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Effect of Central Acute Administration of Cadmium on Drinking Behavior

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DE CASTRO E SILVA, E., H. FERREIRA, M. CUNHA, C. BULCÃO, C. SARMENTO, I. DE OLIVEIRA AND J. B. FREGONEZE. *Effect of central acute administration of cadmium on drinking behavior.* PHARMACOL BIOCHEM BEHAV 53(3) 687-693, 1996.—The effect of acute third ventricle cadmium administration on the drinking behavior of adult male rats under different situations was studied. Injections of cadmium chloride (0.07, 0.7, and 7.0 ng/rat) significantly attenuated water intake in dehydrated rats. Drinking behavior induced by acute intracerebroventricular injections of carbachol (2 μg/rat) or angiotensin II (5 ng/rat) was also inhibited by central cadmium injections. Cadmium-induced blockade in water intake in dehydrated animals was reverted by the previous administration of a 5-HT_2 antagonist (RP62203) in different doses (5 and 10 μg/rat). The data clearly reveal that cadmium elicits very fast actions on the central nervous system. It is suggested that cadmium-induced attenuation of water intake may rely on at least three different mechanisms: impairment of cholinergic and angiotensinergic systems in the brain and stimulation of a central serotonergic drive acting on 5-HT_2 receptors. The study of cadmium neurotoxicity by observation of drinking behavior, a behavioral parameter easy to be recorded and measured, is proposed.

Cadmium Cadmium intoxication Cadmium neurotoxicity Drinking behavior Brain angiotensin Brain cholinergic systems Brain serotonergic systems

CADMIUM, a trace element extremely diffused in the biosphere, seems to be devoid of biological functions in mammals (38). Together with lead and aluminum, cadmium is considered a nonphysiological metal potentially toxic to man. After low-level exposure, cadmium accumulates in the kidneys, liver, adrenal glands, and central nervous system (32,23). Indeed, signs of cadmium toxicity on multiple organs and systems in man have been well documented. Pathological processes like renal dysfunction, testicular tumors, hypertension, and growth inhibition may be associated with cadmium intoxication (11).

The brain is a target for cadmium toxicity. Behavioral disorders and central nervous system (CNS) biochemical dysfunction have been observed in cadmium-exposed laboratory animals (16). Heavy metals like cadmium may alter intracellular calcium metabolism and impair calcium functions as a second messenger in the CNS. Furthermore, it was recently demonstrated that intracerebroventricular (ICV) injections of cadmium in rats, promotes an acute hypertensive response that depends on the action of the metal on brain calcium channels (24).

Disruption of the functional integrity of several neurotransmitters in the CNS has been observed in the course of cadmium intoxication. Catecholaminergic and serotonergic transmission in the brain may be altered by cadmium (34). Cadmium is also able to affect cholinergic transmission because it modifies acetylcholine release (42,6). The central cholinergic systems participate in the control of drinking behavior, and stimulation of cholinergic pathways in the hypothalamus generates a strong dipsogenic effect in rats (1,22). Still, drinking behavior induced by angiotensin II (AII) depends, at least partially, on the function of brain cholinergic pathways (4,39). On the other hand, it has been shown that brain serotonin may exert an inhibitory effect on water intake (30,48) in laboratory animals.

In the present study we analyzed the effects of third ventricle injections of minute amounts of cadmium on water intake induced by three different situations: dehydration, and central

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cholinergic and angiotensinergic stimulation in rats. We also investigated if cadmium-induced alterations in water intake after dehydration could involve brain serotoninergic participation.

METHOD

Animals

In this study we used Wistar male rats (220–250 g) kept under controlled light (lights on from 0600 to 2000 h) and temperature (22–24°C). They had free access to tap water and laboratory chow (Nuvital Nutrientes Ltd., Brazil).

Surgical Procedure

After an overnight fast, the animals were anesthetized with Nembutal (sodium pentobarbital, 40 mg/kg, IP) for stereotaxic cannulation of the third ventricle (1). A 22 gauge stainless steel cannula (15 mm in length) was stereotaxically implanted into the third ventricle (anteroposterior = 0.5 mm behind bregma; lateral = just on the midline and vertical = 8.5 mm below the skull). The cannulas were provided with a mandrill (28 gauge) to prevent its obstruction. Two screws embedded in the skull bone and cemented with dental acrylic firmly anchored the cannulas. After the experiments a small amount of blue Evans dye was injected through the cannula to determine its position in the brain. Only animals whose cannulas were correctly placed in the third ventricle were taken in consideration.

Drugs and Microinjections

The following drugs (purchased from Sigma Co., St. Louis, MO) were used: carbachol, angiotensin II (Asp^1-$Ileu^5$-AII), cadmium chloride ($CdCl_2$) and sodium chloride (NaCl). RP62203, a 5-HT_2 receptor antagonist, (Rhône Poulenc, France) was also used. All drugs were dissolved in saline solution (0.9% NaCl). A volume of 2 μl was injected with a Hamilton microsyringe (10 μl) connected to a Mizzy-Slide-Pak needle (12 × 27 gauge) through polyethylene tubing over a period of 30–60 s. This needle was 1 mm longer than the guide cannula. During injections the syringe was held by a clamp connected to a metal support put outside the cage. The injector was left at least 30 s after each compound was infused.

Experimental Design

We studied the acute effects of $CdCl_2$ ICV injections on drinking behavior in four distinct sets of rats: a) dehydrated; b) carbachol-treated; c) angiotensin II-treated; and d) dehydrated treated with RP62203, a 5-HT_2 antagonist. Dehydration was achieved after an overnight period (14 h) of water deprivation. The groups treated with carbachol or AII were normohydrated. All experiments were carried out between 0800 and 1200 h. At least 12 animals were used in each experimental group. All experimental sets used naive animals.

In the first experimental set we studied the effect of ICV $CdCl_2$ on the drinking behavior of four different groups of dehydrated rats. Three of these groups received a particular dose of $CdCl_2$ (0.07, 0.70, and 7.0 ng/rat) each one being compared to a control group taking NaCl instead of $CdCl_2$. Graduated water bottles were put in the cages immediately after the ICV injections and the cumulative water intake was recorded by the next 60 min.

In the second experimental set we investigated the effect of $CdCl_2$ on carbachol-induced drinking behavior in normohydrated animals. Three different groups of rats received ICV injections of $CdCl_2$ in the same doses employed in the first group (0.07, 0.70, and 7.0 ng/rat) and after 45 min carbachol (2 μg/rat) by the same route. They were compared to a fourth group (controls) receiving ICV NaCl instead of $CdCl_2$. The cumulative water intake record, from graduated bottles already present in the cages, began immediately after carbachol injections and lasted for 60 min.

With the third experimental set the effect of $CdCl_2$ on AII-induced water intake was explored. These animals were also normohydrated. Here, three different groups of animals received $CdCl_2$ in the same doses employed in the first group (0.07, 0.7, and 7.0 ng/rat) and after 45 min AII (5.0 ng/rat) by the same route. They were compared to control animals receiving ICV NaCl instead of $CdCl_2$. Water intake was recorded exactly as in the former experiment.

The fourth experimental set was designed to evaluate the possible involvement of brain 5-HT_2 receptors in the cadmium-induced alterations in drinking behavior in dehydrated rats. Here, we pretreated dehydrated rats with RP62203, a selective 5-HT_2 receptor antagonist, 45 min before the intracerebroventricular injection of 0.7 ng/rat of $CdCl_2$. This compound was used in two different doses, 5.0 and 10 μg/rat.

In the fifth experimental set we treated normohydrated and dehydrated animals RP62203 alone, to verify its effect on water intake in the absence of cadmium. Here we used RP 62203 in a dose of 10 μg/rat.

Statistical Analysis

We used a computer software (GBSTAT, Dynamic Microsystems Inc., Silver Spring, MD) that performs a repeated measures ANOVA applied to each experimental data set, taking drug (A) and time (B) as factors, and subsequently submit the data to the Tukey's test for comparison of each dose of drug with its correspondent time in the control groups. The groups were considered significantly different when $*p < 0.05$ and $**p < 0.01$. All p-values, for each comparison, in each experimental set, are indicated in the figures. The number of animals used are indicated in the legends of the figures and in Table 1. The cumulative water intake was calculated as ml/100 g of body weight and expressed as means ± SEM.

RESULTS

Figure 1 depicts the effect of three different doses of $CdCl_2$ on the drinking behavior of dehydrated rats as compared to dehydrated saline-treated controls. In a dose-dependent fashion, $CdCl_2$ significantly attenuated water intake in this group. In the lowest dose employed (0.07 ng/rat) the inhibitory effect of $CdCl_2$ was small and present only after 45 min. In the highest dose injected (7.0 ng/rat), the inhibitory effect was prompt lasting for the entire duration of the experiment.

The results of the second experimental set are shown in Fig. 2. Here, we studied the effect of $CdCl_2$ or saline on carbachol-induced drinking behavior in normohydrated animals. As expected, saline-treated animals receiving carbachol (2 μg/rat) presented a highly significant increase in water intake as compared to saline-treated animals not receiving carbachol. In the lowest dose employed (0.07 ng/rat), $CdCl_2$ did not modify the dipsogenic effect of carbachol. However, doses of 0.7 and 7.0 ng/rat significantly blocked carbachol-induced water intake. This blockade was evident 30 min after carbachol injection. Even if the inhibitory effect of the highest dose of $CdCl_2$ (7.0 ng/rat) tended to elicit a higher degree of reversion of cadmium blockade in water intake than that observed with

TABLE 1

CUMULATIVE WATER INTAKE (ml/100 g b.wt.) IN NORMOHYDRATED AND DEHYDRATED ANIMALS (14 h OF WATER DEPRIVATION) AFTER THIRD VENTRICULAR INJECTIONS OF SALINE ALONE AND RP62203 10 μg/RAT

	Normohydrated		Dehydrated	
Drug	30 (min)	60 (min)	30 (min)	60 (min)
Saline	0.00 ± 0.00 (n = 12)	0.10 ± 0.07 (n = 12)	4.55 ± 0.45 (n = 13)	5.76 ± 0.69 (n = 13)
RP62203 (10 μg/2 μl)	0.09 ± 0.06 (n = 14)	0.21 ± 0.09 (n = 14)	4.96 ± 0.58 (n = 12)	6.06 ± 0.33 (n = 12)

Data are presented as mean ± SEM. After a repeated measures ANOVA followed by Tukey's test no statistical significant difference was observed among all groups.

the intermediate dose employed (0.7 ng/rat), a statistically significant difference between these two doses was not demonstrated.

Figure 3 condenses the results of the third experimental set where the effect of $CdCl_2$ or saline on water intake induced by third ventricle AII injections (5.0 ng/rat) was studied. The dipsogenic effect of AII on saline-treated rats is evident. Indeed, saline-treated animals receiving AII presented a highly significant increase in water intake as compared to saline-treated animals not receiving the peptide. In the smallest dose used $CdCl_2$ failed to alter AII-induced water intake. In the other doses (0.7 and 7.0 ng/rat) $CdCl_2$ yielded a significant impairment in the drinking behavior elicited by AII. In this case, the blockade was evident 15 min after AII injection. However, in this case, the blockade lasted for only 45 min (in the dose of 7.0 ng/rat) or 30 min (in the dose of 0.7 ng/rat).

In the fourth experimental set (Fig. 4), dehydrated rats were pretreated with RP62203, a selective 5-HT_2 antagonist, in two different doses (5.0 and 10.0 μg/rat) 45 min before $CdCl_2$ injections. Graduated bottles were available as in the previous experimental set. The 5-HT_2 antagonist was able to revert cadmium-induced blockade in water intake in dehydrated rats. The highest dose employed (10 μg/rat) tended to be greater than the lowest (5 μg/rat), yet a significant difference was not observed.

As observed in Table 1, the administration of RP62203 (10

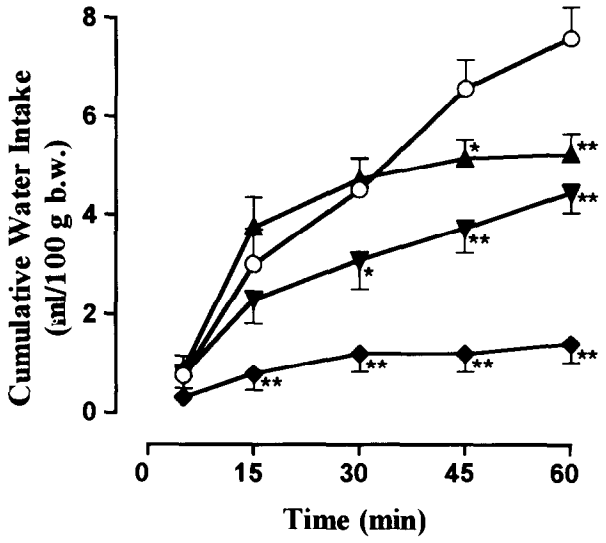

FIG. 1. Cumulative water intake (ml/100 g b.wt.) in dehydrated animals (14 h of water deprivation) after third ventricular injections of several doses of $CdCl_2$. Saline-treated controls, n = 12 (○); $CdCl_2$ 0.07 ng/rat, n = 12 (▲); $CdCl_2$ 0.7 ng/rat, n = 13 (▼); $CdCl_2$ 7.0 ng/rat, n = 13 (♦). Data are presented as mean ± SEM. Repeated measures ANOVA—Factor A (drug), $F(3) = 29.3307$, $p < 0.0001$; Factor B (time), $F(4) = 205.3323$, $p < 0.0001$; Factor A × Factor B, $F(12) = 26.4817$, $p < 0.0001$. Asterisks indicate a statistical significant difference (Tukey's test $*p < 0.05$; $**p < 0.01$) when $CdCl_2$-treated groups are compared to saline-treated controls.

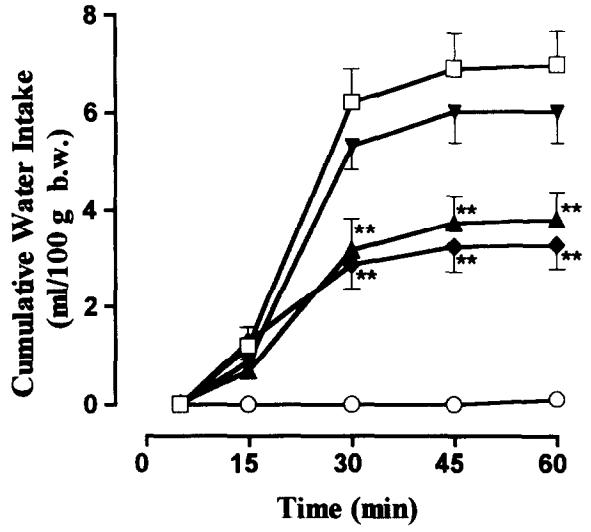

FIG. 2. Cumulative water intake (ml/100 g b.wt.) in normohydrated animals receiving third ventricular injections of saline alone, n = 12 (○); saline + carbachol 4 μg/rat, n = 14 (□); $CdCl_2$ (0.07 ng/rat) + carbachol 4 μg/rat, n = 14 (▼); $CdCl_2$ (0.7 ng/rat) + carbachol 4 μg/rat, n = 12 (▲); $CdCl_2$ (7.0 ng/rat) + carbachol 4 μg/rat, n = 12 (♦). $CdCl_2$ injections were done 45 min before carbachol administration. Data are presented as mean ± SEM. Repeated measures ANOVA—Factor A (drug), $F(4) = 44.2246$, $p < 0.0001$; Factor B (time), $F(4) = 313.0129$, $p < 0.0001$; Factor A × Factor B, $F(16) = 25.3137$, $p < 0.0001$. All groups receiving carbachol are statistically different from the group receiving saline alone. Asterisks indicate a statistical significant difference (Tukey's test $**p < 0.01$) among $CdCl_2$ + carbachol groups and saline + carbachol controls.

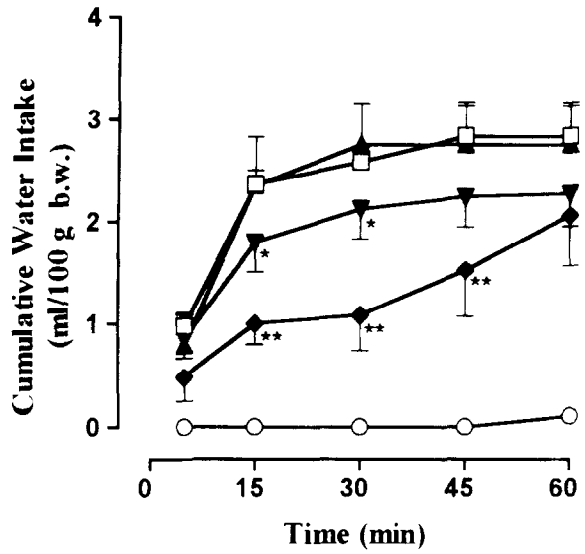

FIG. 3. Cumulative water intake (ml/100 g b.wt.) in normohydrated animals receiving third ventricular injections of saline alone, $n = 13$ (○); saline + AII 5 ng/rat, $n = 14$ (□); $CdCl_2$ (0.07 ng/rat) + AII 5 ng/rat, $n = 13$ (▲); $CdCl_2$ (0.7 ng/rat) + AII 5 ng/rat, $n = 12$ (▼); $CdCl_2$ (7.0 ng/rat) + AII 5 ng/rat, $n = 13$ (♦). $CdCl_2$ injections were done 45 min before AII administration. Data are presented as mean ± SEM. Repeated measures ANOVA—Factor A (drug), $F(4) = 98.7982$, $p < 0.0001$; Factor B (time) $F(4) = 1454.995$, $p < 0.0001$; Factor A × Factor B, $F(16) = 138.5605$, $p < 0.0001$. All groups receiving AII are statistically different from the group receiving saline alone. Asterisks indicate a statistical significant difference (Tukey's test $*p < 0.05$; $**p < 0.01$) among $CdCl_2$ + AII groups and saline + AII controls.

μg/rat) to normohydrated and dehydrated rats was unable to modify water intake as compared to saline-treated controls.

DISCUSSION

The results here obtained clearly demonstrate that third ventricle injections of minute amounts of $CdCl_2$ impair the dipsogenic drive induced by dehydration or central cholinergic and angiotensinergic stimulation in rats. It is also shown that the blockade of water intake in dehydrated rats receiving $CdCl_2$ may be reverted by the previous administration of a 5-HT_2 receptor antagonist.

Industrial procedures and cigarette smoking are the main sources of cadmium in the human environment. Cadmium neurotoxicity has been largely demonstrated. This metal, whose biological half-life is extremely long, accumulates in the CNS following administration by different routes (10). Cadmium may reach the CNS by retrograde axonal transport via hypoglossal (3) and olfactory nerve (17,18) or by crossing the blood-brain barrier using passive or facilitate diffusion mechanisms (19). Divalent metals such as cadmium are bound to plasma proteins and internalized into neurons as metal-protein complexes (2). It is well known that cadmium deposition in the CNS may disrupt different brain physiological mechanisms like blood pressure control (24) and olfactory function (35).

Thirst is the result of multiple signals coming from peripheral and central sites, analyzed, and integrated by different brain structures. At this level, both aminergic and peptidergic pathways cooperate to control drinking behavior (29). In previous articles we demonstrated that central injection of lead or zinc attenuates water intake induced by several procedures (13,14). So, the rational basis of the present article was to investigate if cadmium, another divalent heavy metal potentially toxic to man, could influence water intake induced by physiological and pharmacological stimuli. The results here obtained strongly suggest that cadmium is able to disrupt brain mechanisms controlling thirst.

Central cholinergic induction of water intake is a well-established phenomenon. Indeed, cholinergic activation may be a necessary physiological step in thirst generation (1). Stimulation of the subfornical organ, a circumventricular structure related to hydrosaline control mechanisms, yields a strong dipsogenic response (26) that seems to depend on cholinergic muscarinic activation (36). Cadmium seems to impair peripheral and central acetylcholine transmission. It has been demonstrated that this metal reduces acetylcholine release in rat brain synaptosomes (6,42) as well as at the frog neuromuscular junction (7). Furthermore, it was recently demonstrated that cadmium reduces acetylcholine concentrations in several brain areas, including the hypothalamus, cerebellum, medulla, hippocampus, and midbrain. This effect is more conspicuous in animals submitted to protein restricted diets (8). It is also known that cadmium could impair choline uptake by the brain (21).

In the present article, central cholinergic stimulation by carbachol, a muscarinic agonist, generated a significant increase in water intake saline-treated normohydrated animals. This represents a classical and well-known response. Administration of $CdCl_2$, 45 min before carbachol injection, signifi-

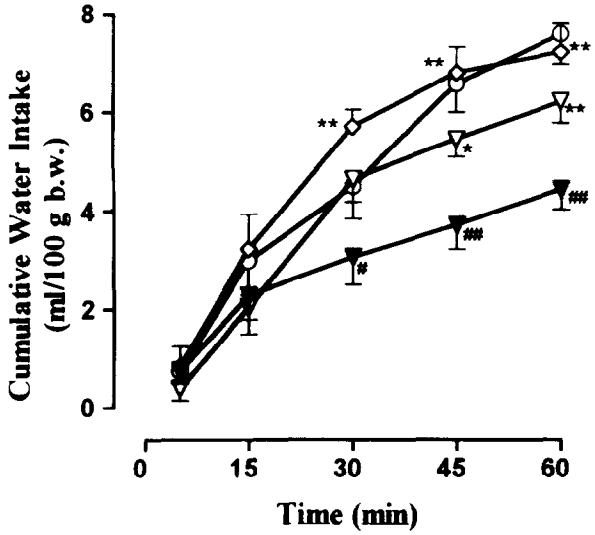

FIG. 4. Cumulative water intake (ml/100 g b.wt.) in dehydrated animals (14 h of water deprivation) after third ventricular injections of saline alone, $n = 12$ (○); saline + $CdCl_2$ 0.7 ng/rat, $n = 13$ (▼); RP62203 5 μg/rat + $CdCl_2$ 0.7 ng/rat, $n = 14$ (▽); RP62203 10 μg/rat + $CdCl_2$ 0.7 ng/rat, $n = 12$ (◇). Data are presented as mean ± SEM. Repeated measures ANOVA—Factor A (drug), $F(4) = 18.2900$, $p < 0.0001$; Factor B (time), $F(4) = 468.2831$, $p < 0.0001$; Factor A × Factor B, $F(16) = 11.81724$, $p < 0.0001$. Asterisks indicate a statistical significant difference (Tukey's test $*p < 0.05$; $**p < 0.01$) among RP62203 + $CdCl_2$ and saline + $CdCl_2$ groups. # Indicates statistical significant difference between saline + saline and saline + $CdCl_2$ group (Tukey's test $\#p < 0.05$; $\#\#p < 0.01$).

cantly blunted the dipsogenic effect of this cholinergic agonist. Thus, it is reasonable to infer that cadmium administration impairs thirst-triggering cholinergic pathways in the brain. The amounts of cadmium injected tried to mimic the brain tissue levels of the metal found in experimental intoxication protocols. As seen in Fig. 2, cadmium-treated animals receiving carbachol exhibit a cumulative water intake that is still higher than those shown by their drug-free controls. So, even in the highest dose used here, cadmium was not able to produce a total blockade in cholinergic-induced mechanisms generating thirst. This probably means that at this concentration cadmium general biochemical derangement affects cholinergic mechanisms activated by carbachol injections. However, we decided not to use doses higher than 7.0 ng/rat just to work within the limits found in human intoxication (15,27).

AII plays an important role as one of the many neurotransmitter systems that cooperate in the mechanisms of thirst generation. This peptide acts on sites located at the circumventricular vicinity to yield a powerful dipsogenic effect (37,44). AII coming from the peripheral blood or from central sources elicits powerful thirst (49). Interestingly, angiotensin-induced water intake may depend on the function of cholinergic pathways in the central nervous system. Indeed, atropine, blocking cholinergic transmission in the brain, impairs drinking behavior observed after AII administration (40). This explains the rational basis of our third experimental set, when we studied the effect of $CdCl_2$ on AII-induced water intake in normohydrated rats. As expected, the group of normohydrated rats pretreated with saline and receiving central injections of a very low dose (5 μg/rat) of AII (controls) exhibit a significantly higher water intake when compared to saline-treated controls. Conversely, $CdCl_2$ injections 45 min before AII, significantly attenuated the dipsogenic effect induced by this peptide. Again, $CdCl_2$ generates only a partial blockade, because animals treated with the metal still display water intake values higher than those observed with drug-free saline-treated controls. It seems that, again, in the concentrations used, cadmium is able to interrupt partially the mechanisms triggered by acute AII injections. Again, general biochemical effects of heavy metal on central nervous system may explain cadmium actions on angiotensinergic transmission observed here. As observed in the results, cadmium blockade of water intake was more delayed in dehydrated and carbachol-treated animals as compared to those treated with AII. Bearing in mind that AII is the most powerful dipsogenic agent, it is rational to suggest that AII induces thirst-triggering brain mechanisms more resistant to cadmium effects.

Locally expressed renin-angiotensin systems seem to regulate individual tissue functions. It has been clearly demonstrated that, in the brain, a local renin-angiotensin system operates. It depends on local AII formation and exerts physiological roles in fluid homeostasis and cardiovascular control (33). Also, blood-borne AII may induce thirst acting on central structures lacking a blood–brain barrier. As far as we known, there are no data associating AII actions in the brain and cadmium neurotoxicity. However, it was recently shown that, in peripheral systems, binding of endothelin I to its receptor was strongly inhibited by cadmium (47). So, it would be interesting to investigate, in future studies, if cadmium inhibition of AII binding to its receptor could be a mechanism explaining how this metal blocks water intake following administration of this peptide.

Several evidences indicate that central serotonergic pathways seem to inhibit water intake. Some studies indicated that serotonergic raphe lesions (45,46), as well as chemical destruction of serotonergic pathways by the neurotoxic agent *p*-chlorophenylalanine (41), increase water intake. In addition, fenfluramine, a serotonin uptake blocker with powerful serotonin releasing effects, inhibits water intake (31).

Data concerning cadmium actions on central serotonin transmission are scanty. In a recent study, cadmium treatment produced a significant reduction in serotonin content in several brain areas in both normal and protein restricted rats (8). This may indicate that cadmium induces serotonin release (reducing intracellular stores in the central nervous system) or, conversely, inhibits its synthesis. We decided to investigate if the central administration of a 5-HT_2 receptor antagonist would modify cadmium-induced impairment of water intake in dehydrated animals.

RP62203 is a selective and potent 5-HT_2 receptor antagonist (9,25). When administered to dehydrated rats 45 min before cadmium injections it reverted, in a dose-dependent fashion, the blockade in drinking behavior elicited by the metal. It is rational to suggest that, adjoining those effects on cholinergic and angiotensinergic systems, cadmium may inhibit water intake in dehydrated rats by enhancing serotonergic transmission, and that 5-HT_2 receptors family is involved with this response. RP62203 in the highest dose employed to revert cadmium inhibition of water intake was unable to modify drinking behavior in normohydrated and dehydrated animals. So, it is rational to propose cadmium effects are at least in part due to an enhancement of brain serotonergic function via 5-HT_2 receptor activation. It is also reasonable to suggest that 5-HT_2 receptors is not normally involved in water intake control in normal (cadmium-free) animals.

We recently demonstrated, using a very similar experimental protocol, that acute central lead injections produce very similar results, reducing water intake in dehydrated and cholinergic and angiotensinergic stimulated rats (13). So, it seems conceivable that lead and cadmium may have a common site of action in the central nervous system.

The extremely acute effects of cadmium observed here are probably due to very fast biochemical effects in the CNS. These rapid biochemical effects may be linked, at least in part, to a cadmium-induced disarrangement of calcium-mediated cellular processes (20). Cadmium may bind to calmodulin (5,43) generating acute derangement of normal biological responses (12,28). Indeed, it has been recently demonstrated, in rats, that injections of cadmium into the lateral ventricles produce a rapid hypertensive response that seems to be mediated by calcium channels (24). Evidently, cadmium actions in the several neurotransmitter pathways studied here may be just the result of biochemical effects shared by other heavy metals.

In summary, the present article shows that third ventricle injections of minute amounts of cadmium attenuates water intake in three different situations: dehydration, and cholinergic and angiotensinergic stimulation. Also, it seems that cadmium-induced reduction in water intake in dehydrated animals may involve stimulation of central serotonergic pathways acting on 5-HT_2 receptors. It is important to note that the present work represents an extremely simple bioassay to study acute heavy metal effects on the central nervous system by the observation of a behavioral parameter (water intake) very easy to be recorded and measured.

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