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Acute Effect of Intracerebroventricular Administration of Lead on the Drinking Behavior of Rats Induced by Dehydration or Central Cholinergic and Angiotensinergic Stimulation

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FREGONEZE, J. B., M. CUNHA, C. BULCÃO, H. FERREIRA AND E. DE CASTRO E SILVA. *Acute effect of intracerebroventricular administration of lead on the drinking behavior of rats induced by dehydration or central cholinergic and angiotensinergic stimulation.* PHYSIOL BEHAV **56**(1) 129–133, 1994.—In the present paper, the acute effect of third ventricle injections of lead acetate (5, 10, 100, 1000 ng/rat) on the drinking behavior of adult, male, Wistar rats was investigated. Lead generates a prompt and significant reduction in water intake induced by three different circumstances: dehydration (14 h of water deprivation) and after carbachol (2 μg/rat, ICV) or angiotensin II (10 ng/rat, ICV) administration. These results show that lead may produce very fast actions in the central nervous system and suggest that inhibition of water intake by lead may depend on impairment of central cholinergic and/or angiotensinergic functions.

Lead Lead intoxication Lead neurotoxicity Drinking behavior Brain angiotensin Brain cholinergic systems

LEAD poisoning is one of the most significant diseases of environmental origin (1,9). The picture of lead neurotoxicity comprises various disorders like idiopathic hyperactivity (11), inferior IQ scores (2), as well as impaired performance on attention, auditory,and language function in children (19). Disturbances of learning processes are also observed (28).

In laboratory animals, lead toxicity impairs the function of several brain neurotransmitters. Indeed, disruption of the functional integrity of serotonergic (43), dopaminergic (36), GABAergic (37), adrenergic (44), and cholinergic (38) pathways in the central nervous system may occur during lead intoxication. Cholinergic transmission is impaired by lead because it inhibits evoked acetylcholine release (40). This effect of lead seems to be due to a blockade of inward Ca^{2+} currents and a slow increase in cytoplasmic-free Ca^{2+} by competition with Ca^{2+} binding sites (5).

Central cholinergic pathways participate in the regulation of water intake. It is very well known that stimulation of cholinergic pathways in the hypothalamus generates a strong dipsogenic effect in rats (4,20). Furthermore, drinking behavior induced by angiotensin II (AII) depends, at least partially, on the function of brain cholinergic pathways (8,34).

In the present study, we decide to investigate the effect of acute intracerebroventricular (ICV) lead injections on water intake induced by dehydration and central cholinergic and angiotensinergic stimulation in rats.

METHOD

Wistar male rats (220–250 g) kept under controlled light (lights on from 0600 to 2000 h) and temperature (26 ± 2°C) conditions were used in this study. They had free access to tap water and laboratory chow (Nuvital Nutrientes Ltda, 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 according to the procedures described elsewhere (4). After surgery the animals were maintained in individual cages for 7 days before the experiments.

Drugs and Microinjections

The following drugs (purchased from Sigma Chemical Co., St. Louis, MO) were used: carbachol, angiotensin II (Asp[1]-Ileu[5]-

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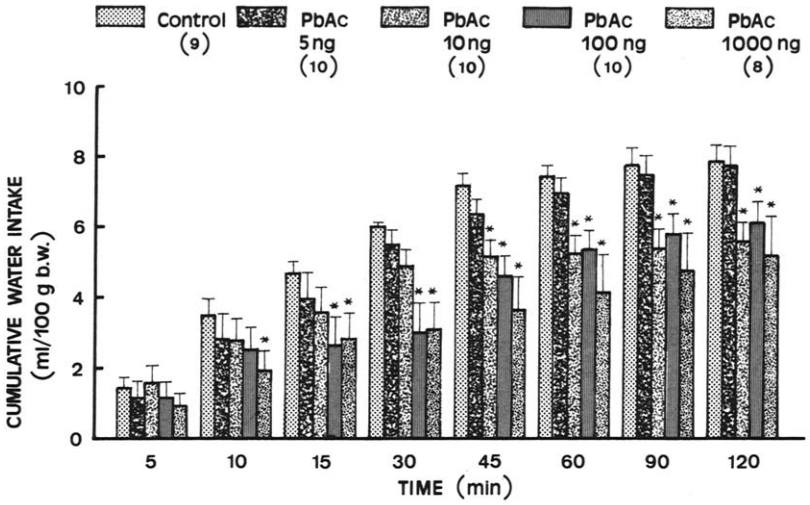

FIG. 1. Cumulative water intake (ml/100 g b.wt.) in dehydrated animals (14 h of water deprivation) receiving third ventricular injections of several doses of PbAc. Control animals received NaAc (1000 ng/rat) by the same route. Data are presented as mean ± SEM. Asterisks indicate a statistical significant difference ($p < 0.01$). The numbers in parentheses show number of animals used in each group.

AII), lead acetate (PbAc), and sodium acetate (NaAc). All drugs were dissolved in saline solution (0.9% NaCl). A volume of 2 μl was injected with a Hamilton microsyringe connected to a Mizzy-Slide-Pak needle through polyethylene tubing over a period of 30–60 s. This needle was 1 mm longer than the guide cannula.

Experimental Design

We studied the effect of ICV injections of PbAc on drinking behavior in three different groups of animals: dehydrated, carbachol-treated, and AII-treated rats. All experiments were performed between 0800 and 1200 h. The animals were considered dehydrated after an overnight period (14 h) of water deprivation. The groups treated with carbachol or AII were normohydrated. The NaAc ICV injections (1000 ng) were used as control to the PbAc treatments. We made experimental groups (data not shown) to ascertain that NaAc does not block water intake in dehydrated rats or induce thirst in normohydrated animals. To confirm this we compared water intake in dehydrated and normohydrated rats after ICV injections of saline solution (0.9% NaCl) and NaAc.

In the first group we studied the effect of several doses (1000, 100, 10, and 5 ng/rat) of ICV PbAc or NaAc on the drinking behavior of dehydrated rats. In this case, graduated water bottles were put in the cages immediately after the ICV injections and the cumulative water intake was recorded for the next 120 min.

The second group of animals was normohydrated. Here, the effect of lead on carbachol-induced thirst was studied. The rats received an ICV injection of PbAc (100 ng) and then received carbachol (2 μg) by the same route 45 min later. This group was compared to another group receiving carbachol (2 μg) alone. The cumulative water intake from graduated bottles already present in the cages was recorded for 120 min.

With the third group of animals we investigated the effect of lead on AII-induced water intake. These animals were also normohydrated. The PbAc (100 ng) was centrally administered 45 min before AII (10 ng) injections by the same route. This group was compared to another group to which AII (10 ng/rat, ICV) was administered alone. Water intake was recorded exactly as in the previous experiment.

Statistical Analysis

We used a computer software (GBSTAT, Dynamic Microsystems Inc., Silver Spring, MD) that performs the analysis of variance (ANOVA) and subsequently submitted the data to the Tukey test. The groups were considered significantly different when $p < 0.01$. The cumulative water intake was calculated as ml/100 g of body weight, expressed as mean ± SEM.

RESULTS

Figure 1 shows the effect of four different doses of ICV PbAc on the cumulative water intake of dehydrated rats. As expected, control animals receiving NaAc exhibited a high water intake in the period studied. In the lowest dose employed (5 ng/rat) PbAc was ineffective. All other doses tested promoted a significant inhibition of water intake in dehydrated animals. However, the onset of the blockade depended on the dose employed; in the dose of 10 ng/rat it begun only after 45 min of the PbAc injection; with the highest dose it was evident just after 10 min. In all efficacious doses, the Pb-induced blockade lasted for the entire duration of the experiment (120 min).

As depicted in Fig. 2, normohydrated animals receiving ICV injections of 2 μg of carbachol, a cholinergic agonist, exhibited a high water intake. This is classical data just reproduced here. However, when the animals were pretreated with PbAc (100 ng/rat, ICV) their water intake was significantly reduced. This blockade was evident 15 min after carbachol injection. We chose the intermediate dose of 100 ng of PbAc because (as shown in Fig. 1) it proved to be efficacious in inhibiting water intake in dehydrated rats.

Figure 3 exhibits the effect of PbAc administration (100 ng/rat, ICV) on water intake induced by AII injections (10 ng/rat, ICV). As expected, ICV AII administration elicits a high water intake. Again, this is a well-known phenomenon. Here, PbAc pretreatment was also able to promote a significant reduction in

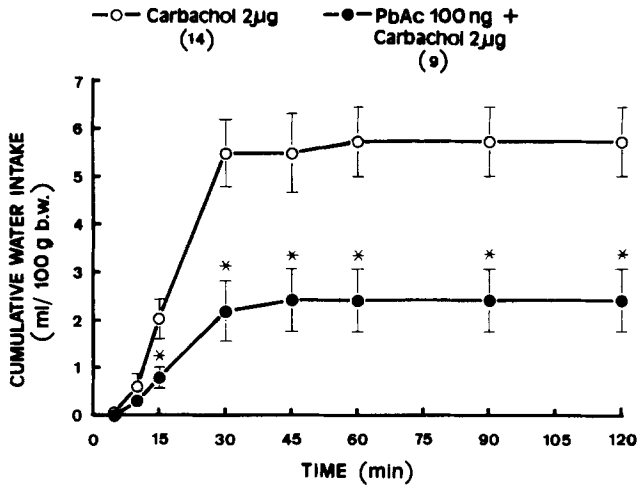

FIG. 2. Cumulative water intake (ml/100 g b.wt.) in normohydrated animals receiving third ventricular injections of carbachol alone (○) or PbAc + carbachol (●). In the PbAc + carbachol group PbAc was administered 45 min before the carbachol injection. Data are presented as mean ± SEM. Asterisks indicate a statistical significant difference ($p < 0.01$). The numbers in parentheses show number of animals used in each group.

AII-induced water intake. As in the previous experiment, this blockade was also evident 15 min after AII injection.

DISCUSSION

The present study clearly demonstrates that acute administration of PbAc into the third ventricle significantly decreases water intake in dehydrated rats. Furthermore, this procedure significantly attenuates the dipsogenic effect of central injections of both carbachol and AII in normohydrated animals.

During experimental intoxication, lead slowly accumulates in the brain of adult rats (32). This is probably due to the blood–brain barrier that restrains the passage of polar molecules into the brain (15). However, in very young animals (humans included), the blood–brain barrier is still immature, allowing lead to accumulate faster and in greater amounts (14). In addition, lead itself may be concentrated in brain endothelial cells (42) and may damage the blood–brain barrier (15). Brain Pb concentrations ranging from 4.9 to 7 µg/g of tissue were determined in rats showing signs of lead intoxication (14,27,32). Neurologic signs of lead poisoning may be evident with Pb plasma levels around 800 µg/dl or even less (7). So, the injections of Pb directly into the third ventricle of rats, in the doses we employed, mimic the arrival of the first toxic burden of lead into the brain. This helps to understand the very acute effects of this metal in the central nervous system. Besides, the third ventricle is closely related to several brain structures controlling drinking behavior (3). Thus, in the investigation of central control of water intake it represents an ideal route of administration for the drugs or other agents studied.

It is well known that lead impairs cholinergic function. Cholinergic transmission in the cat superior cervical ganglion and in the rat diaphragm is blocked by Pb (18,39). In frogs, the amplitude of ionophoretically evoked acetylcholine responses is also reduced by lead (21). In mouse neuroblastoma cells, nanomolar concentrations of lead selectively block nicotinic acetylcholine responses (30). In the central nervous system, it has been demonstrated that Pb induces deficits in cholinergic functions and modifies acetylcholine levels as well as its turnover rate (10,38).

Some data in the literature suggest that activation of brain cholinergic function may participate in the generation of thirst (12). Indeed, ICV atropine administration blocks water intake in dehydrated rats (23), and carbachol, a cholinergic agonist, injected in minute amounts into the brain elicits drinking behavior (24).

Hence, the rational basis of our first experiment was to investigate if PbAc injected into the third ventricle modifies water intake in dehydrated rats. If lead disturbs central cholinergic transmission and if brain cholinergic pathways modulate thirst, dehydrated rats treated with lead might display alterations in their water intake. As Fig. 1 shows, this is exactly what we observed.

Generation of thirst depends on the integration of many different brain systems working altogether. Activation of cholinergic pathways is only part of the whole picture. Thus, we decided to investigate if Pb was able to block water intake specifically induced by the administration of carbachol in the central nervous system. As depicted in Fig. 2, lead promotes a partial blockade in carbachol-induced water intake. This suggests that a particular action on central cholinergic transmission is involved in the mechanism of the antidipsogenic action of lead.

Among the many allied systems that coordinate thirst generation in the brain, angiotensin is one of the most important. Acting on sites located in the circunventricular organs, AII yields a powerful dipsogenic effect (33,41). Interestingly, angiotensin-induced thirst may depend on the function of cholinergic pathways in the central nervous system. Indeed, central atropine administration blocks AII-induced water intake in rats (35). This lead us to investigate if Pb might also interfere with AII-induced drinking behavior. As shown in Fig. 3, this really happens. The dipsogenic effect of AII is significantly lessened in the group of rats pretreated with lead.

All effects of lead observed here are extremely acute. They may be manifested even after a period so brief as 10 min. The knowledge of some mechanisms of action of lead at the cellular level may help to explain why its effects appear so fast. It was

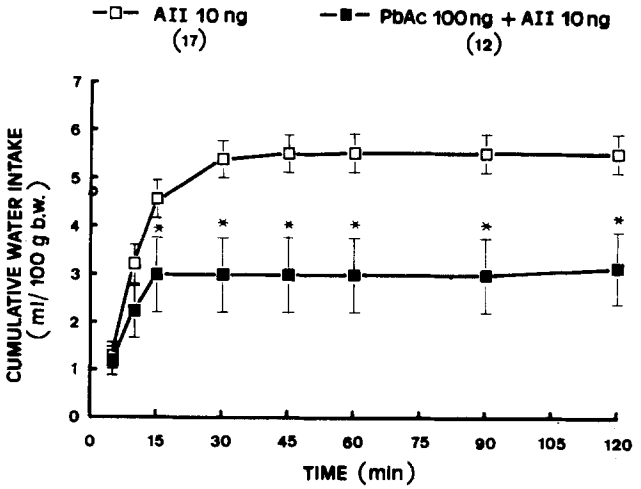

FIG. 3. Cumulative water intake (ml/100 g b.wt.) in normohydrated animals receiving third ventricular injections of angiotensin II alone (□) or PbAc + angiotensin II (■). In PbAc + angiotensin II group PbAc was administered 45 min before the angiotensin II injection. Data are presented as mean ± SEM. Asterisks indicate a statistical significant difference ($p < 0.01$). The numbers in parentheses show number of animals used in each group.

recently demonstrated that lead may substitute calcium in its properties as an intracellular second messenger. Lead might replace calcium in its synergistic action with diacylglycerol, or might directly combine with calmodulin (17), a protein with important second messenger functions in the cell. In both cases, deep alterations in the normal regulation of protein kinase C, an enzyme that controls the release of neurotransmitters in the brain, could be produced. Moreover, a direct action of Pb on protein kinase C has been proposed. Indeed, some authors, using crude fractions of brain protein kinase C, demonstrated that lead increases its activity (22); others, employing purified subtypes of this enzyme (types I, II, and III), observed a calcium-independent and reversible inhibition (26). Lead may also modify the electrophysiological behavior of neurons (6) by altering the function of sodium channels or by activating new types of ion channels in neural tissues (31). These are mechanisms of action that could easily explain the very rapid onset of the lead effects observed here.

In the central nervous system, AII exerts its effects by interacting with receptors of AT_1 type (33). The AII binding to these receptors activates phospholipase C, at cellular membrane level. This breaks phosphoinositide biphosphate, generating diacylglycerol and inositol triphosphate, second messengers able to activate protein kinase C. Furthermore, coupling to this very receptor type, AII may open voltage-dependent Ca^{2+} channels, increasing intracellular calcium concentrations (29). Considering what is said above, Pb could directly interfere with the signal transduction at these levels, decreasing the efficacy of AII action. As mentioned previously, the dipsogenic effect of AII may partially depend on brain cholinergic activation. Because lead may impair central cholinergic transmission, this could be another mechanism by which lead could impair AII functions in the brain.

The data presented here strongly suggest that lead may decrease thirst generation mechanisms in the brain. This is a very acute effect of lead because it is observed minutes after its administration into the third cerebral ventricle of rats. The higher the dose employed the sooner the reduction in water intake was observed. With the dose of 10 ng/rat the blockade was apparent only after 45 min, and with the dose of 1000 ng/rat it was evident after 10 min. This probably means that the velocity of lead diffusion into the circumventricular structures triggering thirst is proportional to the dose employed. Also, the inhibitory effect of lead on water intake in dehydrated rats disappears after 3 h (data not shown). This indicates that the lead-induced disturbance in drinking behavior observed here is a reversible disruption of the functional integrity of brain systems controlling thirst.

Lead attenuated thirst produced by three different procedures: dehydration, and central cholinergic and angiotensinergic stimulation. Dehydration and angiotensin activation are thirst-promoting conditions for which the physiological nature is irrefutable. The essential role of cholinergic neurons in thirst generation may be questioned by some. However, the stimulatory effect of carbachol on drinking behavior is indisputable. We believe we have provided considerable evidence that the lead-induced disturbances in drinking behavior presented here are of physiological pertinence.

Besides giving new information about lead toxicity at the brain level, the data presented here may elicit some other considerations. Severe nephropathy may be part of the clinical picture of lead intoxication (13,16). This evidently disturbs water–electrolyte balance. By inhibiting thirst, lead could further contribute to increase the fluid homeostasis disorder, present in nephropathic patients. Also, the evaluation of water intake is a very convenient, simple, and inexpensive method to investigate acute and chronic brain lead actions, at least in laboratory animals.

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