PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 242–245 (1977)

Increased Responsiveness to LH-Releasing Hormone (LHRH) in Rats with Median Eminence Lesions (39646)

G. ZEBALLOS[1] AND S. M. McCANN[2]

Department of Physiology, The University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas 75235

Lesions in the median eminence of the tuber cinereum inhibit the release and synthesis of LH as evidenced by declines in plasma and pituitary LH measured by both bio- and radioimmunoassay (1–4). The inhibition of LH release and synthesis is presumably caused by the destruction of the neurosecretory system which discharges LH-releasing hormone (LHRH) into hypophyseal portal vessels to stimulate LH release in the intact animal. Alternatively, damage to hypophyseal portal vessels produced by the lesions could reduce adenohypophyseal blood flow which could interfere with the function of gonadotropes and lead to diminished hormone release. The present studies were carried out to determine the responsiveness of the adenohypophysis to synthetic LHRH at various times after median eminence lesions in orchidectomized male rats. To minimize possible damage to the hypophyseal portal vessels, discrete radiofrequency lesions were placed bilaterally in the anterior median eminence.

Materials and methods. Adult male rats weighing approximately 250 g of the Sprague–Dawley strain (Simonsen Laboratories, Gilroy, California) were used. They were fed Purina laboratory chow and given free access to tap water. Rats were caged in group cages in a room with controlled temperature (22°) and lighting (lights on 500–1900 hr). Castrated animals were utilized 1 month after orchidectomy.

Bilateral radiofrequency lesions were placed in the median eminence using a radiofrequency lesion maker (Grass mono LM-3; intensity, 90; duration, 30 sec) as previously described (3). Stainless steel wires 0.3 mm in diameter and insulated except for the flattened tip were used. In sham-operated rats the electrode was lowered 5 mm below the dura without passage of current. One-milliliter heparinized blood samples were removed from the external jugular vein while the animals were lightly anesthetized with tribromoethanol (5). Blood was taken just prior to placement of lesions and just before and at 10 and 20 min after the injection of synthetic LHRH on the 1st, 7th, and 14th post-operative days. Synthetic LHRH was injected sc at a dose of 50 ng in 0.2 ml of physiological saline.

At 21 days postoperatively, animals were weighed, killed by decapitation, and the pituitary gland weighed. The anterior pituitary was then separated from the posterior lobe under a stereomicroscope and weighed separately. Posterior pituitary weight was calculated as the difference between whole pituitay weight and anterior lobe weight.

Plasma and anterior lobes were stored frozen until assayed. Plasma and pituitary LH were measured by radioimmunoassay according to the method of Niswender *et al.* (6) and the results were expressed in terms of the NIH LH-S-1 ovine standard.[3] Significance of differences between means was determined by Student's t test or the paired t test as appropriate.

Results. Effects of lesions on plasma LH in castrated rats. Median eminence lesions uniformly lowered plasma LH in castrated males (Table I). Plasma LH was elevated in the castrated males with intact brains by a factor of 20 above that in intact males as expected, but lesions were associated with a

[1] Fellow of the Ford Foundation. Present address: New York Medical College, Department of Neurosurgery, New York, N.Y.

[2] Supported by grants from NIH (AM 10073 and HD 05151) and the Ford Foundation.

[3] We are indebted to Dr. G. D. Niswender (Colorado State University) for the supplies of antiovine LH serum. Dr. L. Reichert (Emory University) generously provided the purified ovine LH for iodination.

ISSN 0037-9727

decline to approximately 30% of the preoperative values at 24 hr after the operation. Plasma LH remained low in these animals for the duration of the experiment. Sham-operations produced no effect on plasma LH in castrated rats.

The effect of median eminence lesions on the response to LHRH in castrated males. Responsiveness to LHRH was judged by the increment in plasma LH at 10 and 20 min following injection of the neurohormone (Table II). At 24 hr after lesions, there was a marked increase in the size of the increment in response to LHRH on comparison to that of control or sham-operated rats which was apparent at both 10 and 20 min postinjection. The absolute concentration of LH in plasma following LHRH rose from the suppressed levels in the rats with lesions to values which were higher than those found in the castrates with intact brains and already elevated plasma LH; however, the value for plasma LH concentration at 10 and 20 min after injection of LHRH was not significantly higher than that in the animals with intact brains in most experiments.

As previously reported (5), the increment in plasma LH following injection of LHRH was much greater in all castrate groups than in animals with intact testes (not shown).

By 7 days after lesions in castrates, there was a tendency for the responsiveness to LHRH to still be greater than that in sham-operated and intact castrates, but by this time, the increase was no longer significant. By 14 days after lesions, responsiveness was similar in castrate and intact animals whether or not they bore hypothalamic lesions.

Effect of median eminence lesions on body and pituitary weight, and anterior pituitary LH concentration. At sacrifice 1 week after the third LHRH response test, body weights of the animals in the various groups were similar (Table III). Median eminence le-

TABLE I. PLASMA LH IN CASTRATE MALES BEFORE AND AFTER MEDIAN EMINENCE AND SHAM LESIONS.

Type of animal	Plasma LH (ng/ml ± SEM) time postlesion			
	Initial	24 hr	7 days	14 days
Control				
Expt 1 (4)[a]	19.83 ± 6.16[b]	24.56 ± 4.54	16.50 ± 3.77	12.50 ± 2.66
Expt 2 (4)	23.50 ± 1.93	22.00 ± 1.68	20.75 ± 2.25	22.25 ± 1.31
Sham				
Expt 1 (4)	17.00 ± 1.99	21.00 ± 3.46	18.33 ± 3.20	25.26 ± 7.26
Expt 2 (4)	25.25 ± 7.79	20.00 ± 2.70	21.25 ± 3.42	32.00 ± 5.43
Median eminence lesions				
Expt 1 (8)	29.32 ± 4.17	8.24 ± 1.27*	8.30 ± 2.29*	5.15 ± 1.14*
Expt 2 (8)	27.50 ± 2.93	9.00 ± 1.30*	11.66 ± 2.06*	14.36 ± 3.68*

[a] Number of rats.
[b] Mean ± SEM.
* Significantly lower than preoperative values ($P < 0.01$).

TABLE II. INCREMENT IN PLASMA LH FOLLOWING SC LHRH IN CASTRATE RATS WITH ME LESIONS.

Type of animal	Increment in plasma LH (ng/ml ± SEM) time postinjections					
	1 day		7 days		14 days	
	10 min	20 min	10 min	20 min	10 min	20 min
Control						
Expt 1 (4)[a]	47.9 ± 10.0[b]	23.1 ± 9.3	39.3 ± 15.7	52.5 ± 27.5	75.0 ± 21.9	37.3 ± 4.8
Expt 2 (4)	34.0 ± 4.3	22.8 ± 4.3	51.0 ± 3.3	32.5 ± 1.6	68.8 ± 10.5	57.7 ± 2.5
Sham						
Expt 1 (4)	51.1 ± 4.9	51.3 ± 14.5	44.4 ± 5.0	54.7 ± 3.4	70.8 ± 15.9	62.8 ± 11.6
Expt 2 (4)	32.0 ± 4.8	10.6 ± 3.5	41.3 ± 7.1	28.3 ± 9.0	75.2 ± 5.1	42.3 ± 4.2
ME lesions						
Expt 1 (8)	78.7 ± 12.9*	71.8 ± 16.9*	73.0 ± 15.3	54.1 ± 14.3	59.7 ± 10.3	51.5 ± 8.8
Expt 2 (8)	58.5 ± 9.0*	41.8 ± 10.0*	56.7 ± 7.7	37.8 ± 6.6	75.9 ± 9.5	67.9 ± 12.6

[a] Number of rats.
[b] Mean ± SEM.
* $P < 0.05$ vs control or sham.

sions were associated with a significant reduction in anterior pituitary weight on comparison to that of the control or sham-operated animals. Posterior pituitaries of the castrated animals with lesions were also smaller than those of the two control groups; however, this change was only significant in the case of the comparison of rats with lesions versus control castrates. Anterior pituitary LH concentration in the castrates with lesions was not significantly altered in one experiment, but in one experiment it was significantly elevated on comparison to that of sham-operated animals but almost the same as that of the intact controls. For unexplained reasons, pituitary LH values were higher in each group of the second experiment than comparable values in the first experiment.

Localization of median eminence lesions. Location of lesions was determined by stereomicroscopic inspection of the ventral surface of the brain at sacrifice and in some animals was verified by histological examination of serial sections through the lesions. These lesions were similar to those previously reported (2–4) in that they destroyed the anterior portion of the median eminence but left that portion just prior to the emergence of the pituitary stalk more or less intact. Thus, there was relatively little damage to hypophyseal portal vessels.

Discussion. The median eminence lesions in the present series were clearly effective in inhibiting LH release in castrates since they induced a sustained lowering of plasma LH which was apparent on first measurement at 24 hr after lesions and persisted for the 14-day duration of the experiment. The lesions were probably not complete, as evidenced by their failure to decrease pituitary LH concentration significantly in the castrates. There was a minimal decrease in size of the anterior pituitary as previously reported after median eminence lesions (1) and also a reduction in posterior pituitary weight presumably caused by interruption of the supraopticohypophyseal tract. Larger lesions would presumably have produced a greater decline in pituitary LH content as described earlier (1).

As previously reported, the response to LHRH determined by the increment in plasma LH at 10–20 min postinjection was greater in the castrates than in intact rats (5). This response was magnified further by median eminence lesions when the test was carried out 24 hr postlesions. Responsiveness had returned nearly to normal by 7 days and was in the normal range at 14 days after lesions. Clearly, responsiveness to LHRH is not impaired by lesions which inhibit the release of LH in castrate rats, which indicates that there is no obvious impairment in gonadotroph responsiveness caused by possible damage to the hypophyseal portal vasculature. In fact, responsiveness to LHRH was actually increased at 24 hr after these lesions.

Several possibilities suggest themselves as

TABLE III. BODY AND ORGAN WEIGHTS AND PITUITARY LH CONCENTRATIONS IN CASTRATE RATS WITH ME LESIONS.

Type of animal	Body weight	Post pituitary (mg/100 g body wt)	Anterior pituitary (mg/100 g body wt)	Anterior pituitary (mg total weight)	Anterior pituitary LH concentration (μg/mg of wet tissue)
Control					
Expt 1 (4)	407.7 ± 18.0	0.72 ± 0.12	3.34 ± 0.28	15.14 ± 1.06	38.35 ± 4.49
Expt 2 (4)	414.0 ± 15.3	0.53 ± 0.11	3.55 ± 0.20	16.36 ± 0.77	89.49 ± 2.58
Sham					
Expt 1 (4)	421.2 ± 9.9	0.90 ± 0.06	2.95 ± 0.26	13.82 ± 2.62	48.57 ± 7.47
Expt 2 (4)	387.0 ± 12.6	0.79 ± 0.30	3.62 ± 0.40	15.60 ± 2.18	73.92 ± 1.67
Median eminence lesions					
Expt 1 (8)	413.1 ± 21.6	0.47 ± 0.05*	2.61 ± 0.21*	12.0 ± 0.84*	33.38 ± 6.28
Expt 2 (8)	364.5 ± 22.5	0.43 ± 0.05*	2.76 ± 0.15*	11.2 ± 0.83*	90.13 ± 0.71**

* $P < 0.05$ versus control.
** $P < 0.05$ versus sham.

the cause for the increased responsiveness to LHRH in rats with median eminence lesions in the early postlesion period. The first possibility would be that diminished release of LH in the face of continued synthesis of the hormone may cause an increase in the size of a readily releaseable pool of LH which is then discharged following the injection of LHRH.

A second possibility is that in the absence of endogenous LHRH following lesions, the number of unoccupied LHRH receptors on the surface of the gonadotrophs is increased. Following the presentation of exogenous LHRH, there is an increased combination of the neurohormone with these receptors which leads to a greater discharge of LH.

The third possibility would be that following the loss of endogenous LHRH, the number of LHRH receptors on the cell surface increases. Following the injection of LHRH, an increased number of receptors combine with the neurohormone leading to increased LH release. The data provided in this paper cannot distinguish between these three possibilities to explain the increased responsiveness to LHRH at 24 hr postlesion.

The decline in responsiveness back to that observed in the sham-operated and control animals at 7 and 14 days after lesions may be related to a gradual suppression of LH synthesis in the presence of a chronic relative absence of LHRH. This would lead to a diminished releaseable pool of LH in the gland and thereby lead to releases of LH approximately the same as in animals with intact brains. Plasma LH was uniformly lowered after lesions and did not decline further as responsiveness to LHRH declined. This may be explained by increasing release of LHRH with partial recovery from the lesions which, when coupled with lessened responsiveness to LHRH, resulted in maintenance of subnormal plasma LH.

It should be pointed out that there are several examples of an apparent hypersensitivity to hormones following removal of the endogenous hormone. For example, following hypophysectomy, there is an immediate increase in responsiveness of the adrenal cortex to ACTH which is followed by diminished responsiveness later on (7).

Summary. Responsiveness to synthetic LHRH, as measured by increments in plasma LH following its sc injection, was evaluated in castrated rats with median eminence lesions and compared to that in sham-operated controls and controls with intact brains. Median eminence lesions in castrates were followed by a decline in plasma LH to approximately 30% of control values at 1, 7, and 14 days postlesions. This was associated with an increase in responsiveness to LHRH at 1 day, followed by a return to normal responsiveness at 7 and 14 days postlesions. Even though plasma LH was lowered in the animals with lesions, pituitary LH was little changed, which indicates that these lesions, although effective to inhibit LH release, were probably incomplete.

It is concluded that LH release is inhibited by lesions in the median eminence in the face of normal or even increased responsiveness to LHRH. The cause of the increased responsiveness to LHRH which appeared at 1 day after lesions remains to be elucidated.

1. Taleisnik, S., and McCann, S. M., Endocrinology **68,** 263 (1961).
2. Bishop, W., Krulich, L., Fawcett, C. P., and McCann, S. M., Proc. Soc. Exp. Biol. Med. **136,** 925 (1971).
3. Bishop, W., Kalra, P. S., Fawcett, C. P., Krulich, L., and McCann, S. M., Endocrinology **91,** 1404 (1972).
4. Bishop, W., Fawcett, C. P., Krulich, L., and McCann, S. M., Endocrinology **91,** 643 (1972).
5. Zeballos, G., and McCann, S. M., Proc. Soc. Exp. Biol. Med. **145,** 415 (1974).
6. Niswender, G. D., Midgley, A. R., Monroe, S. E., and Reichert, L. E., Proc. Soc. Exp. Biol. Med. **128,** 807 (1968).
7. Sayers, G., and Beall, R. J., Science **179,** 1330 (1973).

Received July 12, 1976. P.S.E.B.M. 1977, Vol. 154.