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Area postrema lesions attenuate LiCl-induced c-Fos expression correlated with conditioned taste aversion learning

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ABSTRACT

Lesions of the area postrema (AP) block many of the behavioral and physiological effects of lithium chloride (LiCl) in rats, including formation of conditioned taste aversions (CTAs). Systemic administration of LiCl induces c-Fos immunoreactivity in several brain regions, including the AP, nucleus of the solitary tract (NTS), lateral parabrachial nucleus (latPBN), supraoptic nucleus (SON), paraventricular nucleus (PVN), and central nucleus of the amygdala (CeA). To determine which of these brain regions may be activated in parallel with the acquisition of LiCl-induced CTAs, we disrupted CTA learning in rats by ablating the AP and then quantified c-Fos-positive cells in these brain regions in sham- and AP-lesioned rats 1 h following LiCl or saline injection. Significant c-Fos induction after LiCl was observed in the CeA and SON of AP-lesioned rats, demonstrating activation independent of an intact AP. LiCl-induced c-Fos was significantly attenuated in the NTS, latPBN, PVN and CeA of AP-lesioned rats, suggesting that these regions are dependent on AP activation. Almost all of the lesioned rats showed some damage to the subpostremal NTS, and some rats also had damage to the dorsal motor nucleus of the vagus; this collateral damage in the brainstem may have contributed to the deficits in c-Fos response. Because c-Fos induction in several regions was correlated with magnitude of CTA acquisition, these regions are implicated in the central mediation of lithium effects during CTA learning.

1. Introduction

Conditioned taste aversion (CTA) is a robust form of associative learning with unique single-trial and long-delay characteristics. A single pairing of a novel taste with a toxic substance such as lithium chloride (LiCl) produces a robust and persistent avoidance of substances containing that taste [1]. Furthermore, CTAs can be formed with a delay of minutes to several hours between the taste and the toxin [2–4]. These unique features make CTA learning a useful experimental model for studying learning and memory at the neuroanatomical level.

c-Fos immunohistochemistry can be used to identify sites of neuronal activation involved in the processing of both taste and toxic stimuli. LiCl is widely used as a toxin to produce CTA in rats, and several investigators have shown that systemic administration of LiCl increases c-Fos protein or mRNA in many brain regions, including the nucleus of the solitary tract (NTS), area postrema (AP), lateral parabrachial nucleus (latPBN), central nucleus of the amygdala (CeA), and the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus [5–11]. Several of these regions also receive input from gustatory pathways, so that they may be important for the integration and association of taste and toxic information during acquisition of a CTA.

Lesion studies have identified some brain regions that are sensitive to LiCl and mediate CTA learning. The acute, central effects of LiCl are thought to require the detection of lithium by the AP, a highly vascularized circumventricular organ in the brainstem. AP neurons are activated by blood-borne toxins, and are sensitive to LiCl [12]. Several studies have demonstrated that lesions of the area postrema block the acquisition of LiCl-induced CTA in rats [13–20] and monkeys [21]. This loss of function is not due to deficits in taste perception or CTA learning because rats with AP lesions are able to express CTAs acquired prior to AP ablation [22], and AP-lesioned rats can form CTAs produced by other drugs such as amphetamine [13,23] or apomorphine [24] or by motion sickness [18,21]. These studies show that the AP is necessary for the central detection of LiCl leading to the acquisition of LiCl-induced CTA. Other behavioral and physiological responses to systemic LiCl such as lying-on-belly, delayed gastric emptying and hypothermia are also blocked by AP ablation [16]. However, not all responses to LiCl are lost in AP-lesioned rats [17,19,25].

The study of c-Fos expression after LiCl administration in AP-lesioned rats would be useful in defining brain regions that may be important for the acquisition of LiCl-induced CTAs. We hypothesized that brain regions involved in the central processing of CTA learning

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would fulfill two complementary criteria: 1) the site should be activated by a stimulus that induces CTA learning (e.g. LiCl), and 2) the site should not be activated under conditions when CTA learning fails (e.g. following AP ablation). Furthermore, because AP lesions do not block all the behavioral and physiological effects of LiCl, we also expected that AP lesions would not attenuate LiCl-induced c-Fos in all brain regions. Thus, in the present study we ablated the AP in rats and verified functional disruption of LiCl-induced CTA learning. We then quantified c-Fos by immunohistochemistry in several brain regions in sham- and AP-lesioned rats 1 h following LiCl or saline injection. We examined the NTS and latPBN which receive direct projections from the AP, and which are critical for CTA learning. We examined the SON and PVN, which mediate the neuroendocrine responses to systemic LiCl (e.g. oxytocin, vasopressin, or CRH release). We also examined the CeA, which receives brainstem projections and has been implicated in CTA learning. Our results demonstrate that LiCl-induced c-Fos expression is attenuated in the NTS, latPBN, PVN and CeA, but not the SON, of AP-lesioned rats, implicating these brain regions in central mechanisms of CTA learning and other effects of LiCl.

2. Materials and methods

2.1. Animals

Forty-two adult male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were housed individually under a 12-h light, 12-h dark cycle at 25 °C. At the start of the experiment the rats weighed between 250 and 300 g. Purina rodent chow and water were provided ad libitum, except as noted below. All experiments were approved by the Institutional Animal Care and Use Committee of Florida State University.

2.2. Lesions of the area postrema

Twenty-six rats received AP lesions and 16 rats received sham lesions. Rats were anesthetized with a cocktail of magnesium sulfate (75 mg/kg), chloral hydrate (153 mg/kg) and pentobarbital (35 mg/kg) and placed in a stereotaxic apparatus with the head in a ventroflexed position. A midline incision was made on the back of the neck and the neck muscles were retracted. With the aid of a dissecting microscope, the atlanto-occipital membrane was punctured and removed to expose the dorsal surface of the medulla. To permit clear visualization of the AP, a small portion of the base of the skull was removed with rongeurs to enlarge the foramen magnum. The membrane covering the AP was carefully removed and a thermal lesion of the AP was made by briefly touching the structure with the tip of a small cautery. Sham-lesioned rats were treated in an identical fashion, but the AP was not touched and left intact. Immediately following the lesion, rats invariably stopped breathing and some required resuscitation by manual compression of the rib cage. Rats were given antibiotics (16 mg sulfamethoxazole/3.2 mg trimethoprim s.c.) and were maintained on sweetened condensed milk (diluted 1:2 with water) with vitamin supplements for 1 week after surgery. Seven lesioned rats did not survive the post-operative period.

2.3. CTA acquisition and expression

Once the rats had surpassed their pre-operative weights and were steadily gaining weight (2–3 weeks following surgery), they were placed on an 18-h water-deprivation schedule. After 3 days, all rats ($n=19$ lesioned rats, $n=16$ sham rats) were given access for 30 min to 5% sucrose and then injected with LiCl (i.p., 0.15 M, 12 ml/kg). Sucrose intake was measured by weighing sucrose bottles before and after the 30 min access. Water was returned for an additional 5.5 h and then rats continued on an 18-h water-deprivation schedule. Forty-eight hours after the pairing of sucrose and LiCl, all rats were again given access to 5% sucrose for 30 min in a 1-bottle test of CTA expression. For each rat, CTA magnitude was calculated from the sucrose intake 48 h after LiCl injection as a percentage of the rat's sucrose intake prior to LiCl injection (% suppression). A positive % suppression indicates a decrease in sucrose intake. The water deprivation schedule was discontinued following behavioral testing. Based on CTA magnitude, lesioned rats were subsequently divided into two groups: an APX group ($n=13$) that failed to acquire a CTA, and a "failed-APX" group ($n=6$) that showed significant CTA expression comparable to sham rats despite their surgical lesion (see Results section).

2.4. Tissue collection and immunohistochemistry

Approximately 1 week after behavioral testing, rats were given an injection of either LiCl or NaCl (i.p., 0.15 M, 12 ml/kg). Three groups of rats received LiCl injections: sham-lesioned rats ($n=8$), APX rats ($n=6$), and failed-APX rats ($n=6$). Two groups of rats received NaCl injections: APX rats ($n=7$) and sham-lesioned rats ($n=8$). Rats were overdosed 1 h later with sodium pentobarbital. When completely unresponsive, the rats were perfused transcardially, first with 100 ml of isotonic saline/0.5% sodium nitrite/1000 U heparin, and then with 400 ml phosphate-buffered 4% paraformaldehyde. The brains were removed, blocked, post-fixed for 2 h and then transferred to 0.1 M phosphate buffer (PB) for storage at 4 °C. Individual blocks of hindbrain and forebrain tissue were transferred into 30% sucrose 24 h to 1 week prior to sectioning. Rats were perfused in 2 cohorts, each containing half the rats from each treatment group. Hindbrain and forebrain tissue from each cohort were processed separately within 1–2 weeks after perfusion. Tissue from all treatment groups was processed in parallel.

Forty micron coronal sections were cut on a freezing, sliding microtome. Alternate sections were processed from the medulla at the level of the NTS (bregma −12.8 mm to −14.3 mm) and the pons at the level of the PBN (bregma −9.16 mm to −10.3 mm). Every fourth section was processed from the forebrain through the hypothalamus and amygdala (bregma −0.8 mm to −3.6 mm). Coordinates were based on Paxinos and Watson's atlas [26]. Sections were immediately processed after cutting for c-Fos immunohistochemistry.

Free-floating tissue sections were washed twice for 15 min in 0.1 M phosphate-buffered saline (PBS) and then incubated for 30 min in 0.2% Triton X-100/1% bovine serum albumin (BSA)/PBS. After two washes in PBS/BSA for 15 min each, sections were incubated overnight with a rabbit anti-c-Fos antiserum (Ab-5, Oncogene Research) at a dilution of 1:20,000. After two 15-min washes in PBS/BSA, sections were then incubated for 1 h with a biotinylated goat anti-rabbit antibody (Vector Laboratories) at a dilution of 1:200. Antibody complexes were amplified using the Elite Vectastain ABC kit (Vector Laboratories), and visualized by a 5-min reaction in 0.05% 3,3-diaminobenzidine tetrahydrochloride (DAB). Sections were stored in 0.1 M PB until mounted onto gelatin-coated glass slides and coverslipped using Permount. Alternate coronal sections through the caudal hindbrain were stained with cresyl violet to evaluate AP lesions.

Cells expressing darkly-positive, nuclear c-Fos immunoreactivity were quantified using a custom software program (MindsEye 1.19b, T. Houpt). Images were digitally captured in a 0.72 mm × 0.54 mm counting frame (0.87 mm × 0.65 mm for latPBN). For the NTS, latPBN and SON, counting was restricted to the area delineated by a hand-drawn outline. Outlining was not necessary for the PVN and CeA because these regions mostly filled the counting frame or had little c-Fos outside the area of interest. Bilateral cell counts were averaged for 3 sections of the PVN (approximately bregma −1.8 mm to −2.12 mm), 4 sections of the SON (bregma −1.3 mm to −1.8 mm), 6 sections of the CeA (bregma −2.3 mm to −3.14 mm),