EFFECTS OF AMYGDALOID LESIONS ON HYPOTHALAMIC–HYPOPHYSIAL LUTEINIZING HORMONE ACTIVITY

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Summary. Bilateral lesions were made by electrocoagulation in one of the following amygdaloid areas: basolateral, cortical or medial in adult male deermice. Animals were killed 20 days after the operation, and determinations were made of pituitary LH concentrations, hypothalamic LH-RF potencies and seminal vesicle fructose concentrations. Data indicate that lesions placed in either the basolateral or cortical amygdaloid areas, but not in the medial, result in significant increases in pituitary LH and plasma LH as interpreted by the significant increases in dry seminal vesicle weight and fructose concentrations. Hypothalamic LH-RF concentrations decline significantly in animals with medial lesions while increasing significantly in animals with basolateral or cortical amygdaloid lesions.

INTRODUCTION

Lesions produced by electrocoagulation in various amygdaloid nuclear groups result in alteration of hypophysial trophic hormone secretion. Thus, lesions in the medial amygdaloid nucleus result in increased secretion of adrenocorticotrophin and adrenal corticosterone (Mason, 1959; Eleftheriou, Zolovick & Pearse, 1966) while causing a transient inhibition of the secretion of thyrotrophin (Eleftheriou & Zolovick, 1968). In addition, lesions placed in the basolateral–lateral amygdaloid nuclear complex result in the continuous secretion of luteinizing hormone in both male and female deermice (Eleftheriou & Zolovick, 1967; Eleftheriou, Zolovick & Norman, 1967) while affecting significantly the hypothalamic activity of both luteinizing hormone-releasing factor and follicular-stimulating hormone-releasing factor (Eleftheriou, 1967; Eleftheriou & Pattison, 1967). These data, combined with other data obtained previously, have given support to the theory that the amygdala exerts a modulating influence on the hypothalamic–hypophysial system for the secretion of certain trophic hormones (Koikegami, Yamada & Usui, 1953; Koikegami, Fuse, Yokoyama, Watanabe & Watanabe, 1955; Bunn & Everett, 1957; Shealy & Peele, 1957; Yamada & Greer, 1960; Taleisnik, Caligaris & DeOlmos, 1962).

Since the amygdala is composed of a number of distinct nuclear groups, the

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The present experiment was designed to study the effects of amygdaloid lesions, produced individually in a number of different nuclear groups, on hypothalamic luteinizing hormone-releasing factor (LH-RF) and hypophysial luteinizing hormone (LH) potency. The data indicate maximal effects on hypothalamic-hypophysial LH interrelationships were produced through lesions placed only in the basolateral and cortical amygdaloid areas but not in the medial amygdaloid nuclear complex.

**MATERIALS AND METHODS**

Adult male deermice (*Peromyscus maniculatus bairdii*), weighing 15 to 19 g, were anaesthetized with sodium pentobarbital, orientated in a stereotaxic instrument, and lesions were induced bilaterally in one of the following amygdaloid nuclei: basolateral (ABL), cortical (ACO) or medial (AME). For purposes of orientation, the stereotaxic atlas for this species was used (Eleftheriou & Zolovick, 1965). In addition, intact control males and sham-operated males were included in the experimental design. Each treatment included 150 subjects with the exception of the intact control group which contained only 100 male animals. The total number of deermice used was 700. The experiment was conducted during the period of September to January. Lesions were produced by electrocoagulation using a Radio Frequency Lesion Maker (Grass), discharging 1·5 mA of current for 10 sec through a monopolar, varnish-coated, stainless steel electrode. Insulation was removed 0·2 mm from the tip to produce a lesion of uniform diameter. A large stainless steel bar, inserted in the anus, served as the indifferent electrode. Following the operation, the deermice were placed four to a cage and were not killed until 21±2 days after the operation.

The animals were killed by cervical dislocation and the pituitaries were removed, weighed and placed in cold saline. The hypothalami were also removed and placed in cold 0·1 N-HCl and the seminal vesicles were removed and frozen for later analysis of the fructose content. After all tissues were collected, the hypophyses were homogenized (10 mg/ml of 0·85% NaCl), and pooled into three major groups each containing fifty hypophyses. Homogenates were centrifuged at 5000 g for 15 min at 3°C, and the supernatant fluid stored at −25°C for later assay of LH activity.

Luteinizing hormone was determined by the one-ovary, 4-hr ovarian ascorbic acid depletion method of Parlow (1961), using five rats (Sprague-Dawley) for each dose level of standard or unknown. LH potencies of unknown pituitaries were measured with 0·2 and 0·8 mg-equivalents of fresh tissue, and these were compared with 0·4 and 1·6 μg of NIH-LH-s11 standard preparation. The procedure of Bliss (1952) for calculating relative potencies was followed. Replicate potency estimates were combined by the procedure of Sheps & Moore (1960). Hypophysial concentrations were subjected to analysis of variance and orthogonal contrasts were used to evaluate treatment differences.

The hypothalami were homogenized, centrifuged at 10,000 g and the supernatant was removed and boiled for 10 min. After the pH had been adjusted to 6·8, it was re-centrifuged and the new supernatant was removed for LH-RF measurement. One ml of hypothalamic extract, containing three hypothalami,
was injected into normal male rats, 290±10 g. Thirty minutes later these assay rats were killed, the pituitaries removed, homogenized (10 mg/ml), and assayed later for their LH content (McCann, Taleisnik & Friedman, 1960; McCann, 1962). Replicate potency estimates were combined and statistical analyses were conducted as described previously.

Fructose determinations in the seminal vesicles were conducted according to the method of Roe (1934). Values were expressed as µg/100 mg of fresh tissue.

RESULTS

The location of the various lesions was confirmed by histological examination and found to be in the intended areas (Plate 1).

The most significant increases ($P<0.01$) in pituitary luteinizing hormone were observed after lesions were placed in the cortical and basolateral amygdaloid nuclei (Table 1). The average value of 0.61 µg-equivalents rose to 1.53 and 1.44 after cortical and basolateral lesions were placed in the amygdala. Although the pituitary level of 1.00 µg-equivalent after medial lesions appears high in comparison with the control values, it was not significantly different from the control ($P = 0.10$). Thus, it appears that significant changes in pituitary LH content occur only after lesions are placed in the basolateral–cortical area, and not in the medial area of the amygdala.

**Table 1**

<table>
<thead>
<tr>
<th>Lesion</th>
<th>No. of animals</th>
<th>LH concentration*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Potency 95% limits</td>
</tr>
<tr>
<td>Unoperated controls</td>
<td>100</td>
<td>0.65 0.28 to 1.21</td>
</tr>
<tr>
<td>Sham-operated controls</td>
<td>150</td>
<td>0.61 0.24 to 1.27</td>
</tr>
<tr>
<td>Cortical amygdaloid nucleus</td>
<td>150</td>
<td>1.53 0.84 to 3.17</td>
</tr>
<tr>
<td>Basolateral amygdaloid nucleus</td>
<td>150</td>
<td>1.44 0.91 to 2.83</td>
</tr>
<tr>
<td>Medial amygdaloid nucleus</td>
<td>150</td>
<td>1.00 0.47 to 1.77</td>
</tr>
</tbody>
</table>

* Each value represents the mean concentration and the 95% confidence limits of three replicate assays. Potency is expressed in µg-equivalents of NIH-LH-s11/mg of fresh pituitary tissue. Combined index of precision was 0.22.

The hypothalamic LH-RF content in deermice with basolateral and cortical lesions was significantly enhanced and significantly depressed ($P<0.01$) after medial lesions (Table 2). This is based on the finding that assay rats exhibited a pituitary LH content of 1.05 µg/mg after injections of 1 ml of SME’s injection from sham-operated controls while exhibiting a value of 4.23, 0.72 and 0.46 µg/mg after injections of extracts from deermice bearing medial, basolateral and cortical lesions, respectively.

The concentration of seminal vesicle fructose increased significantly in animals with lesions in the basolateral or cortical amygdaloid areas while remaining unchanged in animals with lesions in the medial amygdaloid area or in the sham-operated controls. In addition, the dry seminal vesicle weight showed a
significant increase in animals with lesions either in the basolateral or cortical amygdaloid area but remained unchanged in animals having medial lesions or sham lesions (Table 3).

**DISCUSSION**

The results indicate that significant changes occur in animals with lesions in any of the three amygdaloid areas: medial, basolateral, cortical. These changes, however, appear to have a differential quality of direction. Thus, although the pituitary LH concentration increases significantly in animals which have basolateral or cortical lesions, it remains unchanged in animals that have medial lesions or are sham-operated. The hypothalamic LH-RF potency increases significantly in animals with basolateral or cortical lesions, and declines significantly in animals that bear medial amygdaloid lesions. Based on these data, and on previous knowledge of hypothalamic–hypophysial interrelationships, one would assume that a great deal of LH would be present in the plasma of animals with lesions in the medial amygdaloid nuclear complex, while a decline would appear in order in animals with lesions in the basolateral

**Table 3**

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Mean ± S.E.</th>
<th>Weight* (mg)</th>
<th>Concentration (µg/100 mg fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unoperated controls</td>
<td>68 ± 1.4</td>
<td>300 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>Sham operated controls</td>
<td>70 ± 1.6</td>
<td>311 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>Cortical amygdaloid nucleus</td>
<td>85 ± 2.3</td>
<td>379 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>Basolateral amygdaloid nucleus</td>
<td>81 ± 2.1</td>
<td>366 ± 6.6</td>
<td></td>
</tr>
<tr>
<td>Medial amygdaloid nucleus</td>
<td>71 ± 1.8</td>
<td>318 ± 5.8</td>
<td></td>
</tr>
</tbody>
</table>

* Empty seminal vesicle weight.
Diagrammatic and photomicrographic representation of the diencephalon of *P. m. bairdii* with location of various amygdaloid lesions: ABL = basolateral amygdaloid nucleus; AME = medial amygdaloid nucleus; ACE = central amygdaloid nucleus; ACO = cortical amygdaloid nucleus; AL = lateral amygdaloid nucleus. Arrows point to sites of lesions: from above downwards—AME, ABL, ACO.
or cortical amygdaloid areas. However, the data from the seminal vesicle weight response and fructose content of these glands do not support the latter assumption. Indeed, it appears from the data on seminal vesicles that there was an increase in LH release from the pituitaries of animals with basolateral or cortical lesions and no change in LH secretion from the pituitaries of animals with lesions in the medial amygdaloid area. The latter assumption is supported by our previous work in male deermice which has demonstrated not only an increased release of LH, but also an increased synthesis and storage in the pituitaries of male animals with lesions in the basolateral amygdaloid area, but in female deermice an increase in plasma LH and a decrease in pituitary LH (Eleftheriou & Zolovick, 1967; Eleftheriou et al., 1967; Eleftheriou, 1967). Thus, it appears that the response to amygdaloid lesions of the pituitary LH concentrations is dissimilar in the two sexes.

The general conclusion must be reached that lesions placed in the cortical or basolateral amygdaloid areas, in the male, result in a significant increase of pituitary and plasma LH concentrations while the hypothalamic factor for this hormone also increases. For medial lesions, the pituitary LH remains unchanged, but the hypothalamic LH-RF content declines significantly. How this change is brought about is not clear. A possibility, however, exists that, after the initial trauma of the lesions, there may be a tonic discharge of the hypothalamic LH-RF in animals with medial lesions, followed later by an increase in the synthesis and storage of this releasing factor, which is influenced by the presence of the lesion either electrophysiologically or neurohumorally in a manner not clear to us at this time. Conversely, in animals that bear lesions in the basolateral or cortical areas, the initial discharge is sustained by favourable conditions for the elimination of the inhibitory influence of these amygdaloid areas on the hypothalamus for the release of LH-RF, and is supplemented by increases in the synthetic rate of this factor.

The manner in which the amygdala exerts its influence on the hypothalamus for the secretion of LH is not clear. Recent data (Zolovick, 1968), however, indicate that the amygdala is not involved in the initial events leading to LH release, but that it responds only after LH is released. It also appears that the basolateral–lateral amygdaloid complex exerts an inhibitory effect on the medial nucleus, which can be removed by direct electrical stimulation of the medial amygdaloid nucleus. Since additional data (Egger, 1967) indicate that the medial and lateral areas of the amygdala are functionally distinct in their neurophysiological relationships to the hypothalamus, it is not surprising that these two distinct nuclear groups would exert a differential effect upon different areas of the hypothalamus for the release and/or synthesis of the trophic hormone-releasing factors. It is hoped that additional data now being accumulated will lead to some generalizations regarding the rôle of the amygdala in trophic hormone secretion.

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REFERENCES


