Brain angiotensinergic mediation of enhanced water consumption in lactating rats

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Speth, Robert C., M. Susan Smith, and Kevin L. Grove. Brain angiotensinergic mediation of enhanced water consumption in lactating rats. Am J Physiol Regulatory Integrative Comp Physiol 282: R695–R701, 2002; 10.1152/ajpregu.00432.2001.—The mechanism by which lactating rats increase fluid consumption to meet the demands of milk production is unknown. Because ANG II is the most potent dipsogenic stimulus known, this study examined whether angiotensinergic signaling plays a role in enhanced drinking in lactating rats. ANG II administered intracerebroventricularly caused a significantly greater dipsogenic response in lactating rats than in control rats, suggesting that dipsogenic responsivity to ANG II is enhanced in the brains of lactating rats. The angiotensin type 1 (AT1) ANG II receptor subtype antagonist SKF-108566, also given intracerebroventricularly, caused a significant reduction in water consumption in lactating rats, whereas it did not significantly affect water intake in control rats. In contrast, stimulation of drinking by the muscarinic agonist carbachol, also administered intracerebroventricularly, did not differ between lactating and control rats. Inhibition of drinking by the muscarinic antagonist atropine also did not differ significantly between lactating and control rats. These results suggest that the increased drinking in lactating rats involves an increased responsiveness to ANG II in neurons that mediate dipsogenesis, as well as an enhancement in the amount of angiotensinergic input to these ANG II-responsive neurons.

dipsogenesis; angiotensin II; atropine; isoproterenol; food intake

To meet the demands of milk production and secretion, lactating rats greatly increase their fluid intake (16). A number of hormones and neurotransmitters can stimulate drinking; however, ANG II produced by the renin-angiotensin system (RAS) is generally recognized as the most potent dipsogenic stimulus. As little as 0.1 fmol administered into the subfornical organ or organum vasculosum of the lamina terminalis (OVLT) in the brain of rats can cause drinking (6). In response to extracellular dehydration, e.g., hypovolemia or hypotension, or in response to hyponatremia, renin is released from the kidneys into the bloodstream. Renin acts on angiotensinogen (renin substrate) present in the bloodstream, initiating a cascade leading to the formation of ANG II, which acts on circumventricular organs of the brain, primarily the subfornical organ and OVLT, to stimulate a dipsogenic response. ANG II administered into the ventricles of the brain also stimulates drinking (1; for review, see Ref. 5). It has been proposed that ANG II formed locally within the brain contributes to the motivation to drink; however, intracerebroventricular administration of ANG II receptor antagonists does not always block extracellular dehydration or cellular dehydration-induced drinking (for review, see Ref. 6).

The RAS plays a major role in fluid and electrolyte balance. However, little is known about the functionality of the RAS during lactation. There is a decreased pressor response to ANG II in lactating rabbits (15) and goats (10, 11). One study (11) examined the dipsogenic effect of ANG II during lactation. However, no dipsogenic response to ANG II was found in either lactating or control goats adapted to arid conditions. Lactating rats show a diminished dipsogenic response to an intravenous hypertonic saline challenge and also to subcutaneously administered isoproterenol compared with control rats (16). Because isoproterenol-induced drinking is mediated by the RAS, this may indicate a decreased dipsogenic responsivity to ANG II. However, this could also reflect a diminished ability of isoproterenol to stimulate renal renin release during lactation.

The other major regulator of thirst is cellular dehydration. It occurs with the intake of hypertonic solutions, which then draw water out of cells to restore isotonicity in extracellular fluids. In the rat, the thirst induced by cellular dehydration is mediated by acetylcholine acting on muscarinic receptors in the brain (for review, see Ref. 33).

To investigate the possible involvement of the RAS in increased fluid intake during lactation, this study examined the ability of ANG II, administered intracerebroventricularly, to stimulate drinking in lactating rats. In addition, the ability of antagonists of the AT1 ANG II receptor, also administered intracerebroventricularly, to inhibit drinking during lactation was investigated. For comparison, the effects of a musca-
rinic cholinergic agonist and antagonist on drinking in lactating rats were also determined.

**METHODS**

Timed pregnant Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) and age-matched controls (4 mo of age, 240–260 g) were housed individually starting at gestational days 12–15. They were maintained on rat chow (Purina 5100, Purina Mills, St. Louis, MO) and water ad libitum. Lights were on from 0700 to 1900. Animals were checked daily for the presence of pups; the day of delivery was considered postpartum day 0 (P0). The litters were culled to eight pups on P2 to allow for an equivalent suckling stimulus for all lactating rats.

The lactating and nonlactating age-matched control rats were ovarioectomized (Ovx) on P2 or P3 to eliminate any gonadal steroid-associated alterations in fluid consumption (3). A 22-gauge stainless steel guide cannula fitted with an obturator (Plastics One, Roanoke, VA) was also implanted stereotaxically at the same time. The location of the tip of the guide cannula was just dorsal to the left lateral cerebral ventricle, 1 mm caudal to bregma, 1.5 mm lateral to midline, and 2 mm beneath the surface of the brain. The rats were anesthetized with tribromoethanol (77 mg/kg) during the surgical procedures. The rats were allowed to recover for 6 days before testing for dipsogenic and antidipsogenic effects of drugs. All of the rats in the lactating group were able to sustain their litters during the postsurgical and testing periods. Overnight as well as daily food and water intake were monitored starting at P3 or on the day of surgery (control rats) until the completion of each experiment.

The animal procedures used in this study were approved by the Oregon Regional Primate Research Center Institutional Animal Care and Use Committee.

**Experiment 1**

The schedule for the treatments for this experiment is presented in Fig. 1. At 1400 on P8, 5 days after Ovx, 2 μl of artificial cerebrospinal fluid (aCSF) was administered intracerebroventricularly, via an injector cannula that extended 2 mm beyond the level of the guide cannula, to assess baseline water consumption for a 30-min period. At 1400 on P9, 6 days after Ovx, 300 ng of carbachol (Sigma Chemical, St. Louis, MO) in 2 μl of aCSF was administered intracerebroventricularly to assess cholinergic stimulation of water consumption. The dipsogenic response to intracerebroventricular carbachol was determined as the amount of water consumed in excess of that consumed when the rats were given aCSF only. At 1400 on P10, 7 days after Ovx, 1 ml/kg of saline (0.9% NaCl) was administered subcutaneously between the scapulas. Baseline water consumption was determined over a 2-h period. At 1400 on P11, 8 days after Ovx, isoproterenol (Sigma Chemical), 25 μg/ml, in a volume of 1 ml/kg saline, was administered subcutaneously between the scapulas. This dose was selected on the basis of studies of Rowland and Fregly (32) and Kaufman (16). Isoproterenol-induced water consumption was monitored for 2 h. The 2-h monitoring period was based on the observations of Lehr et al. (18). The dipsogenic response to isoproterenol was determined as the amount of water consumed in excess of that consumed when the rats were given saline only.

At 1400 on P13, 10 days after Ovx, a near-maximal dipsogenic dose of ANG II (Bachem, Torrance, CA), 800 pmol in 2 μl of aCSF, was administered intracerebroventricularly. ANG II-induced water consumption was monitored for 30 min. The dipsogenic response to intracerebroventricular ANG II was determined as the amount of water consumed in excess of that consumed when the rats were given aCSF only.

**Experiment 2**

The schedule for the treatments for this experiment is presented in Fig. 1. At 1800–1900 on P9 or P10, 6–7 days after Ovx, 5 μl of SKF-108566 (kindly provided by Dr. J. Weinstock, SmithKline Beecham Pharmaceuticals, King of Prussia, PA) at a concentration of 10 mM was administered intracerebroventricularly. SKF-108566 was used as the ANG II receptor antagonist for these studies because it has a higher affinity for AT1 receptors than the more commonly used antagonist losartan. In competition binding studies, SKF-108566 displayed an IC50 of 292 pM vs. 120 nM for [125I]Sar1, Ile8[ANG II binding to rat liver, while losartan had an IC50 of 1.48 nM (Speth, unpublished observations). The drug was administered over a 2-min period, and the injector cannula was left in place for 1 min after drug administration. To control for the effects of handling on overnight food and water consumption, one-half of the rats in each group were given aCSF the day before SKF-108566 was given, and one-half of the rats in each group were given aCSF the day after SKF-108566. Overnight water consumption was determined because this is the time rats consume most of their water. Because of concerns about disturbing rats during their dark cycle and possibly desynchronizing their diurnal rhythm, water consumption was not measured until the completion of the dark period.

At 1800–1900 on P11 or P12, 8–9 days after Ovx, atropine (Sigma Chemical) was administered intraperitoneally at a dose of 20 mg/kg. This dose has been shown to inhibit drinking in diabetic rats without inhibiting eating for periods of 2–24 h (25). Overnight water and food intake was again monitored for comparison to that on the night before and the night after administration of atropine. To control for the effects of handling on overnight food and water consumption, one-half of the rats in each group were injected with saline the day before atropine, and one-half of the rats in each group were injected with saline the day after atropine. Drug treatments were staggered such that there was a 2-day interval between the SKF-108566 and atropine treatments.

Fig. 1. Schedule of drug treatments for experiments 1 and 2. aCSF, artificial cerebrospinal fluid; SKF, SKF-108566.
RESULTS

Experiment 1

The average daily water intake of lactating rats was 53.7 ml/day on P4 and P7, which corresponds to the 4 days after surgery. The average daily water intake of control rats during the equivalent time period was 20.1 ml/day (Fig. 2A). The water consumption of lactating rats was significantly greater ($P < 0.01$) than control rats on P4–P7.

The average daily food intake of lactating rats was 32.6 g/day on P4–P7. The average daily food intake of control rats during the equivalent time period was 11.7 g/day. The food consumption in lactating rats was also significantly greater ($P < 0.01$) than in control rats on P4–P7 (Fig. 2B). Rats in the lactation group were significantly heavier than the rats in the control group (Fig. 3). To control for this weight difference, fluid consumption in response to treatment with dipsogenic agents was expressed as milliliters of water consumed per 100 g body wt.

ANG II given intracerebroventricularly caused a substantial dipsogenic response in both groups of rats. The response in lactating rats, 5.9 ± 0.4 ml/100 g body wt, was significantly greater ($P < 0.05$) than the response in control rats, 4.4 ± 0.3 ml/100 g body wt (Fig. 4). The muscarinic agonist carbachol given intracerebroventricularly caused a moderate dipsogenic response in both the lactation and control groups (Fig. 4). There was no significant difference in the response to carbachol between the two groups. Isoproterenol, which stimulates drinking indirectly via the stimulation of renin release from the kidney, leading to the production of ANG II in the bloodstream, had a small and inconsistent dipsogenic effect in both groups (Fig. 4). Three of the seven rats in the control group did not show a dipsogenic response beyond that seen in response to subcutaneous administration of an equal volume of saline. Two of the five rats in the lactation

Statistical Analyses

Experiment 1. For analysis of the dipsogenic effects of carbachol, isoproterenol, and ANG II, water intakes of the lactation and control groups were compared by an unpaired t-test. Values presented are means ± SE. $P$ values <0.05 were considered to be significant. To eliminate nonresponding rats due to failure to successfully administer drugs intracerebroventricularly, data from rats that showed no response to both carbachol and ANG II, or that showed less than a 1 ml/100 g body wt dipsogenic response to ANG II, were eliminated from the data set analyzed statistically. Using these criteria, none of the carbachol-treated rats were eliminated from the data analyzed, and one rat from each of the lactation and control groups was eliminated from the ANG II data set.

Simple t-tests were used to compare the food and water consumption and body weights of lactating and control rats on the days corresponding to days 4–7 of lactation.

Experiment 2. Two-way, repeated-measures ANOVAs were used to determine the effects of SKF-108566 and atropine on overnight water and food consumption in the two different groups. A priori paired t-tests were used to compare intakes on the night of SKF-108566 or atropine treatment to the nights before and after SKF-108566 or atropine treatment.

![Graph](image-url)
nonlactation. More water (\(F_{1,16} = 14.3, P < 0.0001\); Fig. 5, C and D). There was a significant group effect. Lactating rats consumed more water (\(F_{1,16} = 36.4, P < 0.0001\)) and more food (\(F_{1,16} = 121.4, P < 0.0001\)) than control rats.

There was a significant treatment-by-group interaction on water intake (\(F_{4,64} = 4.49, P = 0.0015\)) and food intake (\(F_{4,64} = 2.91, P = 0.028\)). SKF-108566 caused a significant (\(P = 0.013\)) 27% reduction in overnight water intake in the lactation group compared with the preceding and subsequent nights (Fig. 5B) but did not significantly affect overnight water intake in the control group (Fig. 5A). SKF-108566 caused a smaller, but still significant (\(P < 0.05\)), 18% reduction in overnight food intake in the lactation group of rats compared with average food intake on the preceding and subsequent nights (Fig. 5D), but it did not significantly affect overnight food intake in the control group of rats (Fig. 5C).

Atropine significantly (\(P < 0.01\)) decreased overnight water intake by 53% in the lactation group (Fig. 5B) and 40% in the control group compared with the average water intake on the preceding and subsequent nights (Fig. 5A). Atropine also caused a smaller, but still significant (\(P < 0.05\)), 36% reduction in overnight food intake in the lactation group (Fig. 5D), and 43% in the control group (\(P < 0.01\)), compared with the average food intake on the preceding and subsequent nights (Fig. 5C).

**DISCUSSION**

Lactating rats, which are in a state of hyperdipsia and hyperphagia, appear to have an enhanced brain angiotensinergic drinking circuitry. ANG II, administered intracerebroventriculatly at a dose sufficient to cause a near-maximal dipsogenic response, caused a greater stimulation of water consumption in lactating rats than in control rats. Water consumption in response to ANG II was measured as milliliters per 100 g body wt. Because lactating rats were on average heavier than nonlactating rats (Fig. 3) while the brain size did not differ, it is possible that this measure slightly underestimated the enhanced responsivity of lactating rats to intracerebroventricularly administered ANG II. This enhanced response could be due to an increase in brain ANG II receptors mediating dipsogenesis, an increased binding affinity for ANG II, or a more efficient transduction of ANG II receptor-mediated responses. Consistent with the first two possibilities, previous work from this laboratory demonstrated increased ANG II receptor binding in the preoptic area of lactating rats compared with diestrous rats (37). The...
preoptic area of the brain is one of many brain regions enriched in AT1 ANG II receptors that has been proposed to participate in intracranial ANG II-induced drinking (6, 13).

Blockade of brain ANG II receptors with the potent AT1 ANG II receptor subtype antagonist SKF-108566 caused significant reductions in water consumption only in lactating rats. This suggests that endogenous brain ANG II plays a role in the enhanced drinking in lactating rats but that endogenous brain ANG II does not play an essential role in maintaining normal fluid consumption. A similar lack of effect of blockade of the brain angiotensinergic system on normal drinking was observed with chronic intracerebroventricular administration of an ANG II antagonist (35) and with intracerebroventricular administration of an anti-ANG II antibody (7). Most studies of ANG II-induced drinking suggest that the receptor subtype mediating this effect is the AT1 subtype. The brain regions most commonly implicated in dipsogenic responses to ANG II, the subfornical organ, OVLT, preoptic area, and paraventricular nucleus of the hypothalamus, all have AT1 receptors with negligible amounts of the angiotensin type 2 (AT2) ANG II receptor subtype (8, 31, 36). The AT1 antagonist losartan completely blocks drinking in response to intracerebroventricularly administered ANG II (29), and intracerebroventricular administration of antisense to the AT1 receptor decreases dipsogenic responses to ANG II (24). In a pilot study, the dose of SKF-108566 used in this study completely and reversibly inhibited intracerebroventricular ANG II-mediated drinking in a female rat (Speth, unpublished observation). However, the possibility that this dose of SKF-108566 might have nonselective effects on other dipsogenic mechanisms was not determined and cannot be excluded.

There has been some suggestion that AT2 receptors may also mediate dipsogenic responses to ANG II (17, 32). The lateral septum has been identified as a possible site at which ANG II may cause a dipsogenic response (40), and the AT2 receptor predominates in this brain region (31). However, others suggest that the AT2 receptor subtype may inhibit ANG II-induced drinking (9). Because it is generally acknowledged that the AT1 receptor is the primary subtype that mediates drinking in response to ANG II (6, 22), the effects of AT2 receptor-selective antagonists on the lactation-induced stimulation of water intake were not examined.

Muscarinic cholinergic mechanisms appear to play a substantial role in normal drinking in both lactating and control rats. Atropine decreased drinking in lactating rats by >50%, while decreasing drinking in control rats by 40%. However, there does not appear to be an enhanced responsivity to muscarinic agonists in lactating rats because intracerebroventricular carbachol stimulated drinking to a similar extent in both lactating and control rats.

The reduction in drinking in lactating rats in response to central AT1 receptor blockade was not sufficient to lower their water intake to that of control rats. This is likely due to the inability of the single dose of SKF-108566 to block AT1 receptors for the 12-h period during which drinking was monitored. It could, however, also be due to the involvement of AT2 receptors or other transmitter systems (for review, see Ref. 6).

Isoproterenol, which stimulates dipsogenesis primarily through the production of ANG II in the bloodstream, had little dipsogenic effect on either group of rats. It has been reported that ovariectomy reduces the dipsogenic response to isoproterenol (3). In addition, the relative ability of isoproterenol to cause renin release from the kidney and subsequent formation of ANG II in lactating and/or Ovx rats is unknown. Because the dipsogenic response to the dosage of isoproterenol used in this study was so low relative to the response to intracerebroventricular ANG II, it may be inappropriate to compare the effects of these two agents. Other investigators [Stocker et al. (39a) and Lehr et al. (18)] have used higher doses of isoproterenol (330 μg/g body wt) and observed a more robust dipsogenic response. Thus it is possible that a higher dose of isoproterenol might have indicated an altered dipsogenic responsivity in lactating rats.

The subfornical organ is thought to be the primary mediator of the dipsogenic responses to blood-borne ANG II (34) and isoproterenol-induced drinking (4). ANG II receptor binding in the subfornical organ was reduced in lactating rats (37), so the lack of an enhanced dipsogenic response to isoproterenol in lactating rats is not unexpected. It should be noted that in our previous study (37), the rats were not Ovx, although they were studied at a time when endogenous ovarian steroids were low.

It is also not known how lactation affects blood-borne ANG II concentration in the rat. Because intracerebroventricularly administered ANG II receptor antagonists can block dipsogenic responses associated with increased blood-borne ANG II (14, 20, 22, 30) it could be hypothesized that the enhanced drinking in lactating rats could be due to an increase in blood-borne ANG II acting on brain ANG II receptors. There are several reasons why this is not likely. Increased blood-borne ANG II, caused by dehydration, upregulates ANG II receptors in the subfornical organ (23). Conversely, chronic inhibition of ANG II formation decreased ANG II receptors in the subfornical organ of spontaneously hypertensive rats (27). Because ANG II receptor binding in the subfornical organ of lactating rats is decreased (37), this suggests that blood-borne ANG II is not increased in lactating rats. Elevated levels of ANG II in the bloodstream cause a reduction in food intake (2), which is contrary to the hyperphagic behavior demonstrated by lactating rats. Dehydration, which increases plasma ANG II levels, attenuates prolactin secretion (26), which would compromise lactation. In addition, lactating Yanomama Indians, who live in a low-salt environment, do not show any increases in plasma renin activity compared with their nonlactating peers (28).

Lactating rats also decreased their food consumption when treated with SKF-108566. This inhibition may be...
secondary to the decrease in water consumption because the reduction in eating was smaller than the reduction in drinking.

Atropine decreased food intake in both lactating and control rats. This inhibition may also be secondary to decreased water intake in the lactating rats because the reduction of food intake was less than the reduction of drinking. However, in the control rats, the decrease in drinking and eating was comparable. Thus it is possible that part of the decrease in drinking and eating in response to atropine resulted from secondary effects of this muscarinic antagonist.

From these studies, it is clear that intracranial angiotensinergic mechanisms mediate at least a portion of the increase in fluid intake in lactating rats. It is not possible to determine the extent to which angiotensinergic mechanisms mediate this effect because the duration of inhibition of AT1 receptors by SKF-108566 was not determined. However, the lack of an enhanced response to a muscarinic cholinergic stimulus or isoproterenol (acting via stimulation of the peripheral RAS) suggests that brain angiotensinergic function is the primary mediator of the increase in fluid intake in lactating rats. There is some parallel between this study and those that examined other models of induced drinking. The selective AT1 receptor antagonist losartan, administered intracerebroventricularly, blocked the dipsogenic response to heat exposure in rats (21). Hemorrhage-induced drinking, which could be blocked with intracerebroventricular losartan, was associated with increased brain ANG II (30). Thus it is clear that a number of peripheral stimuli can activate brain angiotensinergic mechanisms, leading to dipsogenesis.

The suckling stimulus activates neuronal input to a number of brain regions, including the preoptic area (19). Increasing the intensity of the suckling stimulus further enhances water and food intake in lactating rats (12). This neuronal activation may increase local ANG II formation, leading to the stimulation of ANG II receptors in this region of the brain that mediate thirst. These observations suggest that under normal conditions, this pathway is not active and is not a driving force for water consumption. In response to suckling, however, this pathway is activated and stimulates an increase in drinking.

Perspectives

The lactating rat is a model for hyperdipsia, hyperphagia, and suppression of reproductive function. These experiments indicate that activation of brain angiotensinergic function mediates at least a part of the hyperdipsic behavior of lactating rats. In view of the predominant lack of change in angiotensinogen mRNA in lactating rats (38), this suggests that activation of a reninergic neuron or activation of some other proteinase capable of cleaving ANG I or ANG II from angiotensinogen may be the critical factor in activation of brain angiotensinergic drinking. Recent studies (for review, see Llorens-Cortes and colleagues (30a)) suggest that ANG III is the active angiotensin in the brain and that ANG II has no efficacy in the brain (and may therefore be an antagonist). Therefore, another possible explanation is that there is increased formation or reduced degradation of ANG III in the lactating rat brain.

Muscarinic cholinergic drinking, while important for normal water consumption, does not appear to mediate the increase in drinking in lactating rats. Blood-borne ANG II also does not appear to play a role in the stimulation of drinking in lactating rats. However, this should still be tested by measuring plasma and brain ANG II concentrations during lactation. ANG II levels in the preoptic area of the hypothalamus and other brain regions should also be determined to assess the contribution of brain ANG II production in the generation of the heightened dipsogenic behavior of lactating rats. In view of the inhibitory effects of ANG II on prolactin secretion occurring at the level of the arcuate nucleus (39), the increase in brain ANG II should be region specific to accommodate all of the processes required to sustain lactation.

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