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Losartan blocks drinking and cFos expression induced by central ornithine vasotocin in rats

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Abstract

We previously reported that an intracerebroventricular (icv) injection of the oxytocin receptor antagonist ornithine vasotocin (OVT) caused water and saline intakes, a pressor response, and Fos-like immunoreactivity (Fos-IR) in the median preoptic nucleus of the rat brain. In the present report, rats receiving an icv injection of isotonic saline vehicle followed by an icv injection of 10 μg of OVT 20 min later drank 5.5 ± 1.1 ml of total water and saline intake in 60 min after the OVT; rats receiving 10 μg of losartan before the OVT drank only 0.9 ± 0.3 ml of total fluid. In a separate study, rats were treated as above except that they were not allowed to drink and were perfused for analysis of Fos-IR in the median preoptic nucleus at 90 min. Fos-IR in the dorsal part of the median preoptic nucleus was significantly suppressed from 2.69 ± 0.57 cells per 10,000 square μm in vehicle-treated rats to 0.89 ± 0.20 in losartan-treated rats. Losartan alone had no effect on Fos-IR. Losartan did not reduce intake of saccharin in a dessert test. This suggests that the OVT-induced drinking may result from an activation or disinhibition of angiotensin type AT_1 receptors in the median preoptic nucleus.

Keywords: Median preoptic nucleus; Oxytocin; Angiotensin; Salt appetite; Water intake

1. Introduction

An injection of the oxytocin receptor antagonist, ornithine vasotocin (OVT), into the cerebral ventricles of rats has been reported to elicit water and saline drinking responses [1] and food intake [2] with a short latency. In our drinking study, we also observed that OVT caused a brief, moderate rise in blood pressure and an expression of Fos-like immunoreactivity (Fos-IR) along the lamina terminalis of the rat forebrain [1]. We suggested that these effects were all consistent with an activation of the brain renin–angiotensin system (reviews 3–5). The present study sought to test this hypothesis by blocking central angiotensin AT_1 receptors with losartan potassium prior to the administration of OVT. Drinking responses and Fos-IR in the median preoptic nucleus (MnPO) were recorded.

2. Method

2.1. Animals

The subjects were 44 adult male Long–Evans rats that passed a test of cannula patency (see below). They were maintained in hanging wire mesh cages with continuous access to Teklad rodent diet, water, and 0.3 M NaCl for consumption. The lights in the colony room were on 12 h per day beginning at 7 am and the temperature was maintained at 22 ± 1 °C. The animals were handled 2–3 times per week for body weight measurements and for acclimation to handling.

2.2. Surgery

One stainless steel 23 ga cannula was implanted into the left lateral ventricle of each animal under Equi-Thesin anesthesia (0.33 ml/100 g, intraperitoneally) using aseptic technique. The cannula was cemented with methyl methacrylate to three stainless steel anchor screws in the skull. All rats were allowed at least 1 week to recover before the experiments. A stainless

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steel obturator filled the guide cannula when it was not in use for injections.

2.3. Drugs

Ornithine vasotocin (OVT, $d(CH_2)_5$,Tyr(Me)^2,Orn^8-, Lot numbers 0549614, 0557312, and 0557950) and angiotensin II were obtained from Bachem California. Losartan potassium was a gift from Merck and Co., Inc. The drugs were mixed fresh before each use with isotonic saline as the vehicle.

2.4. Injections, infusions and cannula patency

Injectors fashioned from 31 ga stainless steel tubing were inserted into the cannula guides to a point 1 mm beyond the tip of the guide into the target ventricle. These injectors were connected by a 1 m piece of PE-10 tubing to a 10- or 25-μl Hamilton syringe. The entire syringe was filled with sterile isotonic saline except for the very tip which included a sufficient volume of injectate separated from the saline by a 0.5-μl bubble of air.

Rats were given a behavioral test of the patency of the cannulas. ANG II was injected (40 ng in 2 μl) into hand-held rats, and all rats were required to drink 4 ml in 15 min in their home cages to be included in the study.

2.5. Drinking experiment

Seventeen rats were used in this experiment. Two burettes containing water and 0.3 M saline were placed onto each cage for measurement of drinking behavior, and food was removed, at least 30 min before the beginning of the test. Each rat was taken from its cage, the obturator was removed, and an injector was inserted into the cannula guide. A pretreatment of either 10 μg of losartan ($n=7$) or sterile isotonic saline vehicle ($n=10$) in 2 μl volume over 15 s was given to the rats with random assignment to the two treatments. The rats were then returned to their home cages for 20–25 min, after which all rats received a second injection of OVT (10 μg in 5 μl over 25 s). The rats were again returned to their cages and the latency to drink each fluid was recorded and the amount of each fluid consumed was measured at 15, 30 and 60 min.

2.6. Fos-IR experiment

Twenty rats were used in this experiment. For 15 rats, the treatments were the same as the drinking experiment except that only water was available for consumption and the water was immediately removed from the cage of any rat that licked at the tube sufficiently to cause a bubble to rise in the burette. Eight rats received vehicle and 7 rats received losartan before the OVT injection. In an additional 5 rats, two received a vehicle injection and 3 received losartan 20 min before an isotonic saline injection instead of OVT. A drinking latency was recorded, and at 90 min after the OVT injection each rat was perfused for determination of Fos-IR in the median preoptic nucleus. The rats were anesthetized with pentobarbital and perfused through the heart with phosphate buffered saline (PBS, 0.1 M pH 7.4) followed by 4% paraformaldehyde. Fos-IR was demonstrated using the avidin–biotin-peroxidase technique. Brains were removed and post-fixed in 4% paraformaldehyde overnight and then in a 25% sucrose and PBS solution for an additional 24 h for cryoprotection. The tissue was sectioned coronally on a freezing microtome at 50 μm. Sections were rinsed in PBS, soaked in a methanol–hydrogen peroxide solution for 20 min, soaked in 3% goat serum for 60 min, and incubated chilled with primary Fos antibody (rabbit polyclonal IgG, diluted 1:14,000, Santa Cruz Biotechnology), goat serum, and Triton X-100 for 48 h. Rinsed sections were then incubated in biotinylated goat antirabbit antibody for 1 h and processed using the Vectastain ABC kit (Vector laboratories) for 1 h. They were rinsed again, treated with hydrogen peroxide with diaminobenzadine and nickel as the chromogens for 2–4 min, mounted on subbed slides, dried, and coverslipped with Permount.

The lamina terminalis was the region of interest in this experiment because it was the location of increased OVT-induced Fos-IR activity reported in our previous paper. Unfortunately, the circumventricular organs were difficult to count accurately because of technical problems. The dorsal and ventral portions of the MnPO were photographed using a light microscope and digital camera, and were analyzed in a computer running Scion Image processing software. The area of the nucleus was measured by the Image software after tracing its outline with a drawing tool. The density of Fos-IR was then calculated by dividing the total number of Fos-positive cells by the area. The densities are reported as cells per 10,000 square μm (a square 100 μm on a side).

2.7. Dessert test

Seven rats were used in a test to determine if losartan nonspecifically suppresses drinking behavior in the dose used in the drinking and Fos studies. The rats were provided 0.6% saccharin solution for consumption overnight to familiarize them with the taste. For the next 5 days in the middle of the morning the water bottle was removed and saccharin was allowed for 1 h, after which the saccharin was removed and the water was returned. On the 6th and 7th days the rats received a lateral ventricular injection of 2 μl of 10 μg losartan or sterile saline vehicle in counterbalanced orders. Saccharin was provided for drinking 20 min later as in the OVT-injection studies and intakes and latencies to drink were recorded for 1 h.

2.8. Statistics

Intake and Fos-IR data are presented as mean ± S.E.M. and were analyzed using analysis of variance. Planned comparisons were made using Fisher's LSD test following a significant F-ratio. Drinking latency data are presented as medians and were analyzed by nonparametric techniques. A probability of less than .05 was required for significance.