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Blockade of central kappa-opioid receptors inhibits the antidipsogenic effect of interleukin-1β

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Abstract

The objective of the present study was to investigate the role of brain kappa-opioid receptors (KOR) in the antidipsogenic effect promoted by third ventricle injections of interleukin-1β (IL-1β). Wistar male rats were submitted to three different, thirst-inducing, physiological conditions: dehydration induced by water deprivation, hyperosmolarity induced by salt-load and hypovolemia induced by polyethylene glycol subcutaneous injection. Third ventricle injections of IL-1β significantly inhibited the increase in water intake observed in those situations. The pharmacological blockade of central KOR by the selective KOR antagonist nor-binaltorphimine (BNI) at different doses significantly inhibited the antidipsogenic effect induced by the central administration of IL-1β in all conditions tested: dehydration, hypovolemia and hyperosmolarity. The central administration of IL-1β failed to induce any locomotor deficit, as verified in an open field test. Stimulation of the central interleukinergic component did not result in any general suppression of ingestive behavior since no change in saccharin intake was recorded during a dessert test in animals receiving central injections of IL-1β. Furthermore, the inhibitory effects of IL-1β on water intake cannot be attributed to sickness-like effects induced by these compounds, since an aversion test excluded this possibility. In summary, the data shown in the present study clearly show that the antidipsogenic effect observed in rats following third ventricle injections of IL-1β depend on the functional integrity of a brain kappa-opioid-dependent component.

Keywords: Interleukin; Water intake; Opioids; Kappa-opioid receptors; Hypovolemia; Dehydration; Hyperosmolarity

1. Introduction

It is well-known that peripheral and central administration of interleukin-1β (IL-1β), an immunoregulatory cytokine, induces significant inhibition of water intake and that endogenous IL-1β may play a role in the control of thirst (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).

The central opioid system participates in the control of water and salt intake. Intracerebroventricular injections of morphine have been found to decrease water intake in rats (Eidi et al., 2003), while central opiatergic stimulation has been shown to reduce water intake provoked by the activation of central angiotensinergic pathways (Summy-Long et al., 1981, 1983).

In a previous study we showed that central administration of IL-1β inhibits water and salt intake in rats in several different situations, and that this effect seems to depend on the functional integrity of the brain opiatergic system. Indeed, it was shown that, in rats, central administration of IL-1β inhibits water intake after dehydration, hyperosmolarity and hypovolemia, and that this antidipsogenic effect of IL-1β appears to rely on an opioid-dependent mechanism (Luz et al., 2006). We

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have also shown that intracerebroventricular administration of IL-1β decreases salt intake in fluid-deprived and sodium depleted rats. Moreover, in this case, the expression of the antidipsogenic and antinatriorexic effect of IL-1β depends on the activity of brain opioid system since these effects are blocked by treatment with non-specific opioid antagonists (De Castro e Silva et al., 2006).

Distinct opioid receptors mediate drinking behavior. Indeed, both kappa- and mu-opioid receptor subtypes are involved in the control of water intake induced by angiotensin II or hypertonic saline (Ruegg et al., 1994), while mu-opioid receptors are involved in deprivation-induced water intake (Beczkowska et al., 1992), and deprivation-induced water intake is suppressed by the pharmacological blockade of both kappa- and mu-opioid receptors (Bodnar et al., 1995).

The involvement of specific opioid receptors in the antidipsogenic effects resulting from the central administration of IL-1β is the next logical question to be asked. As mentioned above, both mu- and kappa-opioid receptors seem to mediate water intake and both may play a role in the mechanisms leading to the antidipsogenic effects of IL-1β. Since selective KOR antagonists that constitute appropriate pharmacological tools for the evaluation of the physiology of KOR are available, the present study investigated the participation of these receptors in the inhibition of water intake induced by central administration of IL-1β in three different situations: dehydration induced by water deprivation, hyperosmolarity induced by salt-load and hypovolemia induced by subcutaneous injection of polyethylene glycol.

2. Materials and methods

2.1. Animals

Wistar male rats (220 ± 20 g) kept under controlled light (lights on from 7 AM to 7 PM) and temperature (22–24 °C) conditions with free access to tap water and laboratory chow (Nuvital Nutrientes Ltda, Curitiba, Brazil) were used in the present study. All experimental sets were conducted in naïve rats. Groups of rats used in an experimental set were not reused in any other part of the study. The experimental protocols were conducted according to the regulations established by the National Institutes of Health (USA).

2.2. Surgical procedure

Six days prior to the experimental sessions, third ventricles were cannulated under sodium pentobarbital anesthesia (50 mg/kg i.p.). 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; and vertical = 8.0 mm below the skull. To avoid lesions to the brain regions involved in the control of cardiovascular and body fluid homeostasis, the animals were fixed to the stereotaxic apparatus with the head inclined upwards (+2.0 mm). 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. Blue Evans dye injections were 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 – *Escherichia coli* derived) purchased from R&D Systems (catalog number 201-LB); polyethylene glycol (m.w. 15,000–20,000; PEG) and nor-binaltorphimine (BNI), KOR antagonist, purchased from Sigma Co. (catalog number N1771). The drugs were dissolved in sterile isotonic saline solution. Third ventricle injections were given using a Hamilton microsyringe connected to a needle through polyethylene tubing (PE 10). A total volume of 2 μl was slowly injected (60 s).

2.4. Experimental protocols

2.4.1. Dehydration

Different groups of naïve animals received third ventricle injections of the drugs or isotonic saline solution (controls) after 14 h of water deprivation (from 18:00 to 08:00 the night before the experiment). Thirty minutes after the central injections, graduated bottles containing water were reintroduced into the cages. Cumulative water intake was measured for the next 120 min. These groups of animals were also compared to an additional normohydrated group not submitted to water deprivation, which received third ventricle injections of saline.

2.4.2. Hypovolemia

A 30% PEG solution was prepared in 0.15 M sodium chloride by heating the mixture to approximately 50 °C while stirring constantly. This solution was administrated subcutaneously (2 ml/100 g) 4 h before the icv injections of drugs or isotonic saline solution (controls). Graduated bottles were removed from the cages immediately before PEG administration and reintroduced 30 min after the icv injections. Cumulative water intake was measured over the next 120 min. These groups of

animals were also compared to an additional group receiving subcutaneous injections of isotonic saline at the same volume as the PEG solution followed by third ventricle injections of saline.

2.4.3. Hyperosmolarity

Animals were fasted for 14 h, between 18:00 and 08:00, the night before the experiment. Ten minutes after third ventricle injections of drugs or isotonic saline solution (controls) the animals received an intragastric salt-load. This was achieved by administrating 1 ml/100 g of a hypertonic saline solution (1.5 M) via orogastric tubing. Twenty minutes after the salt-load, graduated bottles containing water were reintroduced into the cages and the cumulative water intake was recorded over the next 120 min. In this experimental set, the graduated bottles were removed from the cages immediately before the intracerebroventricular (icv) injections, and were reintroduced 30 min later. These groups of animals were compared to an additional group receiving intragastric administration of isotonic saline solution followed by third ventricle injections of saline.

2.4.4. Dessert test

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), was investigated to evaluate whether the inhibition of water intake induced by the central administration of IL-1β was due to a non-specific, general inhibition of the central nervous system or to a locomotor deficit. In this experiment, after third ventricle cannulations, 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 training period, 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. The intake of water and saccharin was then recorded during the following 120 min.

2.4.5. Aversion test

To ascertain that IL-1β was devoid of non-specific inhibitory "illness-like" effects on water intake, a taste aversion test was performed. The protocol employed was based on the experimental design proposed by Nachman (1970) in which lithium chloride is used to make rats ill in temporal association with the novel taste of saccharin. Seven days after third ventricle cannulation, the animals' access to water was restricted to 15 min/day (between 12:00 and 12:15 h) for four consecutive days. The rats were divided into three different groups on the fifth day. The first group (controls) received two immediately consecutive injections of isotonic saline solution, the first being intraperitoneal (0.6% b.w.) and the second into the third ventricle (2 μl). In the second group of animals, intraperitoneal injections of lithium chloride 0.15 M (0.6% b.w.) were followed by third ventricle injections of isotonic saline solution. The third group of animals received intraperitoneal injections of isotonic saline solution in the same amount used in the previous group, followed by third ventricle injections of IL-1β (8 ng). On this same fifth day, all groups of animals had access to bottles containing saccharin (0.25%) for 1 min immediately before the injections, and for an additional 14 min immediately after. On the next day, at the same time that bottles had been available on the previous days (12:00–12:15 h), saccharin-containing bottles were introduced into all cages and the volume ingested recorded.

2.4.6. Body temperature measurement

To measure the effect of the central administration of IL-1β 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 continuously shows body core temperature (T_c) on a digital display.

2.4.7. Open field test

Different groups of rats receiving third ventricle injections of IL-1β or saline were submitted to an open field test to test whether the central administration of IL-1β was capable of inducing a reduction in locomotor activity significant enough to explain the inhibition of water intake observed here.

The apparatus consisted of a circular wooden box (60 cm in diameter and 60 cm in height) 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 by the number of floor units into which the animals entered with all four paws).

The experiments took place in a sound-attenuated, temperature-controlled (24 ± 1 °C) room between 7 AM and 12 noon. 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.

2.5. Statistical analysis

A computer software package (GraphPad, San Diego, CA) was used to perform two-way (treatment and time as factors) analysis of variance for repeated

measures in each experimental set. The post-hoc Bonferroni test was used for comparison of each treatment. Differences between the groups were considered statistically significant when $p < 0.05$. The data are presented as means ± SEM. Unpaired Student's *t*-test was used to analyze the data obtained from the open field and dessert tests. Data from the aversion tests were treated using the one-way analysis of variance followed by the post-hoc Bonferroni test.

3. Results

Fig. 1 depicts the antidipsogenic effect of the central administration of IL-1β in dehydrated rats and the inhibition of this effect by central pretreatment with BNI, a specific kappa-opioid receptor antagonist, at different doses. Analysis of variance showed a significant treatment x time effect and significant treatment × time interaction. [Factor A = treatment $F(5,343) = 85.47$; $p < 0.0001$; Factor B = time $F(7,343) = 137.64$; $p < 0.0001$; treatment × time interaction $F(35,343) = 15.05$; $p < 0.0001$]. As expected, there was a significant increase in water intake in the control, water-deprived rats (those receiving saline + saline) compared to normohydrated rats receiving saline + saline. Intracerebroventricular administration of IL-1β (8 ng) to water-deprived rats induced a significant decrease in water intake (saline + -saline compared to saline + IL-1β). At all doses used (30, 60 and 120 ng), pretreatment with BNI impaired the antidipsogenic effect of the central administration of IL-1β (saline + IL-1β compared to BNI + IL-1β) in a dose-dependent fashion.

Fig. 2 shows the antidipsogenic effect of the central administration of IL-1β in hypovolemic rats and the inhibition of this effect by central pretreatment with BNI at different doses. [Factor A = treatment $F(5,378) = 80.80$; $p < 0.0001$; Factor B = time $F(7,378) = 211.40$; $p < 0.0001$; treatment × time interaction $F(35,378) = 15.40$; $p < 0.0001$]. Here, a significant increase occurred in water intake in the control hypovolemic rats (those receiving saline + saline) compared to the euvolemic animals receiving saline + saline. The cen-

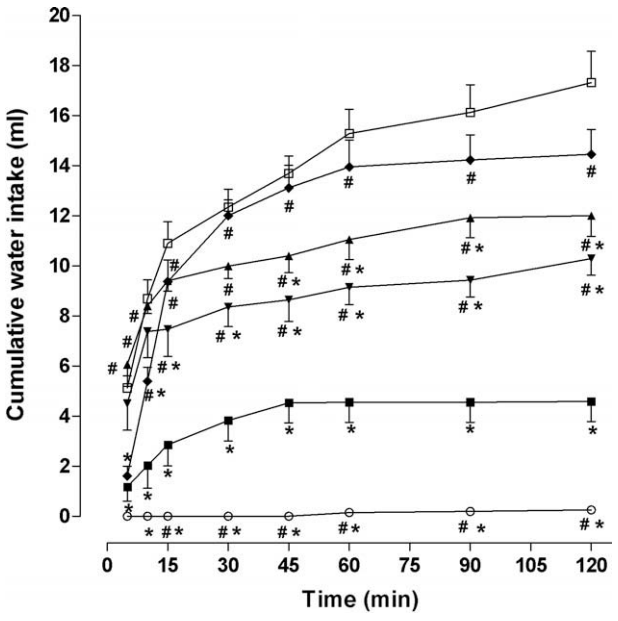

Fig. 1. Cumulative water intake in dehydrated animals pretreated with third ventricle injections of BNI at different doses before receiving central administration of 8 ng of IL-1β [▼: BNI 30 ng + IL-1β ($n = 7$); ▲: BNI 60 ng + IL-1β ($n = 9$); ♦: BNI 120 ng + IL-1β ($n = 9$); or ■: saline + IL-1β ($n = 10$)], and in control animals receiving two subsequent third ventricle injections of saline [□: saline + saline ($n = 8$)]. Data are presented as mean ± SEM. An additional group of normohydrated animals receiving third ventricle injections of saline is also shown (○; $n = 12$). ★ indicates a statistically significant difference (two-way ANOVA followed by post-hoc Bonferroni test; $p < 0.05$) when all groups of animals are compared to controls (saline + saline). #Indicates a statistically significant difference when the groups of dehydrated rats receiving BNI + IL-1β are compared to the group of rats receiving saline + IL-1β. Each curve in the graph was obtained from a naïve group of animals.

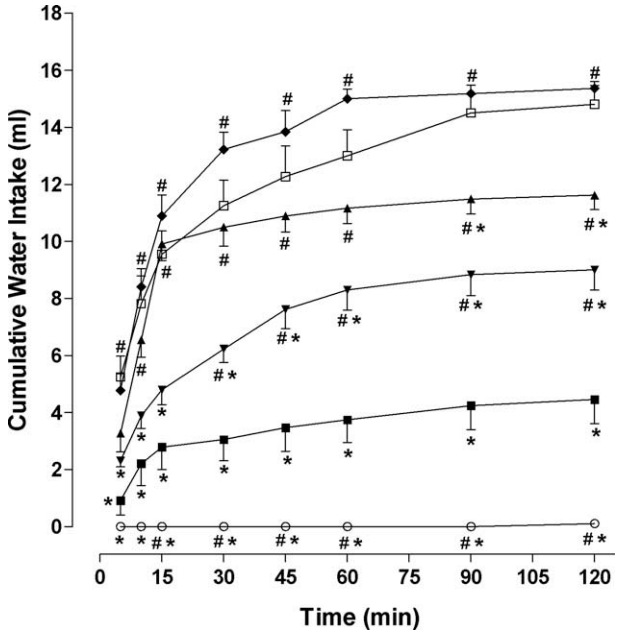

Fig. 2. Cumulative water intake in hypovolemic animals pretreated with third ventricle injections of BNI at different doses before receiving central administration of 8 ng of IL-1β [▼: BNI 30 ng + IL-1β ($n = 8$); ▲: BNI 60 ng + IL-1β ($n = 11$); ♦: BNI 120 ng + IL-1β (n=11); or ■: saline + IL-1β ($n = 12$)], and in control animals receiving two subsequent third ventricle injections of saline [□: saline + saline ($n = 8$)]. Data are presented as mean ± SEM. An additional group of normovolemic animals receiving third ventricle injections of saline is also shown (○; $n = 9$). ★ indicates a statistically significant difference (two-way ANOVA followed by post-hoc Bonferroni test; $p < 0.05$) when all groups of animals are compared to controls (saline + saline). #Indicates a statistically significant difference when the groups of hypovolemic rats receiving BNI + IL-1β are compared to the group of rats receiving saline + IL-1β. Each curve in the graph was obtained from a naïve group of animals.

tral administration of IL-1β (8 ng) to hypovolemic rats induced a significant decrease in water intake compared to that of control hypovolemic rats treated with saline (saline + saline compared to saline + IL-1β). As in the previous experimental set, pretreatment with BNI at all doses used (30, 60 and 120 ng) blocked the antidipsogenic effect of the central administration of IL-1β (saline + IL-1β compared to BNI + IL-1β) in a dose-dependent way.

Fig. 3 shows the antidipsogenic effect of the central administration of IL-1β in salt-loaded rats and the blockade of this effect by central pretreatment with BNI at different doses. [Factor A = treatment $F(5,315) = 43.63$; $p < 0.0001$; Factor B = time $F(7,315) = 203.85$; $p < 0.0001$; treatment × time interaction $F(35,315) = 13.66$; $p < 0.0001$]. In this case, there was a significant increase in water intake in the control salt-loaded rats (those receiving saline + saline) compared to the rats not submitted to salt-load that received saline + saline. The central administration of IL-1β (8 ng) to salt-loaded rats induced a significant decrease in water intake compared to that of control salt-loaded rats treated with saline (saline + saline compared to saline + IL-1β). Central pretreatment with BNI reversed the antidipsogenic effect evoked by the central administration of IL-1β; however, in this situation the dose-dependent mode seen in the two preceding experimental sets was not observed.

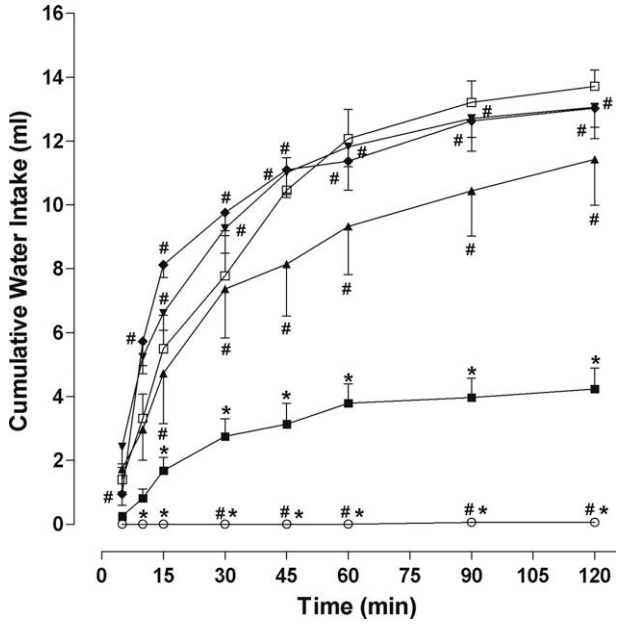

Fig. 3. Cumulative water intake in salt-loaded rats pretreated with third ventricle injections of BNI at different doses before receiving central administration of 8 ng of IL-1β [▼: BNI 30 ng + IL-1β ($n = 13$); ▲: BNI 60 ng + IL-1β ($n = 7$); ♦: BNI 120 ng + IL-1β ($n = 7$); or ■: saline + IL-1β ($n = 12$)], and in control animals receiving two subsequent third ventricle injections of saline [□: saline + saline ($n = 7$)]. Data are presented as mean ± SEM. An additional group of rats not submitted to salt-load receiving third ventricle injections of saline is also shown (○; $n = 8$). ★ indicates a statistically significant difference (two-way ANOVA followed by post-hoc Bonferroni test; $p < 0.05$) when all groups of animals are compared to controls (saline + saline). #Indicates a statistically significant difference when the groups of salt-loaded rats receiving BNI + IL-1β are compared to the group of rats receiving saline + IL-1β. Each curve in the graph was obtained from a naïve group of animals.

Fig. 4 shows the percentage change in overall (120 min) water intake in dehydrated, hypovolemic and hyperosmotic animals receiving IL-1β (8 ng) and pretreated with saline or with BNI at several doses (30, 60 and 120 ng) relative to the mean water intake presented by the drug-free control groups in each of the thirst-inducing physiological conditions studied. It is possible to observe that the action of BNI in inhibiting the antidipsogenic effect of IL-1β in dehydrated and hypovolemic rats, in which the dose response pattern is clear, is completely different from that seen in hyperosmotic rats, in which BNI, at all doses used, successfully blocked the antidipsogenic effect of IL-1β at the same magnitude. It is also interesting to note that BNI, at the lowest dose used (30 ng), led to a reduction in the antidipsogenic effect of IL-1β in hyperosmotic animals that was significantly greater than that observed in dehydrated and hypovolemic rats ($p < 0.001$).

Fig. 5 shows that the effect of third ventricle injections of BNI alone at the highest dose used in the previous experimental sets (120 ng) was unable to modify water intake in water-deprived, hypovolemic and salt-loaded rats. Statistical values for this set of experiments are summarized in the legend.

Fig. 6 shows the effect of intracerebroventricular injections of IL-1β or saline on body temperature. Analysis of variance showed a significant treatment and time main effect and significant treatment × time interaction [$F(1,56) = 28.61$, $p = 0.0007$; $F(7,56) = 5.05$, $p = 0.0002$; and $F(7,56) = 9.17$, $p < 0.0001$, respectively]. Body temperature increased significantly in animals receiving third ventricle injections of IL-1β (8 ng) compared to saline-treated controls.

Fig. 7 (upper panel) depicts the effects of the aversion test carried out to investigate whether the inhibition of water intake observed after the central administration of IL-1β could be ascribed to non-specific "illness-like" side effects. Analysis of variance showed a significant treatment effect [$F(2,19) = 22.08$, $p < 0.0001$]. Animals that associate intraperitoneal lithium chloride injections with saccharin intake drink significantly less saccharin the next day compared to controls ($p > 0.05$). The association of IL-1β injections with saccharin intake failed to induce any significant decrease in saccharin intake the next day, apparently indicating that third ventricle injections of IL-1β do not induce illness-like effects.

Fig. 7 (lower panel) shows the results of the dessert test. As expected, saline-treated control animals drank more saccharin than water. This indicates the well-known hedonic behavior represented by the preferential

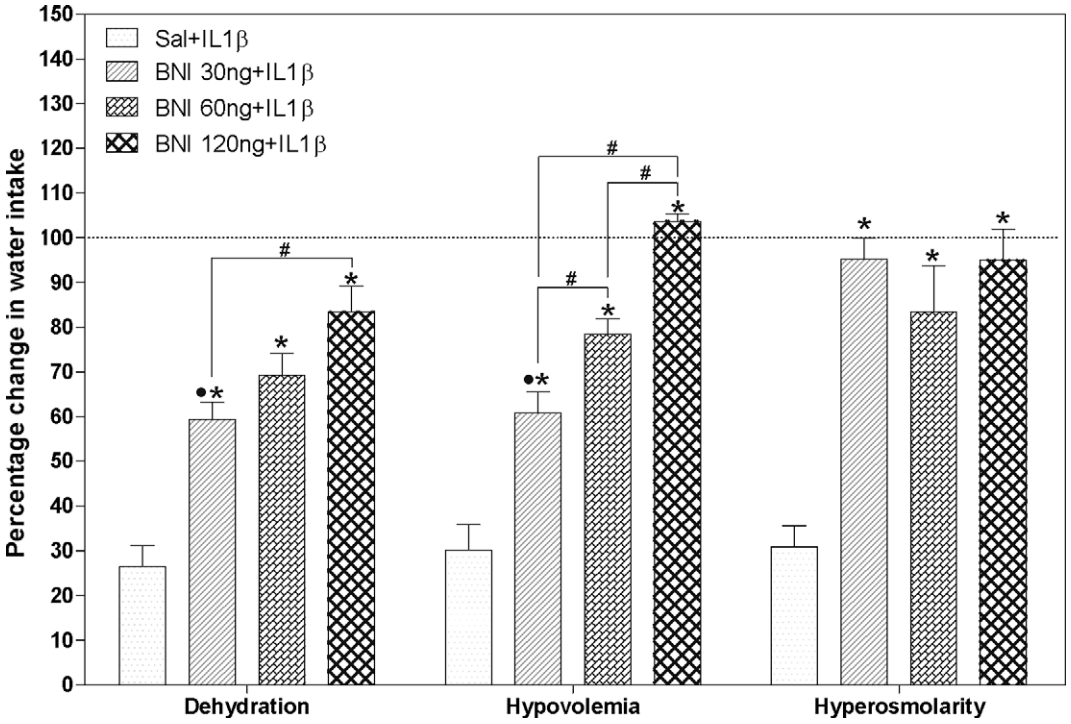

Fig. 4. Percentage change (mean ± SEM) in overall (120 min) water intake in dehydrated, hypovolemic and hyperosmotic animals receiving IL-1β (8 ng) and pretreated with saline or with BNI at several doses (30, 60 and 120 ng) relative to the mean water intake presented by drug-free control groups (saline + saline) in each of the thirst-inducing conditions. The data were submitted to two-way ANOVA (drug treatment – $F(3,102) = 93.40$; $p < 0.0001$; thirst-inducing condition – $F(2,102) = 9.25$; $p = 0.0002$; interaction – $F(6,102) = 3.84$; $p = 0.0017$) followed by post-hoc Bonferroni test ($p < 0.05$). Asterisks denote significant change in water intake induced by BNI pretreatment (BNI + IL-1β) relative to the corresponding saline + IL-1β under the three different thirst-inducing conditions. The symbols (#) indicate a significant ($p < 0.05$; post-hoc Bonferroni test) difference between the doses of BNI under the same thirst-inducing condition. The symbols (●) indicate significant change in water intake induced by BNI pretreatment, at the dose of 30 ng, when dehydrated and hypovolemic groups were compared to the hyperosmotic group.

intake of a "tasty" solution. This hedonic preference was not modified by third ventricle injections of IL-1β (8 ng). Indeed, animals receiving IL-1β drank the same amount of saccharin [$t = 0.3884$; degree of freedom = 9; $p = 0.7068$] as saline-treated controls, although there was a decrease in water intake [$t = 3.021$; degree of freedom = 9; $p = 0.0145$] compared to saline-treated controls.

No significant changes occurred in locomotor activity in animals receiving third ventricle injections of IL-1β at the same dose used in the previous experiment compared to saline-treated controls in the open field test. Indeed, values expressing the number of areas entered are very similar in both groups (controls = 44.8 ± 2.75 IL-1β-treated = 42.67 ± 2.61; $t = 0.56$; df = 9; $p = 0.5891$).

4. Discussion

The data in the present study show that in rats the functional integrity of brain KOR is essential for the antidipsogenic effect observed after third ventricle injections of IL-1β in three different situations: dehydration induced by water deprivation, hypovolemia produced by subcutaneous PEG administration and hyperosmolarity promoted by intragastric salt-load.

Studies carried out recently by our group showed that the inhibition of water intake after third ventricle injections of IL-1β in the same doses used in the present study relied on an opioid-dependent mechanism, since the blockade of central opioid receptors by naloxone, a non-selective opioid antagonist, was able to suppress the antidipsogenic effect observed after central administration of this cytokine (Luz et al., 2006). A thorough literature review revealed no data on the subtypes of opioid receptors that could be mediating this behavioral effect of IL-1β at central level. The present data add new information indicating that central KOR are essential in this phenomenon.

In addition to their key role in the control of immune response, cytokines such as IL-1β exert a myriad of important behavioral effects. Indeed, peptides released by immune cells set off behavioral changes in infected animals, and IL-1β acts on central sites to induce behavioral changes such as increased sleep and loss of appetite (Tizard, 2008). Experimental conditions that reproduce some characteristics present in infected animals, such as intraperitoneal LPS injections, induce synthesis of

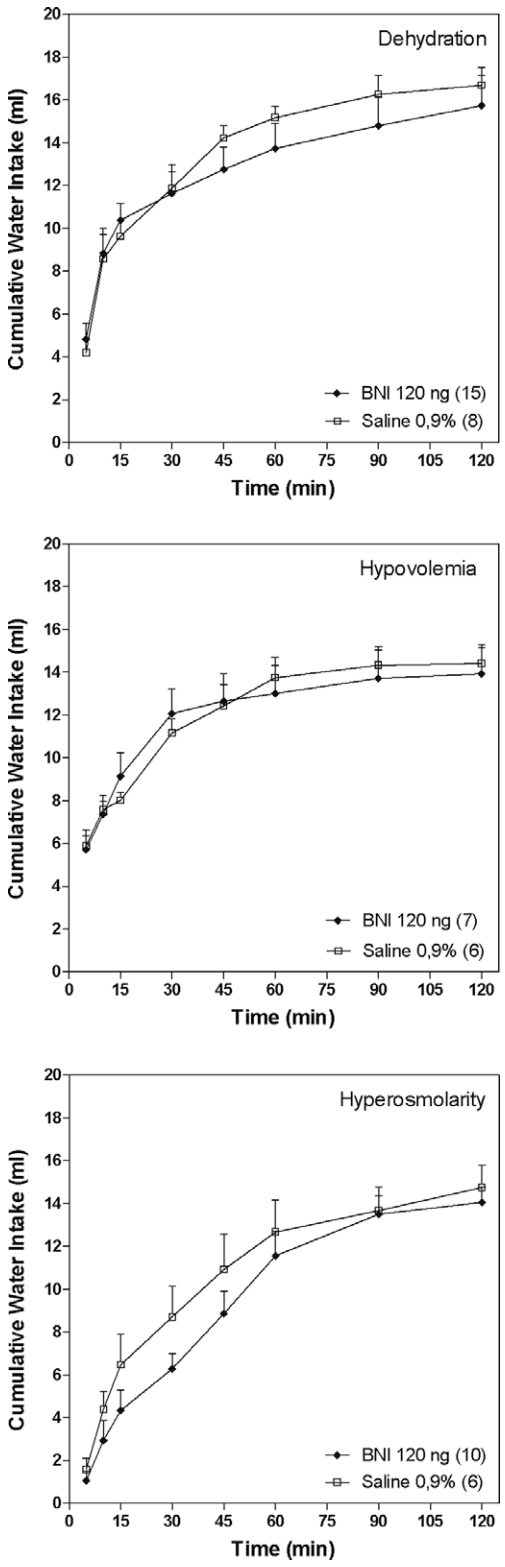

Fig. 5. Cumulative water intake in distinct groups of dehydrated (upper panel), hypovolemic (middle panel) or hyperosmotic (bottom panel) rats following third ventricle injections of BNI 120 ng alone or saline. Data are presented as mean ± SEM. Two-way ANOVA showed no statistically significant differences between treatments [dehydrated – $F(7,147) = 0.84$; $p = 0.5542$; hypovolemic – $F(7,77) = 0.49$; $p = 0.8379$; hyperosmotic – $F(7,84) = 0.55$; $p = 0.7948$].

IL-1β and other cytokines in the central nervous system (Watkins et al., 1995).

It has been previously established that administration of IL-1β induces a significant decrease in water intake, and that endogenous IL-1β may play an active role in water intake regulation in conditions in which this peptide is physiologically synthesized and released (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).

The mechanisms by which IL-1β inhibits water intake at central level have yet to be adequately investigated and at the present time remain poorly understood. It is possible that the peptide by itself exerts an inhibitory drive on the higher integrative areas connected to motor pattern generators that trigger the behaviors leading to the acquisition and ingestion of water. Alternatively, IL-1β may affect these centers by modulating the activity of some neurotransmitters in central areas related to thirst control. The interplay among these neurotransmitters and areas is complex and has been extensively reviewed elsewhere. Briefly, brain angiotensin II, cholinergic, adrenergic, serotonergic and several peptidergic pathways are involved in the central control of water and salt intake and the brain opioid system plays a significant role in this process (Johnson and Thunhorst, 1997; McKinley and Johnson, 2004).

It is well-documented that the endogenous central opioid system plays a role in the control of water intake. However, the nature of these actions may be extremely variable, depending on the brain areas in which opioids are located, the subtype of opioid receptor involved and the pharmacological doses of the opiatergic drugs used (Antunes-Rodrigues et al., 2004; Bodnar, 2007). It seems that the administration of a great number of opioid antagonists induces significant decreases in water intake (Brown and Holtzman, 1979; Brown et al., 1980; Czech and Stein, 1980; Maickel et al., 1977; Ostrowski et al., 1981; Rowland and Bartness, 1982), while the effects obtained with opioid agonists are rather variable. Anyway, increases in water intake have been observed after the administration of mu agonists (Cooper, 1981; Gosnell et al., 1986; Ukai and Holtzman, 1988) and the same dipsogenic effect has been demonstrated after the selective pharmacological stimulation of KOR by kappa-opioid agonists (Sanger and McCarthy, 1981; Turkish and Cooper, 1984). Increases in water intake may also be obtained after the administration of selective delta opioid receptor agonists (De Caro et al., 1979; Jackson and Sewell, 1985).

In the present study, the pharmacological blockade of brain KOR by the selective kappa-opioid antagonist BNI suppressed the antidipsogenic effect induced by third ventricle injections of IL-1β in all the physiological circumstances studied: dehydration, hypovolemia and hyperosmolarity. It seems reasonable to suggest that

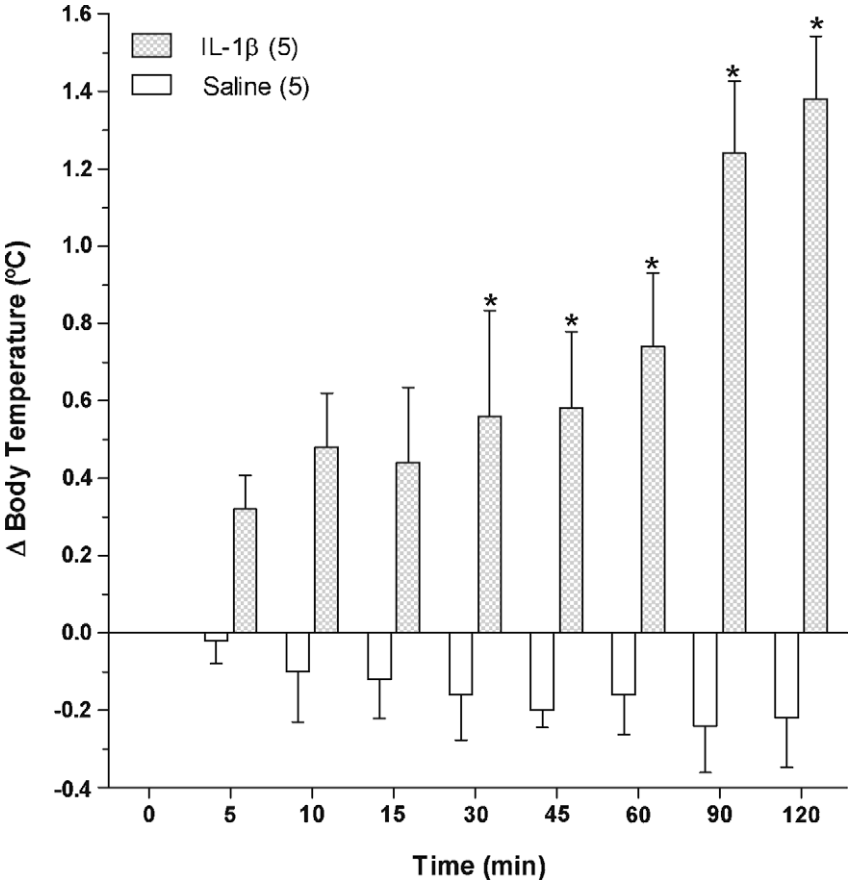

Fig. 6. Change in body temperature of rats following third ventricle injections of IL-1β 8 ng or saline. Data is presented as mean ± SEM. ★ indicates a statistically significant difference (two-way ANOVA followed by post-hoc Bonferroni test; $p < 0.05$) when the groups of animals receiving IL-1β are compared to controls.

the existence of a functional, central kappa-opioid-dependent component is essential for the expression of the antidipsogenic effect of IL-1β. This may involve either an IL-1β-stimulated kappa-opioid component exerting a negative drive on water intake or an IL-1β-inhibited kappa-opioid component imposing a positive drive on drinking.

It is interesting to note that the dipsogenic effect of angiotensin II also seems to depend on a kappa-opioid component since the selective blockade of central KOR reduces angiotensin II-induced drinking (Ruegg et al., 1994). Therefore, it is possible to speculate that central kappa-opioid components may mediate the effects of distinct peptides on water intake.

Nevertheless, it is important to note that central mu-opioid receptors are also involved in the control of water intake in many physiological situations. These receptors are essential for the induction of water intake promoted by isoproterenol injections (Glass et al., 1994) and deprivation-induced water intake in sham drinking rats is significantly reduced by the blockade of mu-opioid receptors (Leventhal and Bodnar, 1996), a pharmacological condition in which water intake in water-deprived rats is also impaired (Bodnar et al., 1995). In our previous papers (Luz et al., 2006; De Castro e Silva et al., 2006), naloxone, a general opioid antagonist that promotes a significant blockade of mu-opioid receptors, also reduced the antidipsogenic and antinatriorexigenic effects of IL-1β. Hence, it is possible that these effects of IL-1β may be centrally mediated by both kappa- and mu-opioid receptors.

BNI suppresses the antidipsogenic effect of IL-1β more easily in hyperosmotic animals compared to dehydrated and hypovolemic rats. This means that the antidipsogenic effect of IL-1β in conditions of hyperosmolarity is extremely dependent on the KOR component, while this component seems to be less essential to the expression of the antidipsogenic effect of IL-1β in the presence of dehydration or hypovolemia.

In addition, the suppression of the antidipsogenic effect of IL-1β by BNI followed a dose–response pattern in animals submitted to dehydration and to hypovolemia. However, in hyperosmotic animals BNI successfully blocked the antidipsogenic effect of IL-1β at all doses used. This may mean that in the course of hyperosmolarity the modulation of the brain kappa-opioid

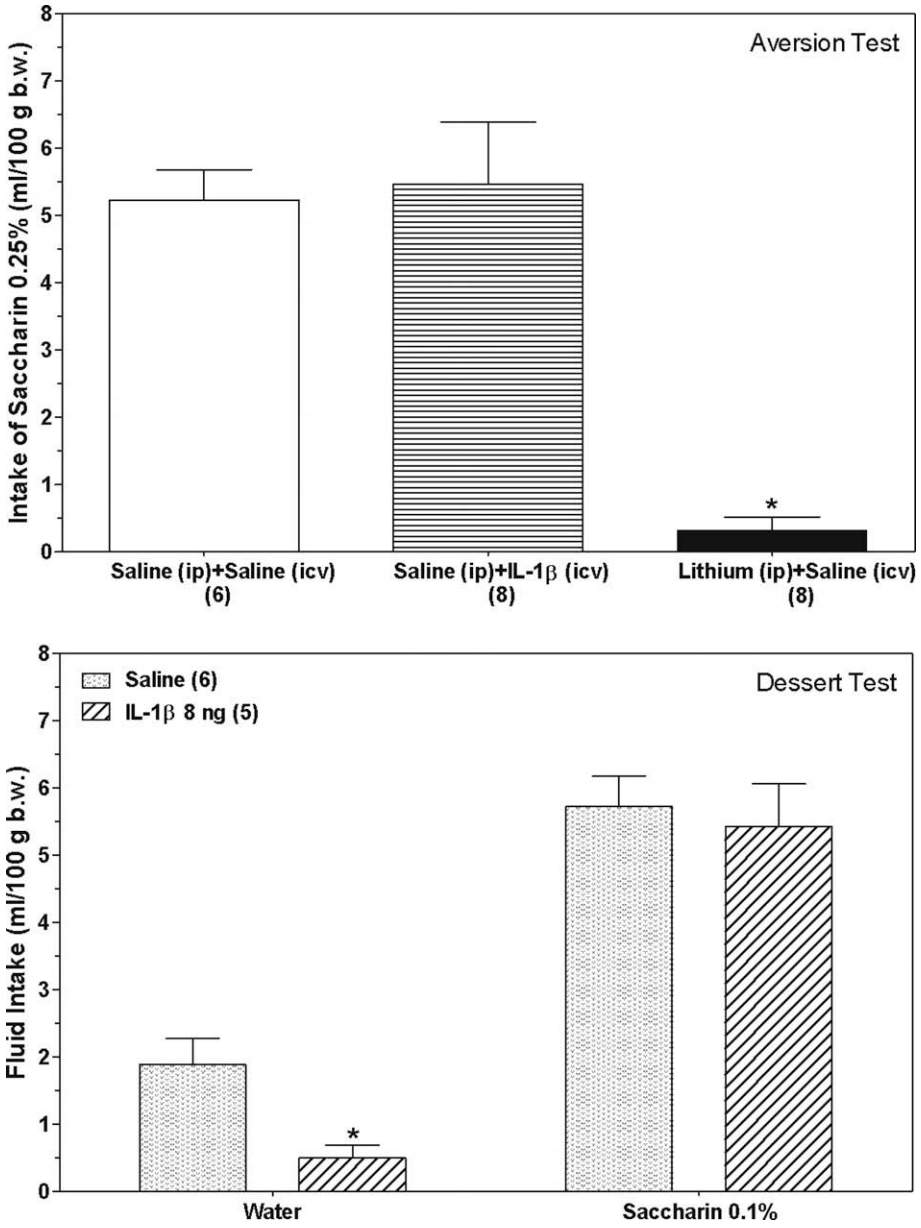

Fig. 7. Cumulative saccharin 0.25% intake in the aversion test (upper panel) and saccharin 0.1% and water intakes in the dessert test (lower panel) in rats receiving third ventricle injections of IL-1β (8 ng) or isotonic saline solution (controls). Data are expressed as mean ± SEM. In the aversion test the data were treated by one-way analysis of variance followed by post-hoc Bonferroni test; ★ indicates a statistically significant difference when groups of animals receiving lithium + saline or saline + IL-1β were compared to saline + saline. In the dessert test, the data were analyzed using the unpaired Student's *t*-test; ★ indicates a statistically significant difference when groups of animals receiving IL-1β were compared to controls.

system related to water intake control by IL-1β is crucial, and that even very small reductions in its activity may suppress the capacity of IL-1β to express its antidipsogenic effect.

Administration of the selective KOR antagonist BNI alone at the highest dose used in the present study, a dose that successfully blocked the antidipsogenic effect of IL-1β (120 ng), failed to modify water intake in any of the situations studied. This indicates that, at the doses used here, BNI was unable to alter the central, kappa-opioid-dependent effect on water intake that is normally present when pharmacological stimulation of the central interleukinergic component is absent. Similar results have been found indicating that BNI have no effect on water intake induced by hyperosmolarity or during water deprivation (Beczkowska et al., 1992; Ruegg et al., 1994).

Reciprocal interactions are present in the immune and opioid systems by which opioids participate in the control of immune function and the immune system modulates the activity of some opioid systems (Ruzicka and Akil, 1997). Some data have shown that hypothalamic

concentrations of opioids are increased by IL-1β (Murphy et al., 1983), which also intensifies opioid production by central nervous system glial cells (Ruzicka et al., 1996). Furthermore, IL-1β contributes to up-regulation of KOR mRNA in neural tissues (Puehler et al., 2006) and the release of opioids from immune cells by IL-1β may be an important step in the generation of analgesia in the course of immune response (Rittner et al., 2003). Therefore, the interaction observed here between the interleukinergic and kappa-opiatergic components at central level is plainly feasible and justifiable.

We have demonstrated in previous papers that inhibition of water intake by IL-1β is not due to a non-specific general central nervous system depression or to a decrease in locomotor activity (De Castro e Silva et al., 2006; Luz et al., 2006). However, the potency of the effect of peptides from different batches may vary greatly even when originating from the same supplier, resulting in major differences in the magnitude of a specific effect at a given dose. For this reason, we decided to retest the locomotor activity of the animals receiving IL-1β from the same batch used in the present study. With the batch of IL-1β used here, the test confirmed that third ventricle injections of IL-1β do not decrease the locomotor activity of rats, thereby excluding the hypothesis that the inhibition in water intake induced by this procedure could be merely a non-specific general central nervous system depression. The absence of locomotor deficits suppressing any ingestive behavior is further confirmed by the fact that animals receiving central injections of IL-1β or saline solution drank the same amounts of saccharin in the dessert test.

An investigation was also carried out to evaluate whether the IL-1β contained in this new batch would provoke any aversive behavior that could explain its inhibitory effects on water intake. The aversion test performed in the present paper has indisputably shown that the association between third ventricle injections of IL-1β and saccharin consumption does not reduce saccharin intake the following day, while in animals associating saccharin intake with a known sickness-inducing procedure (intraperitoneal lithium chloride injections), this reduction is quite significant.

The present paper also shows that the IL-1β contained in the batch used in this study successfully increased body temperature, confirming that the peptide supplied reproduces the cytokine actions of IL-1β. Third ventricle injections of IL-1β affect body temperature and water intake independently. As the increase in body temperature normally elicits water intake (Dinarello, 2004), the present data allow us to speculate that central activation of IL-1β receptors by IL-1β neutralizes the thirst-inducing stimulus represented by increased body temperature.

We have previously demonstrated that intragastric salt-load in the same conditions used in the previous experiments efficaciously induce hyperosmolarity (Luz et al., 2006). Therefore, it is reasonable to consider that the increase in water intake observed in salt-loaded animals in the present study is a consequence of this condition.

In summary, the data shown in the present study clearly demonstrated that the antidipsogenic effect observed in rats after third ventricle injections of IL-1β depends on the functional integrity of a brain kappa-opioid-dependent component.

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