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Antidipsogenic effects of central adenosine-5'-triphosphate

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Besides other physiological functions, adenosine-5'-triphosphate (ATP) is also a neurotransmitter that acts on purinergic receptors. In spite of the presence of purinergic receptors in forebrain areas involved with fluid-electrolyte balance, the effect of ATP on water intake has not been investigated. Therefore, we studied the effects of intracerebroventricular (*icv*) injections of ATP (100, 200 and 300 nmol/µL) alone or combined with DPCPX or PPADS (P1 and P2 purinergic antagonists, respectively, 25 nmol/µL) on water intake induced by water deprivation. In addition, the effect of *icv* ATP was also tested on water intake induced by intragastric load of 12% NaCl (2 mL/rat), acute treatment with the diuretic/natriuretic furosemide (20 mg/kg), *icv* angiotensin II (50 ng/µL) or *icv* carbachol (a cholinergic agonist, 4 nmol/µL), on sodium depletion-induced 1.8% NaCl intake, and on food intake induced by food deprivation. Male Holtzman rats (280-320 g, N = 7-11) had cannulas implanted into the lateral ventricle. *Icv* ATP (300 nmol/µL) reduced water intake induced by water deprivation (13.1 ± 1.9 *vs* saline: 19.0 ± 1.4 mL/2 h; $P < 0.05$), an effect blocked by pre-treatment with PPADS, but not DPCPX. *Icv* ATP also reduced water intake induced by NaCl intragastric load (5.6 ± 0.9 *vs* saline: 10.3 ± 1.4 mL/2 h; $P < 0.05$), acute furosemide treatment (0.5 ± 0.2 *vs* saline: 2.3 ± 0.6 mL/15 min; $P < 0.05$), and *icv* angiotensin II (2.2 ± 0.8 *vs* saline: 10.4 ± 2.0 mL/2 h; $P < 0.05$), without changing *icv* carbachol-induced water intake, sodium depletion-induced 1.8% NaCl intake and food deprivation-induced food intake. These data suggest that central ATP, acting on purinergic P2 receptors, reduces water intake induced by intracellular and extracellular dehydration.

Key words: Water intake; ATP *icv*; Purinergic receptors; Angiotensin II; Carbachol; Ingestive behavior

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Introduction

A role for adenosine-5'-triphosphate (ATP) as an extracellular signaling molecule and neurotransmitter was first proposed by Burnstock (for a review, see Ref. 1). ATP binds to two classes of purinergic receptors: the ionotropic P2X and the metabotropic P2Y receptors (2). However, ATP can be easily converted to adenosine that binds to P1 purinergic receptors (classified as A1, A2, and A3) (3,4). Functional studies have shown that central purinergic mechanisms are involved in cardiac, respiratory and thermal regulation (5-16). However, there is little information on the role of purinergic mechanisms in behavioral responses (17), including pathways involved in intracellular and extracellular thirst.

Immunohistochemical studies have demonstrated the presence of purinergic receptors in several areas of the central nervous system (CNS), which are involved in fluid-electrolyte balance (18,19), including circumventricular organs that are areas strongly involved in the control of sodium and water intake (20). Therefore, an important question that remains is whether purinergic receptors in forebrain areas are involved in the control of water and sodium intake. In the present study, we investigated the

effects of intracerebroventricular (*icv*) injections of ATP alone or combined with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, an adenosine A1 receptor antagonist) or pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS, a purinergic P2 receptor antagonist) on water intake induced by 24 h of water deprivation (a model that mixes intra- and extracellular dehydration) (21,22). In addition, we also tested the effects of *icv* ATP on water intake induced by intragastric gavage with 12% NaCl (a model of intracellular dehydration) (23), acute treatment with the diuretic/natriuretic furosemide (a model of extracellular dehydration) (24,25), *icv* angiotensin II (ANG II, the main mechanism activated by extracellular dehydration to induce thirst) (21), and *icv* carbachol (a cholinergic agonist suggested to activate the same central mechanisms involved in intracellular dehydration-induced thirst) (26,27). To determine the specificity of central ATP for the control of water intake in comparison to another type of ingestive behavior, we also tested the effects of *icv* ATP on sodium depletion-induced 1.8% NaCl intake and on food deprivation-induced food intake.

Material and Methods

Animals

Male Holtzman rats weighing 280-310 g were used. The animals were housed in individual stainless steel cages with free access to a normal sodium diet (Guabi Rat Chow, Brazil), water and 1.8% NaCl solution and maintained under controlled conditions (23 ± 2°C, humidity at 55 ± 10% and on a 12-h light/dark cycle with lights on at 7:00 am). Standard Guabi rodent pellets (0.5% sodium) and tap water were available *ad libitum* unless otherwise stated. All experiments were started between 8:00 and 9:00 am at least 5 days after surgery. The experimental protocols used in the present study were approved by the Ethics Committee for Animal Care and Use of the Dental School of Araraquara, UNESP, Brazil, and followed the recommendations of the Brazilian College of Animal Experimentation (COBEA) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 80-23, 1996, USA). All efforts were made to minimize animal discomfort and the number of animals used.

Surgery for cerebral cannulas

Rats were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg body weight; Cristália, Brazil) combined with xylazine (7 mg/kg body weight; Agener União, Brazil), and placed in a Kopf stereotaxic instrument. The skull was leveled between the bregma and lambda. A stainless steel guide cannula (10 x 0.7 mm OD) was implanted into the lateral ventricle (LV) using the following coordinates: 0.3 mm caudal to the bregma, 1.6 mm lateral to the midline, and 3.6 mm below the bone. The cannula was secured to the top of the skull with dental acrylic resin and two jeweler screws. Insertion of a close-fitting stylet kept the lumen free of debris and clots. A prophylactic presurgical dose of penicillin (30,000 IU) was given intramuscularly. Immediately after surgery, the rats received intramuscular injections of 1% cetoprophen, an analgesic drug (30 µL). Rats were allowed to recover from surgery for at least 5 days.

Intracerebral injections

Single-pulse intracranial injections were made after gently removing the animal from its cage, replacing the stylet with an injector that protruded 2.0 mm beyond the tip of the guide cannula and that was connected by PE-10 tubing to a 10-µL syringe, and injecting a total volume of 1.0 µL over a period of 20 s. Stylet and injector were always wiped with cotton soaked in 70% alcohol between injections. After the injection, the injector was removed and replaced with the stylet, and the animal was returned to its cage for observation of its behavior.

Drugs

ATP (a natural P2 receptor agonist), DPCPX (an adenosine A1 receptor antagonist), PPADS (a P2 purinergic receptor antagonist), ANG II, and carbachol were purchased from Sigma. All drugs were freshly dissolved in 0.9% saline, except DPCPX that was dissolved in saline containing 5% dimethyl sulfoxide (Sigma, USA). ATP was administered into the LV at the dose of 100, 200, and 300 nmol/µL in 2 protocols (water deprivation and sodium depletion) and ATP was administered at the dose of 300 nmol/µL in the other protocols. PPADS and DPCPX were administered into the LV at the dose of 25 nmol/µL only in water-deprived rats. ANG II and carbachol were administered into the LV at the doses of 50 ng/µL and 4 nmol/µL, respectively. Doses of ATP, PPADS, and DPCPX were chosen on the basis of pilot experiments. Doses of ANG II and carbachol were based on a previous study (28).

Furosemide (Sigma) was administered subcutaneously (*sc*) at 20 mg/kg body weight and was dissolved in alkaline saline (pH adjusted to 9.0 with NaOH), as described previously (29).

Histology

At the end of the experiments, the animals received an injection of 1 µL 2% Evans blue solution into the LV. They were then deeply anesthetized with an intraperitoneal

injection of sodium thiopental (80 mg/kg body weight; Cristália) and perfused transcardially with saline followed by 10% formalin. The brains were removed, fixed in 10% formalin, frozen, cut into 50-µm sections, stained with Giemsa, and analyzed by light microscopy to confirm that the injection sites were in the LV. Only data from animals in which injections reached the LV were considered in the analysis.

Statistical analysis

Data are reported as means ± SEM. Two-way repeated-measures analysis of variance (ANOVA) or one-way ANOVA followed by the Newman-Keuls test was used for group comparison. The level of significance was set at $P < 0.05$ in all tests.

Experimental protocols tested

a) Water deprivation-induced water intake. Water and 1.8% NaCl were removed from the cage and only food remained available for 24 h. After this period, ATP (100, 200, or 300 nmol/µL) or vehicle (saline) was injected into the LV 10 min before the burettes containing water and 1.8% NaCl became available to the animals. Cumulative water and 1.8% NaCl intake was measured for the next 2 h (at 0, 15, 30, 45, 60, and 120 min) in the absence of food. In another group of rats, PPADS or DPCPX (P2 and P1 purinergic antagonists, respectively, 25 nmol/µL) or vehicle was injected into the LV 10 min before ATP (300 nmol/µL) or saline. Each rat was submitted to four experimental tests. In each test, the group of rats was divided into two subgroups and each half of the group received one of the treatments. The sequence of the treatments in different tests was randomized and at the end of four tests all animals had received all treatments. Water, 1.8% NaCl and standard food were returned to the animals at the end of the intake test. A recovery period of at least 3 days was allowed between tests.

b) Water intake induced by intragastric load of 12% NaCl. Another group of animals was trained daily for at least 3 days to receive the intragastric load (gavage). The training was performed once a day and consisted of carefully holding the animal and injecting 2 mL 0.9% NaCl through PE-200 polyethylene tubing from a 5-mL syringe into the stomach. On the day of the experiment, food, water and 1.8% NaCl were removed from the cages, and the animals received an intragastric load (gavage) of 12% (2 M) NaCl or 0.9% NaCl (2 mL/rat). The intragastric load of 12% NaCl produces a 4% elevation of both plasma osmolality and sodium concentration, inducing cell dehydration and thus water intake (23). Concurrent reduction of plasma renin activity and no alteration in plasma volume indicate that the procedure does not induce extracellular dehydration (23). Fifty minutes after gavage with 12% NaCl or 0.9% NaCl (control), ATP (300 nmol/µL) or vehicle (saline) was injected *icv*. Half the group received ATP and the other half received vehicle (saline) into the LV. Ten minutes after ATP, the animals had access to both water and 1.8% NaCl in 0.1-mL graduated glass burettes. Cumulative water and 1.8% NaCl intake was recorded at 15, 30, 45, 60, 90, and 120 min. This procedure was repeated in a counterbalanced design in a second experimental session performed 2 days later.

c) Acute furosemide-induced water intake. Food, 1.8% NaCl and water were removed from the cages and the rats were injected *sc* with the diuretic/natriuretic furosemide (20 mg/kg body weight). This dose of furosemide induces a rapid and maximum loss of water and electrolytes, including sodium and potassium, within 1 h (24,25). Fifty minutes after the *sc* injection of furosemide, ATP (300 nmol/µL) or vehicle (saline) was injected *icv*. Ten minutes later, water and 1.8% NaCl were offered to the animals in 0.1-mL graduated glass burettes fitted with stainless steel spouts. Cumulative water and 1.8% NaCl intake was recorded at 15, 30, 45, 60, 90, and 120 min after the access to these fluids. At the end of the test, water, food and 1.8% NaCl were made available to the animals until the next test. This procedure was repeated in a counterbalanced design in a second experimental test performed 2 days later.

d) Central ANG II- and carbachol-induced water intake. In another group of normohydrated rats, water intake was induced by *icv* injection of ANG II (50 ng/µL) or carbachol (4 nmol/µL). Cumulative water and 1.8% NaCl intake was recorded for 2 h (at 15, 30, 45, 60, 90, and 120 min) immediately after the injection of ANG II or carbachol. ATP (300 nmol/µL) or vehicle (saline) was injected *icv* 10 min before the injection of ANG II or carbachol. The same procedure was repeated in a counterbalanced design in a second experimental test performed 2 days later.

e) Sodium depletion-induced 1.8% NaCl intake. The rat's cage was rinsed with water to eliminate any environmental sodium and sodium depletion was induced by an *sc* injection of furosemide (20 mg/kg body weight) followed by animal access to only water and sodium-deficient food (powdered corn meal; 0.001% sodium and 0.33% potassium) for 24 h. Then, food was removed and the injections of ATP (100, 200, and 300 nmol/µL) or vehicle (saline) into the LV were performed. Ten minutes after the injection of ATP, water and 1.8% NaCl were provided to the animals in 0.1-mL graduated glass burettes fitted with stainless steel spouts. Cumulative water and 1.8% NaCl intakes were measured at 15, 30, 45, 60, 90, and 120 min. In each test, the group of rats was divided into two equal subgroups,

each receiving one of the treatments. The sequence of the treatments in different tests was randomized and at the end of four tests all animals had received all treatments. Each rat was submitted to four experimental depletion tests. A recovery period of at least 3 days was allowed between tests. Treatment with furosemide induces a 1.5 to 2.0 mEq loss of sodium and consistent intake of hypertonic sodium solutions (24,30-32).

f) Food deprivation-induced food intake. Another group of rats was submitted to 24 h of food deprivation with water available. Food deprivation started at 8:00 am on the day before the test. Ten minutes before the beginning of the meal test, the animals received an *icv* injection of ATP (300 nmol/µL) or vehicle (saline). Food intake was measured at 15, 30, 60, 90, and 120 min from the beginning of the test.

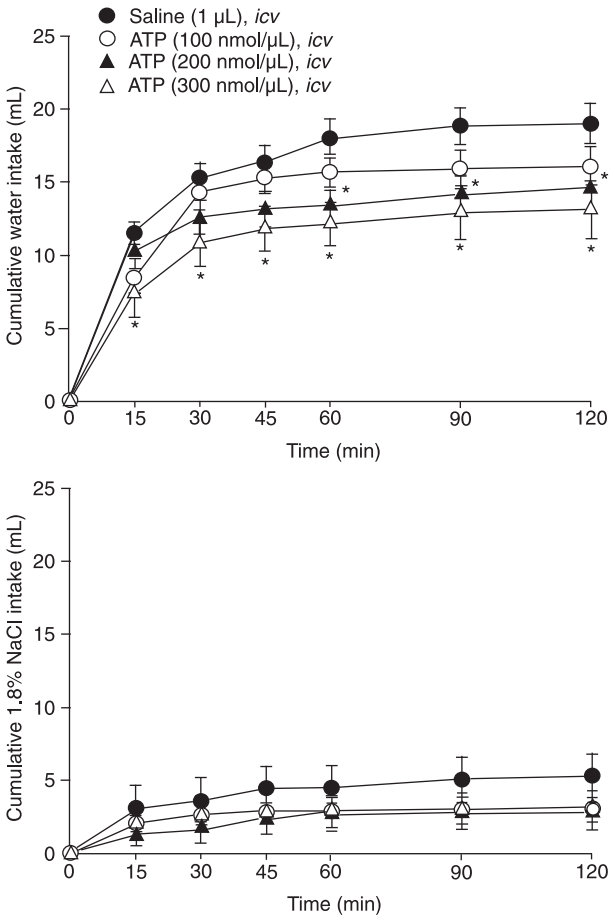

Figure 1. Cumulative water (upper panel) and 1.8% NaCl intake (lower panel) of 24-h water-deprived rats treated with *icv* injections of ATP (100, 200, and 300 nmol/µL) or saline. Data are reported as means ± SEM for 9 rats in each group. *P < 0.05 compared to saline *icv* (one-way ANOVA and Newman-Keuls test).

The same procedure was repeated in a counterbalanced design in a second experimental test performed 2 days later. The measurement of food intake was accurate to 0.1 g.

Results

Effects of *icv* injection of ATP alone or combined with *icv* PPADS or DPCPX on water and 1.8% NaCl intake in water-deprived rats

Icv injection of ATP (200 and 300 nmol/µL) reduced water intake in water-deprived rats in a dose-dependent manner during the entire 2-h test (14.6 ± 1.5 and 13.1 ± 1.9 mL/2 h, respectively, *vs* saline: 19.0 ± 1.4 mL/2 h, N = 9; P < 0.05, Figure 1, upper panel). The low dose of ATP (100 nmol/µL) had no effect on water intake (16.1 ± 1.4 *vs* saline: 19.0 ± 1.4 mL/2 h, Figure 1, upper panel). None of the doses of ATP tested (100, 200 and 300 nmol/µL) had an effect on 1.8% NaCl intake in water-deprived rats (3.0 ± 0.5, 2.71 ± 1.1 and 3.2 ± 1.1 mL/2 h, respectively, *vs* saline: 5.3 ± 1.5 mL/2 h, Figure 1, lower panel).

Pre-treatment with *icv* PPADS (25 nmol/µL) reduced the inhibitory effect of *icv* ATP (300 nmol/µL) on water intake in water-deprived rats at 15 and 30 min into the test (9.4 ± 1.2 and 14.4 ± 0.9 mL/15 and 30 min, respectively, *vs* saline + ATP: 4.4 ± 0.8 and 9.1 ± 1.2 mL/15 and 30 min, respectively, N = 10; P < 0.05, Figure 2). PPADS (25 nmol/µL) alone produced no significant change in the water intake induced by water deprivation (Figure 2).

Pre-treatment with *icv* DPCPX (25 nmol/µL) produced no significant change on the antidipsogenic action of ATP (Figure 3).

Effects of *icv* injection of ATP on water and 1.8% NaCl intake in rats treated with an intragastric load of 12% NaCl

In rats treated with an intragastric load of 12% NaCl (2 mL/rat), *icv* injection of ATP (300 nmol/µL) reduced water intake (5.6 ± 0.9 *vs* saline: 10.3 ± 1.4 mL/2 h, N = 7; P < 0.05, Figure 4, upper panel), without changing 1.8% NaCl intake (1.0 ± 0.5 *vs* saline: 0.9 ± 0.5 mL/2 h, Figure 4, lower panel).

When rats were treated with an intragastric load of 0.9% NaCl (2 mL/rat), *icv* injection of ATP (300 nmol/µL) did not change water intake (0.4 ± 0.2 *vs* saline: 1.7 ± 0.7 mL/2 h, N = 7) or 1.8% NaCl intake (0.5 ± 0.1 *vs* saline: 0.7 ± 0.5 mL/2 h) (data not shown).

Effects of *icv* injection of ATP on water and 1.8% NaCl intake in rats acutely treated with furosemide

In rats acutely treated with *sc* furosemide (20 mg/kg body weight), *icv* injection of ATP (300 nmol/µL) reduced

water intake during the first 15 min of the experimental period (0.5 ± 0.2 *vs* saline: 2.3 ± 0.6 mL/15 min, N = 8; P < 0.05, Figure 5, upper panel), without changing 1.8% NaCl intake at any time during the test (Figure 5, lower panel).

Effects of *icv* injection of ATP on ANG II- or carbachol-induced water intake in rats

Icv injection of ATP (300 nmol/µL) reduced water intake induced by *icv* ANG II (50 ng/µL) throughout the test (2.2 ± 0.8 *vs* saline: 10.4 ± 2.0 mL/2 h, N = 8; P < 0.05, Figure 6, upper panel), without changing 1.8% NaCl intake (3.4 ± 2.9 *vs* saline: 4.2 ± 1.5 mL/2 h, Figure 6, lower panel).

Icv injection of ATP (300 nmol/µL) had no effect on *icv* carbachol (4 nmol/µL)-induced water intake (6.0 ± 1.6 *vs* saline: 7.4 ± 1.5 mL/2 h, N = 8), or on 1.8% NaCl intake (2.2 ± 0.7 *vs* saline: 2.0 ± 0.7 mL/2 h) (data not shown).

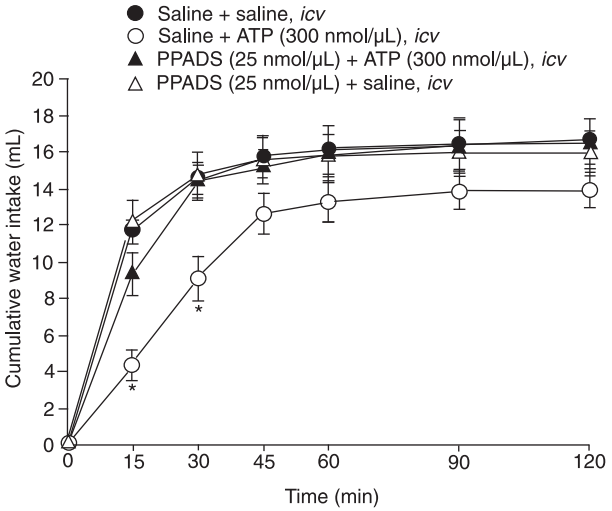

Figure 2. Cumulative water intake of 24-h water-deprived rats treated with *icv* injections of PPADS (25 nmol/µL) or saline combined with *icv* ATP (300 nmol/µL) or saline. Data are reported as means ± SEM for 10 rats in each group. PPADS = pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid. *P < 0.05 compared to saline + saline *icv* (two-way ANOVA and Newman-Keuls test).

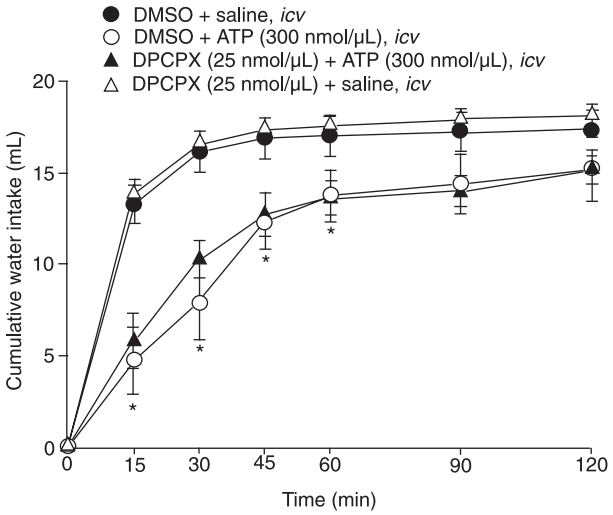

Figure 3. Cumulative water intake of 24-h water-deprived rats treated with *icv* injections of DPCPX (25 nmol/µL) or DMSO (vehicle) combined with *icv* ATP (300 nmol/µL) or saline. Data are reported as means ± SEM for 8 rats in each group. DPCPX = 1,3-dipropyl-8-cyclopentylxanthine; DMSO = dimethyl sulfoxide. *P < 0.05 compared to DMSO + saline *icv* (two-way ANOVA and Newman-Keuls test).

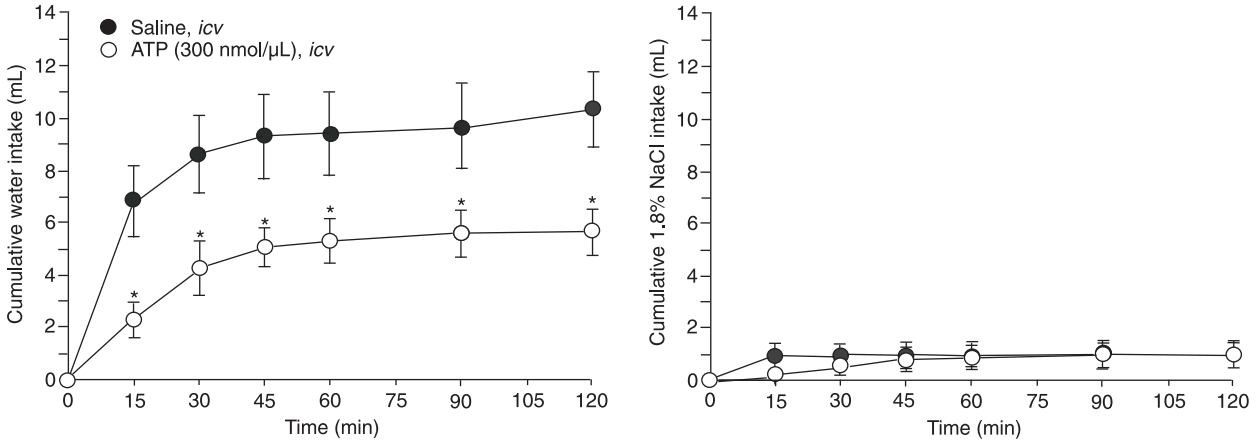

Figure 4. Cumulative water (left panel) and 1.8% NaCl intake (right panel) induced by an intragastric load of 12% NaCl (2 mL) in rats treated with *icv* injections of ATP (300 nmol/µL) or saline. Data are reported as means ± SEM for 7 rats in each group. *P < 0.05 compared to saline *icv* (one-way ANOVA and Newman-Keuls test).

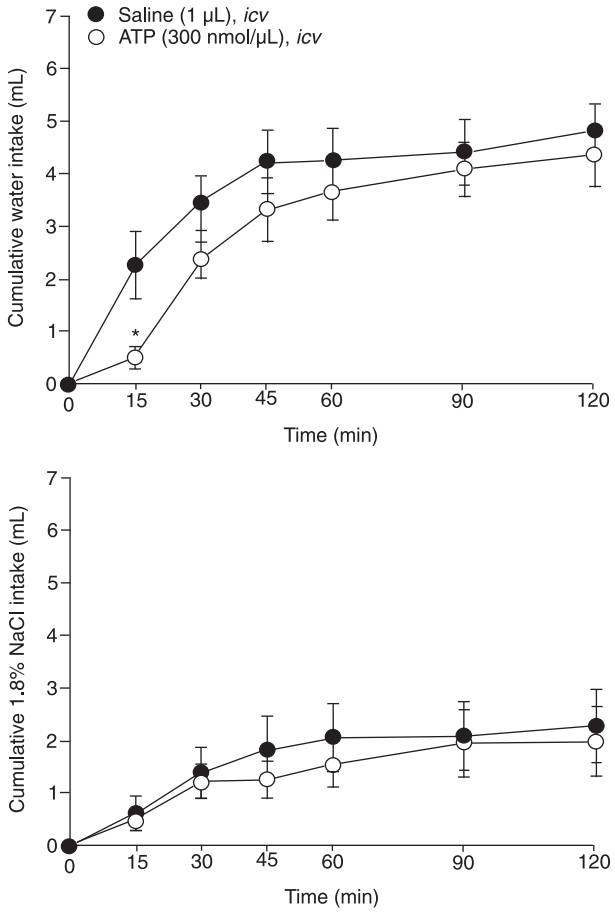

Figure 5. Cumulative water (upper panel) and 1.8% NaCl intake (lower panel) induced by acute treatment with *sc* furosemide (20 mg/kg body weight) in rats treated with *icv* injections of ATP (300 nmol/µL) or saline. Data are reported as means ± SEM for 8 rats in each group. *P < 0.05 compared to saline *icv* (one-way ANOVA and Newman-Keuls test).

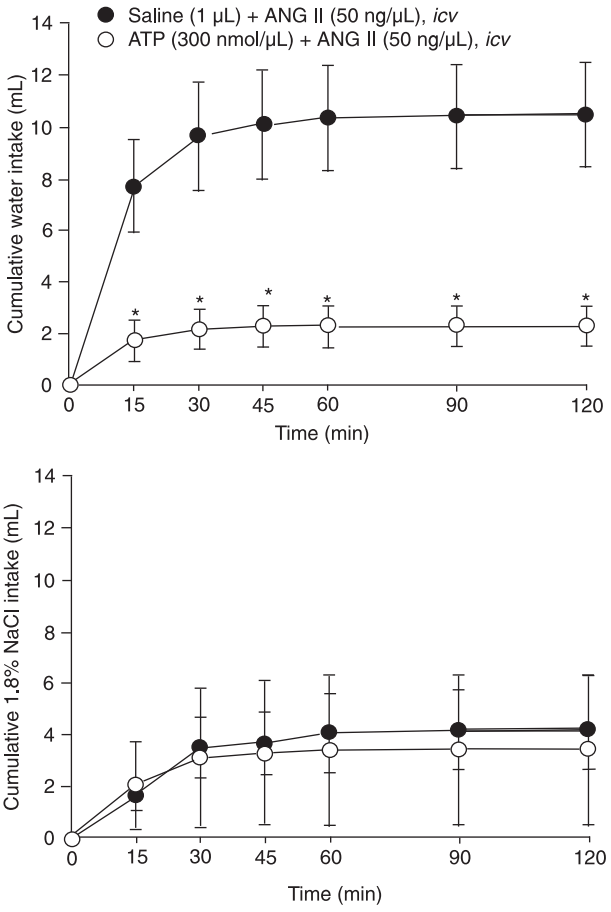

Figure 6. Cumulative water (upper panel) and 1.8% NaCl intake (lower panel) induced by *icv* injection of ANG II (50 ng/µL) in rats previously treated with *icv* injection of ATP (300 nmol/µL) or saline. Data are reported as means ± SEM for 8 rats in each group. *P < 0.05 compared to saline + ANG II *icv* (one-way ANOVA and Newman-Keuls test).

Effects of *icv* injection of ATP on sodium depletion-induced 1.8% NaCl and water intake

None of the ATP doses (100, 200, and 300 nmol/µL) had any effect on sodium depletion-induced 1.8% NaCl intake (19.8 ± 1.4, 17.0 ± 2.2, 17.5 ± 1.2 mL/2 h, respectively, *vs* saline: 16.4 ± 2.4 mL/2 h, N = 7) or water intake (0.6 ± 0.2, 1.1 ± 0.3 and 2.5 ± 0.8 mL/2 h, respectively, *vs* saline: 1.5 ± 0.4 mL/2 h, N = 7).

Effects of *icv* injection of ATP on food intake in food-deprived rats

Icv injection of ATP (300 nmol/µL) did not change food intake induced by 24 h of food deprivation throughout the test (7.7 ± 0.6 *vs* saline: 8.2 ± 0.7 g/2 h, N = 11).

Discussion

Terrestrial animals have evolved several complex mechanisms to ensure constancy of body fluid. The brain receives and integrates different types of hydration-related input, and the result of this integrative process determines the probability for the animal to ingest water and/or sodium (for a review, see Ref. 33).

The present results show that central injections of ATP experimentally inhibit induced water intake in rats. *Icv* injection of ATP reduced water intake induced by intracellular dehydration (intragastric load of 12% NaCl), extracellular dehydration (acute *sc* furosemide), a mix of intra- and extracellular dehydration (water deprivation), and also *icv*

ANG II that mimics extracellular dehydration. Therefore, the activation of central purinergic receptors with ATP inhibits dipsogenic responses to different stimuli, except water intake produced by central cholinergic activation or the low water intake in a protocol more related to sodium ingestion (sodium depletion). Central injection of ATP did not affect the ingestion of hypertonic NaCl or food, suggesting that the inhibitory effect of ATP on water intake is not the result of nonspecific inhibition of all ingestive behaviors.

The present study provides the first evidence for the involvement of central purinergic mechanisms in the control of fluid and electrolyte balance. However, because ATP in the CNS can be quickly converted to adenosine by the enzymatic action of adenosine triphosphatase and ecto-5'-nucleotidase (3,4), the antidipsogenic responses described in the present study might be due to ATP acting on central purinergic P2 receptors (classified as P2X and P2Y) or adenosine acting on central purinergic P1 receptors (classified as A1, A2 and A3). To identify the receptor involved in the antidipsogenic response to ATP, we tested the effects of *icv* ATP after pretreatment with PPADS (a purinergic P2 receptor antagonist) or DPCPX (a purinergic P1 receptor antagonist more specific for adenosine A1 receptors; 34) on water intake induced by 24 h of water deprivation. PPADS, but not DPCPX, abolished the antidipsogenic effect of ATP, suggesting that the inhibitory effect of ATP on water intake is due to central purinergic P2 receptor activation. It is important to emphasize that our results suggest that the antidipsogenic action of ATP is not due to A1 activation, but do not rule out a possible action of ATP via adenosine A2 or A3 receptors. Thus, further studies are necessary to determine if other kinds of adenosine receptors are involved in the inhibitory effects of central ATP on water intake.

ANG II acting centrally is the main facilitatory mechanism activated by extracellular dehydration (21) to induce water intake and central cholinergic mechanisms are suggested to play a role in intracellular dehydration-induced dipsogenic responses (26,27). Central ATP reduced *icv* ANG II-induced water intake and all other models of extracellular dehydration-induced water intake tested, clearly suggesting that central purinergic mechanisms inhibit water intake induced by extracellular dehydration. Central ATP also reduced water intake induced by intracellular dehydration (intragastric load of 12% NaCl), but failed to reduce carbachol-induced water intake. The intragastric load of 12% NaCl induces intracellular dehydration, increasing plasma osmolality and sodium concentration and reducing plasma renin activity (23). Therefore, an intragastric load of 12% NaCl is a typical model that induces intracellular dehydration-induced water intake, suggesting that central ATP also reduces water intake induced by this experimental condition. The reasons why ATP did not affect carbachol-induced water intake are not clear. Perhaps central cholinergic activation-induced water intake does not totally mimics intracellular dehydration-induced water intake as suggested by Levitt and Fisher (35). Or, although central cholinergic mechanisms are part of the mechanisms activated by intracellular dehydration to induce thirst, ATP might act in one of the first steps (or synapse) of the pathway before the activation of the cholinergic mechanisms.

In addition to the absence of any effect of ATP on carbachol-induced water intake, the inhibitory effects of ATP on water intake in other protocols tested were also variable. Central ATP produced a strong reduction of water intake induced by intragastric load of 12% NaCl (intracellular dehydration-induced thirst) or *icv* ANG II (extracellular dehydration-induced thirst). However, *icv* ATP produced only a partial reduction of water deprivation-induced water intake and weakly reduced water intake only at 15 min into the test in rats acutely treated with furosemide. The different effects of ATP in different protocols of water intake may be the result of the different interactions between facilitatory and inhibitory mechanisms activated in each situation. For example, rats acutely treated with furosemide have increased renin activity together with hypovolemia (24,25) that is an additional signal to increase water intake, while central injection of ANG II induces water intake in spite of the pressor response that is an inhibitory signal for water intake.

The effects of ATP on water intake are very similar to those produced by treatment with moxonidine and clonidine (alpha$_2$-adrenergic/imidazoline agonists), that are typical antidipsogenic drugs (28,36-40). Taken together, our data and those of the literature suggest that activation of central alpha$_2$-adrenergic receptors (28,36-40) and central purinergic receptors with ATP (present data) inhibits the excitatory mechanisms related to the control of water intake. The effects of activation of central alpha$_2$-adrenergic and central P2 purinergic receptors on water intake are not the result of a nonspecific inhibition of all ingestive behaviors because food intake was not affected by *icv* injections of norepinephrine (40) or ATP (present data). Because ATP may act as a co-transmitter with different neurotransmitters (17), including norepinephrine, or that ATP may also release norepinephrine (17), an interaction may exist between ATP and norepinephrine in the forebrain to control water intake, which deserves further investigation.

The present results suggest that inhibitory purinergic

mechanisms in the forebrain may contribute to the control of water intake. However, further studies are necessary to demonstrate which areas of the brain are involved in the effects of ATP on water intake. Therefore, a complete evaluation of the role of central purinergic mechanisms in the control of water intake may make an important contribution to the understanding of the complex neural network that maintains fluid balance.

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