Transection of the stria terminalis without damage to the medial amygdala does not alter behavioural sodium regulation in rats

Richard M. Black\textsuperscript{1}, Harvey P. Weingarten\textsuperscript{1}, Alan N. Epstein\textsuperscript{2}, Reiko Maki\textsuperscript{2}, and Jay Schulkin\textsuperscript{2}

\textsuperscript{2}Departments of Anatomy and Biology, Mahoney Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA, 19104, USA and \textsuperscript{1}Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1

Abstract. Damage to the medial region of the amygdala has been shown to impair mineralocorticoid-induced sodium appetite, while leaving intact sodium appetite induced through sodium depletion. This effect may result from the interruption of the flow of information through the stria terminalis (ST), a neural pathway linking the medial amygdala with the ventral forebrain. We determined the effect of transecting the ST of the rat, at a point remote from the medial amygdala, on sodium appetite induced with the administration of mineralocorticoids and with the natriuretic furosemide. Similar to control and amygdala lesioned rats, rats with ST knife-cuts displayed a normal sodium appetite following treatment with furosemide. However, unlike medial amygdala lesions, transection of the ST alone did not block mineralocorticoid-induced sodium appetite. Therefore, the inability of mineralocorticoids to induce a salt appetite in medial amygdala lesioned rats does not result from damage to the stria terminalis.

Key words: Stria terminalis, amygdala, mineralocorticoid, furosemide, sodium appetite
INTRODUCTION

Total ablation of the amygdala reduces or completely blocks sodium appetite induced by sodium deprivation or mineralocorticoid injection (Cox et al. 1978). However, in an earlier set of studies, we found that damage to a specific area of the amygdala, the medial region, effectively blocked mineralocorticoid-induced sodium appetite while leaving sodium depletion-induced sodium appetite intact (Nitabach et al. 1989; Schulkin et al. 1989). This finding was of interest because the medial amygdala has been shown to be a primary receptor site for the mineralocorticoid aldosterone (Birmingham et al. 1979; Coirini et al. 1983; Coirini et al. 1985; Funder, 1986; McEwen et al. 1986), which participates in the behavioural and physiological regulation of body sodium homeostasis (Braun-Mendez 1952; Wolf 1965; Fregly and Waters 1966; Denton 1982). More recent work has shown that ablation of the central nucleus of the amygdala also impairs mineralocorticoid-induced salt appetite (Galverna et al. 1990). Furthermore, these findings demonstrate that sodium appetite elicited through stimulation of the angiotensin system, i.e. through sodium depletion, depended upon a neural network which was anatomically distinct from that sensitive to aldosterone (Nitabach et al. 1989; Schulkin et al. 1989). The anatomy of the aldosterone neural circuit is not known.

There are two major pathways linking the amygdala with the ventral forebrain and other aldosterone concentrating regions such as the medial preoptic-hypothalamic region and the bed nucleus of the stria terminalis (Birmingham et al. 1979; Cox et al. 1978; McEwen et al. 1986; Simerly and Swanson 1986): the ventroamygdalo-fugal pathway (VAF) and the stria terminalis (ST). However, anatomical studies have shown that only the ST carries collaterals originating in the medial amygdala (de Olmos and Ingram 1972; de Olmos et al. 1985). Also, electrical stimulation of the medial amygdala activates fibres in the ST without any observable activation of VAF fibres (Watson et al. 1983). Therefore, the electrolytic lesions which block mineralocorticoid-induced sodium appetite not only damaged the medial region of the amygdala, but most likely also disrupted the stria terminalis pathway (Nitabach et al. 1986; Schulkin et al. 1989). Thus, we were unsure whether the sodium appetite impairments previously observed were a direct result of (1) the medial amygdala damage or (2) interruption of the stria terminalis pathway. If the effects observed were attributable to ST damage, then severing the ST at a point remote from the amygdala should also disrupt mineralocorticoid-induced sodium appetite. In addition, sodium depletion induced sodium appetite should remain unimpaired. We report herein the effects of transection of the stria terminalis, leaving the medial amygdala intact, on the behavioural regulation of body sodium, specifically sodium appetites elicited by mineralocorticoid administration and acute sodium depletion.

METHODS

Animals and housing

Forty-four male Sprague-Dawley rats weighing between 300 and 450 grams at the start of the experiment were used. Subjects were divided into three groups: stria terminalis knife-cut animals (ST), medial amygdala lesion animals (AMY), and controls. All subjects were individually housed in stainless steel, wire-mesh cages in a temperature- and humidity-controlled room on a 14:10 light:dark cycle. Unless otherwise specified by test protocol, rats had ad libitum access to Purina Rat Chow (Na content 0.5-1.0%). Tap water and a 3% NaCl solution were provided in individual graduated drinking tubes attached to the front of each cage.

Surgery and recovery

Twenty-one rats received stereotaxically guided knife cuts of the stria terminalis under ketamine hydrochloride (40 mg/kg administered intramuscularly (i.m.) and acepromazine maleate (15 mg/kg), or sodium pentobarbital (45 mg/kg, administered intraperitoneally (i.m.))). Before surgery, a prophylactic dose of gentamicin sulfate was administered (i.m.). Knife cuts were produced using a spring-loaded brain knife (Hamilton et al. 1973), a 30 gauge tungsten wire which fed through a 23 gauge guide-tube. The knife was positioned (with the incisor bar 3.0 mm below the horizontal) at 1.3 mm posterior to bregma, 4.7 mm lateral to the midline and 5.2 mm below the skull surface. The tungsten knife was extended 3.0 mm in a caudal-medial direction, 45 degrees to the midline, and the spring-loaded catch was released allow-
Stria terminalis knife-cuts and salt appetite

ing the knife to travel 4.0 mm vertically, after which the wire was retracted and the knife was removed from the brain. In control rats, the knife was lowered to the same coordinates and the trigger released, but the tungsten wire was not extended. All ST rats and controls recovered with no apparent adipsia or aphagia.

Seven rats received stereotaxically guided electrolytic lesions of the medial amygdala under anaesthetic as described above. These animals received two lesions on each side of the brain. An insulated tungsten electrode, with 0.5 mm bared at the tip, was positioned at coordinates 2.8 mm and 3.8 mm caudal to bregma, 3.6 mm lateral from the midsagittal sinus, and 8.2 mm in depth from the dural surface. An anodal current of 2.0 Ma was delivered for 15 seconds to create each lesion. Animals sustaining amygdala damage generally showed a mild adipsia and aphagia for the first 3 to 5 days following surgery. During this time their body weights dropped approximately 15 grams. They were given wet mash for 3 to 4 days following surgery, until they resumed normal feeding and drinking. All animals were allowed a 2-week recovery period following stereotaxic surgery, prior to any testing, during which they regained their preoperative weights.

Part A: mineralocorticoid-induced sodium appetite

Each subject received a subcutaneous injection of 2 mg deoxycorticosterone (DOCA) in 1 ml of propylene glycol vehicle, for three successive days. The 3% NaCl intakes were recorded for three days prior to the treatment and the three days during the DOCA treatment. A second course of DOCA administration and monitoring of 3% NaCl intake was conducted one week after the first exposure to DOCA.

Part B: Depletion-induced sodium appetite

Two sodium depletion trials, separated by one week, were conducted seven days after the second DOCA test. The rats were sodium depleted by combining pharmacological natriuresis with removal of ambient sodium. For two days prior to each depletion trial, Teklad sodium deficient pellets (sodium content 0.02% to 0.03%) were introduced into the home cage along with the normal diet (Purina 5001). This was done to familiarize the rat with the sodium deficient food thereby eliminating any neophobic response to the food. Familiarization with the sodium deficient diet also reduced the possibility of the acquisition of a conditioned taste aversion based on the association of the deficient diet with acute sodium depletion.

In each experimental trial, furosemide was administered to each rat following removal of the 3% NaCl buret and a thorough washing of the cage to remove ambient sodium. The furosemide, a potent diuretic and natriuretic agent that produces rapid sodium loss (see Wolf 1982), was injected subcutaneously in two injections (5 mg furosemide in 0.5 ml isotonic vehicle) separated by 2 h. The rats were allowed access to only sodium-deficient diet and water for the subsequent 24 h. The following day, (18 to 24 h after the first furosemide injection), 3% NaCl was returned to the front of the cages in graduated cylinders. The rats were alerted to the return of the 3% NaCl by gentle prodding of their mouths with the spout of the 3% NaCl buret. Latency to the onset of drinking 3% NaCl and the cumulative intakes of water and 3% NaCl were recorded (to the nearest 0.5 ml) at 15, 30, 60, and 120 min.; this two hour access was called the appetite test. Subsequent overnight water and 3% NaCl intakes were recorded; normal sodium replete pellets were then returned.

Histology

At the completion of the experiments, the animals were anaesthetized with 0.5 ml of Euthanasia-6 (i.p) or 1.0 ml of 50% chloral hydrate (i.p) and perfused intracardially with isotonic saline followed by 10% buffered formalin. Each brain was removed and stored in 10% buffered formalin. Forty micron frozen sections, taken in the coronal plane, were mounted on gelatin coated slides and subsequently stained with luxol fast blue and cresyl violet. Subjects were assigned to groups based upon the location and extent of tissue damage, assessed by a rater blind to the experimental results.

Statistical analysis

All data were analyzed using analysis of variance (ANOVA), with an alpha level of 0.05. When justified, post hoc tests were conducted using the Newman-Keuls procedure and the Studentized range statistic, q.
RESULTS

Histology

Rats in the medial amygdala group had significant damage to the medial portion of the amygdala. In addition, the lesion typically damaged portions of the cortical and central nuclei of the amygdala and interfered with the stria terminalis and the ventroamygdalofugal pathway as well (Fig. 1, panels A and B). Of the seven rats undergoing lesions, six sustained sufficient damage to the medial amygdala to be included in this group.

In order to be included in the ST group, rats had to have complete bilateral transection of the stria terminalis; 18 of the original 21 ST rats met the criterion. The stria terminalis was cut at its most dorsal excursion as it coursed along with the fimbria. At this location, the stria terminalis is a discrete fiber bundle and, severing it here, interrupts all four of its subsequent component parts (de Olmos and Ingram 1972) without damage to the amygdala (Figure 1, panels I and 2). The cuts extended in a medial-caudal direction from the caudate putamen through the globus pallidus to the anterior portion of the lateral posterior and posterior thalamic nuclei. Typically, the fimbria and the CA3 layer of the hippocampus were also damaged. All 16 rats in the control group completed the study and were included in all analyses.

Mineralocorticoid-induced sodium appetite

For each rat, results from the two DOCA tests were collapsed, all analyses being conducted on the collapsed data. There were group differences in sodium consumption ($F(2,37)=3.52, P<0.05$), and DOCA administration affected sodium consumption, ($F(1,37)=24.6, P<0.001$). A significant Group x Drug interaction indicated that the three experimental groups did not respond in a uniform manner to DOCA administration ($F(2,37)=8.57, P<0.01$) (Fig. 2). Post-hoc analyses (37 df) showed that DOCA increased the 24 hour sodium intake of ST rats ($q_2=4.8, P<0.01$) and controls ($q_4=7.7, P<0.01$), but not that of AMY rats ($q_2=0.43$). Furthermore, the sodium intakes of ST rats and controls following DOCA administration were not significantly different from each other ($q_2=1.8$), and both exceeded the intake of medial amygdala damaged rats (ST: $q_5=6.8, P<0.01$; control: $q_6=8.5, P<0.01$).

Fig. 1. Panels A and B: Coronal section of a successful medial amygdala lesion. Panels I and 2 Coronal brain slice depicting a successful ST knife-cut. The knife-cut can be seen to pass directly through the ST (indicated by an arrow), with some damage to the fimbria, at about A.5340 in the König and Klippel (1963) atlas. Abbreviations: CA3, CA3 of the hippocampus; CP, caudate putamen; fi, fimbria; ST, stria terminalis.

Sodium depletion-induced sodium appetite

One of the AMY rats began to rapidly lose weight and subsequently died prior to the completion of the furosemide-induced sodium depletion tests, and so was not
included in these analyses. Sodium depletion produced a significant increase above baseline levels of 3% NaCl intake in both the first ($F(1,36)=106.4, P<0.0001$) and second ($F(1,36)=115.4, P<0.0001$) depletion tests (Fig. 3). An analysis of the NaCl intake profile over time revealed no group differences from either the first or second depletion ($F(2,36)=0.23$ and $F(2,36)=1.06$ respectively) (Fig. 4). Finally, there were no group differences in the 24 h water consumption following sodium depletion ($F(2,36)=0.22$). For all three groups, need-free sodium intakes (i.e. ad libitum 3% NaCl intake in the absence of any sodium appetite induction) following sodium depletion treatments were elevated compared to need-free sodium intake measured two weeks postoperatively ($F(2,36)=4.20, P<0.05$). However, no group differences existed ($F(2,36)=0.1$).
DISCUSSION

In these experiments, we showed that similar to control and amygdala lesioned rats, rats with ST transections displayed a normal sodium appetite following treatment with furosemide. However, unlike medial amygdala lesions, transection of the ST alone did not block mineralocorticoid induced sodium appetite. Therefore, stria terminalis disruption is not involved in medial amygdala lesion-induced alterations of sodium appetite.

Two possibilities present themselves in regard to these findings. Destruction of neural tissue in the medial amygdala (and the subsequent loss of mineralocorticoid receptors found therein) may be responsible for the impaired expression of mineralocorticoid-induced sodium appetite. Alternatively, neural communications not dependent upon an intact ST may be disrupted by amygdala lesions, thereby blocking the mineralocorticoid-induced sodium appetite (e.g. the VAF).

Since aldosterone receptors have been documented in the medial amygdala (Birmingham et al. 1979; Coirini et al. 1985; Funder 1986; McEwen et al. 1986), and more recent evidence has shown that damage to the central nucleus of the amygdala also impairs DOCA-induced NaCl appetite (Galverna et al. 1990), it is conceivable that these cells act in some direct manner to promote sodium seeking behaviour, or to promote ingestion when sodium is encountered. Consistent with this view is the observation that amygdala lesions reduce exploratory behaviour in the rat (White and Weingarten 1976). In addition, it has been suggested that lesions of the amygdala reduce a rat's ability to attach motivational significance to sensory stimuli (Cormier 1981), such as the importance of a salty taste when sodium appetite has been stimulated. The observation that sodium depletion-induced salt appetite is unaffected by medial amygdala lesions does not necessarily argue against this view, but rather underscores the extent to which the two systems governing salt appetite, i.e. angiotensin and aldosterone, are separated at both the anatomical and the behavioural level.

A second possibility is that interruption of information flow from the amygdala via the VAF contributes in part (or entirely) to the effects of medial amygdala damage in DOCA-induced sodium appetite. The VAF is a diffuse fiber system which connects the amygdala to the bed nucleus of the stria terminalis, the medial preoptic area and the ventromedial hypothalamus (de Olmos et al. 1985). While stimulation of the medial amygdala does not produce activation of VAF fibres (Watson et al. 1983), the chance remains of secondary or tertiary activation of VAF fibres via other amygdaloid nuclei adjacent to the medial amygdala, for example via the cen-
central nucleus of the amygdala. However, the complex interconnections of the amygdaloid nuclei (de Olmos et al. 1985) make it difficult to assess this possibility.

A series of studies examining the effects of small restricted lesions of nuclei within the amygdala would lead to a better understanding of aldosterone neural circuitry. In addition, studies which ablate only the cell bodies of the medial amygdala, for example through the use of ibotenic acid, would allow the distinction to be made between the role of cell bodies in the amygdala, and efferent neural fibres arising from the amygdala, in mineralocorticoid-induced sodium appetite in the rat.

ACKNOWLEDGEMENT

This research was supported by NIMH Research Career Development Award MH00678 to J.S., by NIMH Grant NH43787 and NIH Grant NS 03469 to A.N.E., an NSERC PGs to R.M.B. and an NSERC grant to H.P.W. R.M.B. is now at the departments of Nutritional Sciences and Psychiatry, University of Toronto. We thank Callie Schewchuk for excellent technical assistance.

REFERENCES


Received 7 October 1991, accepted 22 January 1992