferroni-corrected *t*-test that was used in the one way analysis of variance (ANOVA). P values < 0.05 were considered to be significant.

Results

Data of weight and tissue Cd^{2+} levels in control and Cd^{2+}-treated rats

Animals injected with Cd^{2+} (0.25, 0.5 and 1 mg/kg, i.p.) experienced significant weight loss compared to age-matched controls at the end of 3 months (Table 1). There was not a significant difference between the control and Cd^{2+} treated groups in terms of blood Cd^{2+} levels, whereas the tissue Cd^{+2} levels were significantly increased in the Cd^{2+}-treated rats in a dose-dependent manner except for the 0.25 mg/kg Cd^{2+}-treated group (Table 1).

Contractions induced by ACh

ACh-induced contractions at all concentrations were significantly attenuated in only 1 mg/kg Cd^{2+}-treated rats (Fig. 1) at the end of 3 months. Treatment with 0.25 and 0.5 mg/kg Cd^{2+} did not have a significant effect on the contractions caused by ACh as compared to the control groups (Fig. 2).

Contractions induced by EFS

In all Cd^{2+}-treated groups the contractions induced by EFS at all frequencies were significantly decreased, but there was no significant difference between them (Figs. 1, 3). Atropine (0.2 μM) caused a significant decrease in the contractions elicited by EFS (16 Hz) in the control group. In the 0.25 mg/kg Cd^{2+}-treated group, atropine elicited a significant inhibition on the contractions to EFS (16 Hz) compared to the control group. However, this significant decrease was not observed in the 0.5 or the 1 mg/kg Cd^{2+}-treated groups (Table 2).

Contractions induced by KCl solution

KCl-induced contractions were not significantly different between control and Cd^{2+}-treated groups of rats (Table 3).

Table 1. Weight gain at the end of 3 months as well as blood and tissue Cd^{2+} levels of detrusor muscles. The weight gain is presented as a percentage of the initial weight. * indicates a significant difference from the control group ($P < 0.05$). ($n = 10$ for each group)

Treatment (mg/kg Cd^{2+})	Body weight gain (%)	Blood Cd^{2+} level (μg/l)	Tissue Cd^{2+} level (μg/g tissue)
Control group	148.7 ± 11.2	0.410 ± 0.09	0.363 ± 0.035
0.25	120.1 ± 9.23*	0.370 ± 0.08	0.373 ± 0.019
0.50	124.1 ± 6.35*	0.400 ± 0.04	0.457 ± 0.021*
1	119.0 ± 7.21*	0.349 ± 0.07	0.476 ± 0.013*

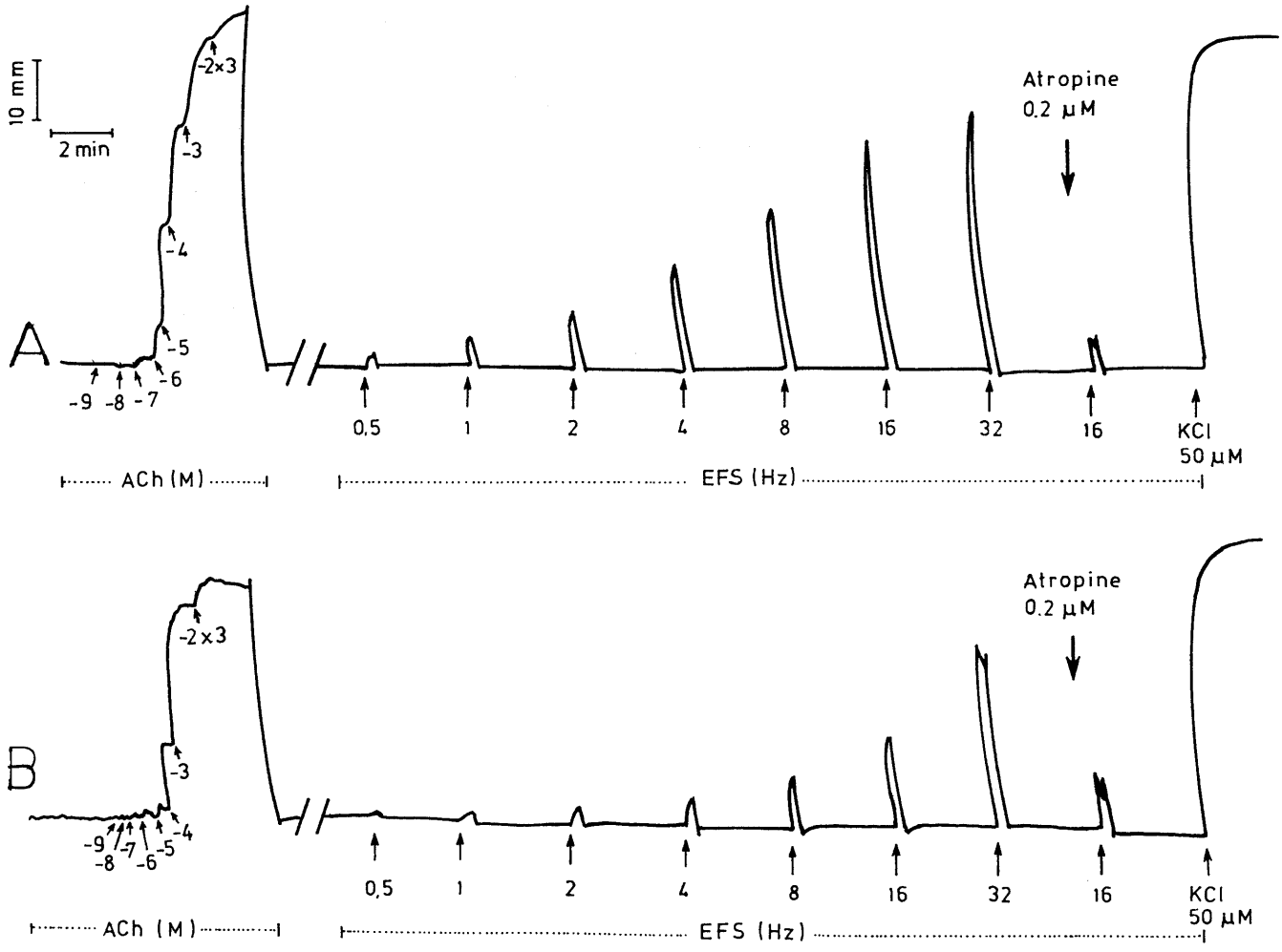

Fig. 1. Representative tracings of the contractile responses of detrusor to ACh and electric field stimulation (*EFS*) from control (**A**) and Cd^{2+} (1 mg/kg) treated (**B**) rats

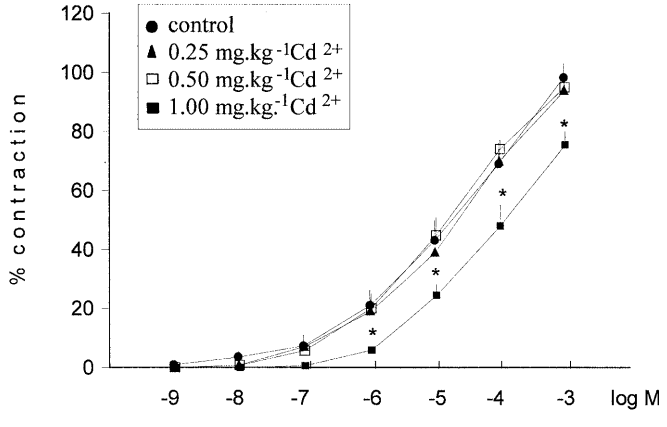

Fig. 2. The contractile responses to ACh in the rat detrusor from control and Cd^{2+}-treated rats. Each *asterisk* represents a significant difference between the control and treated group for the mean contractile response expressed as percentage of KCl-induced contraction ($P < 0.05$) ($n = 10$ for each group)

Discussion

Our study demonstrated that Cd^{2+} treatment for 3 months may cause a suppression on the neurogenic and myogenic contractions in rat detrusor muscle. The tissue Cd^{2+} levels were significantly increased in the Cd^{2+}-treated rats in a dose-dependent manner in comparison to the Cd^{2+} level of the control groups. These results indicate that Cd^{2+} toxicity may impair the derusor contractility due to Cd^{2+} accumulation in the tissue.

Cadmium toxicity can reduce body weight in rats [10]. In our study, the observation of a significant decrease in the body weight for the Cd^{2+}-treated group may indicate cadmium toxicity. In addition, no significant differences between the blood Cd^{2+} levels in the control group and the Cd^{2+}-treated groups were observed, but the tissue Cd^{2+} levels were significantly elevated in the two high-dose Cd^{2+} groups. This result is in accordance with previous studies in which Cd^{2+} accumulation was observed in various tissues such as liver, kidney and reproductive organs (testis, ovary and uterus) [14, 16]. Although the accumulation of Cd^{2+} in detrusor muscle was not mentioned in previous studies,

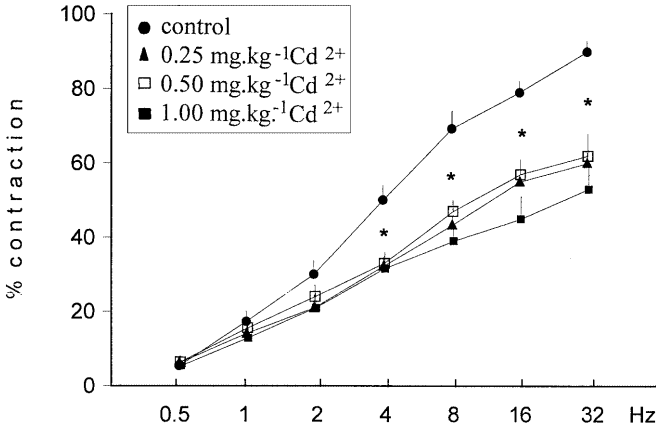

Fig. 3. The contractile responses to electric field stimulation in the rat detrusor from control and Cd^{2+} treated rats. Each *asterisk* represents a significant difference between the control and treated group for the mean contractile response expressed as percentage of KCl-induced contraction ($P < 0.05$) ($n = 10$ for each group)

Table 2. The effect of atropine-induced muscarinic receptor blockage on the contractile responses of the rat detrusor muscle to electric field stimulation (EFS) (16 Hz) in control and Cd^{2+}-treated groups. The results are presented as percentage of the contraction elicited by EFS (16 Hz) in the absence of atropine (mean ± SE). * indicates a significant difference from control group ($P < 0.05$). ($n = 10$ for each group)

Treatment (mg/kg Cd^{2+})	%
Control group	37.3 ± 7.8
0.25	53.1 ± 7.9
0.5	62.8 ± 5.0*
1	80.0 ± 12*

high tissue levels can be expected because the urine level of Cd^{2+} is significantly raised in Cd^{2+}-exposed people [13]. No correlation was observed between blood and tissue levels of Cd^{2+}. This may be due to the cumulative increase of Cd^{2+} in the tissues.

In the highest dose of the Cd^{2+}-treated group, the myogenic contractile responses induced by ACh were significantly attenuated, whereas lower doses of Cd^{2+} were ineffective. In all Cd^{2+}-treated groups, there was a significant impairment in the neurogenic contractions induced by EFS. However, there was no significant difference between these groups. In the two groups treated with high doses of Cd^{2+}, the ratio of the non-cholinergic component to the total contraction induced by EFS in the absence of atropine was higher. Therefore there may be a decrease in the cholinergic component of the EFS-induced contraction. Cadmium can also be a potent neurotoxic agent for the peripheral nervous system [25, 26] and it was possible that Cd^{2+} treatment might have inhibited the response to other types of nerves within the detrusor muscle, such as purinergic or adrenergic nerves. It has been suggested that atropine-resistant contractions evoked by EFS in normal human detrusor tissue have been caused by ATP [8]. In the present study, no significant decrease was observed in the noncholinergic component of the contraction to EFS. It was also suggested that Cd^{2+} exposure for 30 days impairs the nitrergic relaxation of mouse corpus cavernosum to EFS [4]. The finding that the lower doses of Cd^{2+} affected the neurogenic contraction but not the myogenic contraction to ACh indicate that Cd^{2+} may exhibit a partial selectivity on nerve-mediated contractile activity in low doses. However, a high dose of Cd^{2+} also affected the myogenic contraction induced by ACh. This may be partly due to an impairment of the cholinergic muscarinic system or to a toxic effect leading to damage to the smooth muscle. This toxic effect of Cd^{2+} may be due to the induction of a lipid peroxidation process and an increase in the production of reactive oxygen species leading to oxidative injury. This may play a role in the development of neurological complications [7, 9, 20, 21]. Oxidative injury may also affect the myogenic contractile mechanism. On the other hand, a high dose of KCl acts directly on the detrusor smooth muscle and depolarizes it. This is followed by contraction. Our study showed that this agent appears to have no effect on the depolarization-inducing contraction in rats treated with Cd^{2+}, since differences in the contractile effect of KCl between control and Cd^{2+}-treated groups were statistically insignificant. In a recent study, Cd^{2+} treatment did not cause any significant action on the contractions elicited by a high dose of KCl in rat mesenteric vessels [23].

Conclusion

This study showed that Cd^{2+} exposure for 3 months impairs neurogenic and myogenic contractile activity in the rat detrusor muscle. This action seems to be due, at least in part, to an inhibition of the cholinergic muscarinic system which may have clinical implications in people who are exposed to Cd^{2+}. However, further studies are needed to show the responses to other neurotransmitters or agonists in order to determine whether Cd^{2+} may be selectively active on the nerves or smooth muscles.

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Table 3. The contractile effects of KCl solution (50 mM) on the rat detrusor muscle obtained from control and Cd^{2+}-treated groups ($n = 10$ for each group)

	Control	0.25 mg/kg Cd^{2+}	0.5 mg/kg Cd^{2+}	1 mg/kg Cd^{2+}
KCl-induced contraction (mm)	36.1 ± 4	34.7 ± 5	35.28 ± 6	33.7 ± 4.3

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