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Differential effects of mineralocorticoid and angiotensin II on incentive and mesolimbic activity

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ABSTRACT

The controls of thirst and sodium appetite are mediated in part by the hormones aldosterone and angiotensin II (AngII). The present study examined the behavioral and neural mechanisms of altered effort-value in animals treated with systemic mineralocorticoids, intracerebroventricular AngII, or both. First, rats treated with mineralocorticoid and AngII were tested in the progressive ratio operant task. The willingness to work for sodium versus water depended on hormonal treatment. In particular, rats treated with both mineralocorticoid and AngII preferentially worked for access to sodium versus water compared with rats given only one of these hormones. Second, components of the mesolimbic dopamine pathway were examined for modulation by mineralocorticoids and AngII. Based on cFos immunohistochemistry, AngII treatment activated neurons in the ventral tegmental area and nucleus accumbens, with no enhancement by mineralocorticoid pretreatment. In contrast, Western blot analysis revealed that combined hormone treatment increased levels of phospho-tyrosine hydroxylase in the ventral tegmental area. Thus, mineralocorticoid and AngII treatments differentially engaged the mesolimbic pathway based on tyrosine hydroxylase levels versus cFos activation.

Introduction

Fluid depletion can be life threatening, and animals must carefully titrate their intake of water and sodium to restore and maintain osmotic and volemic balance. Sodium replacement requires the goal-directed behavior known as sodium appetite (Andersson, 1977), a behavior that can be prompted by mineralocorticoids, such as aldosterone, and angiotensin II (AngII) (Johnson and Thunhorst, 1997). AngII acts in the brain to elicit water intake and sodium intake (Epstein et al., 1969). During fluid depletion, suppression of either central AngII or aldosterone action does not eliminate sodium appetite, but blocking the central actions of both hormones abolishes the behavior (Buggy and Jonklaas, 1984; Sakai et al., 1986). Conversely, when both aldosterone and AngII are given exogenously, sodium appetite is potentiated (Fluharty and Epstein, 1983). The behavioral and neural basis for the combined effect of aldosterone and AngII on sodium appetite remains undefined.

The behavioral effects aldosterone and AngII to promote sodium ingestion may involve parallel behavioral mechanisms. For example, sodium ingestion could be enhanced by a change in the hedonic strength of the sodium tastant. Indeed, in rats placed on a sodium deficient diet, which increases aldosterone and AngII levels, sodium ingestion is preferred to moderately reinforcing brain stimulation, which suggests sodium appetite involves the modulation of the pleasurable properties of sodium intake (Conover et al., 1994). In parallel to altered taste value, sodium appetite may involve a recalibration of incentive-based effort. In this regard, rats treated with both aldosterone and AngII run faster on a runway to gain access to sodium, compared with rats treated with either hormone alone (Zhang et al., 1984), which suggests that the combined hormone treatment increases the incentive value of sodium. The progressive ratio task is a quantitative assay for incentive-based effort, but the effects of combined mineralocorticoids and AngII on this behavioral test have not been reported.

The mesolimbic dopamine system has been widely implicated in effort-related behaviors (Barbano and Cador, 2006; Floresco et al., 2008; Kelley et al., 2005; Phillips et al., 2007; Salamone et al., 2009). Dopamine neurons in the ventral tegmental area (VTA) project to the accumbens, which in turn projects to brain regions such as the ventral pallidum to generate goal directed movement (Carelli, 2002). Previous work has implicated this brain system in sodium appetite. For example, the accumbens receives multisynaptic input from aldosterone-sensing and sodium-sensing neurons in the hindbrain (Miller and Loewy, 2014; Shekhtman et al., 2007). Sodium depletion alters the level of dopamine transporters and opioid peptides in the accumbens (Grondin et al., 2011; Lucas et al., 2003; Roitman et al., 1999). In addition, sodium

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depletion modifies the dendritic arbor of ventral striatum neurons (Roitman et al., 2002). Although these studies have suggested a role for mesolimbic activity in sodium appetite, the separate effects of aldosterone and AngII on mesolimbic activation have not been studied.

The present studies tested the overall hypothesis that mineralocorticoids and AngII recalibrate the willingness to work for sodium versus water. In particular, the willingness to work was measured with the progressive ratio task. In addition, the activity of the mesolimbic dopamine pathway was assessed with immunohistochemistry for cFos and Western blot analysis for tyrosine hydroxylase. Although it is recognized that sodium depletion is a complex physiological state that is imperfectly mimicked with mineralocorticoid and central AngII treatments, this preparation has yielded useful insights into the neuroendocrine actions that influence motivated behavior.

Materials and methods

Animals

Adult male Sprague–Dawley rats (weight between 225 and 250 g) were obtained from Charles River Laboratories (Wilmington, MA, USA). Rats were pair-housed in plastic tubs with standard bedding and with food and water available *ad libitum*, except during experimental procedures. The temperature in the colony was maintained at 22 °C with a 12:12 h reversed light/dark cycle. Behavioral testing, described below, was conducted during the lights-out phase. Animals were allowed at least one week to acclimate to the colony before any procedures were performed. The Institutional Animal Care and Use Committee of the University of Pennsylvania approved all procedures with animals.

Surgery

Surgeries were performed in aseptic conditions. Animals were anesthetized with inducted and maintained with isofluorane anesthesia for stereotaxic surgery. A 26-gauge guide cannula (Plastics One, Roanoke, VA, USA) was aimed at the lateral ventricle using these coordinates: 0.48 mm caudal to bregma, 1.6 mm from mid-line and 4.2 mm ventral to dura mater. The cannulae were fixed in place with dental cement and bone screws. Upon completion of surgery procedures, animals were injected with yohimbine (0.11 mg/kg, ip, Ben Venue Laboratories Bedford, OH), and upon awakening animals were returned to the housing facility and singly housed. The animals were allowed at least five days to recover before verification procedures were performed.

Prior to undergoing experimental treatments, animals were tested for correct lateral ventricle cannula placement and patency. They were given an *icv* injection of 20.0 ng of AngII diluted in artificial cerebrospinal fluid (aCSF) via a Hamilton syringe connected with PE-10 tubing to an injector that terminated 1 mm beyond the guide cannula. Animals were excluded from the experiment if they failed to demonstrate a drinking response in less than 30 s, consuming at least 3 ml of water, in two separate AngII challenges. Experiments began three days after these *icv* test injections.

Experimental Design

In all experiments, animals were assigned to one of four treatments in a 2 × 2 design, with a crossover in behavioral experiments. Animals were first pre-treated twice daily (10 h apart) for three days with a subcutaneous injection of sesame oil or an aldosterone analog, deoxycorticosterone acetate (DOC; 0.25 mg/0.2 ml sesame oil; Sigma, St. Louis, MO). DOC penetrates the blood brain barrier more easily than aldosterone due to its low capacity for hydrogen bond formation (Kraulis et al., 1975). Animals then were injected *icv* with either artificial cerebrospinal fluid (aCSF; R&D Systems, Minneapolis, MN) or 20.0 ng AngII in a volume of 2.0 ul (Bachem, King of Prussia, PA). The treatment groups will be referred to as follows: Veh/Veh, DOC/Veh, Veh/AngII, DOC/AngII. Using this experimental design and identical doses, the DOC/AngII treatment has been shown to elicit a greater than additive effect on sodium intake, but not water intake (Grafe et al., 2014).

Experiment 1. Progressive Ratio Task

Rats were acclimated to wire mesh cages for one hour with two 25-ml bottles containing tap water and 3% saline, each marked with 0.2 ml graduations. These bottles were then removed, and rats were water restricted for 23 h per day for the next six days. During these six days, rats underwent operant lever pressing training in conditioning boxes for 30 min per day (Med Associates; MDPC IV Software, St. Albans, Vermont). The conditioning boxes contained levers for both water (right lever) and 3% saline (left lever), both simultaneously present. A lever press lowered a syringe pump, which delivered a 0.1 ml drop of the appropriate liquid into a cup available to the rat. The saline and water each had their own syringe pump and their own cup. During the first two training days, to facilitate learning, an aliquot was dispensed every 300 s that elapsed without bar pressing. In addition, animals could earn a 0.1 ml of water or saline for each bar press, depending on which of the two levers was pressed. During the subsequent two training days, the animals earned a 0.1 ml water or saline for each lever press, followed by two training days during which three lever presses were required for each aliquot of water or saline. Animals were considered to have learned the lever-fluid contingencies when they had made at least 10 lever presses for water during the 30-min session. Once this occurred, rats were given ad libitum access to water again. Rats were then assigned to treatment groups, as described above, and given no further operant training while they received their three days of pretreatment injections (vehicle or DOC). After pretreatment was complete, 24 h after the last DOC treatment, rats were administered their assigned *icv* injection (vehicle or 20 ng AngII), and immediately given a test with a progressive ratio (PR) schedule. Thus, animals were water replete at the beginning of the PR test. The response requirement of the PR schedule increased progressively for both saline and water, as previously described (Davis et al., 2011). The breakpoint for each animal was defined as the final reinforced bar pressing set that preceded a 10-min period without earning a reinforcment, with a two-hour limit total. Food was not available during this task.

Experiment 2: Hormone-Induced cFos expression

To observe brain activation after DOC and AngII treatments, rats were assigned to treatment groups, as explained above. Sixty minutes after the last *icv* injection, each rat was anesthetized with 50 mg/kg ketamine and 20 mg/kg xylazine, intraperitoneally. As discussed below, there were group differences in the effort for sodium versus water during the first few minutes of the progressive ratio task, making 60 min post-treatment a reasonable time to expect differences in cFos levels. Rats were perfused transcardially with 100 mL of heparinized saline followed by 200 ml 4% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA). The brains were isolated, post-fixed in paraformaldehyde overnight at 4 °C, and then submerged in 20% sucrose in 0.1 M phosphate buffer for three days. Coronal sections were cut on a freezing microtome into three serial sets of 40-um-thick sections. These sectons encompassed the VTA and the shell and core of the accumbens. One set of sections from each animal underwent immunohistochemical staining and analysis; the other two sets of sections were preserved in cryoprotectant as reserve material.

Sections were washed in Tris-buffered saline (TBS; pH 7.4) and then incubated with a cFos antibody (1:500, sc-52, rabbit; Santa Cruz Biotechnology, Santa Cruz, CA) in TBS with 0.2% TritonX-100 and 3% normal donkey serum (Jackson Immunoresearch; West Grove, PA) overnight at 4 °C. After several washes, sections were incubated with a