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Opiatergic participation in the thirst-inhibiting effect of acute third ventricle injections of cadmium (Cd^{2+}) and lead (Pb^{2+})

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Abstract

We have previously demonstrated that acute third ventricle injections of both lead and cadmium prevent the dipsogenic response elicited by dehydration or by central injections of dipsogenic agents such as angiotensin II, carbachol and isoproterenol in rats. We have also shown that the antidipsogenic action of cadmium may be due, at least in part, to activation of thirst-inhibitory central serotonergic pathways. In the present paper we show that in Wistar male rats the antidipsogenic effect of both lead acetate (3.0 nmol/rat) and cadmium chloride (3.0 nmol/rat) may be partially dependent on the activation of brain opiatergic pathways since central injections of naloxone (82.5 nmol/rat), a non-selective opioid antagonist, blunt the thirst-inhibiting effect of these metals. One hundred and twenty minutes after the second third ventricle injections, dehydrated animals (14 h overnight) receiving saline + sodium acetate displayed a high water intake (7.90 ± 0.47 ml/100 g body weight) whereas animals receiving saline + lead acetate drank 3.24 ± 0.47 ml/100 g body weight. Animals receiving naloxone + lead acetate drank 6.94 ± 0.60 ml/100 g body weight. Animals receiving saline + saline drank 8.16 ± 0.66 ml/100 g body weight whilst animals receiving saline + cadmium chloride drank 1.63 ± 0.37 ml/100 g body weight. Animals receiving naloxone + cadmium chloride drank 8.01 ± 0.94 ml/100 g body weight. It is suggested that acute third ventricle injections of both lead and cadmium exert their antidipsogenic effect by activating thirst-inhibiting opioid pathways in the brain.

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Heavy metal intoxication is a current public health topic. Cadmium (Cd^{2+}) and lead (Pb^{2+}) have been shown to induce a wide variety of toxic effects in humans and animals. The normal function of multiple organs and systems is disturbed by both metals. These metals induce major neurological disorders by disrupting the functional integrity of several central nervous system circuitries linked to brain control of innumerable physiological processes (1,2). At the level of the brain, the toxic effects of both metals are related to their capacity to elicit a wide range of physiological, biochemical and behavioral dysfunctions. After absorption by many routes, cadmium and lead enter the brain by damaging the blood-brain barrier or via alternative pathways such as retrograde axonal transport (2,3). Once in the brain, the metals penetrate the cellular elements (both neurons and glia), altering countless biochemical processes related to normal cell machinery function. Both cadmium and lead interfere with a number of calcium-dependent events at the subcellular level. They alter neurotransmitter kinetics, block voltage-dependent calcium channels, substitute calcium in calcium-sodium ATP pumps and bind to second messenger calcium receptors such as calmodulin, phosphodiesterases and protein kinases (4-6).

We have demonstrated that both cadmium and lead induce a significant antidipsogenic effect in rats whose water intake was stimulated by dehydration or by third ventricle injections of dipsogenic agents such as angiotensin II, carbachol and isoproterenol (7-9).

Drinking behavior is regulated by a complex integrated central system. This thirst-controlling system involves many brain regions and distinct neurotransmitters exerting positive and negative drives, whose balance induces water intake or water satiety. At the brain level, angiotensinergic, cholinergic and ß-adrenergic stimulation induces thirst whilst α-adrenergic, serotonergic, ANPergic and opiatergic activation provokes an antidipsogenic effect (10,11).

In the present paper we investigate whether the antidipsogenic effect exerted by acute third ventricle injections of cadmium and lead may be due to enhancement of a central opiatergic drive.

We used adult male Wistar rats weighing 230 ± 20 g. Each experimental group consisted of 10 animals. The animals were housed under controlled temperature (26 ± 2°C) and light conditions (lights on from 6:00 to 20:00 h). Seven days before the experimental sessions the animals had the third ventricle cannulated as described elsewhere (12). Briefly, after an overnight fast, the animals were anesthetized with Nembutal (sodium pentobarbital, 40 mg/kg, *ip*) for stereotaxic cannulation of the third ventricle. 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 mandril (28 gauge) to prevent obstruction. Two screws embedded in the skull bone and cemented with dental acrylic firmly anchored the cannulas. After the experiments 2 µl of 5% Evans blue 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 into consideration. All drugs used (lead acetate (PbAc), sodium acetate (NaAc), cadmium chloride ($CdCl_2$) and naloxone) were obtained from Sigma Chemical Co., St. Louis, MO. The drugs were dissolved in isotonic saline solution and injected into the third ventricle using a Hamilton syringe connected to a Mizzy-Slide-Pak needle through polyethylene tubing. The volume of the third ventricle injections was 2 µl and the injections were performed over a period of 90 s. Naive animals were used in all experiments (always performed between 8:00 and 11:00 a.m.).

Cadmium chloride or lead acetate was injected into the third ventricle of dehydrated animals (overnight, 14-h water deprivation) and their water intake was measured for the next 120 min. The animals receiving cadmium chloride (3.0 nmol/rat) were compared to saline-treated controls whereas animals receiving lead acetate (3.0 nmol/rat) were compared to controls treated with sodium acetate (3.0 nmol/rat). We have previously ascertained that, when compared to isotonic saline, third ventricle injections of sodium acetate are devoid of any effect on water intake. To investigate the role of central opiatergic pathways in the antidipsogenic effect of both lead and cadmium we pretreated animals receiving lead or cadmium with two different doses (10.3 and 82.5 nmol/rat) of naloxone, an opioid antagonist, into the third ventricle. Pretreatment with naloxone or saline was applied 30 min before the injections of the metals. The effect of naloxone on animals free from cadmium or lead injections was also tested in a separate group.

Data were analyzed statistically by repeated measures analysis of variance (ANOVA) followed by the Scheffé test using the GBSTAT computer software (Dynamic Microsystems Inc., Silver Spring, MD). Differences were considered significant when $P<0.01$. The cumulative water intake is presented as ml/100 g body weight (mean ± SEM).

Figure 1 (panel A) shows that dehydrated control animals receiving NaAc (3.0 nmol/rat) 30 min after saline (saline + NaAc) presented, as expected, a high water intake. Animals receiving PbAc (3.0 nmol/rat) and pretreated with saline (saline + PbAc) exhibited a significant inhibition of water intake compared to controls (saline + NaAc). Pretreatment with naloxone blocked this antidipsogenic effect of PbAc. Indeed, animals receiving PbAc but pretreated with naloxone at the highest dose (naloxone 82.5 nmol/rat + PbAc) displayed a cumulative water intake that was indistinguishable from that of control animals (saline + NaAc). The group receiving PbAc but pretreated with naloxone at the lowest dose (naloxone 10.3 nmol/rat + PbAc) showed a water intake similar to that of animals receiving PbAc but pretreated with saline (saline + PbAc). Thus, at the lowest dose employed, naloxone was unable to reverse the antidipsogenic effect of PbAc.

Figure 1 (panel B) shows that dehydrated control animals receiving a double saline injection separated by a 30-min interval (saline + saline) displayed a high water intake, as predicted. Animals receiving $CdCl_2$ (3.0 nmol/rat) pretreated with saline (saline +

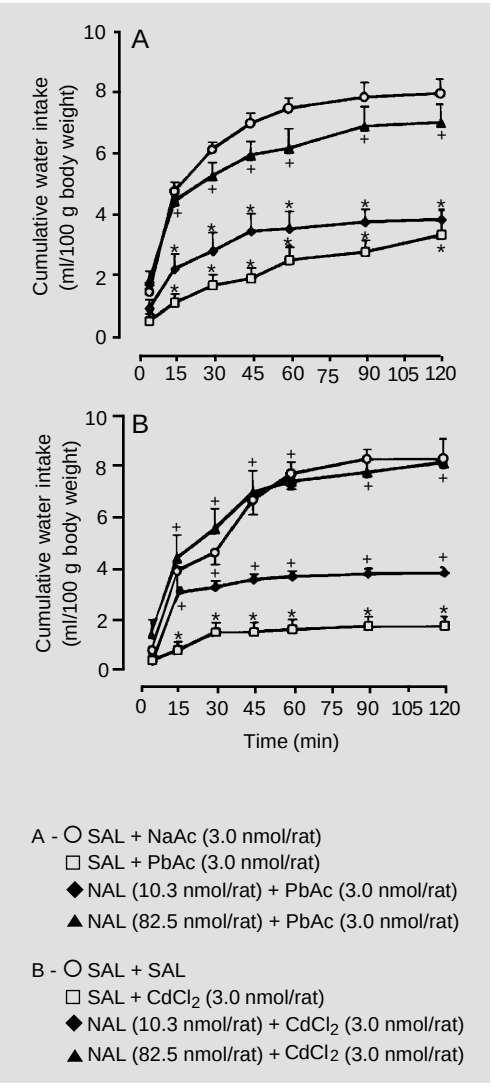

Figure 1 - Effect of third ventricle injections of naloxone on water intake in dehydrated (overnight, 14 h) rats receiving PbAc or $CdCl_2$. Cumulative water intake (ml/100 g body weight) data are reported as mean ± SEM for 10 animals in each group; *$P<0.01$ compared to saline + NaAc group (A) or saline + saline group (B). $^{+}P<0.01$ compared to saline + PbAc group (A) or saline + $CdCl_2$ group (B) (repeated measures ANOVA followed by Scheffé test). SAL, Saline; NAL, naloxone.

$CdCl_2$) presented a significant decrease in water intake compared to controls (saline + saline). Naloxone administered as pretreatment at both doses used here was able to block the antidipsogenic effect of $CdCl_2$. The group receiving $CdCl_2$ and pretreated with naloxone at the highest dose (naloxone 82.5 nmol/rat + $CdCl_2$) presented a water intake very similar to that of control animals (saline + saline). The group receiving $CdCl_2$ but pretreated with naloxone at the lowest dose (naloxone 10.3 nmol/rat + $CdCl_2$) exhibited a lower water intake than that of controls (saline + saline) but still significantly higher than that of animals receiving $CdCl_2$ but pretreated with saline (saline + $CdCl_2$).

Table 1 shows that control animals receiving a double injection of saline (saline + saline), animals receiving NaAc pretreated with saline (saline + NaAc) and animals pretreated with naloxone but receiving saline (naloxone 82.5 nmol/rat) presented very similar values of water intake, with no significant differences among them.

The present data clearly show that acute third ventricle injections of two distinct divalent cationic heavy metals (cadmium and lead) block the dipsogenic response normally observed after a period of dehydration in rats. The present paper also shows that the antidipsogenic effect of both cadmium and lead injections relies on a mechanism dependent on the functional integrity of central opiatergic pathways. In fact, central pretreatment of animals receiving cadmium or lead with naloxone, a non-selective opioid antagonist, is able to reverse the antidipsogenic action of both metals.

Table 1 - Effect of treatment with saline, NaAc and naloxone (82.5 nmol/rat) on water intake (ml/100 g body weight) in dehydrated animals free of heavy metals pretreated with saline.

Data are reported as mean ± SEM for 10 animals in each group. Statistical analysis (repeated measures ANOVA followed by the Scheffé test) showed no difference among groups.

Time (min)	Cumulative water intake (ml/100 g body weight)		
	Saline + Saline	Saline + NaAc	Saline + Naloxone
5	0.75 ± 0.21	1.45 ± 0.30	1.36 ± 0.16
15	3.81 ± 0.70	4.70 ± 0.32	4.77 ± 0.25
30	4.51 ± 0.58	6.08 ± 0.24	5.20 ± 0.28
45	6.58 ± 0.58	6.94 ± 0.35	6.11 ± 0.25
60	7.60 ± 0.62	7.44 ± 0.34	6.89 ± 0.29
90	8.16 ± 0.66	7.80 ± 0.48	6.89 ± 0.31
120	8.16 ± 0.66	7.90 ± 0.47	7.42 ± 0.31

Cadmium and lead are actively transported into brain cells. By interfering with the normal function of many cellular processes, both metals may, in some situations, induce sustained release of aminergic and peptidergic neurotransmitters in the brain. Conversely, in different circumstances the metals may inhibit proper release of brain neurochemicals. As a consequence of these biochemical events, lead and cadmium disturb the normal function of central cholinergic, GABAergic, dopaminergic, serotonergic, glutamatergic and opiatergic pathways (2,13,14).

Thirst is a motivational behavior regulated by a complex integration of many central regions ceaselessly receiving information concerning the balance and distribution of body fluids (10). Many circumventricular structures participate in the control of water intake. These include the subfornical organ, the organum vasculosum laminae terminalis, the anteroventral region of the third ventricle (AV3V) as well as the area postrema. The hypothalamic preoptic, paraventricular and septal areas are additional thirst-controlling areas (15). We injected both metals into the third ventricle, a route that allows a prompt transit to most of the mentioned areas.

The brain areas related to thirst control use different neurotransmitters exerting opposing drives (water seeking *vs* water satiety) whose final balance yields normal drinking, a behavior scheduled to repair physiological and pathological oscillations in blood volume and plasma osmolarity. The main neurochemical components that stimulate thirst are angiotensin II, acetylcholine and

catecholamines acting on ß-adrenergic receptors. On the other hand, serotonin, ANP, α-adrenergic receptor stimulation and opioids inhibit drinking (10,11).

The injection of both metals generated an indisputable reduction of water intake in dehydrated animals. This means that cadmium and lead act on the central nervous system stimulating pathways that exert an inhibitory drive on water intake or, alternatively, that the metals block thirst-inducing pathways. Evidently, both things may concomitantly occur. We have previously shown that both cadmium and lead disrupt the thirst-generating drive exerted by brain angiotensin II as well as by muscarinic and ß-adrenergic activation (7-9). We have also demonstrated that the antidipsogenic effect of cadmium could be due to 5-HT_2 serotonin receptors activation (8).

The results of the present paper clearly indicate that both metals trigger an additional mechanism related to thirst inhibition, i.e., the activation of central opiatergic pathways. Indeed, in animals pretreated with naloxone, a non-selective opioid antagonist, cadmium and lead were unable to impair the dipsogenic response induced by dehydration. Opioids in the central nervous system participate in the control of water-salt balance. Opiatergic activation inhibits renal electrolyte excretion and water intake (16). Thus, our data demonstrating the role of inhibitory opiatergic circuits in the antidipsogenic effects of cadmium and lead agree with the information available in the literature. In the present study third ventricle injections of naloxone in dehydrated animals free of heavy metals did not alter water intake. This further supports our hypothesis that central administration of cadmium and lead increases the release of endogenous opioids able to inhibit drinking. Indeed, it is legitimate to suppose that in normal dehydrated animals the natural drives inducing thirst are activated, while those causing water satiety, among which opioids are included, are suppressed.

The present data confirm previous work from our laboratory showing that the thirst-inhibiting effect of cadmium is more potent than that of lead. Naloxone at the lowest dose employed was unable to abolish the antidipsogenic effect of cadmium as observed with the antidipsogenic action of lead. Thus, it seems conceivable that, as compared to lead, cadmium induces a more prominent activation of central opioid circuits related to thirst-inhibition.

In summary, the present data indicate that acute third ventricle injections of cadmium and lead provoke an antidipsogenic effect in dehydrated rats that seems to depend on the release of endogenous opioids.

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