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Lesions of the posterior basolateral amygdala block feeding induced by systemic 8-OH-DPAT

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Abstract

We have recently reported that bilateral electrolytic lesions of the posterodorsal amygdala (PDA) in female rats which induce protracted overeating and weight gain also attenuate short-term feeding stimulated by intraraphe infusions of the serotonin (5-HT) 1A agonist, (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin, (8-OH-DPAT). Bilateral lesions of the posterior basolateral amygdala (pBLA) in male rats have also been reported to enhance feeding and weight gain, but much less so than PDA lesions do in female rats. The present study was performed to determine if pBLA lesions in female rats might attenuate 8-OH-DPAT feeding and what, if any, relationship exists between 8-OH-DPAT-induced feeding and lesion-induced weight gain. Lesioned rats showed reliable increases in 24-h food intake and weight gain relative to shams during the days between surgery and acute drug-induced feeding tests. 8-OH-DPAT (0, 60, 120 or 240 μg/kg in saline) increased feeding of shams in a dose-dependent manner over 2 h. Feeding at the most effective dose (120 μg/kg) was reduced to vehicle levels in lesioned rats. The feeding induced by this dose correlated inversely ($r = -.59$, $P < .01$) with the magnitude of weight gained following lesions. Feeding at the highest dose (240 μg/kg) showed a biphasic effect of feeding inhibition over the first vs. second hour that was unaffected by lesions. These findings imply that either fibers of passage and/or cellular elements in both the PDA and pBLA normally inhibit overeating and weight gain via intact serotonergic mechanisms.

Keywords: Feeding; Body weight; Serotonin; Electrolytic lesions; 8-OH-DPAT; Subcutaneous; Amygdala

1. Introduction

Systemic injections of (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) produce short-term feeding increments in otherwise satiated rats (Bendotti and Samanin, 1987; Dourish et al., 1985). This effect appears to be mediated by stimulating somatodendritic serotonin (5-HT) 1A autoreceptors located on cell bodies of 5-HT-containing neurons in the midbrain raphe nuclei (Fletcher and Coscina, 1993; Hutson et al., 1986, 1988). Local administration of 8-OH-DPAT by reverse dialysis decreases the release of extracellular 5-HT in both the raphe and at distal forebrain sites (Bosker et al., 1994). This suggests that the anatomical locus of feeding induced by this drug occurs because of decreased serotonergic neurotransmission in brain sites controlling ingestive behaviors.

Past literature has linked several hypothalamic nuclei to 5-HT's control over feeding in general (Hoebel et al., 1989; Leibowitz and Shor-Posner, 1986; Waldbillig et al., 1981) as well as the effects of 8-OH-DPAT on feeding being temporally associated with altered 5-HT levels (Schwartz et al., 1990). However, previous work from our group has shown that infusions of 5-HT directly into the hypothalamus do not block 8-OH-DPAT-induced feeding (Fletcher and Coscina, 1993). This suggests that the neuroanatomical locus of such feeding is produced by decreased 5-HT release in extra-hypothalamic sites. Supporting this contention, earlier work by Fletcher (Fletcher, 1991a,b) has implicated dopaminergic mechanisms in the neostriatrum as well as opiate mechanisms in both the neostriatum and nucleus accumbens as contributing to 8-OH-DPAT-induced feeding.

Another extra-hypothalamic region that may contribute to 8-OH-DPAT-induced feeding is the amygdala. It has

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been known for some time that bilateral amygdalar damage in dogs (Fonberg, 1971) or rats (Korczynski and Fonberg, 1979) can produce increased food intake and body weight gain. More recently, Ganaraj and Jeganathan (1998) have reported that male rats bearing electrolytic lesions of the basolateral amygdala (BLA) show small but reliable increases in food intake over 3 weeks, producing weight gains that average 23 g (+10%). Contrasting to this, an extensive series of studies by King and coworkers has presented a variety of data suggesting that the posterodorsal portion of the amygdala (PDA) is the most effective lesion locus to produce enhanced feeding and weight gain (King et al., 1996a, 1994; Rollins and King, 2000). Electrolytic damage to that site produces rapid, significant increases in the food intake and weight gain in female rats (King et al., 1996b, 1999). King's rats typically gain 20–30 g during the first three postoperative days, with overall weight gains varying from 50 to 100 g by 20 days postsurgery (King et al., 1996b).

We have recently reported that PDA-lesioned female rats supplied by the King lab show attenuated feeding after 8-OH-DPAT infusions directly into the dorsal raphe nucleus (Coscina et al., 2000). Since most investigators in this field study feeding following peripheral injections, we also tested one systemic dose (250 μg) in these lesioned animals and found suggestive evidence that it too was less effective in driving feeding. However, the number of animals tested was relatively small, so these findings were inconclusive.

The purpose of the present experiment was extend our previous observations (Coscina et al., 2000) in three ways: (1) to determine if pBLA lesions in female rats would show enhanced feeding and weight gain, (2) to perform a dose–response study of feeding induced by systemic 8-OH-DPAT in such lesioned animals, and (3) to assess the potential relationship of pBLA lesion-induced weight gain to feeding induced by systemic 8-OH-DPAT.

2. Method

2.1. Animals

Twenty-one female Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were housed singly in plastic cages with sawdust bedding and overhead steel tops that also served as holders for food pellets (LabDiet, PMI Nutrition Intl., Brentwood, MO) and water bottles. Cages were located in a temperature-controlled (21–23°C) colony room illuminated 12 h daily (0600–1800). Average animal weight at surgery was 243 g (S.E. = 17.4; range = 220–278 g). Throughout these studies, rats had ad libitum access to food and water. Daily measurements of food intake and body weight were made prior to surgery as well as during postoperative recovery. All procedures were approved by the Wayne State University Animal Investigation Committee as being in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Surgery

Rats were anaesthetized with 43 mg/kg ip sodium pentobarbital (Butler, Columbus, OH) and positioned in a Kopf small animal stereotaxic frame with the incisor bar horizontal to interaural line. The 1 mg/kg atropine sulfate (Radix Labs., Eauclaire, WI) was injected intraperitoneally 30 min previously and buprenorphine (BUPRENEX, Reckitt & Colman, Hull, UK) 0.1 mg/kg administered subcutaneously. A No. 0 insulated insect pin was lowered to the following stereotaxic coordinates: bregma −2.1 mm, lateral ±4.5 mm, skull surface −8.4 mm. Bilateral electrolytic lesions were produced by passing 1.5 mA anodal DC (Stoetling, Chicago, IL) for 20 s between the 0.5 mm bared tip of the insect pin and a rectal cathode wrapped in saline-soaked gauze. Sham lesions were performed using the same procedure except that the insect pin was lowered to 1 mm above the electrolytic lesion coordinates and no current was passed. Following surgery, scalp incisions were sutured closed and treated with Bacitracin zinc antiseptic ointment (Fougera, Melville, NY). Two animals failed to recover from anesthesia, resulting in 8 lesioned and 11 shams being available for testing.

2.3. 8-OH-DPAT feeding tests

Mock tests were first conducted to accustom rats to the separate stainless steel wire-mesh cages as well as the regimen of food exchanges used during the feeding tests. Rats were moved to test cages in the same colony room in which fresh food was made available and measured every hour of a 3-h test session (1300–1600 h). Water was also freely available. For mock tests, subcutaneous injections of saline (1 ml/kg) were given after the first hour of feeding (presatiation). 8-OH-DPAT feeding tests began 8 days after surgery, by which individual body weights had recovered to at least presurgery levels and the weight of food consumed during mock tests had stabilized. Using the same procedure as during mocking tests, each rat was removed after the first feeding hour and injected subcutaneously with 0 (saline), 60, 120 or 240 μg/kg/ml (±) 8-OH-DPAT HBr (RBI, Natick, MA). Each rat was tested once every 2–3 days using a counterbalanced design to control for order effects.

2.4. Histological analysis

After completion of testing, all rats were euthanized with 120 mg/kg sodium pentobarbital, decapitated, then their brains removed and stored in 10% formalin. The 40-μm sections were cut 24–48 h later and analyzed using a light microscope to visualize the extent of lesions. Animals showing a lesion in either hemisphere extending beyond

the area described in King et al. (1996a) were removed from further analysis.

2.5. Statistical analysis

Planned comparisons were used to compare the effect of the lesion on total food intake at each dose of 8-OH-DPAT tested. Feeding data were further analyzed using a multivariate analysis of variance (MANOVA) followed by Tukey post hoc tests. A three-way MANOVA was performed with one between variable, lesion, and two within variables, hour (two levels) and dose (four levels). Body weight data were analyzed using *t* tests for independent samples and Pearson Product Moment Correlations. All statistical analyses were performed using STATISTICA '98 edition (StatSoft, Tulsa, OK).

3. Results

3.1. Histological analysis

Each lesioned animal retained sustained bilateral damage no wider than 0.25 mm and extending no more than 0.75 mm in length. Only one animal whose lesions extended dorsally to the optic tract and the caudate was excluded from the body weight and behavioral analysis. All lesions included the dorsal portion of the pBLA. Lesions did not extend caudally into the ventral hippocampus. The extent of a typical lesion is represented in Fig. 1.

3.2. Body weight analysis

Individual body weights were averaged over 4 days prior to and 8 days after surgery. Mean ± S.E. changes in body weights of sham ($n = 11$) and pBLA-lesioned ($n = 7$) rats were 5.1 ± 1.6 g and 15.1 ± 3.1 g, respectively. Lesions produced significant weight gains compared to presurgery (*t* test for independent samples $t_{16} = 3.11$, $P < .01$). There was no significant difference in the variances between the unbalanced ns (11 sham, 7 lesion: Brown–Forsythe's Test $P = .32$). There was also a significant increase in daily food consumption in the 8 days following surgery in the lesion group (*t* test for independent samples $t_{16} = 2.41$, $P < .05$, see Fig. 2). Mean ± S.E. daily food consumption was 20.1 ± 0.7 g and 22.3 ± 0.6 g, for the sham and lesion groups, respectively.

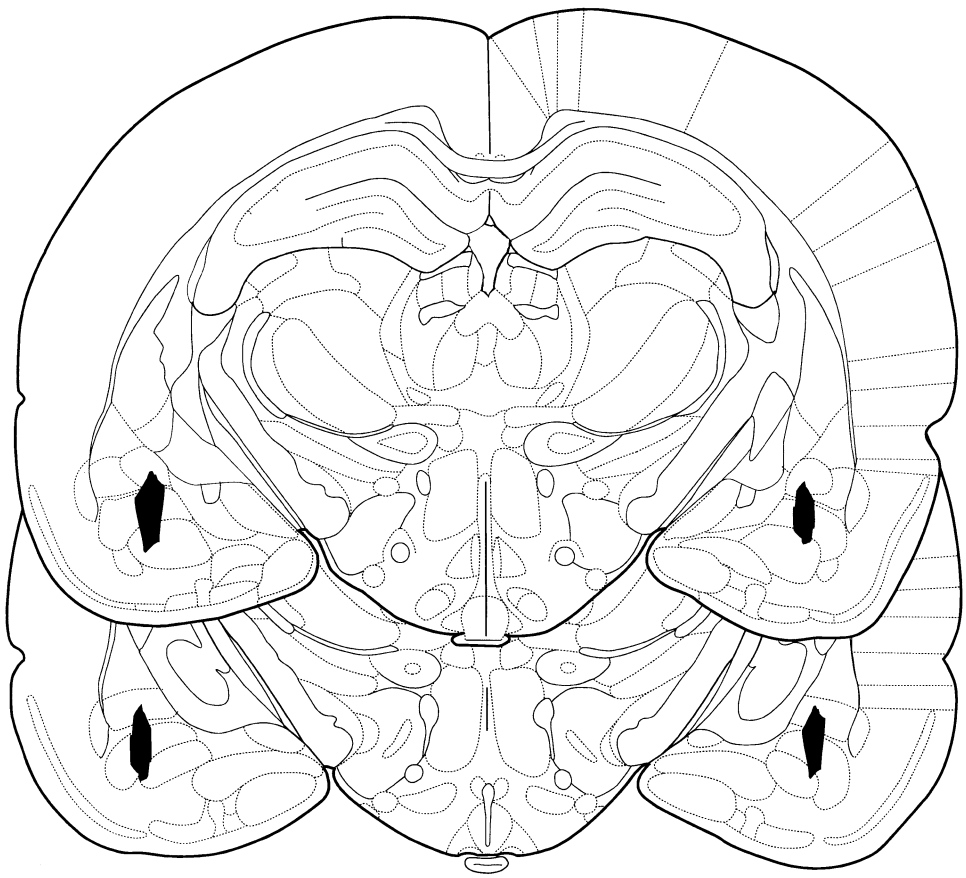

Fig. 1. The shape and extent of a typical lesion. The dark shaded area represents the outer limit of the damaged tissue. The plates are edited images of bregma − 3.60 and − 3.80 mm (Paxinos and Watson, 1998).

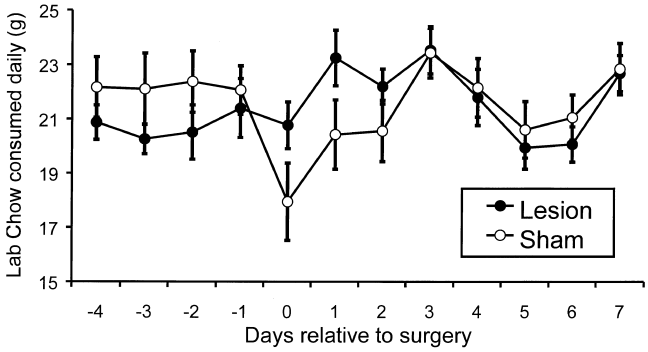

Fig. 2. Mean weight of daily food (lab chow) consumed in the home cage in rats receiving a sham or bilateral electrolytic lesion of the pBLA. Filled and open circles represent lesion ($n=7$) and sham-lesioned ($n=11$) animals, respectively.

3.3. 8-OH-DPAT feeding tests

Total feeding following systemic injections of 8-OH-DPAT (Fig. 3) showed a significant effect of lesion at only the 120 μg dose [$F(1,16)=15.22$, $P<.01$]. No other dose showed a significant lesion effect. The three-way MANOVA (Fig. 3) confirmed there was significantly more feeding in the sham group [$F(1,16)=9.08$, $P<.01$] and that more feeding occurred during the second hour across dose and lesion conditions [$F(1,16)=4.92$, $P<.05$]. There was also a significant interaction of Dose × Hour [$F(3,48)=3.94$, $P<.05$]. Post hoc testing revealed that feeding was significantly less during the first hour at the highest dose tested (240 μg) than at the lowest dose (60 μg) during the first hour and at the highest dose during the second hour (both $P<.01$). The main effect of dose was barely significant [$F(3,48)=2.85$, $P<.05$], but there was a strong effect of lesion group on the dose–response [Lesion × Dose $F(3,48)=4.95$, $P<.01$]. Levene's test for homogeneity of

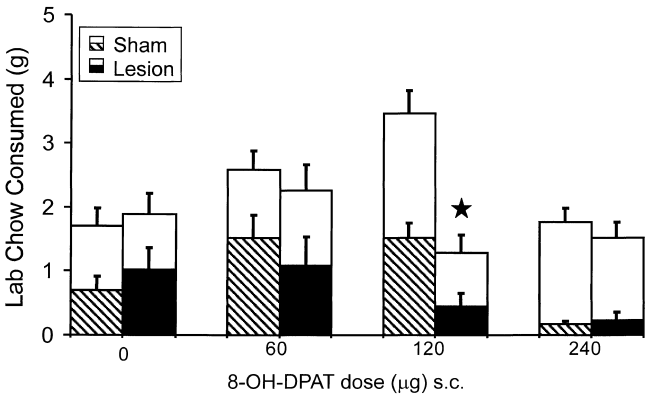

Fig. 3. Mean weight of food (lab chow) consumed during the first and second hour (filled and open stacked bars, respectively) following a systemic (subcutaneous) injection of 8-OH-DPAT (0, 60, 120 or 240 μg/kg in saline) in rats bearing a sham or bilateral electrolytic lesion of the pBLA. ★ $P<.01$ as compared to the sham group (planned comparisons at each dose).

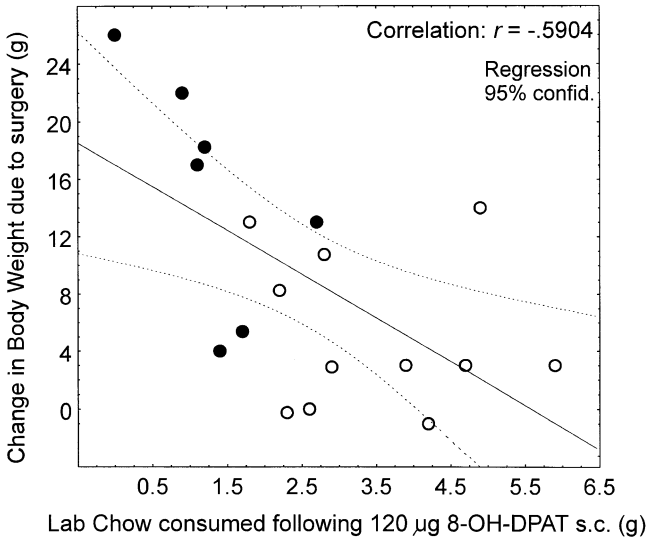

Fig. 4. Scatterplot and regression line describing the relationship between the weight gained following surgery (8 days post- and 4 days presurgery) and the feeding response over 2 h to 120 μg 8-OH-DPAT (subcutaneous), Pearson's $r=-.59$, $P<.01$, $n=18$). Filled and open circles represent individual lesion and sham-lesioned animals, respectively.

variance indicated the unbalanced n across the lesion variable did not produce a significant difference at any combination of dose and hour cells [$F(1,16)$, all $P<.17$].

A correlation was calculated to explore whether the effect of lesion on body weight might predict the feeding response to the 120 μg dose of 8-OH-DPAT. The difference in body weight 2 days prior to initiating 8-OH-DPAT feeding tests (8 days postsurgery) and 4 days prior to surgery was calculated for each animal. Separate analyses by lesion group failed to reach significance. However, their combined distribution suggested the lesion (r=.635) might have shifted rats to one end of a continuum of effect reflective of an underlying mechanism. This was confirmed by the similarity of the combined correlation between this weight difference and the feeding response to 120 μg 8-OH-DPAT showing a significant negative relationship (Pearson's $r=-.59$, $P<.01$, $n=18$, Fig. 4). Separate regression analyses run showed change in body weight was in no way predictive of feeding tests at the other doses of 8-OH-DPAT used (0, 60, 240 μg, all $P>.15$).

4. Discussion

4.1. Histology results

An examination of the damage produced by electrolytic lesions revealed consistent bilaterally symmetric injury to the pBLA. In addition, this damage consistently included parts of the posterior extent of the basomedial amygdaloid nucleus in its ventral extent, and dorsally to the ventromedial part of the lateral amygdaloid nucleus. The extent of our lesions was more restricted than those typically reported by

the King group (Coscina et al., 2000; King et al., 1999). However, some of the most dramatic increases in body weight have been seen in animals bearing relatively focal lesions (Rollins and King, 2000). The presentation of the histology by Ganaraj and Jeganathan (1998) make it difficult to accurately compare the localization of their lesion loci with ours. However, the physical extent of the damage appears similar.

4.2. Body weight results

Our pBLA lesioned female rats showed small but significant increases in body weight compared to sham operated controls. These animals clearly did not show the dramatic weight gains reported by King et al. (1996b) following PDA lesions but instead showed more modest gains in keeping with the results of Ganaraj and Jeganathan (1998) in male rats as well as similar pBLA lesions in female rats more recently described by Rollins and King (2000). Regardless of lesion grouping, the negative correlation reported here between postoperative weight gain and the feeding induced by 8-OH-DPAT suggests that the variance in the extent of our lesions produces effects distributed along a naturally occurring continuum.

4.3. 8-OH-DPAT tests

Systemic administration of the 5-HT1A agonist, 8-OH-DPAT, produced dose-dependent increases in feeding in control subjects. This increase was attenuated in pBLA-lesioned rats to the levels observed following vehicle injections in controls. Such an attenuation occurred with no change in feeding to a control injection as compared to sham animals, demonstrating the lesions did not simply impair the ability to eat. The lesion-induced increase in weight gains relative to controls, the increase in daily food intakes, and our informal observations of home cage behavior all support the conclusion that pBLA lesions do not appear to produce any generalized ingestive or motoric impairments.

The ability of 8-OH-DPAT as an orexigen is dependent on animals being in a nonfood-deprived state (Bendotti and Samanin, 1987; Currie and Coscina, 1993). The state-dependent characteristic of 5-HT-related feeding appears to reflect its role as a modulator of feeding rather than as a primary neurochemical signal to initiate or cease eating. The ability of larger amygdalar lesions to produce more dramatic changes in feeding and body weight suggest the recruitment of other transmitter systems, or the influence of 5-HT on those other systems. Our previous investigation in animals with much larger and less localized PDA lesions produced an attenuation of 8-OH-DPAT's feeding stimulatory effects (Coscina et al., 2000). That the pBLA lesions employed here essentially abolished such feeding suggests that this amygdalar region may be a highly circumscribed zone that mediates this effect.

Separate regression analyses of postsurgical body weight gain against 8-OH-DPAT-induced feeding (see Fig. 4) for the lesion and sham groups failed to reach significance. However, both suggested that an inverse relationship existed between these two variables. The significant result of the combined regression analysis is of particular interest since it is consistent with the existence of an orderly inverse process mediating the degree of 5-HT tone in the pBLA and the magnitude of body weight gain. This tantalizing possibility merits further investigation.

The role of forebrain dopamine in the 8-OH-DPAT feeding effect investigated by Fletcher (1991a,b) can presently be seen in parallel or in series with the present results. Without further experiments manipulating both the pBLA and, for example, accumbens DA we will be unable to resolve whether our effects are a result of inhibiting output to that region.

In conclusion, the present experiment provides confirmatory evidence that damage restricted to the pBLA can induce small but reliable overeating and weight gain. It additionally shows that normal neural processes either in or projecting through this region appear necessary for the induction of feeding induced by systemic 8-OH-DPAT (Dourish et al., 1985). The latter finding supports our recent work showing that PDA injury also impedes feeding induced by direct infusion of 8-OH-DPAT drug into the dorsal raphe nucleus (Coscina et al., 2000). However, it seems instructive that the suppression of 5-HT release produced by this drug, which only attenuated feeding after less circumscribed PDA lesions, was completely abolished by the more restricted pBLA lesions described here. This implies that the pBLA is a novel yet potentially important extra-hypothalamic zone meriting further study surrounding the role of 5-HT and its control over feeding. In that context, it is important to acknowledge that different subtypes of 5-HT receptors have been ascribed different roles in the initiation vs. cessation of feeding (Bendotti and Samanin, 1987; DeVry and Schreiber, 2000). For this reason, we are currently pursuing additional work to clarify the specificity of 5-HT receptor subsystems in feeding controls that seem to reside in and/or interact with this small but apparently important neural region of the amygdala.

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