Centrally administered ascorbic acid induces antidiuresis, natriuresis and neurohypophyseal hormone release in rats

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Submitted: 2009-06-06 Accepted: 2009-07-18 Published online: 2010-02-16

Key words: **vasopressin; oxytocin; diuresis; natriuresis**

Neuroendocrinol Lett 2010; **31**(1):87–91 **PMID:** 20150870 NEL310110A08

Abstract

OBJECTIVE: Ascorbic acid represents one of the most important antioxidants and neuromodulators, and plays an important role in the cerebral system. In the present study, we investigated the central effect of ascorbic acid on fluid regulation and electrolyte homeostasis.

METHODS: Male Wistar rats were implanted with stainless steel cannulas into the lateral ventricle, and sodiun excretion and urinary volume were measured after intracerebroventricular (i.c.v.) injection of ascorbic acid (200 or 600 nmol/rat). In another set of experiments, vasopressin and oxytocin plasma levels were evaluated 10, 20 and 30 minutes after ascorbic acid i.c.v. injection.

RESULTS: The administration of ascorbic acid to conscious rats resulted in a significant decrease in urinary volume and an increase in the renal excretion of sodium, with a concomitant increase in the plasma levels of vasopressin and oxytocin.

CONCLUSIONS: These results suggest that ascorbic acid may play a significant role in the central regulation of fluid and electrolyte homeostasis.

ORIGINAL ARTICLE

INTRODUCTION

Ascorbic acid is present in high concentrations in the mammalian brain. In addition to its functions as an antioxidant, a considerable number of studies have suggested that ascorbic acid is an important neuromodulator in the brain (Spector & Lorenzo, 1973; Grunewald, 1993; Harrison & May, 2009).

A large amount of evidence has showed that the extracellular concentration of ascorbic acid in the brain changes rapidly in response to drugs and to changes in plasma osmolality, and affects neural activities (Manson *et al.* 1988; Brazell *et al.* 1990;

Abbreviations

aCSf	- artificial cerebrospinal fluid
AUC	- area under curve
i.c.v	- intracerebroventrucular
LV	- lateral cerebral ventricle
NO	- nitric oxide
PVN	- paraventricular nucleus
SON	- supraoptic nucleus

Wu, 1994; Manson *et al.* 1995; Enrico *et al.* 1997, Miele *et al.* 2000; Gu *et al.* 2006; Harrison & May, 2009).

It has been demonstrated that, in water-restricted rats, the extracellular concentration of ascorbic acid in the lateral hypothalamus rises with the decrease of plasma osmolality (Manson *et al.* 1995). This data suggest that the ascorbic acid might also be involved in the integrative control of body fluid homeostasis.

The increase in plasma osmolality stimulates the neurohypophysial secretion of oxytocin, a natriuretic hormone, and vasopressin, the antidiuretic hormone (Antunes-Rodrigues *et al.* 2004). There neurohypophysial hormones are produced and secreted from the magnocellular neuronal terminal of the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN). Several different factors can influence the release of vasopressin and oxytocin from the neurohypophysis, but the osmotic regulation of vasopressin secretion is one of the most finely tuned homeostatic mechanisms (Dunn *et al.* 1973; Cunningham & Sawchenko, 1991).

On the basis of the above mentioned observations, in the present study, we examined the effect of intracerebroventricular injection of ascorbic acid on neurohypophysial secretion of oxytocin and vasopressin, and the effect on urinary excretion of sodium and water in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (280–350 g) from the Central Animal Facility of the Federal University of Alfenas were kept in individual cages in an animal room with controlled temperature (23 ± 2 °C) and a 12/12 hour light/dark cycle (light on from 06:00 to 18:00 hours) with free access to food pellets and tap water. All experiments were conducted between 08:00 and 11:00 A.M. The experimental procedures were approved by the Ethical Committee for Animal Use of the Federal University of Alfenas and carried out in accordance with the Declaration of Helsinki.

Intracerebroventricular (i.c.v.) surgery

The rats were anesthetized with tribromoethanol (250 mg/kg of body weight, intraperitoneal) and placed in a stereotaxic instrument (Kopf, model 900). Their skulls were leveled between bregma and lambda. Stainless steel guide cannulas (10.0 mm long, 0.6 mm o.d., 0.4 mm i.d.) were implanted unilaterally into the right lateral cerebral ventricle (LV), at the following coordinates: 0.6 mm caudal to the bregma, 1.5 mm lateral to the midline and 3.6 mm below the dura mater. The cannula was fixed to the cranium using dental acrylic resin and two jeweler's screws. A 30-gauge metal wire filled the cannulas except during the injections, as previously described (Giusti-Paiva *et al.* 2003; Giusti-Paiva *et al.* 2005). After surgery, the rats received a prophylactic injection of penicillin and were allowed to recover for 5–6 days, during which they were daily handled and habituated to the removal of the obturator of the guide cannula and to the gavage procedures, so as to minimize stress during the experimental phase.

Experimental Protocols

Experiment 1: Effects of ascorbic acid in urinary sodium and water excretion

To evaluate the effects of ascorbic acid on urinary sodium and water excretion, on the day of the experiment the rats received two water loads (5% of body weight each, 37 °C) by intragastric gavages at 60 min interval, with the purpose of increasing and producing a continuous urine flow, as previously described (Margatho *et al.* 2007; Reis *et al.* 2007). Immediately after the second water load, the animals received injections of ascorbic acid (200 or 600 nmol) or vehicle (2 µl). At the time of testing, the rats were removed from their home cages, and the injection cannulas were introduced into the guide cannulas. The injections took 60 seconds. Thereafter, they were kept in the metabolic cages without access to chow or water. Urine samples were then collected at 20-min intervals over a 120 min period. Complete voiding of urine was manually induced by gently pressing the suprapubic region of the animal at the end of each interval. Urinary volume was determined using 100 µl graduated tubes and expressed as ml/100g body weight. Urinary sodium excretion was determined by flame photometer (Micronal, model b262).

Experiment 2: Effects of ascorbic acid in vasopressin and oxytocin secretion

To determinate the effect of ascorbic acid in vasopressin and oxytocin release, the rats received an i.c.v. injection (2 µl) of vehicle or ascorbic acid (200 nmol) and were decapitated 0, 10, 20 or 30 minutes afterwards. Trunk blood was collected into cooled plastic tubes containing heparin for the measurement of vasopressin and oxytocin. Blood samples were centrifuged (3,000 rpm for 20min at 4 °C), and plasma was kept in a freezer at −20 °C. For the vasopressin and oxytocin determinations, samples were extracted from 1ml of plasma with acetone and petroleum ether. Plasma levels of vasopressin and oxytocin were measured by specific radioimmunoassays as described by Elias *et al.* (1997) and Haanwinckel *et al.* (1995), respectively. The assay sensitivity and the intra- and inter-assay coefficients of variation were, respectively, 0.8 pg/ml, 7.7% and 11.9% for vasopressin, and 0.9 pg/ml, 7.0% and 12.6% for oxytocin.

Drugs

The drugs were injected into the LV using a Hamilton microsyringe (10 µl) connected to a PE-10 polyethylene tube to an injector needle (0.3 mm o.d), which was introduced into the guide cannula. Ascorbic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in artificial cerebrospinal fluid (aCSF).

The composition of aCSF in mM was: 135.0 Na^+, 2.6 K^+, 0.9 Mg^{2+}, 1.3 Ca^{2+}, 122.7 Cl^-, 21.0 HCO_3^-, 2.5 HPO_4^{2-} and 3.87 glucose. Before use, all solutions administered through the fiber were bubbled with a 5% CO_2/95% O_2 mixture and pH was adjusted to 7.2–7.4.

Statistical analysis

The results are reported as means ± SE, and their statistical significance was assessed by analysis of variance (ANOVA) followed by the Newman-Keuls post hoc test. The level of significance was set at $p<0.05$.

RESULTS

Injection of ascorbic acid (200 and 600 nmol) into the LV increased sodium excretion at 120 min (43.3 ± 6.8 and 67.8 ± 11.2 μEq/100 g bw; $p<0.05$) compared to the values observed in rats injected with vehicle (17.7 ± 2.9 μEq/100 g bw; Figure 1). The area under curve (AUC) response to 200 and 600 nmol of ascorbic acid was significantly higher than the response to vehicle. The cumulative urinary volume from this set of experiments is presented in Figure 2. Intracerebroventricular administration of ascorbic acid resulted in a significant decrease ($p<0.05$) in urine volume and in AUC during the first 80 min compared to the control group ($p<0.05$), returning toward the control levels after this period.

Figure 3 shows the increase in plasma vasopressin and oxytocin levels over time after 200 nmol of ascorbic acid i.c.v. administration. A significant increase in plasma vasopressin and oxytocin was observed 10, 20 and 30 minutes after i.c.v. injection of ascorbic acid, compared with the control group. In control animals, vasopressin and oxytocin levels did not change from the baseline during the entire experimental period.

DISCUSSION

The present results show that injection of ascorbic acid into the LV decreased the urinary volume and increased the renal excretion of sodium, with a concomitant increase in the plasma levels of vasopressin and oxytocin. It is well established that the increase of vasopressin plasma levels is related to the water reabsorption in the kidney and the increase of urinary osmolality (Nielsen *et al.* 1995). In addition, the increase in oxytocin plasma levels results in natriuretic effects (Verbalis *et al.* 1991; Haanwinckel *et al.* 1995).

The highest concentrations of ascorbate in the body are found in the brain. Ascorbate is transported into the brain and neurons via the sodium-dependent vitamin C transporter 2 (SVCT2), which causes accumulation of ascorbate within cells against a concentration gradient. This trans-cellular transport generates a fourfold plasma-to-CSF ascorbate gradient in rats, resulting in CSF concentrations of about 200–400 μM, compared to plasma concentrations of 60 μM or less (Harrison & May 2009). The solution of 200 nmol of ascorbic acid in 2 μl (100 mM) i.c.v. administrated was dissolved in CSF of lateral ventricle, so that the estimated final concentration increases 25 fold the concentration of ascorbic acid in CSF, or even greater and is 10 times lower than that intraneuronally and probably many fold lower than inside synaptic vesicles of glutamergic neuron. In addition, pH of artificial CSF with ascorbic acid was adjusted to 7.2 the same to vehicle. This suggests that i.c.v. administration of 200 nmol did not led to artifacts, but to a pharmacological effect. On the other hand, the peripheral doses that evoked behavioral changes peripherally administered (suggesting a central effect) were 1–2 g/kg in rats, that induces 1000-fold increase in ascorbic acid plasma concentration. This dose certainly

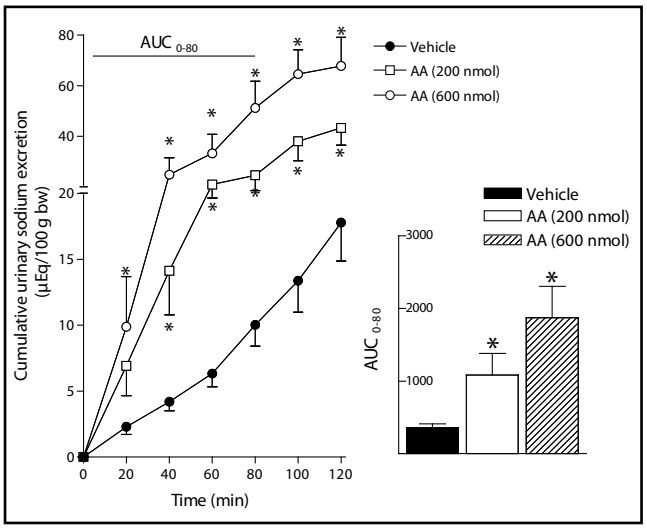

Fig. 1. The changes (plotted over time) and the area under curve (AUC) in sodium excretion after i.c.v. injection of ascorbic acid (AA). Data represent the mean ± S.E., n=8 per group. *$p<0.05$ vs. vehicle-treated group.

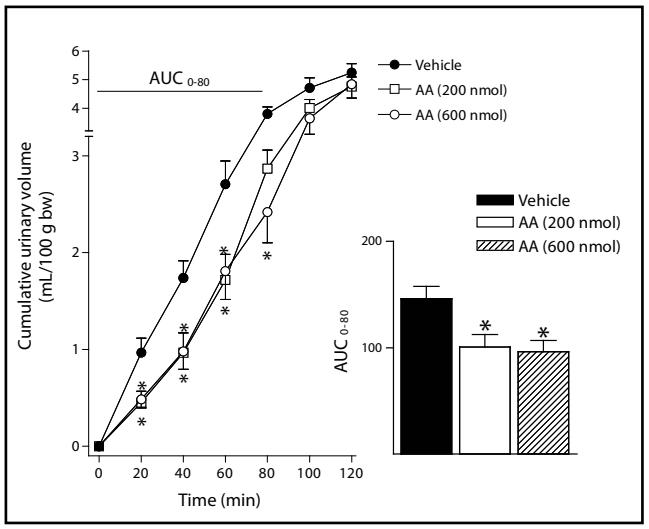

Fig. 2. The changes (plotted over time) and area under curve (AUC) in cumulative urinary volume, after i.c.v. injection of ascorbic acid (AA). Data represent the mean ± S.E., n=8 per group. *$p<0.05$ vs. vehicle-treated group.

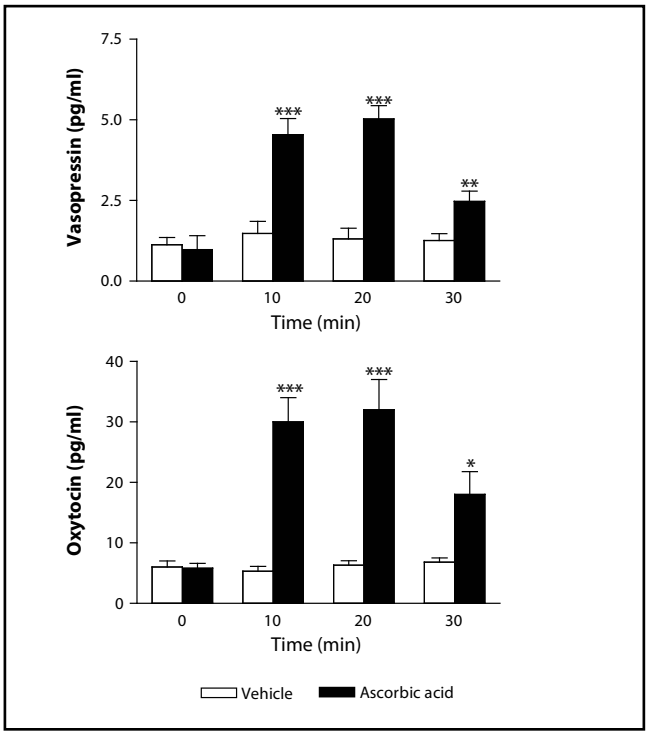

Fig. 3. Temporal effect of vehicle or ascorbic acid (200 nmol/5 µl; i.c.v) on plasma vasopressin (top) and oxytocin (bottom) levels. Bars represent the mean ± S.E., n = 8 per group. *p<0.05; **p<0.01; ***p<0.001 vs. vehicle group.

provokes hydroelectrolytic disorder. Thence, to evaluate the effect of ascorbic acid on neurohypophysial secretion of oxytocin and vasopressin, and the effect on urinary excretion of sodium and water in rats, the ascorbic acid was administrated intracerebroventricularly.

In animal studies, ascorbic acid modulates dopaminergic and noradrenergic activity (Majewska *et al.* 1990; Grunewald 1993; Rebec & Pierce 1994; Rebec *et al.* 2005), exhibits an antinociceptive effect (Rosa *et al.* 2005), provides protection from scopolamine-induced impairment of memory (Parle & Dhingra 2003), and potentiates the inhibitory effect of dopamine on prolactin release (Shin *et al.* 1997). In the present study, we demonstrated that ascorbic acid applied in the brain ventricle modulates neurohypophyseal hormone secretion.

Several studies have shown that various neurotransmitter systems, such as the dopaminergic, glutamatergic, cholinergic, and serotonergic systems, may be involved in the central ascorbic acid release (Rice, 2000; Harrison & May, 2009). The mechanisms and the physiological implication of neurotransmitter induced ascorbic acid release are not yet well understood.

Vasopressin and oxytocin are released from the neurohypophysis by axon terminals of magnocellular neurones located mainly in the SON and PVN of the hypothalamus. The release of these hormones is controlled by hypothalamic neuronal activity, which is influenced by body fluid volume, osmolarity, extracellular sodium concentrations, blood pressure and other stimuli (Antunes-Rodrigues *et al.* 2004; McKinley *et al.* 2004). PVN and SON magnocellular neurones are innervated by afferent projections from many areas of the brain, and many neuromodulators, such as glutamate and nitric oxide, can influence vasopressin and oxytocin release (Kadekaro 2004; Durand *et al.* 2008).

Previous reports have demonstrated that corticostriatal glutamate transmission is sensive to the level of ascorbic acid, and the effects induced by increased ascobic acid in extracellular fluid are similar to those observed with glutamate uptake inhibitors (Rebec *et al.* 2005). Similar to its role in other parts of the brain, glutamate is the main excitatory neurotransmitter in the hypothalamus, and in this way may be involved in the oxytocin and vasopressin release stimulated by ascorbic acid.

Ascorbic acid may not only alter physiological neurotransmission, but it also may play a very important role in scavenging free radicals, including nitric oxide (NO). The antioxidative action of ascorbic acid may be due not only to the direct trapping of NO (at elevated levels), but also to an attenuation of (patho)physiological NO levels (Rose & Bode 1993; Armour *et al.* 2001). Ascorbic acid counterbalances NO levels through various nonenzymatic pathways, that is, scavenging by NO-depleting reactive nitrogen-oxygen species, and through trapping of NO by ascorbate-derived products (Kytzia *et al.* 2006). It has been demonstrated that NO may act as an inhibitory regulator of vasopressin and oxytocin (Giusti-Paiva *et al.* 2003; Kadekaro 2004; Ventura *et al.* 2005; Reis *et al.* 2007). Thus, ascorbic acid can promove increment in vasopressin and oxytocin plasma levels by scavenger, an inhibitory gaseous neuromodulator of magnocellular neurons in the hypothalamus. Indeed, the effect of ascorbic acid on neurohypophysial hormone release can be attributed to a rise in excitatory neurotransmiters or to a decrease in inhibitory neuromodulators.

In conclusion, the intracerebroventricular administration of ascorbic acid to conscious hydrated rats resulted in a significant decrease in the urinary volume and an increase in the renal excretion of sodium, with a concomitant increase in the plasma levels of vasopressin and oxytocin, and suggests that ascorbic acid may play a significant role in the central regulation of fluid and electrolyte homeostasis. Additional studies are required for a better understanding of these complex neuromodulatory mechanisms at the hypothalamic level.

ACKNOWLEDGMENTS

This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG CBB-1311/06 and CDS-APQ-01272-08) and Conselho Nacional de Desenvolvimento Científico (CNPq #479584/2007-9). A Giusti-Paiva received a research fellowship from CNPq. The authors thank Dr. José Antunes-Rodrigues for providing laboratory facilities.

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