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NON-NMDA RECEPTORS IN THE LATERAL PARABRACHIAL NUCLEUS MODULATE SODIUM APPETITE

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Abstract

Glutamatergic mechanisms have been implicated in the control of fluid ingestion. In the present study, we investigated whether non-N-methyl-D-aspartate (NMDA) glutamatergic receptors in the lateral parabrachial nucleus (LPBN) are involved in the control of water and sodium intake. Male Sprague-Dawley rats had cannulas implanted bilaterally into the LPBN. They were acutely depleted of water and sodium by injections of the diuretic furosemide (Furo; 10 mg/kg, bw) and given a low dose of the angiotensin-converting enzyme inhibitor, captopril (Cap; 5 mg/kg, bw). Bilateral LPBN injections of the non-NMDA receptor antagonist DNQX (2 and 5 nmol/0.2 μ l) increased the ingestion of 0.3 M NaCl and water of Furo/Cap treated rats. The increased ingestion produced by DNQX was abolished by pretreating the LPBN with α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), a non-NMDA receptor agonist. AMPA injected alone into the LPBN reduced water and 0.3 M NaCl intake. Injections of DNQX (5 nmol/0.2 μ l) into the LPBN also produced ingestion of 0.3 M NaCl after sc injections of the β -adrenoceptor agonist, isoproterenol, a hypotensive drug that typically produces only water intake. Food intake, arterial blood pressure and heart rate were not altered by DNQX LPBN injections. We conclude that agonists acting on non-NMDA receptors in the LPBN exert an inhibitory influence on sodium intake during acute fluid depletion with hypotension and after isoproterenol treatment. A possible interaction of serotonin with glutamate within the LPBN is discussed.

Keywords

glutamate; α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA); fluid and electrolyte balance; sodium appetite/intake; thirst

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1. Introduction

Glutamate is the major excitatory amino acid neurotransmitter in many species and has been implicated in the control of drinking behavior. Glutamate activates both metabotropic and ionotropic receptors (van den Pol et al., 1990). Ionotropic glutamate receptors have been categorized as N-methyl-D-aspartate (NMDA) and non-NMDA receptor subtypes. In turn, non-NMDA receptors are divided into those that bind either α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) or kainate and are referred to as AMPA and kainate receptors, respectively. Intracerebroventricular (icv) injections of either NMDA or kainate cause drinking in pigeons (Baron and Woods, 1993). In rats, icv injections of NMDA receptor antagonists reduce water intake caused by angiotensin II (ANG II) or dehydration (Xu et al., 1997; Xu and Herbert, 1998). Central (i.e., icv) administration of the non-NMDA receptor antagonists, DNQX or CNQX, in rats produces drinking that is antagonized by pretreatment with icv injections of the non-NMDA glutamate receptor agonist, AMPA (Xu and Johnson, 1998).

The lateral parabrachial nucleus (LPBN) is a structure located in the pons that plays a significant role in the control of saline and water intake. Rats with electrolytic or neurotoxic ibotenic acid lesions of the LPBN have increased water intake in response to central or peripheral administration of ANG II and to isoproterenol (Edwards and Johnson, 1991; Ohman and Johnson, 1986; Ohman and Johnson, 1989; Ohman and Johnson, 1995). Several studies have demonstrated the presence of a serotonergic mechanism in the LPBN that modulates sodium and water intake (Colombari et al., 1996; De Gobbi et al., 2000; De Gobbi et al., 2001; Menani et al., 1996; Menani et al., 1998; Menani et al., 2000; Menani et al., 2002; Menani and Johnson, 1995). In general, the localization of serotonin (5-HT) at a site in the brain is accompanied by the presence of glutamate (Maione et al., 1997; Meller et al., 2002; Scruggs et al., 2000; Singewald et al., 1998). Glutamate has been identified by immunocytochemistry to be present in the parabrachial nucleus (PBN) (Gill et al., 1999). Activation of vagal afferent neurons releases glutamate within the PBN (Saleh et al., 1997). An ascending pathway from the nucleus of the solitary tract (NTS) to the PBN has been described in which both NMDA and non-NMDA receptors in the PBN modulate excitatory signals from the NTS (Jhamandas and Harris, 1992). *In vitro* studies show that glutamate agonists like quisqualate, kainate and NMDA depolarize neurons in the PBN (Zidichouski and Jhamandas, 1993), and LPBN stimulation causes local glutamate release, which depolarizes LPBN neurons by non-NMDA and NMDA receptors (Zidichouski et al., 1996). Recently, it was demonstrated that NMDA receptors modulate 5-HT release in the LPBN during fluid depletion (Tanaka et al., 2006).

The present studies investigated if non-NMDA glutamate receptors in the LPBN affect water and sodium intake after acute fluid depletion with hypotension. We also determined the effects of the glutamatergic treatment on food intake, mean arterial blood pressure (MAP) and heart rate (HR).

2. Results

2.1. Histological analysis

The LPBN injection sites were comparable to those of previous studies (Ciriello et al., 1984; De Gobbi et al., 2007; De Gobbi et al., 2001; De Gobbi et al., 2005; Menani et al., 2000; Menani et al., 2002; Ohman and Johnson, 1986; Ohman and Johnson, 1989) investigating the effects of injections of serotonergic drugs on NaCl and water intake. Figure 1 depicts the spread of Evans Blue dye from injection sites of a typical rat with cannula tips terminating in both the LPBN. The injections were centered in the central lateral and dorsal lateral portions of the LPBN [see Fulwiler and Saper (1984) for definitions of LPBN subnuclei]. Injections reaching the ventral lateral and external lateral portions, as well as the Kölliker-Fuse nucleus, were

observed in some rats and the results from these rats were included in the analysis. Results from rats in which injections did not reach the LPBN, or did so only unilaterally, were analyzed separately and the results are presented in Table 1.

2.2. Water and sodium intakes after furosemide/captopril (Furo/Cap) treatment

The data were analyzed as rate of intake (i.e., ml/30 min), but for ease of presentation they are presented as cumulative intakes in Fig. 2. In the first experiment, bilateral injections of the non-NMDA receptor antagonist DNQX (2 and 5 nmol/0.2 μ l) dose-dependently increased 0.3 M NaCl intake ($F[3, 15] = 7.38$; $p < 0.05$) in conjunction with Furo/Cap treatment. Saline intakes were elevated within 30 min at both doses of DNQX, and were significantly greater at the higher dose compared to the lower dose at this time (interaction, $F[9, 45] = 3.02$; $p < 0.05$). An overall analysis of variance indicated that water intake was not statistically affected by DNQX treatment.

In a second experiment, the capacity of DNQX to significantly increase ingestion of 0.3 M NaCl was again demonstrated to occur in conjunction with Furo/Cap treatment. DNQX significantly increased ingestion of saline compared to the other treatment conditions which included the DNQX + AMPA treatment condition ($F[3, 30] = 9.97$; $p < 0.05$). DNQX treatment significantly increased the ingestion of water ($F[3, 30] = 10.68$; $p < 0.05$) compared to control, vehicle treatment. Increased water intake was apparent by 60 min of testing (interaction, $F[9, 90] = 2.36$; $p < 0.05$). The non-NMDA agonist AMPA administered along with vehicle significantly reduced water intake compared to control treatment throughout. AMPA pretreatment abolished the capacity of DNQX to increase water intake, and reduced intakes compared to controls in the first h (Fig. 3).

2.3. Water and sodium intakes after isoproterenol treatment

Bilateral injections of DNQX (5 nmol/0.2 μ l) into the LPBN produced a 7-fold increase in ingestion of 0.3 M NaCl after isoproterenol treatment ($F[1, 9] = 14.13$; $p < 0.05$, Fig. 4). A significant treatment $\times$ time interaction ($F[5, 45] = 6.94$; $p < 0.05$) indicated that water intake was initially reduced by DNQX treatment in the first 15 min of testing (DNQX: 0.8 ± 0.4 vs. vehicle: 3.1 ± 1.2 ml/15 min) but not for the remainder of the testing period.

2.4. Food and water intakes after food deprivation

Bilateral injections of DNQX (5 nmol/0.2 μ l) into the LPBN did not affect either food intake ($F[1, 5] = 1.06$; $p > 0.05$) or water intake ($F[1, 5] = 3.02$; $p > 0.05$) of rats after overnight food deprivation (Fig. 5).

2.5. Arterial pressure and heart rate recording

Bilateral injections of DNQX (5 nmol/0.2 μ l) into the LPBN did not significantly change either MAP ($F[3, 9] = 1.80$; $p > 0.05$), or HR ($F[3, 9] = 1.56$; $p > 0.05$) of conscious, freely-moving rats. The data for MAP and HR are presented in Table 2.

2.6. Injection site specificity

Rats in which one or both injections did not reach the LPBN (misplaced sites; i.e., these were generally above or below the LPBN) were excluded from the analyses of data presented in Figure 2, Figure 3 and Figure 4 and the values of their intakes are presented in Table 1. The treatments with DNQX alone (2 and 5 nmol) in misplaced injections did not change 0.3 M NaCl intake ($F[2, 6] = 0.06$; $p > 0.05$) or water intake ($F[2, 6] = 3.95$; $p > 0.05$) after Furo/Cap treatment. The combined treatment of AMPA with DNQX in animals with injections missing the LPBN also did not affect 0.3 M NaCl intake ($F[3, 12] = 3.00$; $p > 0.05$), or water intake ($F[3, 12] = 3.22$; $p > 0.05$) after Furo/Cap administration. Treatment with DNQX in rats with

misplaced LPBN cannulas did not change 0.3 M NaCl intake ($F[1,4] = 0.01$; $p > 0.05$) or water ingestion ($F[1,4] = 1.32$; $p > 0.05$) after isoproterenol.

3. Discussion

The results show that non-NMDA receptors in the LPBN exert inhibitory control over sodium and water intake after acute water and sodium depletion accompanied by mild hypotension produced by Furo/Cap treatment (Thunhorst and Johnson, 1994). Bilateral injections of the non-NMDA receptor antagonist DNQX into the LPBN increased ingestion of both sodium and water after Furo/Cap treatment, and dramatically increased ingestion of sodium after isoproterenol. Injections of AMPA, a non-NMDA receptor agonist, into the LPBN reduced both water and sodium intake of Furo/Cap treated animals and attenuated the increased consumption of water and sodium caused by DNQX. Activation of non-NMDA receptors with DNQX did not significantly affect food or water intake of food deprived animals and did not affect MAP or HR of otherwise undisturbed animals.

Multiple studies suggest that the LPBN importantly modulates sodium and water intake. Water and/or NaCl intake is increased after electrolytic or neurotoxic lesions of the LPBN (Edwards and Johnson, 1991; Ohman and Johnson, 1986; Ohman and Johnson, 1989) or treatments which antagonize serotonergic, cholecystikinin or corticotropin-releasing hormone action in the LPBN (De Castro e Silva et al., 2006; De Gobbi et al., 2001; Menani et al., 1996; Menani et al., 1998; Menani et al., 2000; Menani et al., 2002; Menani and Johnson, 1998). The present work extends these observations by implicating glutamate as another neurotransmitter acting in the LPBN to control thirst and salt appetite responses.

Previous work has provided evidence for the existence of complex serotonergic mechanisms in the LPBN that are related to the control of water and sodium ingestion. As part of this mechanism, 5-HT acting on 5-HT_{2A/2C} receptors is responsible for inhibiting sodium intake (De Gobbi et al., 2000; De Gobbi et al., 2001; Menani et al., 1996), and both 5-HT_{1A} (De Gobbi et al., 2005) and 5-HT₃ (De Gobbi et al., 2007) receptors modulate further release and actions of 5-HT. The present results fit a model in which 5-HT release in the LPBN acts via 5-HT_{2A/2C} receptors to release glutamate that acts on non-NMDA receptors to inhibit sodium intake.

Isoproterenol is a β -adrenergic receptor agonist that causes hypotension, renin release, and water intake upon peripheral administration, but does not stimulate sodium intake (Hosutt et al., 1978; Kirby et al., 1994). It is curious that isoproterenol does not cause sodium ingestion because reductions in arterial blood pressure and increases in renin secretion of the kinds observed after this adrenoceptor agonist are normally associated with the stimulation or facilitation of sodium intake (Avrith and Fitzsimons, 1980; Thunhorst and Johnson, 1994). In the present study, a non-NMDA receptor antagonist injected into the LPBN markedly increased sodium intake after sc injections of isoproterenol. Studies by Menani and colleagues (2000) have shown that antagonism of 5-HT receptors in the LPBN by methysergide (a non-specific serotonergic receptor antagonist) greatly increases NaCl intake after isoproterenol (Menani et al., 2000). It was suggested that the actions of isoproterenol on the heart stimulated vagal afferent c-fibers which actively suppress sodium intake during isoproterenol treatment (Menani et al., 2000). A similar hypothesis might explain the present results. In short, during isoproterenol treatment there is active inhibition of sodium intake. After the blockade of either glutamatergic non-NMDA or serotonergic receptors in the LPBN, sodium intake and enhanced drinking are expressed.

However, it is likely that several factors and mechanisms associated with the LPBN may be involved in switching an animal's ingestion (or preference) from water to saline. For example,

central or peripheral treatment with relaxin, a hormone produced by the ovary during pregnancy, also produces only water intake when administered by itself (Sinnayah et al., 1999; Thornton and Fitzsimons, 1995). However, when central injections of relaxin are administered in conjunction with methysergide into the LPBN, there is an increase in hypertonic saline intake (Menani et al., 2004). In the case of relaxin, it is unlikely that the enhanced ingestion of hypertonic saline is associated with altered afferent input.

Bilateral injections of DNQX into the LPBN did not affect MAP or HR of replete, undisturbed animals so the sodium and water intake observed after antagonism of non-NMDA receptors in the LPBN was not secondary to changes in blood pressure which might influence the behavior. The increased sodium intake in this case may reflect a direct effect of blocking non-NMDA receptors in the LPBN. However it is possible that DNQX might potentiate changes in the MAP and HR of depleted animals while not producing changes by itself in the same way it increases the ingestion of saline in animals primed to drink either water or saline.

DNQX administered bilaterally into the LPBN did not affect food and water intake after an overnight food deprivation. The fasted animals had water continuously available throughout the period that access to food was restricted. Therefore, the motivation to drink water (thirst) was probably minimal in animals that were in all likelihood close to euhydrated. These results suggest that a LPBN glutamatergic inhibition of water and/or saline intake is similar to that observed for the inhibitory actions of 5-HT, cholecystokinin and corticotropin-releasing hormone within the LPBN where enhancement of increased water intake or increased hypertonic saline intake is seen only when there is also a reasonably strong stimulus to drink or ingest salt (Colombari et al., 1996; De Castro e Silva et al., 2006; De Gobbi et al., 2007; De Gobbi et al., 2000; De Gobbi et al., 2001; De Gobbi et al., 2005; Menani et al., 1996; Menani et al., 1998; Menani et al., 2000; Menani et al., 2002; Menani et al., 2004; Menani and Johnson, 1995; Menani and Johnson, 1998).

Recent evidence suggests that release of 5-HT in some brain structures is under glutamatergic control. For example, serotonergic neurons in the locus coeruleus are tonically modulated by excitatory amino acids via NMDA and AMPA/kainate receptors (Singewald et al., 1998). AMPA infusions into the striatum of rats increase 5-HT release and decrease the concentrations of its metabolite, 5-hydroxyindoleacetic acid, suggesting that glutamate is a modulator of 5-HT release (Maione et al., 1997). Activation of 5-HT_{2A/2C} receptors on thalamocortical neurons increases glutamate release, which in turn drives enhanced expression of the immediate early gene, *c-fos*, in cortical neurons through an AMPA receptor-dependent mechanism (Scruggs et al., 2000). In cultured glia cells, activation of 5-HT_{2A/2C} receptors stimulates efflux of glutamate (Meller et al., 2002). Recently, it was demonstrated that NMDA receptors also modulate 5-HT release in the LPBN after Furo/Cap treatment (Tanaka et al., 2006).

Evidence indicates that the LPBN has an important role within the central neural network that controls body fluid and cardiovascular homeostasis. The LPBN is a strategic integrative region for ascending visceral-related information at the level of the pons and is reciprocally connected with important medullary and forebrain areas involved in behavioral and autonomic responses (Ciriello et al., 1984; Krukoff et al., 1993). A glutamatergic pathway from the NTS to the PBN has been described (Jhamandas and Harris, 1992) and similarly a serotonergic pathway from the NTS to the LPBN has also been identified (Lança and van der Kooy, 1985). The NTS is the primary site in the central nervous system that receives important afferents from visceral receptors involved in the control of fluid and electrolyte balance (Krukoff et al., 1993). From the NTS the signals from visceral receptors may reach the LPBN through glutamatergic and serotonergic pathways. Therefore, blockade of 5-HT and/or glutamate receptors in the LPBN

would decrease the inhibition of water and sodium intake, thus releasing the ingestion of water and hypertonic NaCl.

In summary, the present data show that non-NMDA receptors within the LPBN exert inhibitory control of sodium intake. The inhibitory mechanisms that control sodium intake in the LPBN are complex, and in all likelihood use more than one neurotransmitter system.

4. Experimental procedures

4.1. Animals

Male Sprague-Dawley derived rats (Harlan, Indianapolis, IN) weighing 280–300 g were used. The animals were housed in individual stainless steel cages with free access to standard diet (Purina Rat Chow), tap water and 0.3 M NaCl solution. Rats were maintained on a 12:12 light/dark cycle (light onset at 0730 h) in a room with controlled temperature ($23 \pm 1^\circ\text{C}$). All experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and approved by the University of Iowa Animal Care and Use Committee.

4.2. Cerebral cannulas

Rats were anesthetized with an Equithesin[®]-like anesthetic cocktail (composed of 0.97 g of sodium pentobarbital and 4.25 g of chloral hydrate/100 ml distilled water prepared by The University of Iowa Hospitals and Clinics Pharmacy; 0.33 ml/100 g bw) and placed in a Kopf stereotaxic instrument. The skull was leveled between bregma and lambda. Stainless steel 23 gauge cannulas were implanted bilaterally into the LPBN at 9.5 mm caudal to bregma, 2.2 mm lateral to the midline and 4.1 mm below the dura mater (Paxinos and Watson, 1998). The tips of the cannulas were positioned at points 2 mm above each LPBN. The cannulas were fixed to the cranium using dental acrylic resin and jewelers' screws. A 30-gauge metal obturator filled the cannulas between tests. After the surgery, the rats were allowed to recover six days before beginning LPBN drug injections.

4.3. Injections into the LPBN

Bilateral injections into the LPBN were made using 10 μl Hamilton syringes connected by polyethylene tubing (PE-10) to 30 gauge injection cannulas. At the time of testing, rats were taken from the home cage, obturators removed, and injection cannulas introduced into the brain. Upon insertion, the injection cannulas extended 2 mm beyond the tips of the guide cannulas. The animals were handheld as drugs were injected. The injection volume was 0.2 μl per site. After the injections, the obturators were replaced and the rats were placed back into their cages.

4.4. Drugs

A diuretic/natriuretic, furosemide (Furo), an angiotensin converting enzyme inhibitor, captopril (Cap), and a non-selective adrenergic receptor agonist, isoproterenol, were purchased from Sigma-Aldrich (St. Louis, MO). A non-NMDA receptor antagonist, DNQX, and a non-NMDA receptor agonist, AMPA, were purchased from Research Biochemicals International (RBI; Natick, MA). DNQX was dissolved in dimethyl sulfoxide (DMSO; 50%) and DMSO in saline (50%) was used as the vehicle control. Saline was used as the vehicle for AMPA and as the control for AMPA injections.

4.5. Water and 0.3 M NaCl intake

Rats were tested in their home cages. Water and 0.3 M NaCl were provided from burettes with 0.1 ml divisions that were fitted with metal drinking spouts.

4.5.1. Experimental design—The experiments used within-subjects designs. Separate groups of rats were used to test the effects of DNQX and AMPA on water and saline intakes after Furo/Cap or isoproterenol treatment. Other groups of rats were used to measure the effects of DNQX on food intake, MAP and HR. Each animal received all doses of a drug and its vehicle in counterbalanced order in tests separated by at least 48 h.

4.5.2. Acute water and sodium depletion with hypotension (Furo/Cap treatment)

—Two experiments determined the effects of activating or inhibiting non-NMDA receptors on water and 0.3 M NaCl intakes after Furo/Cap treatment. Acute depletion of water and sodium accompanied by mild hypotension was produced by sc injections of the diuretic Furo (10 mg/kg bw) followed by Cap (5 mg/kg bw) as described previously (De Gobbi et al., 2007; Fitts and Masson, 1989; Ohman and Johnson, 1986; Thunhorst and Johnson, 1994). The rats were removed from their home cages, injected with Furo and Cap and then returned to their home cages from which water and saline had been removed. One h later, burettes of 0.3 M NaCl and water were replaced on the cages and intakes of both were measured at 30, 60, 90 and 120 min. Injections of drugs into the LPBN were made 10 min before water and 0.3 M NaCl were returned to the cages. In the first experiment, rats received bilateral injections of the non-NMDA antagonist, DNQX (2 and 5 nmol/0.2 μ l) or vehicle into the LPBN. There were two drug injections and two vehicle injections during the course of testing. In the second experiment, the acutely depleted animals received two central injections into the LPBN before the burettes were returned. The animals received the agonist, AMPA (0.2 nmol/0.2 μ l) or isotonic saline (vehicle) into the LPBN followed 10 min later by bilateral injections of DNQX (5 nmol/0.2 μ l) or vehicle. Ten min after the second injection, burettes were returned to their cages for measurement of intakes.

4.5.3. Isoproterenol treatment—One group of rats was used to study the effects of DNQX on water and sodium ingestion after isoproterenol treatment. On the day of testing, rats were removed from their home cages, injected with isoproterenol (30 μ g/kg bw, sc) followed immediately by injections of DNQX (5 nmol/0.2 μ l) or vehicle bilaterally into the LPBN. They were returned to their cages, where water and 0.3 M NaCl were available. Water and 0.3 M NaCl intakes were recorded at 15, 30, 60, 90, 120 and 150 min.

4.5.4. Food deprivation—One group of rats was used to test the effects of DNQX on food intake after food deprivation. This group of rats had bilateral cannulas implanted in the LPBN at least 5 days before the tests. The rats were deprived of food overnight, but had water available. The next morning, about 18–20 h later, they received bilateral injections of DNQX (5 nmol/0.2 μ l) or vehicle (order randomized) into the LPBN. Ten minutes later the rats were presented with a preweighed quantity of standard diet and water from a burette (0.1 ml divisions). The intakes of both were measured at 15, 30, 60, 90, and 120 min. Food intake measures took spillage into account. Three days separated the two tests.

4.6. Arterial pressure and heart rate recording

In this experiment, MAP and HR were recorded in conscious, freely-moving rats. Catheters were constructed of a short piece of PE-10 tubing heat welded to a longer piece of PE-50. Catheters were implanted the day before the experiment. To implant the catheters, rats were anesthetized with the Equithesin[®]-like anesthetic cocktail (0.33 ml/100 g bw). The PE-10 end of the catheter was inserted about 4 cm into the abdominal aorta through the femoral artery. The PE-50 end of the catheter was tunneled subcutaneously to the scruff of the neck and exteriorized. The next day, rats were brought to a separate room for testing. Arterial catheters for MAP and HR determinations were connected to a pressure transducer coupled to a digital multichannel recorder (PowerLab/AD Instruments, Colorado Springs, CO). The rats were allowed approximately 1 h for adaptation. Then, each rat received bilateral injections of vehicle

(0.2 μ l) into the LPBN followed 20 min later by bilateral injections of DNQX (5 nmol/0.2 μ l) into the LPBN. Measures of MAP and HR continued for another 20 min.

4.7. Histology

At the end of the experiments, the animals received bilateral injections of 2% Evans blue dye (0.2 μ l/injection site) into the LPBN. They were then deeply anesthetized with sodium pentobarbital (80 mg/kg, bw) and perfused transcardially with isotonic saline followed by 10% formalin. The brains were removed, fixed in 10% formalin, frozen, cut in 50 μ m sections, stained with cresyl violet and analyzed by light microscopy to confirm the injection sites in the LPBN.

4.8. Statistical analysis

Data were first analyzed using repeated measures analysis of variance (ANOVA). Newman-Keuls tests were used for pairwise comparisons when the global F was significant. Differences were considered significant at $p < 0.05$. The results are reported as mean $\pm$ SEM.

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Figure 1. Photomicrograph showing site of injection into the lateral parabrachial nucleus (LPBN, arrows). Dots outline the superior cerebellar peduncles.

Figure 2.

A: Cumulative 0.3 M NaCl intake; B: individual 0.3 M NaCl intakes (120 min); C: Cumulative water intake; and D: individual water intakes (120 min) in Furo/Cap treated rats after bilateral injections of DNQX (2 and 5 nmol/0.2 μ l) or vehicle (veh) into the lateral parabrachial nucleus. A, C: values are mean $\pm$ SEM. n = number of rats. * = different from vehicle.

Figure 3.

A: Cumulative 0.3 M NaCl intake; B: individual 0.3 M NaCl intakes (120 min); C: Cumulative water intake; and D: individual water intakes (120 min) in Furo/Cap treated rats after pretreatment of the lateral parabrachial nucleus (LPBN) with α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) (0.2 nmol/0.2 μ) or isotonic saline (sal) before bilateral injections of DNQX (5 nmol/0.2 μ l) or vehicle (veh) into the LPBN. A, C: values are mean $\pm$ SEM. n = number of rats. * = different from saline + vehicle. + = different from saline + DNQX.

Figure 4.

A: Cumulative 0.3 M NaCl intake; B: individual 0.3 M NaCl intakes (120 min); C: Cumulative water intake; and D: individual water intakes (120 min) in subcutaneous isoproterenol treated rats after bilateral injections of DNQX (5 nmol/0.2 μ l) or vehicle into the lateral parabrachial nucleus. A, C: values are mean $\pm$ SEM. *n* = number of rats. * = different from vehicle.

Figure 5.

A: Cumulative food intake; B: individual food intakes (120 min); C: Cumulative water intake; and D: individual water intakes (120 min) in food deprived rats that received bilateral injections of DNQX (5 nmol/0.2 μ l) or vehicle into the LPBN. A, C: values are mean $\pm$ SEM. n = number of rats.

Table 1

Cumulative intakes of water and 0.3 M NaCl after furosemide/captopril (Furo/Cap) treatment or isoproterenol treatment in rats in which one or both of the central injections of DNQX, AMPA or control solutions were outside the LPBN.

LPBN treatments	n	0.3 M NaCl intake (ml/2 h)	Water intake (ml/2 h)
<i>1st Furo/Cap treatment experiment</i>			
vehicle	5	3.1 ± 0.8	4.6 ± 0.8
DNQX 2 nmol	4	3.5 ± 1.1	5.4 ± 0.9
DNQX 5 nmol	5	3.2 ± 0.7	7.9 ± 0.4
<i>2nd Furo/Cap treatment experiment</i>			
saline + vehicle	5	5.7 ± 0.6	6.6 ± 0.7
AMPA 0.2 nmol + vehicle	5	0.1 ± 0.1	1.9 ± 0.7
saline + DNQX 5 nmol	5	2.0 ± 1.2	10.4 ± 2.1
AMPA 0.2 nmol + DNQX 5 nmol	5	1.9 ± 1.0	4.6 ± 2.2
<i>Isoproterenol treatment</i>			
Vehicle	4	0.2 ± 0.1	3.6 ± 0.9
DNQX 5 nmol	4	1.2 ± 0.2	3.4 ± 1.3

Values are mean ± SEM, n = number of rats

Table 2

Mean arterial pressure (MAP) and heart rate (HR) in rats after bilateral injections of DNQX (5 nmol/0.2 μ l) or vehicle into the LPBN.

	baseline	vehicle	baseline	DNQX
MAP (mmHg)	139 $\pm$ 2	144 $\pm$ 2	143 $\pm$ 2	144 $\pm$ 2
HR (bpm)	391 $\pm$ 16	416 $\pm$ 14	400 $\pm$ 10	400 $\pm$ 12

Values are mean $\pm$ SEM. Baseline measures were averaged over the last 20 minutes before injections. Vehicle and DNQX results were averaged over 20 minutes after injection. There were no significant differences in either MAP or HR after treatments. Number of rats = 4. bpm = beats per minute.