Brazilian Journal of Medical and Biological Research (1999) 32: 1413-1416
ISSN 0100-879X

Effect of injection of L-NAME on drinking response

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Abstract

The drinking behavior responses to centrally administered N^G-nitro-L-arginine methyl ester (L-NAME; 10, 20 or 40 µg/µl), an inhibitor of nitric oxide synthase, were studied in satiated rats, with cannulae stereotaxically implanted into the lateral ventricle (LV) and subfornical organ (SFO). Water intake increased in all animals after angiotensin II (ANG II) injection into the LV, with values of 14.2 ± 1.4 ml/h. After injection of L-NAME at doses of 10, 20 or 40 µg/µl into the SFO before injection of ANG II (12 ng/µl) into the LV, water intake decreased progressively and reached basal levels after treatment with 0.15 M NaCl and with the highest dose of L-NAME (i.e., 40 µg). The water intake obtained after 40 µg/µl L-NAME was 0.8 ± 0.01 ml/h. Also, the injection of L-NAME, 10, 20 or 40 µg/µl, into the LV progressively reduced the water intake induced by hypertonic saline, with values of 5.3 ± 0.8, 3.2 ± 0.8 and 0.7 ± 0.01 ml/h, respectively. These results indicate that nitric oxide is involved in the regulation of drinking behavior induced by centrally administered ANG II and cellular dehydration and that the nitric oxide of the SFO plays an important role in this regulation.

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Presented at the Meeting "NO Brazil, Basic and Clinical Aspects of Nitric Oxide", Foz do Iguaçu, PR, Brazil, March 10-13, 1999.

Research supported by FAPESP and CNPq.

Received June 17, 1999
Accepted July 26, 1999

Key words
• Nitric oxide
• Water intake
• SFO
• Dehydration
• CNS

Introduction

Recognition of the role of nitric oxide (NO) in cell-to-cell communication has changed the concept of traditional neurotransmission. N-methyl-D-aspartate receptors mediate the dipsogenic response and c-Fos expression induced by intracerebroventricular (*icv*) infusion of angiotensin II (ANG II) (1). It has been demonstrated that NO may facilitate the release of excitatory transmitters, possibly through a presynaptic cyclic GMP-dependent mechanism (2). NO, a unique biological messenger molecule, has been identified as the endothelium-derived relaxing factor (EDRF) (3). Many biological activities of EDRF such as smooth muscle relaxation and inhibition of platelet aggregation (4), as well as regulation of the immune system (5) have been attributed to NO. Moreover, the presence of immunoreactive nitric oxide synthase (NOS) or NADPH diaphorase, a histochemical marker of NOS, has been demonstrated in the subfornical organ

(SFO) and organum vasculosum laminae terminalis (OVLT) (6). These circumventricular organs and their anatomically related structures in the lamina terminalis, the nucleus medianus, periventricular area and paraventricular nucleus represent a circuit in the anteroventral part of the third ventricle, the so-called AV3V area, which has an important role in the regulation of blood pressure, drinking behavior, and secretion of neurohypophyseal hormones (7).

The objectives of the present study were to investigate the effect of a more potent blocker of NOS, N^G-nitro-L-arginine methyl ester (L-NAME), injected into the SFO on the water intake response of rats with thirst experimentally induced by decreasing intracellular volume, and on the thirst induced by ANG II injected into the lateral ventricle (LV).

Material and Methods

Animals

Adult male Holtzman rats (200-300 g) were housed in individual home cages in a temperature (22°C)- and humidity-controlled environment, with a 12:12-h light:dark cycle, with food and water available *ad libitum* for at least 7 days before surgery.

Surgery

On the day of surgery, each animal was anesthetized with 2,2,2,-tribromoethanol (20 mg/100 g body weight, *ip*) and a 20-gauge stainless steel guide cannula was stereotactically implanted into the right lateral cerebroventricle and into the SFO according to the coordinates of the Paxinos and Watson atlas (8).

Experimental design

Two series of experiments were performed: 1) osmotic stimulation. One group of rats received a subcutaneous injection of hypertonic saline (1.0 M NaCl) at a dose of 15 ml/kg body weight. 2) One group with cannulas implanted into the LV and SFO received an injection of L-NAME into the SFO followed 10 min later by ANG II, 12 ng/0.5 µl, into the LV. Water intake was measured every 20 min for 60 min. Twenty-five minutes after osmotic stimulation, a 30-gauge stainless steel injector, which extended 0.5 mm below the tip of the *icv* guide cannula, was inserted into each animal and left in place for 5 min before delivering 1 µl of saline (0.15 M NaCl), or 10, 20 or 40 µg/µl of L-NAME (Sigma Chemical Co., St. Louis, MO, USA) dissolved in 0.9% saline into the LV. Ten minutes after *icv* injection of the solutions, water was made available to the animals in graduated burettes with a metal spout and the cumulative intake (ml/h) was measured 20, 40, and 60 min after the injection. In another group, L-NAME was injected into the SFO 10 min after ANG II into the LV.

At the end of the experiment the animals were killed and Evans blue was injected *icv*. The animals were perfused with buffered saline followed by 10% formalin. The brains were removed, fixed in 10% buffered neutral formalin solution for at least 2 days, and then cut frontally with a brain slicer. Under a dissecting microscope, the placement of the tip of the internal cannula and the distribution of the dye in the ventricular system and the site of implantation of the SFO cannula were analyzed. Only data from animals with the dye distributed throughout the ventricular system and with injection of L-NAME inside the SFO were used.

Statistical analysis

The results are reported as mean ± SEM and were analyzed by two-way analysis of variance for multiple comparisons between groups. The Dunnett *t*-test was used to assess differences between individual means.

Results

Effects of L-NAME injected into the LV on water intake induced by decreasing intracellular volume

As shown in Figure 1, subcutaneous injection of hypertonic saline induced an increase in water intake when compared to baseline values (12.0 ± 1.2 and 0.5 ± 0.1 ml/h). A previous injection of L-NAME, 10, 20 or 40 µg/µl, into the LV produced a progressive decrease in the water intake induced by hypertonic saline, with values of 5.3 ± 0.8, 3.2 ± 0.8 and 0.7 ± 0.1 ml/h, respectively. These results clearly demonstrate the influence of NO on water intake induced by changes in intracellular volume.

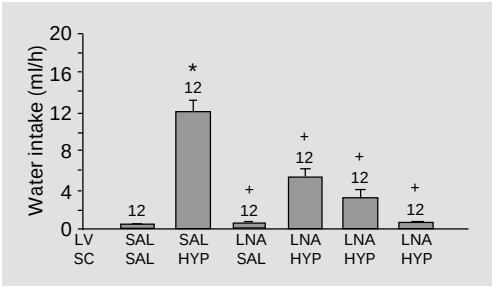

Figure 1 - Water intake after injection of saline (SAL) (control - subcutaneous (SC) and lateral ventricle (LV)), hypertonic saline (HYP) (SC) and L-NAME (LNA) (LV). L-NAME was used at the doses of 10, 20 and 40 µg/µl. The number of animals is indicated at the top of each column. *P<0.05 compared to saline; +P<0.05 compared to hypertonic saline (SC) (Dunnett *t*-test).

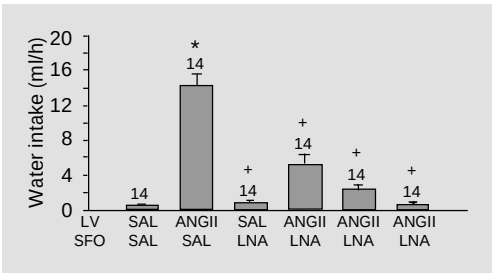

Figure 2 - Water intake after injection of saline (SAL) (control - lateral ventricle (LV) and subfornical organ (SFO)), ANG II injection into the LV, and L-NAME (LNA) injection into the SFO. L-NAME was used at doses of 10, 20 and 40 µg/µl. The number of animals is indicated at the top of each column. *P<0.05 compared to saline; +P<0.05 compared to ANG II injected into the LV (Dunnett *t*-test).

Effects of L-NAME injected into the SFO on water intake induced by ANG II injection into the LV

As shown in Figure 2, isotonic saline injection into the LV and SFO produced no changes in water intake when compared to baseline values (0.6 ± 0.1 ml/h). The injection of ANG II into the LV induced an increase in water intake, with values of 14.2 ± 1.4 ml/h. After injection of L-NAME at the doses of 10, 20 or 40 µg/µl into the SFO, the water intake decreased progressively and reached basal levels at the highest dose of L-NAME (40 µg/µl). The values were 5.3 ± 1.0, 2.4 ± 0.4 and 0.8 ± 0.01 ml/h, respectively. These results show that NO influences the water intake induced by ANG II injected into the LV.

Discussion

The present results show that L-NAME progressively reduced the water intake induced by thirst stimuli. It has been demonstrated that *icv* injection of N^G-monomethyl-L-arginine (NMMA), a blocker of NOS synthase, attenuates water intake (9) and preferentially increases plasma concentration of oxytocin and vasopressin in rats deprived of water for 24 h (10). Our results demonstrated that water intake increased after *sc* administration of hypertonic saline and that the L-NAME previously injected into the LV progressively reduced this ingestion. We also demonstrated that the electrolytic lesion of lateral preoptic area (LPO) and SFO in the same animal abolished the daily water intake induced by hypertonic saline (11). Our results also show that the injection of ANG II into the LV induced an increase in 1-h water consumption by satiated animals. Previous injection of L-NAME into the SFO produced a progressive reduction in water intake induced by ANG II. These results agree with those of Liu et al. (12) and can be explained as follows: a) NO plays an important role in neurotransmission in the CNS; b) the AV3V region plays an important role in the effect of NO on water intake involving the LPO and SFO and other areas; c) ANG II as a thirst-inducing hormone needs NO to produce thirst.

In conclusion, our results show that drink-

ing behavior induced by *sc* injection of hypertonic saline or by ANG II injection into the LV is attenuated by central inhibition of NOS with L-NAME and that the highest dose of L-NAME abolished the water intake induced by cellular dehydration and by ANG II injected into the LV. These results clearly demonstrate that ANG II needs NO to produce the dipsogenic effect, and that the SFO is an important area for this behavior. Future studies should be carried out to identify other areas of the CNS involved in this behavior and the specific mechanism of NO action.

Acknowledgments

The authors appreciate the technical assistance of Reginaldo C. Queiróz, Silas P. Barbosa, Alexandre A. Vieira, Silvia Fóglia and Silvana A.D. Malavolta. They also thank Ana V. Oliveira for animal care.

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