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Role of the central opioid system in the inhibition of water and salt intake induced by central administration of IL-1β in rats

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Abstract

In the present study we investigated, the effect of third ventricle injections of IL-1β on water and salt intake in fluid-deprived and sodium-depleted rats. Central administration of IL-1β significantly reduced water and salt intake in fluid-deprived animals and decreased salt intake in sodium-depleted rats. The antidipsogenic and antinatriorexic effects elicited by the central administration of IL-1β were suppressed by pretreatment with central injections of the non-selective opioid antagonist naloxone (10 μg) in the two different experimental protocols used here (water deprivation and sodium depletion). In addition, central administration of IL-1β failed to modify the intake of a 0.1% saccharin solution when the animals were submitted to a "dessert test" or to induce any significant locomotor deficit in the open-field test. The present results suggest that the activation of the central interleukinergic component by IL-1β impairs the increase in water and salt intake induced by water deprivation and the enhancement in sodium appetite that follows sodium depletion. The data also support the conclusion that the antidipsogenic and antinatriorexic effects resulting from the activation of the central interleukinergic component rely on an opioid-dependent, naloxone-blockable system.

Keywords: Interleukin-1β; Opioids; Water intake; Salt intake; Naloxone

1. Introduction

Activated macrophages and other immune cells produce interleukin-1β (IL-1β), an immunoregulatory cytokine, during acute and chronic pathological processes. In the central nervous system, several cell types including neurons, astrocytes, microglia and endothelial cells also synthesize and release IL-1β (Cunningham and De Souza, 1993; Rothwell and Luheshi, 2000). A great variety of visceral and behavioral responses are produced by both blood-borne and brain-originated IL-1β, including hyperthermia, changes in blood pressure, hypothalamo-pituitary axis hyperfunction, sleep induction, sickness behavior and anorexia associated with shifts in the energy balance of the organism (Turnbull and Rivier, 1999).

Peripheral and central administration of IL-1β has previously been shown to elicit a significant inhibition in water intake, and endogenous IL-1β may participate in thirst regulation in conditions in which this cytokine is naturally synthesized and released from its original sources (Masotto et al., 1992; Osaka et al., 1992; Plata-Salamán and Ffrench-Mullen, 1992; Nava et al., 1996; Kannan et al., 1997; Sonti et al., 1997; Karádi et al., 2005).

Water and salt intake are regulated by a complex interactive network of inhibitory and stimulatory inputs that involve many different brain areas and various neurotransmitters. Central circuits stimulating water intake may also stimulate the intake of sodium, whereas brain regions whose activation inhibits thirst may also diminish salt intake. However, some important exceptions may occur in which a particular circuit/neurotransmitter may stimulate the intake of water without modifying the search and acquisition of salt (Johnson and Thunhorst, 1997; Stricker and Sved, 2000).

Depending on the anatomical location and the type of opioid receptor involved, the brain opioid system exerts complex effects on the control of water and salt intake. Stimulatory and inhibitory effects of the central opioid system on water and salt intake have been described, and these effects are dependent on the doses of

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the pharmacological agents and experimental protocols used (Fitzsimons, 1998). It would appear that the central opioidergic systems particularly influence the intake of palatable solutions. The intake of hypo- or isotonic saline solutions, considered palatable to rats since they are consumed by salt-replenished animals, is reduced by opioid antagonists such as naloxone (Cooper and Gilbert, 1984; Levine et al., 1982). This is confirmed by a more recent work (Bodnar et al., 1995) that has shown that central administration of naltrexone, a non-specific opioid antagonist, inhibits the intake of hypotonic saline, whereas specific μ, κ and δ antagonists are devoid of effects.

It is also important to note that opioids may interact with the immune system modulating its effects and, conversely, some immunological responses may be regulated by the opioid system (Bodnar and Klein, 2005). It has been well-established that pyrogens increase hypothalamic opioid release (Tsai et al., 2003) and that endogenous opioids may mediate IL-1β effects on the central nervous system.

The facts that IL-1β may increase central opioid release and that central opioids may exhibit inhibitory effects on water and salt intake prompted us to investigate whether the antidipsogenic effect of IL-1β was dependent on the functional integrity of the brain opioid system. To achieve this objective we investigated the effects of the central administration of IL-1β on water and salt intake in two different experimental protocols: water deprivation and sodium depletion.

2. Materials and methods

2.1. Animals

In the present study, male Wistar rats (220±20 g), kept under controlled light (lights on from 7 A.M. to 7 P.M.) and temperature (22–24 °C) conditions, were used. All animals had free access to tap water and laboratory chow (Nuvital Nutrientes Ltda, Curitiba, Brazil) in the days immediately prior to the experimental sessions. The experimental protocols used here were conducted according to the recommendations of the National Institutes of Health (USA) and approved by a Local Committee Regulating the Use of Animals in Research Laboratories. The number of animals used in each experimental set varied between 6 and 14.

2.2. Surgical procedure

Third ventricles were cannulated under sodium pentobarbital anesthesia (50 mg/kg i.p.) six days before the experimental sessions. Briefly, after positioning the rat in a stereotaxic apparatus (David Kopf Instruments, USA), a chronic 28-gauge guide cannula was implanted. The following coordinates were used: anteroposterior=0.5 mm behind the bregma; lateral=0.0 mm; vertical=8.0 mm below the skull. The animals were fixed to the stereotaxic apparatus with the head inclined upwards (+2.0 mm) to avoid lesions to the brain regions involved in the control of cardiovascular and body fluid homeostasis. The cannulas were fixed to the skull bone by two screws embedded in dental acrylic. After the experimental sessions, the position of the cannulas was verified. The animals were sacrificed by CO_2 inhalation and a Blue Evans dye injection was given through the cannula in order to confirm whether its tip was in the proper place. Only the data from animals whose cannulas were strictly inside the third ventricle were considered.

2.3. Drugs and microinjections

The following drugs were used: interleukin-1β (rhIL-1β, recombinant human — *E. Coli* derived) was purchased from R&D Systems (catalog number 201-LB). Naloxone hydrochloride was acquired from Sigma Chemical, Co., St. Louis, MO. Furosemide, a loop diuretic, was purchased from Aventis Pharma Ltd., São Paulo, Brazil. All drugs were dissolved in sterile isotonic saline solution. Third ventricle injections were given with the use of a Hamilton microsyringe connected to a Mizzy-Slide-Pak needle through polyethylene tubing. A total volume of 2 μl was slowly injected (60 s).

2.4. Experimental protocols

2.4.1. Fluid deprivation

To induce fluid deprivation, bottles were removed from the individual cages 24 h prior to the onset of the experiments. Euhydrated animals served as controls for this experimental group. To investigate the effect of the central administration of IL-1β on water and salt intake in fluid deprivation, different groups of fluid-deprived rats received third ventricle injections of IL-1β at different doses (1, 2, 4 and 8 ng). To study central opioidergic participation in the effects produced by third ventricle injections of IL-1β on water and salt intake, a different group of animals was pretreated with third ventricle injections of naloxone (10 μg) or saline 30 min before receiving the central administration of IL-1β. A distinct group of animals that received third ventricle injections of saline were pretreated with naloxone or saline in order to investigate the effects of the central blockade of opioidergic receptors alone on water and salt intake. The bottles containing 1.5% saline solution and distilled water were reintroduced into the cages 15 min after the third ventricle injections. A bottle holder in the cages maintained the bottles in the proper position for fluid intake during all the experimental sessions. Fluid intake was recorded 5, 15, 30, 45, 60, 90 and 120 min after the reintroduction of the bottles into the cages. Fluid-deprived control animals received third ventricle injections of isotonic saline solution. All groups were compared to a group of rats not submitted to fluid deprivation.

2.4.2. Sodium depletion

To induce sodium depletion, the animals were submitted to an experimental protocol in which they had simultaneous access to two bottles (distilled water and 1.5% saline solution) and standard rat chow from the period immediately after third ventricle cannulation until the moment of furosemide administration. To provoke the renal sodium loss that induces sodium depletion, the rats received a subcutaneous injection of furosemide (20 mg/kg) 24 h prior to the experimental sessions.

Access to 1.5% saline ceased immediately after the furosemide injection. From that moment on, the animals continued to have free access to distilled water, and normal rat chow was replaced by a low sodium diet (0.001% Na^+ and 0.33% K^+). Control animals not submitted to sodium depletion received subcutaneous injections of isotonic saline solution instead of furosemide. We have previously demonstrated that furosemide administration, at the dose used here, effectively increases urine output and renal sodium excretion and produces hyponatremia (Castro et al., 2003). To test the effect of central injections of IL-1β on water and salt intake in sodium-depleted rats, different groups of sodium-depleted animals received third ventricle injections of different doses of IL-1β (1, 2, 4 and 8 ng). Sodium-depleted control animals received third ventricle injections of isotonic saline solution. To study central opioidergic participation in the effects produced by third ventricle injections of IL-1β on water and salt intake in sodium-depleted rats, a different group of animals was pretreated with third ventricle injections of naloxone (10 μg) or saline 30 min before receiving the central administration of IL-1β. A separate group of sodium-depleted rats that received third ventricle injections of saline were pretreated with naloxone or saline in order to investigate the effects of the central blockade of opioidergic receptors alone on water and salt intake. The bottles containing 1.5% saline solution and distilled water were reintroduced into the cages 15 min after the third ventricle injections. A bottle holder in the cages maintained the bottles in the proper position for fluid intake during all the experimental sessions. Fluid intake was recorded 5, 15, 30, 45, 60, 90 and 120 min after the reintroduction of the bottles into the cages. All groups were compared to a normonatremic control group of animals.

2.4.3. Dessert test

To investigate whether the central administration of IL-1β was able to modify water intake through a non-specific, general inhibition of the central nervous system or by a locomotor deficit, we investigated the effect of third ventricle injections of IL-1β or saline on the intake of a 0.1% saccharin solution, a well-established example of hedonic behavior in rats (Johnson and Schwob, 1975). In this experiment, after third ventricle cannulation, two different groups of animals, kept in the usual individual cages where the only fluid available was water, were transferred (for 2 h each day, for seven consecutive days) to a different cage (the test cage) in which two bottles, one containing water and the other containing a 0.1% saccharin solution, were accessible. After this period of training, two different groups of fluid-deprived animals received third ventricle injections of IL-1β (8 ng) or saline (controls) 30 min before being transferred to the test cage. Water and saccharin intake was recorded for the next 120 min.

2.4.4. Open field test

To test whether the central administration of IL-1β was able to induce a significant reduction in locomotor activity that could explain the inhibition of water intake observed here, we submitted different groups of rats receiving third ventricle injections of IL-1β or saline to an open field test.

The apparatus consisted of a circular wooden box (60 cm in diameter and 60 cm high) with an open top. The floor was divided into eight areas of equal size with a circle at the center (42.43 cm). Hand operated counters and stopwatches were used to score locomotion (measured as the number of floor units entered with all four paws).

The behavioral experiments took place in a sound-attenuated temperature-controlled (24±1 °C) room between 7 AM and 12 PM. Two 40 W fluorescent lights placed 1.50 m away from the apparatus illuminated the environment. A white-noise generator provided constant background noise and the apparatus was cleaned with 70% ethanol and dried before each session to minimize olfactory cues.

2.4.5. Body temperature determination

To measure the effect of the central administration of IL-1β and naloxone on body temperature, a flexible thermistor probe was inserted 6–7 cm into the colon and taped to the base of the tail. The thermistor probe was connected to a temperature-recording device (Minipa Thermometer, MOD: MT-520) that provides body core temperature (Tc) continuously on a digital display.

2.5. Statistical analysis

We used a computer software package (SigmaStat for Windows, Jandel Scientific, San Rafael-CA) to perform two-way (treatment and time as factors) analysis of variance for repeated measures in each experimental set. Post hoc Student–Newman–Keuls test was used for comparison of each treatment with its corresponding time in the control groups. The data related to the effects of the various treatments on body temperature were analysed by one-way analysis of variance. The groups were considered significantly different when $P<0.05$. The data are presented as mean±SEM. Student's t test was used to analyze the data from the open field and dessert tests.

3. Results

Fig. 1 (panel A) shows the effect of third ventricle injections of IL-1β at different doses on water intake in fluid-deprived rats. Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [$F(5,29)=36.54$; $P<0.0001$; $F(7,35)=26.96$; $P<0.0001$; $F(35,203)=6.50$, $P<0.0001$, respectively]. As expected, there was a significant increase in water intake in saline-treated, fluid-deprived rats when compared to saline-treated, normohydrated controls. Third ventricle injections of IL-1β at the doses of 2 and 4 ng were unable to modify the high water intake displayed by fluid-deprived rats. At the highest dose used (8 ng) the central administration of IL-1β significantly reduced water intake in fluid-deprived animals.

Fig. 1 (panel B) depicts the effect of third ventricle injections of IL-1β at different doses on salt intake in fluid-deprived rats. Analysis of variance indicated significant treatment and time main effects and no significant treatment×time interaction [F

(5,29)=9.79; $P<0.0001$; $F(7,35)=10.17$, $P<0.0001$; $F(35,203)=3.54$, $P<0.0001$, respectively]. In this case, a cumulative intake of 1.5% hypertonic saline was observed for saline-treated, fluid-deprived rats which was significantly higher than the intake of this same fluid by saline-treated, normohydrated controls. Third ventricle injections of IL-1β at the lowest dose used (2 ng) failed to alter salt intake in fluid-deprived animals. There was a significant reduction in salt-intake in fluid-deprived animals receiving IL-1β at the two other doses used (4 and 8 ng) as compared to saline-treated, fluid-deprived control animals.

Fig. 1 (panel C) also shows the percentage of total fluid consumed as water and saline at the end of the experimental session (120 min after the reintroduction of the bottles into the cages) in the same groups shown in panels A and B. One-way ANOVA values are $F(7,48)=54.70$, $P<0.0001$. At the dose of 4 ng, central administration of IL-1β significantly reduced the animals' preference for hypertonic saline. For each individual group of animals, the amount of water intake was significantly different from the amount of hypertonic saline consumed.

Fig. 2 (panel A) shows the effect of pretreatment with naloxone (10 μg) on the antidipsogenic response induced by third ventricle injections of IL-1β (8 ng) in fluid-deprived rats. Analysis of variance showed a significant treatment and time main effects and significant treatment × time interaction [$F(3,25)=49.14$, $P<0.0001$; $F(7,21)=37.29$, $P<0.0001$; $F(21,175)=7.63$, $P<0.0001$, respectively]. Dehydrated rats receiving two consecutive third ventricle injections of saline solution (fluid-deprived saline + saline) drank significantly more than normohydrated controls also receiving central injections of saline (saline + saline). A significant reduction in water intake, similar to that observed in the previous experimental set, was detected in fluid-deprived animals receiving third ventricle injections of IL-1β (8 ng), but pretreated with saline (fluid-deprived saline + IL-1β). On the other hand, there was a significant blockade in the antidipsogenic effect of IL-1β (8 ng) in animals pretreated with naloxone (fluid-deprived naloxone + IL-1β).

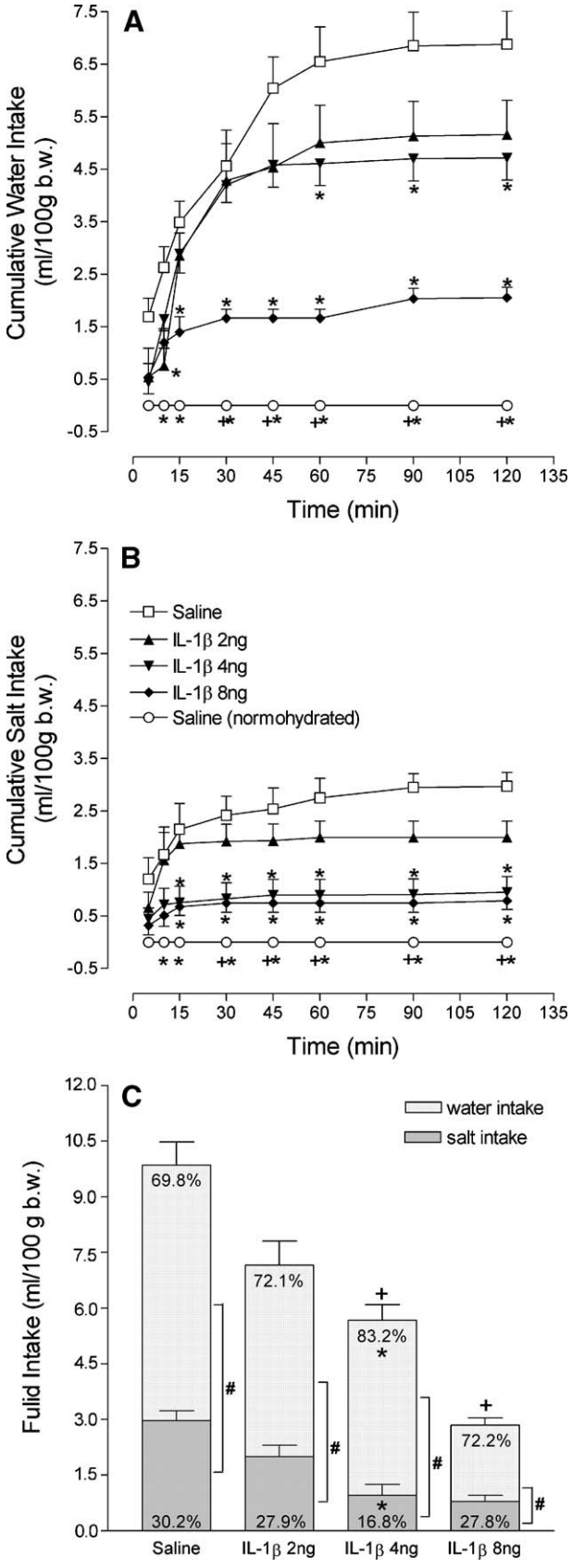

Fig. 1. Cumulative water (Panels A) and salt intake (Panel B) following third ventricle injections of IL-1β at different doses [▲2 ($n=5$); ▼4 ($n=9$); ◆8 ng ($n=7$)] or saline (□; $n=7$) in fluid-deprived rats. An additional group of animals not submitted to water deprivation receiving third ventricle injections of saline is also shown (○; $n=8$). Data is presented as mean±SEM. ⋆ indicates a statistically significant difference (two-way ANOVA followed by Newman–Keul's test; $P<0.05$) when the distinct groups of fluid-deprived animals treated with IL-1β are compared to fluid-deprived animals receiving saline. + indicates a statistically significant difference when the group of animals not submitted to water deprivation is compared to the groups of fluid-deprived rats receiving 2 and 4 ng of IL-1β. # indicates a statistically significant difference when the group of animals not submitted to water deprivation is compared to the groups of fluid-deprived rats receiving 2 ng of IL-1β. Each curve in the graph has been obtained from a naïve group of animals. Panel C displays the percentage of total fluid consumed as water and saline at the end of the experimental session (120 min after the reintroduction of the bottles into the cages) in the same groups shown in panels A and B. ⋆ indicates a statistically significant difference (one-way ANOVA followed by Newman–Keul's test; $P<0.05$) when the group of fluid-deprived animals treated with IL-1β at the dose of 4 ng is compared to all other fluid-deprived groups. + indicates a statistically significant difference when the total amount of fluid intake in the groups treated with IL-1β are compared to the saline-treated control group. # indicates a statistically significant difference when the percentage of water intake is compared with the percentage of hypertonic saline intake in each of the groups.

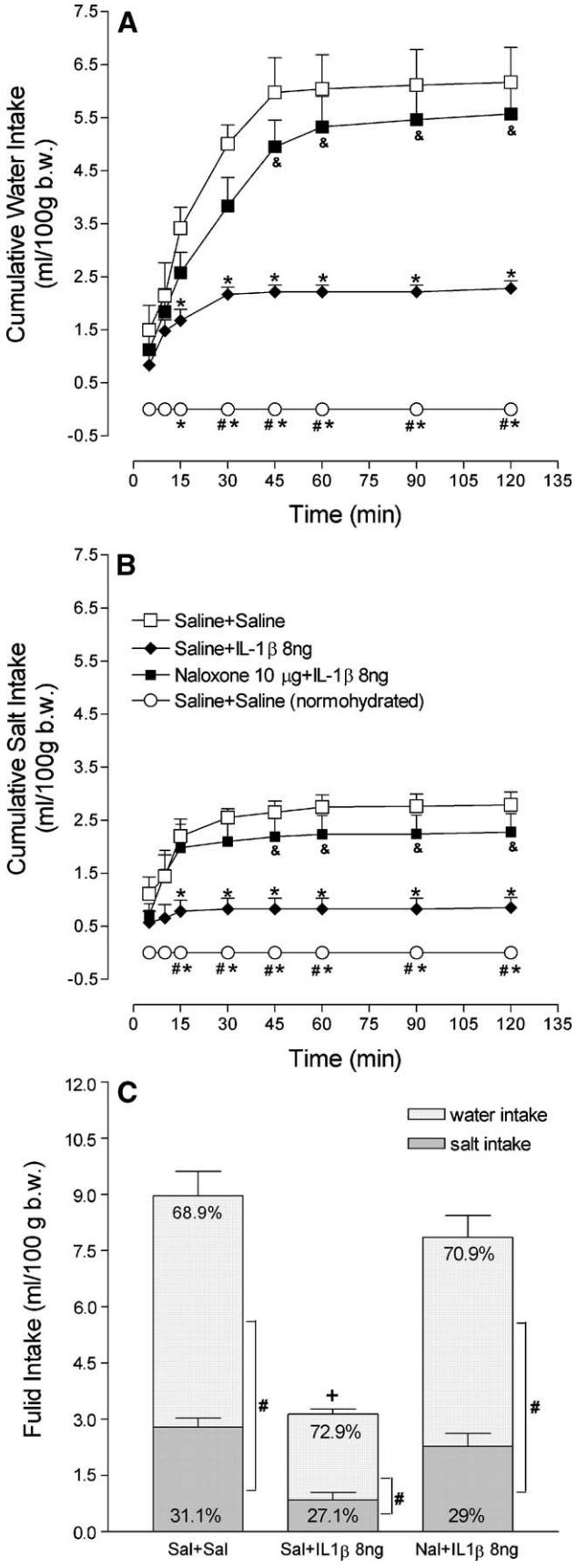

Fig. 2 (panel B) presents the effect of pretreatment with naloxone (10 μg) on the antinatriorexic response induced by third ventricle injections of IL-1β (8 ng) in fluid-deprived rats. Analysis of variance showed a significant treatment and time main effects and significant treatment × time interaction [$F(3,25)=23.68$, $P<0.0001$; $F(7,21)=10.57$, $P<0.0001$; $F(21,175)=2.73$, $P=0.0002$, respectively]. After two consecutive third ventricle injections of saline solution, dehydrated rats (fluid-deprived saline + saline) drank significantly more hypertonic saline than normohydrated controls also receiving central injections of saline (saline + saline). A significant reduction in salt intake, similar to that observed in the previous experimental set, was seen in fluid-deprived animals receiving third ventricle injections of IL-1β (8 ng), but pretreated with saline (fluid-deprived saline + IL-1β). On the other hand, animals receiving IL-1β (8 ng) but pretreated with naloxone (fluid-deprived naloxone + IL-1β) had a significant blockade in the antinatriorexic effect of IL-1β.

Fig. 2 (panel C) also shows the percentage of total fluid consumed as water and saline at the end of the experimental session (120 min after the reintroduction of the bottles into the cages) in the same groups shown in panels A and B. One-way ANOVA values are $F(5,40)=49.80$, $P<0.0001$. There was no significant difference in the preferences for water or hypertonic saline among all treatments used. However, within each individual group of animals, the amount of water intake was significantly different from the amount of hypertonic saline consumed.

Fig. 3 (panel A) illustrates the effect of third ventricle injections of IL-1β at different doses on water intake in sodium-depleted animals. Analysis of variance indicated no significant treatment and time main effects and no significant treatment × time interaction [$F(6,30)=0.56$; $P=0.7586$; $F(7,42)=0.263$, $P=0.9673$; $F(42,210)=0.560$, $P=0.9866$, respectively].

Fig. 2. Cumulative water (Panels A) and salt intake (Panel B) in fluid-deprived animals pretreated with third ventricle injections of naloxone (10 μg) or saline before receiving central administration of IL-1β (8 ng), and in control animals receiving two subsequent third ventricle injections of saline. Data are presented as mean ± SEM. The following groups are presented: Saline + Saline (□; $n=8$), Saline + IL-1β (◆; $n=7$) Naloxone + IL-1β (■; $n=8$). An additional group of normohydrated animals receiving two subsequent third ventricle injections of saline is also shown (○; $n=6$). ⋆ indicates a statistically significant difference (two-way ANOVA followed by Newman–Keul's test; $p<0.05$) when the distinct groups of animals are compared to controls fluid-deprived animals (saline + saline). # indicates a statistically significant difference when the group of normohydrated rats is compared to fluid-deprived animals receiving Naloxone + IL-1β. & indicates a statistically significant difference when the group of fluid-deprived rats receiving Naloxone + IL-1β is compared to the group of rats receiving Saline + IL-1β. Each curve in the graph has been obtained from a naïve group of animals. Panel C displays the percentage of total fluid consumed as water and saline at the end of the experimental session (120 min after the reintroduction of the bottles into the cages) in the same groups shown in panels A and B. + indicates a statistically significant difference (one-way ANOVA followed by Newman–Keul's test; $P<0.05$) when the total amount of fluid intake in the group treated with saline + IL-1β is compared to all other groups. # indicates a statistically significant difference when the percentage of water intake is compared with the percentage of hypertonic saline intake in each of the groups.

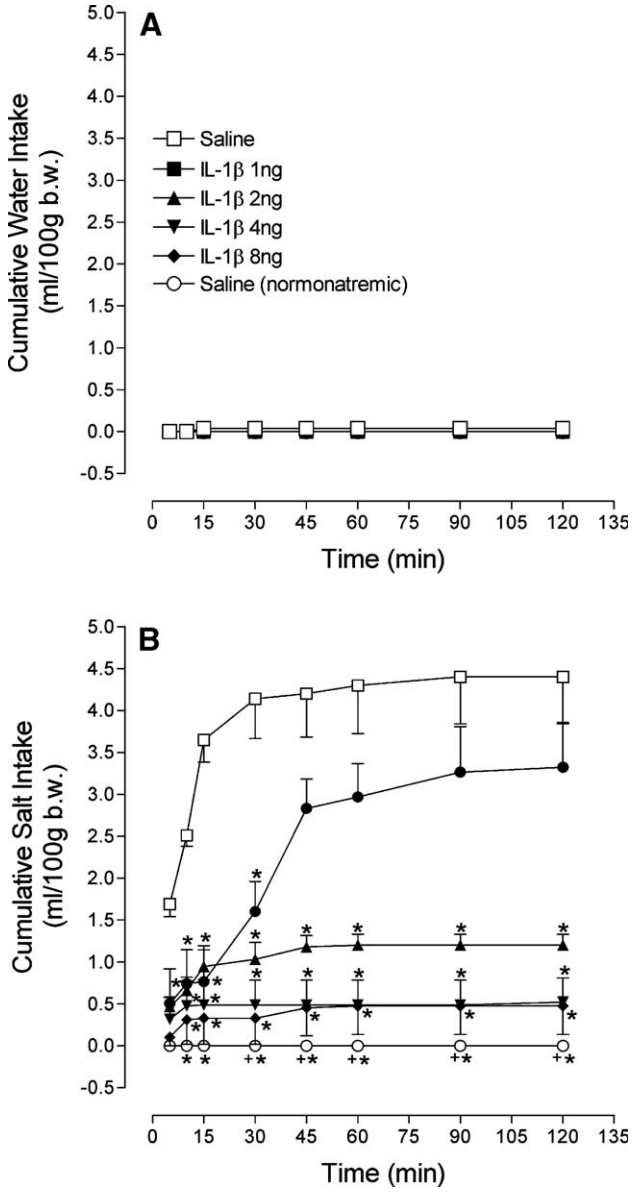

Fig. 3. Cumulative water (Panels A) and salt intake (Panel B) following third ventricle injections of IL-1β at different doses [●1 (n=5); ▲2 (n=5); ▼4 (n=9); ◆8 ng (n=7)] or saline (□; n=7) in sodium-depleted rats. An additional group of animals not submitted to sodium-depletion receiving third ventricle injections of saline is also shown (○; n=8). Data is presented as mean±SEM. ⋆ indicates a statistically significant difference (two-way ANOVA followed by Newman–Keul's test; P<0.05) when the distinct groups of sodium-depleted animals treated with IL-1β are compared to sodium-depleted animals receiving saline. + indicates a statistically significant difference when the group of animals not submitted to sodium-depletion is compared to the groups of sodium-depleted rats receiving 1 ng. Each curve in the graph has been obtained from a naïve group of animals.

Sodium-depleted rats normally reduce their water intake in order to avoid further dilution of the already low plasma sodium concentrations, and in these animals, as expected, there was no water intake.

Fig. 3 (panel B) shows the effect of third ventricle injections of IL-1β at different doses on salt intake in sodium-depleted animals. Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [F(6,30)=48.8; P<0.0001; F(7,42)=172.6, P<0.0001; F(42,210)=229.7, P<0.0001, respectively]. Furosemide-treated, sodium-depleted rats receiving third ventricle injections of

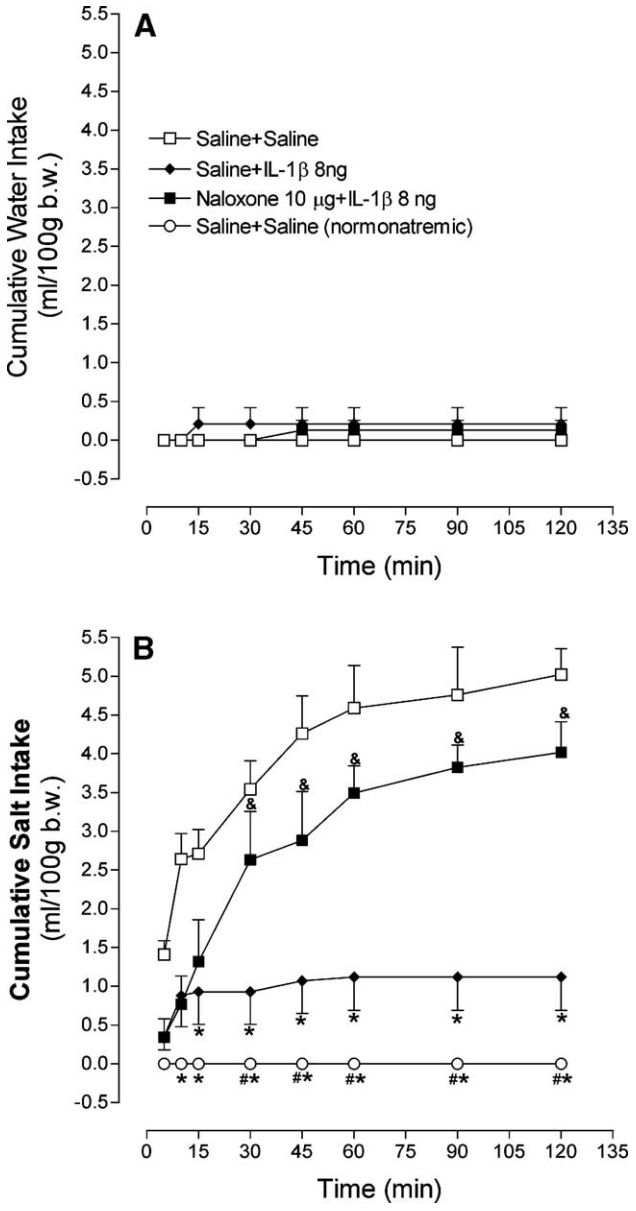

Fig. 4. Cumulative water (Panels A) and salt intake (Panel B) in sodium-depleted animals pretreated with third ventricle injections of naloxone (10 μg) or saline before receiving central administration of IL-1β (8 ng), and in control animals receiving two subsequent third ventricle injections of saline. Data are presented as mean±SEM. The following groups are presented: Saline+Saline (□; n=6), Saline+IL-1β (◆; n=7) Naloxone+IL-1β (■; n=6). An additional group of animals not submitted to sodium-depletion receiving two subsequent third ventricle injections of saline is also shown (○; n=6). ⋆ indicates a statistically significant difference (two-way ANOVA followed by Newman–Keul's test; P<0.05) when the distinct groups of animals are compared to controls sodium-depleted animals (saline+saline). # indicates a statistically significant difference when the group of rats not submitted to sodium-depletion is compared to sodium-depletion animals receiving Naloxone+IL-1β. & indicates a statistically significant difference when the group of sodium-depleted rats receiving Naloxone+IL-1β is compared to the group of rats receiving Saline+IL-1β. Each curve in the graph has been obtained from a naïve group of animals.

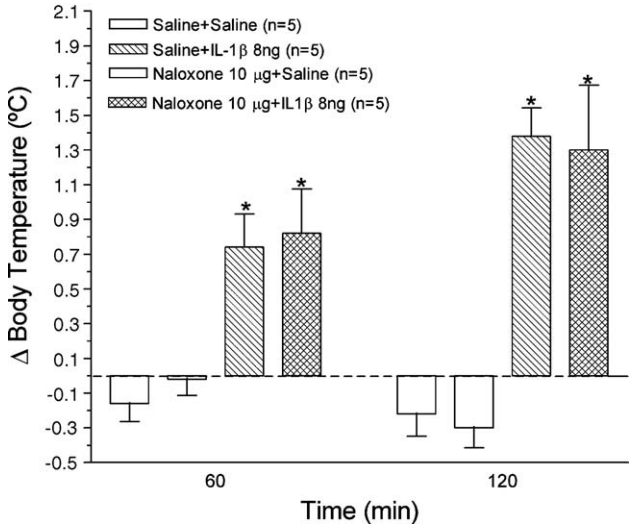

Fig. 5. Change in body temperature of rats following third ventricle injections of IL-1β (8 ng), saline, naloxone (10 μg)+saline and naloxone (10 μg)+IL-1β (8 ng). Data is presented as mean±SEM. ⋆ indicates a statistically significant difference (one-way repeated measure ANOVA followed by Newman–Keul's test; $P<0.05$) when the groups of animals receiving IL-1β, naloxone+saline or naloxone+IL-1β are compared to controls. Mean basal temperature (°C) in the various groups were: IL-1β, 38.02±0.19; Saline, 38.52±0.15; naloxone+saline, 38.3±0.12; naloxone+IL-1β, 38.2±0.2.

saline had a significantly higher salt intake compared to normonatremic animals. Here, third ventricle injections of IL-1β at the dose of 1 ng reduced salt intake in sodium-depleted animals only after 15 and 30 min. At the doses of 2, 4 and 8 ng, the central administration of IL-1β significantly inhibited salt intake for the entire duration of the experiment in sodium-depleted animals.

Fig. 4 (panel A) shows the effect of the pretreatment with naloxone (10 μg) on water intake after third ventricle injections of IL-1β (8 ng) in sodium-depleted rats. Analysis of variance indicated no significant treatment and time main effects and no significant treatment×time interaction [$F(4,20)=0.60$; $P=0.6669$; $F(7,28)=0.284$, $P=0.9593$; $F(28,140)=0.60$, $P=0.9424$, respectively]. Here, as in the previous experiment, sodium-depleted rats presented a negligible water intake that remained unchanged following all treatments.

Fig. 4 (panel B) shows the effect of pretreatment with naloxone (10 μg) on the antinatriorexic response induced by third ventricle injections of IL-1β (8 ng) in sodium-depleted rats. Analysis of variance indicated significant treatment and time main effects and significant treatment×time interaction [$F(4,20)=20.06$; $P<0.0001$; $F(7,28)=13.76$, $P<0.0001$; $F(28,140)=6.69$, $P<0.0001$, respectively].

After two consecutive third ventricle injections of saline solution sodium-depleted rats (sodium-depleted saline+saline) drank significantly more hypertonic saline than normonatremic controls also receiving central injections of saline (saline+saline). There was a significant decrease in salt intake, similar to that observed in the previous experimental set, in sodium-depleted animals receiving third ventricle injections of IL-1β (8 ng), but pretreated with saline (sodium-depleted saline+IL-1β). On the other hand, in animals receiving IL-1β (8 ng) but pretreated with naloxone (sodium-depleted naloxone+IL-1β) there was a significant blockade in the antinatriorexic effect of IL-1β.

Fig. 5 shows the effect of the central administration of IL-1β (8 ng) or naloxone on body temperature. The effect of pretreatment with naloxone (10 μg) or saline on the increase in body temperature induced by third ventricle injections of IL-1β is also shown. Analysis of variance indicated significant differences among the distinct treatments [$F(7,4)=10.0$; $P<0.0001$]. As expected, there was a significant increase in body temperature in animals receiving third ventricle injections of IL-1β compared to saline-treated controls. The central administration of naloxone alone was unable to modify body temperature and the hyperthermic effect of IL-1β was not altered by the pretreatment with naloxone.

Table 1 shows that when either fluid-deprived or sodium-depleted animals receiving third ventricle injections of saline solution pretreated with central injections of naloxone are compared to those receiving the same third ventricle injections

Table 1
Effect of third ventricle injections of naloxone (10 μg) or saline on water and salt intake in fluid-deprived and sodium-depleted rats

Time		Treatment							
		Fluid-deprived				Sodium-depleted			
		Water intake		Saline intake		Water intake		Saline intake	
		Saline (8)	Naloxone (9)	Saline (8)	Naloxone (9)	Saline (10)	Naloxone (7)	Saline (10)	Naloxone (7)
5		1.49±0.47	0.38±0.26	1.11±0.32	0.70±0.20	0.00±0.00	0.00±0.00	1.47±0.21	0.36±0.21
10		2.14±0.62	1.13±0.38	1.42±0.41	0.94±0.27	0.00±0.00	0.03±0.03	2.19±0.27	0.81±0.33
15		3.42±0.39	2.15±0.36	1.46±0.41	1.28±2.29	0.02±0.02	0.03±0.03	3.10±0.44	1.12±0.38
30		5.01±0.36	4.33±0.65	1.81±0.41	1.97±0.39	0.02±0.02	0.03±0.03	3.52±0.58	1.68±0.53
45		5.97±0.65	5.42±0.78	2.22±0.36	2.04±0.35	0.02±0.02	0.03±0.03	3.64±0.55	2.50±0.59
60		6.04±0.65	6.05±0.85	2.75±0.22	2.18±0.35	0.02±0.02	0.03±0.03	4.08±0.51	2.96±0.59
90		6.12±0.67	6.41±0.87	2.76±0.23	2.56±0.28	0.02±0.02	0.05±0.05	4.22±0.49	3.95±0.33
120		6.19±0.66	7.29±0.74	2.79±0.24	3.35±0.53	0.02±0.02	0.05±0.05	4.22±0.49	3.97±0.34
ANOVA	Factor A (drug)	$F_{(1,15)}=0.437$ $P=0.5187$		$F_{(1,15)}=0.361$ $P=0.5568$		$F_{(1,15)}=0.0911$ $P=0.7670$		$F_{(1,15)}=4.519$ $P=0.491$	
	Factor B (time)	$F_{(7,7)}=43.695$ $P<0.0001$		$F_{(7,7)}=12.813$ $P<0.0001$		$F_{(7,7)}=1.5480$ $P=0.1593$		$F_{(7,7)}=23.74$ $P<0.0001$	
	drug×time	$F_{(7,105)}=1.448$ $P=0.1942$		$F_{(7,105)}=0.751$ $P=0.6296$;		$F_{(7,105)}=0.3231$ $P=0.9420$		$F_{(7,105)}=1.86$ $P=0.0834$	

Data are expressed as mean±SEM. The number of animals used in each experiment is indicated in parenthesis.

Table 2
Number of areas entered during 15 min in the "open field" test cage carried out in rats receiving third ventricle injections of IL-1β (8 ng) or isotonic saline solution

Treatment	Number of areas entered
Saline (6)	45.0±2.25
IL-1β (8)	41.9±3.70

The number of animals used is indicated in parenthesis. Data are expressed as mean±SEM. There was no statistically significant difference (t test; $p<0.05$) between the group of animals receiving IL-1β compared to saline-treated animals.

of saline but pretreated with saline, no statistically significant difference in water and salt intake was detected.

Table 2 shows that third ventricle injections of IL-1β (8 ng) failed to alter the pattern of locomotor activity exhibited by animals receiving third ventricle injections of saline solution in the open-field test.

Table 3 summarizes the results of the dessert test. In this experimental protocol, saline-treated control animals drank more saccharin than water, indicating the hedonic behavior represented by the preferential intake of a "tasty" solution. Third ventricle injections of IL-1β (8 ng) failed to alter this hedonic preference. Indeed, animals receiving IL-1β drank the same amount of saccharin as saline-treated controls, although water intake decreased in this group compared to saline-treated controls.

4. Discussion

The present data clearly show that third ventricle injections of IL-1β induce a significant decrease in water and salt intake in rats submitted to fluid deprivation, and significantly reduced salt intake in sodium-depleted rats. This study also shows that both the antidipsogenic and the antinatriorexic effects generated by the central administration of IL-1β seem to require the functional integrity of the brain opioidergic system, since pretreatment with the non-specific opioid antagonist naloxone was able to block these effects.

Several brain mechanisms controlling both visceral and behavioral responses may be affected by peripheral and central cytokines such as IL-1β. Peripheral IL-1β may influence the central nervous system through a number of mechanisms including signaling to the brain via peripheral nerves, direct action on brain parenchyma after crossing the blood-brain barrier using specific transporters, or interaction with brain areas in which this barrier is lacking (Banks and Kastin, 1996). Conversely, IL-1β endogenously produced by neurones and astrocytes may explain some aspects of the cytokinergic modulation of brain function (Licinio and Wong, 1997; Rothwell and Luheshi, 2000). In the present study, we were particularly interested in this aspect and this prompted us to use an experimental protocol in which IL-1β was always injected directly into the brain. Therefore, it seems logical to suggest that the inhibitory action of IL-1β on water and salt intake observed here is a consequence of the activation of an intracerebral interleukinergic system. It is noteworthy that this specific component activates brain areas that are different from those allegedly reached by blood-borne cytokines and is independently activated during peripheral conditions associated with inflammation and other pathological situations. Indeed, the increase in brain synthesis of IL-1α, IL-1β and TNF-α by intraperitoneal LPS injections is a well-known phenomenon (Watkins et al., 1995).

Fever, lethargy, slow wave sleep, hypomotility and decreased food intake are among the behavioral and visceral effects triggered by the actions of cytokines in the brain (Rothwell et al., 1996; Plata-Salamán, 1998). Some authors have reported a significant inhibition of water intake by cytokines (Chance and Fisher, 1991; Karádi et al., 2005; Osaka et al., 1992; Plata-Salamán and Ffrench-Mullen, 1992; Nava et al., 1996). The data presented here would seem to reinforce the inhibitory role of brain IL-1β on water intake.

We failed to find any study that had investigated the effects of the central administration of IL-1β on salt intake in rats. A study conducted by Osaka et al. (1992) showed that, in rats not submitted to sodium depletion, sodium intake remains unaffected by peripheral (intraperitoneal) injections of IL-1β. As we stated above, brain areas activated by blood-borne cytokines are different from those stimulated by cytokines endogenously produced by the brain. Therefore, it may be possible that the brain areas reached by third ventricle injections of IL-1β are involved in the antidipsogenic and antinatriorexic effects of this peptide, whereas central sites reached by IL-1β after intraperitoneal administration are not linked to the control of salt intake. It is important to emphasize that the present study is the first to analyze the effects of the central administration of IL-1β on salt appetite while, at the same time, establishing a clear link between the central interleukinergic and opioidergic systems in the control of fluid intake behaviors. Nevertheless, considering the scarcity of data on the effects of IL-1β on water intake and sodium appetite, additional investigation is certainly necessary.

Water and salt intake is regulated by complex interactions involving various interconnected brain areas in which different neurotransmitters binding to a variety of selective receptors lead to the perception of specific sensations related to the necessity to restore water and salt, and trigger the motor activities involved in the acquisition of those vital elements (Johnson and Thunhorst, 1997; Stricker and Sved, 2000). The data presented here seem to provide further evidence of the inhibitory role of brain IL-1β on water intake and additionally show that the

Table 3
Cumulative water and saccharin intakes (ml/100 g body weight) during 2 h in the test cage in rats receiving third ventricle injections of IL-1β (8 ng) or isotonic saline solution

Treatment	Fluid intake	
	Water	Saccharin
Saline (8)	1.89±0.42	5.40±0.41
IL-1β (7)	0.64±0.18*	5.38±0.66

The number of animals used is indicated in the parenthesis. Data are expressed as mean±SEM. Asterisk indicates a statistically significant difference (t test; $P<0.05$) when the groups of animals receiving IL-1β are compared to controls.

activation of the central interleukinergic system seems to induce a significant antinatriorexic effect.

The total fluid intake of control, dehydrated animals receiving third ventricle injections of saline comprised 69.8% of water and 30.2% of hypertonic saline. The total fluid intake decreased significantly following third ventricle injections of IL-1β, at the doses of 4 and 8 ng. The central administration of IL-1β, at the dose of 4 ng, selectively decreased the animals' preference for hypertonic saline as shown by the significant reduction in the percentage of consumption of this solution in this group of animals compared to all the other groups. At the highest dose used, IL-1β caused a remarkable reduction in the total fluid intake although the preference for saline remained unchanged compared to drug-free controls. This may mean that, at certain doses, the stimulation of the central interleukinergic system provokes an inhibitory drive in salt intake that overcomes the water intake inhibitory drive. It seems that the stimulation of this system by IL-1β at higher doses yields an inhibitory drive that acts with similar intensity on the subsystems controlling both water and salt intake.

In the present study, the inhibitory effects of third ventricle injections of IL-1β on water and salt intake seem to rely on opioid-dependent mechanisms. The brain opioid system exerts complex effects in the control of water and salt intake that depend on the anatomical location and the type of opioid receptor involved in that particular response. Both stimulatory and inhibitory effects of the central opioid system on water and salt intake have been described, depending on the doses of the pharmacological agents and experimental protocols used (Fitzsimons, 1998). Based on the experimental protocol employed here, we suggest that the action of IL-1β on the central nervous system activates an opioid-dependent, naloxone-blockable inhibition of water and salt intake. Opioid inhibition of water and salt intake has been reported by several groups (Summy-Long et al., 1981, 1983; Ruegg et al., 1994) and data showing that intracerebroventricular injections of morphine decrease water intake in rats have also been published (Eidi et al., 2003).

The immune and opioid systems seem to be functionally linked by reciprocal interactions that allow opioids to regulate immune functions and the immune system to modulate some actions of the opioid systems (Ruzicka and Akil, 1997). Indeed, the concentration of opioids in the hypothalamus is increased by IL-1β (Murphy et al., 1983). On the other hand, this peptide increases opioid production by glial cells in the central nervous system (Ruzicka et al., 1996). Therefore, opioid-dependent inhibition of water and salt intake may be, at least in part, one of the mechanisms explaining the antidipsogenic and antinatriorexic effects of IL-1β demonstrated here.

At the dose used here, naloxone was unable to block water and salt intake in fluid-deprived and sodium-depleted rats not treated with third ventricle injections of IL-1β. This may mean that, in the experimental conditions used here, in which animals have to replenish water and salt, the brain opioid system related to water and salt intake control is silent. However, under these same conditions, despite the need for water and sodium, central activation of the interleukinergic system triggers an opioid-dependent, inhibitory drive that impairs drinking behavior and sodium appetite.

We have used two different experimental protocols in which distinct physiological stimuli are activated. It is important to note that fluid-deprived animals drink both water and hypertonic saline, while sodium-depleted rats drink only hypertonic saline. Water-deprived animals normally present clear-cut natriuresis that induces sodium depletion (McKinley et al., 1983). Also, there in an increase in water and salt intake in animals submitted to a 24-h period of dehydration that is consequent to the release of angiotensin II induced by hypovolemia. Animals submitted to sodium depletion have a significant increase in salt intake that also depends on angiotensin II. However, during sodium depletion, hyponatremia strongly inhibits water intake to avoid a further dilution of plasma sodium concentration. Therefore our data, in which fluid-deprived animals exhibited a significant increase both in water and salt intake while sodium-depleted rats drank only hypertonic saline, are in accordance with the data published by other groups (Johnson and Thunhorst, 1997).

We chose the doses of IL-1β used in the present paper based on a detailed study conducted by Plata-Salamán et al. (1996) that accurately associates IL-1β administration with the endogenous brain levels of this cytokine in physiopathological conditions.

In the present study, central administration of IL-1β induced a significant increase in body temperature. The effect of enhanced body temperature on water intake is complex. To cope with the increase in core temperature, many species utilize evaporative heat loss mechanisms (panting, sweating and saliva spreading) that lead to electrolyte and water loss both from cellular and extracellular compartments. In the early stages of heating, plasma volume is normally preserved and the main stimulus that triggers water intake is the increase in plasma osmolality (Barney and West, 1990; Barney, 1997; Kregel et al., 1990). On the other hand, heating increases the temperature of blood reaching hypothalamic areas related to the control of thermoregulatory responses and water intake and may lead to drinking behavior even in the absence of dehydration (Andersson and Larsson, 1961). In view of the association between body temperature and water intake, it is important to consider that studies exploring the effect of heating on drinking behavior normally use heat exposure protocols to induce increases in body temperature. These protocols vary in the degree of heat exposure to which the animals are submitted, leading to significant differences in water and electrolyte losses and consequently producing remarkable differences in the amount of fluid intake, as well as in the time of rehydration after the increase in body temperature.

In the present study, pretreatment with naloxone was unable to modify the increase in body temperature induced by third ventricle injections of IL-1β. This is in agreement with data produced elsewhere showing that the intracerebroventricular injections of naloxone did not alter fever induced by a large number of cytokines, including IL-1β (Romanovsky and Blatteis, 1998; Clark and Harris, 1978). However, it is important to note that other investigators have shown that fever induced

by central administration of IL-1β was attenuated by selective μ-opioid receptor antagonists (Chio et al., 2005). It seems that the ability of naloxone to block cytokine-induced fever results from its interaction with peripheral opioid receptors (Romanovsky and Blatteis, 1998; Zawada et al., 1997).

Nonetheless, dehydrated animals receiving IL-1β reduced their water intake. This indicates that the central mechanisms controlling water intake and body temperature are independently affected by third ventricle injections of IL-1β and that central activation of IL-1β receptors by IL-1β neutralizes the thirst-inducing stimulus normally represented by increased body temperature.

The actions of cytokines such as IL-1β in the central nervous system result in a group of coordinated responses that reduce the animal's energy expenditure. These responses may include decreased locomotor activity and exploratory behavior, diminished social interaction, slow-wave sleep, anorexia and inhibition of water intake, a wide-ranging collection of behavioral and visceral responses that facilitates survival following many pathological states by promoting energy conservation. The inhibition of water and salt intake elicited by the central administration of IL-1β observed in the present study does not seem to be due to a reduction in locomotor activity, since animals receiving third ventricle injections of IL-1β at the doses used here did not modify their locomotor patterns in the open-field test as compared to saline-treated controls.

Animals receiving central injections of IL-1β or saline solution drank the same amounts of saccharin in the "dessert test". This further confirms that IL-1β-induced water and salt intake inhibition does not represent a non-specific inhibition of all ingestive behaviors.

In summary, the data presented here show that the central administration of IL-1β exert significant antinatriorexic and antidipsogenic effects that seem to require the functional integrity of the brain opioidergic system.

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