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Lead (Pb^{2+}) and cadmium (Cd^{2+}) inhibit the dipsogenic action of central beta-adrenergic stimulation by isoproterenol

J.B. Fregoneze[1], C.A. Marinho[2], T. Soares[1], L. Castro[1], C. Sarmento[2], M. Cunha[2], V. Gonzalez[2], P. Oliveira[1], T. Nascimento[2], C.P. Luz[1], P. Santana Jr.[2], I.R. De-Oliveira[2] and E. De-Castro-e-Silva[2]

[1]Departamento de Zoologia, Instituto de Biologia, Universidade Federal da Bahia, 40170-110 Salvador, BA, Brasil
[2]Departamento de Fisiologia, Instituto de Ciências da Saúde, Universidade Federal da Bahia, 40110-100 Salvador, BA, Brasil

Correspondence
J.B. Fregoneze
Departamento de Zoologia
Instituto de Biologia
Universidade Federal da Bahia
40170-110 Salvador, BA
Brasil

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Abstract

We have previously demonstrated that acute third ventricle injections of both Pb^{2+} and Cd^{2+} impair the dipsogenic response elicited by three different situations: dehydration and central cholinergic or angiotensinergic stimulation. ß-Adrenergic activation is part of the multifactorial integrated systems operating in drinking behavior control in the central nervous system. In the present study acute third ventricle injections of Pb^{2+} (3, 30 and 300 pmol/rat) or Cd^{2+} (0.3, 3 and 30 pmol/rat) blocked the dipsogenic response induced by third ventricle injections of isoproterenol (ISO; 160 nmol/rat) in a dose-dependent manner. Normohydrated animals receiving ISO + NaAc (sodium acetate) or saline (controls) displayed a high water intake after 120 min (ISO + saline = 5.78 ± 0.54 ml/100 g; ISO + NaAc = 6.00 ± 0.6 ml/100 g). After the same period, animals receiving ISO but pretreated with PbAc at the highest dose employed (300 pmol/rat) drank 0.78 ± 0.23 ml/100 g while those receiving ISO and pretreated with the highest dose of $CdCl_2$ (30 pmol/rat) presented a water intake of 0.7 ± 0.30 ml/100 g. Third ventricle injections of $CdCl_2$ (3 nmol/rat) or PbAc (3 nmol/rat) did not modify food intake in rats deprived of food for 24 h. Thus, general central nervous system depression explaining the antidipsogenic action of the metals can be safely excluded. It is concluded that both Pb^{2+} and Cd^{2+} inhibit water intake induced by central ß-adrenergic stimulation.

Key words
- Lead
- Cadmium
- Water intake
- Isoproterenol

Human exposure to heavy metals such as cadmium (Cd^{2+}) and lead (Pb^{2+}) may bring about several adverse reactions. Neurotoxicity induced by heavy metals is well-documented in humans and experimental animals (1). Both Cd^{2+} and Pb^{2+} reach the central nervous system either by crossing the blood-brain barrier or via retrograde axonal transport (2,3).

The presence of heavy metals in the central nervous system disrupts the functional integrity of several neurotransmitter pathways. Many independent reports confirm alterations in brain monoaminergic receptors and uptake after Pb^{2+} (4) and Cd^{2+} (5,6) intoxication.

We have recently used a new approach to study the acute actions of heavy metals on

the central nervous system by analyzing the effects of third ventricle injections of very small amounts of Cd^{2+} or Pb^{2+} on water intake, a very easy and simple parameter to measure in rats. Using this methodology we demonstrated that acute intracerebroventricular (*icv*) injections of lead acetate (7) or cadmium chloride (8) block the dipsogenic response induced by three different situations, i.e., water deprivation and central angiotensinergic or cholinergic stimulation, in a dose-dependent manner. In the particular case of Cd^{2+}, its antidipsogenic effect seems to be also due to a serotonergic activation of central 5-HT_2 receptors (8).

Brain control of thirst is achieved by a complex integrated network of neurotransmitters acting in concert. It is generally accepted that central catecholaminergic pathways exert a dual role on drinking behavior regulation, since α-adrenergic stimulation decreases (9) and ß-adrenergic (10,11) stimulation enhances water intake. However, more detailed studies have suggested that α-adrenergic stimulation may yield dipsogenic or antidipsogenic responses depending on the area studied and the doses employed.

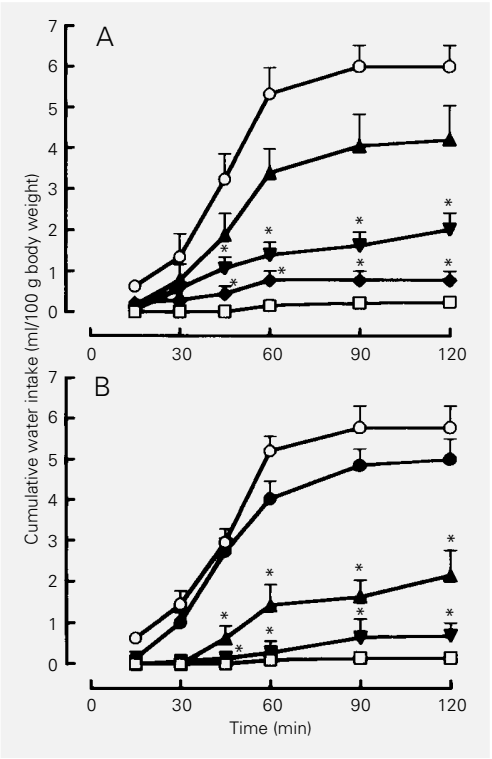

Figure 1 - Effect of PbAc and $CdCl_2$ on water intake induced by third ventricle ISO injections. Data are reported as cumulative water intake (ml/100 g body weight). *Panel A*, Water intake in animals receiving NaAc (300 pmol/rat) + saline (□); NaAc (300 pmol/rat) + ISO (160 nmol/rat) (○); PbAc (3 pmol/rat) + ISO (160 nmol/rat) (▲); PbAc (30 pmol/rat) + ISO (160 nmol/rat) (▼); PbAc (300 pmol/rat) + ISO (160 nmol/rat) (◆). Data are reported as mean ± SEM for 12 animals in each group. *P<0.01 compared to the respective NaAc + ISO controls (repeated measures ANOVA followed by the Scheffé test). *Panel B*, Water intake in animals receiving saline + saline (□); saline + ISO (160 nmol/rat) (○); $CdCl_2$ (0.3 pmol/rat) + ISO (160 nmol/rat) (●); $CdCl_2$ (3.0 pmol/rat) + ISO (160 nmol/rat) (▲); $CdCl_2$ (30.0 pmol/rat) + ISO (160 nmol/rat) (▼). Data are reported as mean ± SEM for 12 animals in each group. *P<0.01 compared to the respective saline + ISO controls (repeated measures ANOVA followed by the Scheffé test).

The aim of the present study was to determine if the dipsogenic response elicited by central ß-adrenergic stimulation by isoproterenol (ISO) could be blocked by acute *icv* injections of cadmium chloride or lead acetate.

Adult male Wistar rats kept under controlled light and temperature conditions (lights on from 6:00 to 20:00 h, 26 ± 2ºC) were used in the experiments. The animals were implanted with a cannula into the third ventricle as described elsewhere (12) 5 days before the experimental sessions. The following substances were used: lead acetate (PbAc), cadmium chloride ($CdCl_2$) and isoproterenol (Sigma Chemical Co., St. Louis, MO). All substances were dissolved in saline solution. Microinjections were made using a 10-µl Hamilton syringe connected to a Mizzy-Slide-Pak needle (12 x 27 gauge) through a polyethylene extension (PE10). The volume injected was 2 µl during a period of 90 s. The experiments were always performed between 8:00 and 11:00 a.m. After sacrifice a third ventricle injection of a vital dye was used for the macroscopic localization of the cannula. Data from only those animals whose cannulae were correctly placed into the third ventricle were considered.

Normohydrated animals received third ventricle injections of ISO at a dose of 160 nmol/rat or saline. To study the effects of the heavy metals on isoproterenol-induced dipsogenic response, distinct groups of animals were pretreated with $CdCl_2$ (0.3, 3.0 and 30.0 pmol/rat) or PbAc (3, 30 and 300 pmol/rat) 45 min before ISO injections. Control animals compared to PbAc-treated groups received *icv* injections of NaAc, whereas control animals compared to $CdCl_2$-treated groups received *icv* injections of saline. We have previously demonstrated no significant alterations in water intake after *icv* injections

of saline or NaAc.

To test if the antidipsogenic effect of Cd^{2+} and Pb^{2+} were not simply due to a general central nervous system depression, we briefly determined the effect of these metals on food intake. After 24 h of food deprivation, the animals received *icv* injections of $CdCl_2$ (control group receiving saline) or PbAc (control group receiving NaAc). Immediately after the injections, the animals were allowed to enter the food compartment within the metabolic cage. The food consumption of a powder chow was monitored during the same time intervals as used to record water intake. The amount of chow dispersed on the floor of the cage was taken into account both in control and experimental animals.

We used a computer software (GBSTAT, Dynamic Microsystems Inc., Silver Spring, MD) that performs the repeated measures analysis of variance (ANOVA) followed by the Scheffé test. Differences were considered to be significant when $P<0.01$. The cumulative water intake is reported as ml/100 g body weight (mean ± SEM).

As shown in Figure 1 (panel A), normohydrated animals receiving NaAc exhibited a very low water intake while those receiving NaAc 45 min prior to ISO (160 nmol/rat) exhibited a significant increase in drinking behavior. Pretreatment with PbAc (3, 30 and 300 pmol/rat) reduced the dipsogenic effect of ISO injections in a dose-dependent fashion.

Figure 1 (panel B) shows the result of $CdCl_2$ injections on water intake induced by *icv* ISO injections. As in the previous experiment, normohydrated rats treated with saline drank very small amounts of water. ISO (160 nmol/rat) induced a powerful increase in drinking behavior. The central administration of $CdCl_2$ (0.3, 3.0 and 30 pmol/rat) reduced the dipsogenic response elicited by ISO injections in a dose-dependent manner.

Table 1 summarizes food intake data for fasted (24 h) animals treated with $CdCl_2$ (3 nmol/rat; controls receiving saline) or PbAc (3 nmol/rat; controls receiving NaAc). There were no significant statistical differences between groups treated with the metals and their respective controls.

Table 1 - Effect of $CdCl_2$ (3 nmol/rat) and PbAc (3 nmol/rat) on food intake by food-deprived (24 h) rats compared to control groups receiving either NaAc or saline.

Data are reported as mean ± SEM for 10 animals in each group. Statistical analysis showed no significant differences among groups.

Time	Cumulative food intake (g)			
	Saline	$CdCl_2$	NaAc	PbAc
15 min	1.86 ± 0.16	1.75 ± 0.24	1.52 ± 0.19	2.65 ± 0.24
30 min	2.46 ± 0.25	3.27 ± 0.29	2.92 ± 0.21	4.08 ± 0.41
45 min	3.08 ± 0.32	4.32 ± 0.40	3.83 ± 0.35	4.88 ± 0.56
60 min	3.68 ± 0.39	4.91 ± 0.55	4.36 ± 0.39	5.43 ± 0.65
90 min	4.09 ± 0.51	5.46 ± 0.76	4.92 ± 0.63	6.62 ± 0.69
120 min	4.11 ± 0.52	6.15 ± 0.84	5.17 ± 0.73	7.50 ± 0.65
180 min	4.11 ± 0.52	6.65 ± 0.72	5.17 ± 0.73	7.27 ± 0.80
24 h	18.54 ± 2.94	20.85 ± 2.18	19.02 ± 1.86	22.66 ± 1.13

The data presented here clearly demonstrate that both Cd^{2+} and Pb^{2+} acutely injected into the third ventricle of male rats impair the dipsogenic response elicited by the central administration of ISO. This response was not related to a general depressive status of the central nervous system after the injection of the metals, since food intake after fasting was normally maintained in animals receiving *icv* injections of Cd^{2+} or Pb^{2+}. In addition, animals receiving Pb^{2+} or Cd^{2+} at the doses used here did not present motor disturbances that could explain the reduced water intake. Circling, hyperactivity, or sickness-like postures or behaviors were not observed.

It is well established that the presence of heavy metals in the central nervous system hampers the function of several neurotransmitter pathways. Recent reports have shown that Pb^{2+} selectively reduced muscarinic receptors and cholineacetyltransferase in some brain areas (13). Dopamine concentration and synthesis regulation seem to be altered by Pb^{2+} (14). Pb^{2+} at extremely low concentrations affects the glutamatergic system in

the brain. Glutamate synthesis is significantly reduced and NMDA-evoked currents are inhibited by Pb^{2+} (15). After postnatal Pb^{2+} exposure noradrenaline levels are increased in many brain regions (16) while the noradrenergic turnover rate may be decreased (17).

Cd^{2+} is also able to alter the function of many brain neurotransmitters. Catecholaminergic and serotonergic transmission in the central nervous system may be affected by heavy metal exposure, with both stimulation and inhibition being reported by different researchers (1). Also, the level and distribution of several biogenic amines in the central nervous system are modified by Cd^{2+} (5).

We have recently reported that acute injections of PbAc (7) or $CdCl_2$ (8) into the third ventricle of rats block the dipsogenic response induced by three different situations, i.e., water deprivation and central cholinergic or angiotensinergic stimulation. The data now included in the present paper clearly show that both Cd^{2+} and Pb^{2+} are also capable of decreasing water intake following central ß-adrenergic stimulation.

Catecholaminergic control of drinking behavior is well documented. Indeed, periventricular noradrenergic systems are crucial for angiotensin-induced water intake (18) and selective destruction of discrete noradrenergic areas by the neurotoxic agent 6-hydroxydopamine reduces drinking (19). Central ß-adrenergic stimulation is an undisputable stimulus generating water intake (10,11).

The data presented here show that the previous third ventricle injection of minute amounts of Cd^{2+} or Pb^{2+} impairs the dipsogenic effect of central ß-adrenergic stimulation by ISO. So it is clear that the presence of these metals in the central nervous system disrupts the capacity of ISO to induce water intake. However, we cannot rule out the possibility that both Cd^{2+} and Pb^{2+} may inhibit a commom mechanism activated by angiotensin II, noradrenaline or acetylcholine.

The effects of the metals observed here are probably due to very fast biochemical effects in the central nervous system which may be related, at least in part, to derangement of calcium-mediated cellular processes. A challenge that lies ahead will be to exploit the relationship between calcium metabolism in the brain in response to acute heavy metal injections, and the effects on drinking behavior observed here. It is important to note that $CdCl_2$ was much more potent than PbAc in blocking the isoproterenol-induced dipsogenic response.

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