PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 168, 38–44 (1981)

Effects of Hypothalamic Arcuate Nucleus Lesions on Pulsatile Luteinizing Hormone Concentration in Ovariectomized Rats[1,2] (41231)

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Abstract. We investigated the effects of hypothalamic arcuate nuclei destruction on the postovariectomy rise in plasma luteinizing hormone (LH) concentration and the pulsatile LH release mechanism in ovariectomized rats. Rats were injected with 1, 2, or 4 mg/g body wt of L-monosodium glutamate (MSG) on Days 1, 3, 5, 7, and 9 of life to lesion the arcuate nuclei. They were then ovariectomized as adults and used 2 or 4–8 weeks later. Serial blood sampling at 10-min intervals and measurement of plasma LH concentration revealed elevated plasma LH levels which fluctuated in a pulsatile fashion in all control and 1 and 2 mg/g MSG-treated rats. In 4 mg/g MSG-treated rats, plasma LH levels were lower than in controls at both time periods after ovariectomy due to a decrease in the amplitude and/or frequency of LH pulses. Phenobarbital was administered to all 4- to 8-week ovariectomized rats to block endogenous pulsatile LH release. In phenobarbital-blocked rats, three sequential iv injections of LH releasing hormone (LHRH) caused substantial elevations in plasma LH concentrations even in the 4 mg/g MSG-treated animals which had low plasma LH concentrations during control bleedings prior to the injection of phenobarbital. The results indicate that the postovariectomy rise in plasma LH concentration and the associated pulsatile LH release mechanism are functional in rats with extensive arcuate nucleus lesions. The diminution in the rise in plasma LH levels and the decreased amplitude and/or frequency of LH pulses in the MSG-treated rats is likely due to a diminution in hypothalamic LHRH release.

In the rat, plasma luteinizing hormone (LH) concentrations rise after ovariectomy and fluctuate in a pulsatile fashion (1) within an elevated range throughout the 24-hr day (2, 3). These responses can persist after anterior or complete surgical deafferentation of the medial basal hypothalamus from the rest of the brain (4–7). It was suggested that the hypothalamic arcuate nucleus may be involved in pulsatile LH release since anterior hypothalamic cuts which lesioned the anterior portion of the arcuate nuclei suppressed pulsatile LH release (4). However, bilateral electrolytic lesions of the arcuate nuclei were without effect unless they were combined with anterior hypothalamic deafferentation (7). This observation led to the conclusion that two separate neural pathways are involved in pulsatile LH release, one involving the arcuate nucleus and the other extrahypothalamic which enters the hypothalamus from an anterior direction (7).

Injection of L-monosodium glutamate (MSG) to neonatal rodents has been reported to destroy selectively neuronal cell bodies restricted to the arcuate nucleus and the inner layer of the retina (8–11). The extent of MSG-induced lesions is related to the dose of MSG injected and to the age of the animals when treated (12–14). In the present study, we used MSG to lesion the arcuate nuclei and have reevaluated the role of the arcuate nucleus in the pulsatile LH release mechanism.

Materials and Methods. We purchased Sprague–Dawley rats from Simonsen Laboratories, Inc. (Gilroy, Calif.), kept them in a temperature-controlled room (24–27°) with automatically controlled lighting (lights on 0500 to 1900 hr daily), and gave them free access to Wayne Laboratory Rat Chow and water. The female rats

[1] This work was supported by a grant from the NIH (HD 11011).

[2] A portion of this study has been presented in abstract form: R. Sridaran, and C. A. Blake, Physiologist 21, 114 (1978).

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0037-9727/81/090038-07$01.00/0

were mated with the males, and on the day of birth (Day 0) each litter was reduced to seven pups. Some female pups were injected with 1, 2, or 4 mg of MSG/g body wt on Days, 1, 3, 5, 7, and 9 of life while control females were untreated. The female pups were weaned on Day 21 and housed two per cage in the vivarium. At 2–4 months of age rats were bilaterally ovariectomized under brief ether anesthesia and then returned to their cages.

In the first series of experiments, control and MSG (4 mg/g)-treated rats were used at 2 weeks after ovariectomy. We inserted a cannula into the right atrium of the heart under brief ether anesthesia as described by Terkel (15). Starting about 4 hr later, we collected blood samples (0.15–0.20 ml) into heparinized tubes at 10-min intervals for 60–90 min and injected an equal volume of heparinized saline (10 units/ml) after each bleeding.

In the second series of experiments, control and MSG (1, 2, and 4 mg/g)-treated rats were used at 4–8 weeks after ovariectomy. We inserted cannulas and bled the rats as described above for a total of eight collections. After the eighth blood collection, we injected rats with sodium phenobarbital (75 mg/kg, ip). This procedure inhibits pulsatile LH release in ovariectomized rats and does not interfere with LH releasing hormone (LHRH)-induced LH release (16). We collected a blood sample at 90 min after phenobarbital and injected 10 ng of LHRH (Beckman Lot No. C0212) in 0.1 ml saline through the cannula. Additional blood samples were collected at 10, 20, and 30 min after LHRH. We then repeated the injection of LHRH and the 30 min of blood collections two more times.

Plasma was stored at −20° until assayed for LH as described previously (17). All samples taken from any individual rat were assayed on 2 or more vol of plasma in a single assay. The volume of plasma which caused nearest 40% displacement of the iodinated LH from the anti-ovine LH sera was used to calculate LH concentration. The intra- and interassay coefficients of variation were less than 10%. Values are expressed as nanograms per milliliter plasma in terms of NIAMDD-rat LH-RP-1 which has a biological potency equivalent to 0.03 × NIH-LH-S1.

Postovariectomy plasma LH concentrations prior to phenobarbital injection were compared in two ways. First, all plasma LH values for an individual rat were averaged to obtain a single mean value for each rat. These mean values for all rats in a group were then used to test for statistical significance between groups (comparison of average levels). Second, we used only the highest plasma LH value measured for each individual rat. These LH values for all rats in a group were then used to test for statistical significance between groups (comparison of highest levels). Values obtained for the two groups in the first series of experiments were analyzed by Student's t test while those obtained for the four groups in the second series were analyzed by analysis of variance followed by Newman–Keuls tests to test for significance. A P value < 0.05 was considered statistically significant.

Results. We have previously reported that our protocol for treatment with 4 mg/g MSG causes extensive lesions in the arcuate nucleus-median eminence region in the strain of rat we used (18). Whereas others (11) have found that MSG can cause lesions to extend anteriorly to the preoptic area and posteriorly to the interpeduncular nucleus, we did not observe such extensive damage in our rats (18).

Plasma LH concentrations in nine individual control rats ovariectomized for 2 weeks are plotted in Fig. 1. At all times in all rats the plasma LH concentration was raised above levels measured in eight cannulated cyclic rats on the morning of estrus (range 24–42 ng/ml; not illustrated). The plasma LH concentration fluctuated in individual rats, showing two to three episodic increases (arbitrarily defined as a >10% fall followed in 10 min by a >10% rise) during the 60–90 min of bleeding.

Rats in the 4 mg/g MSG group which were ovariectomized for 2 weeks had plasma LH concentrations (average and highest values) significantly lower than those of the controls (cf. Figs. 2 and 1, re-

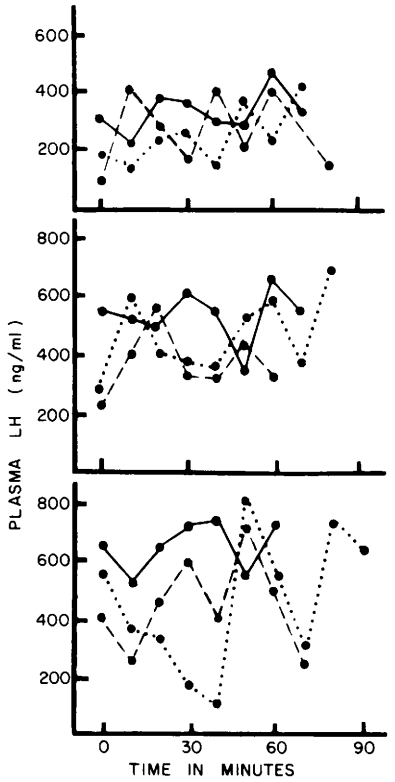

FIG. 1. Plasma LH concentrations are plotted at 10-min intervals for nine serially bled adult control rats ovariectomized for 2 weeks.

spectively). In four of nine rats the plasma LH concentration was not different from that observed in cyclic rats on estrus and episodic increases in plasma LH levels were not seen. In the other five rats, two showed one, two showed two, and one showed three episodic increases in plasma LH concentration during the 60- to 90-min bleeding period. In three of the five rats showing pulsatile plasma LH levels, the plasma LH concentration at times reached levels which were in the range of values observed in the controls. However, the magnitude of the rises in the five rats displaying pulsatile LH levels was generally less than that observed in controls.

Plasma LH concentrations in individual control rats and in animals in the 1, 2, and 4 mg/g MSG groups which were ovariectomized for 4–8 weeks are shown in Figs. 3 and 4. We observed two to three episodic increases in plasma LH concentration during the first 70 min of bleeding in all control rats and in all rats in the 1 and 2 mg/g MSG groups. In the 4 mg/g MSG group, three rats had one, two had two, and one had four episodic increases. In some but not all of the rats in the 4 mg/g MSG group, the magnitude of the pulses was clearly less than that observed in the other three groups. These factors resulted in rats in the 4 mg/g MSG group having plasma LH concentrations (average and highest values) significantly lower than those of the rats in the other three groups (cf. Figs. 4 and 3, respectively). The plasma LH concentration increased between 2 and 4–8 weeks postovariectomy in control rats (average and highest values; cf. Figs. 1 and 3) and in animals in the 4 mg/g MSG group (average values only; cf. Figs. 2 and 4).

Plasma LH concentrations were lower at 90 min after the injection of phenobarbital than that observed just prior to injection in all but four rats (Figs. 3 and 4). These four rats were given the high dose of MSG neonatally and their plasma LH levels were too low just prior to and 90 min after injection of phenobarbital to ascertain if the barbiturate had any effect.

Rats in the 4 mg/g MSG group which had low plasma LH concentrations and a reduced magnitude of LH pulses prior to the injection of phenobarbital responded to LHRH as well as did rats in the other groups (Fig. 3). There were no differences in the magnitudes of the LH responses at 10 min after the first or second injection of LHRH between any of the four groups. In fact, the plasma LH concentration in rats in the 4 mg/g MSG group was elevated ($P < 0.05$) above that in control rats at 30 min after the second injection of LHRH. This may account, at least in part, for the delayed LH response to the third injection of LHRH in four of six rats in the 4 mg/g MSG group; i.e., LH concentration declined before it rose.

Discussion. We conclude from the results of this study that the mechanisms responsible for a postovariectomy rise in plasma LH concentration and pulsatile LH

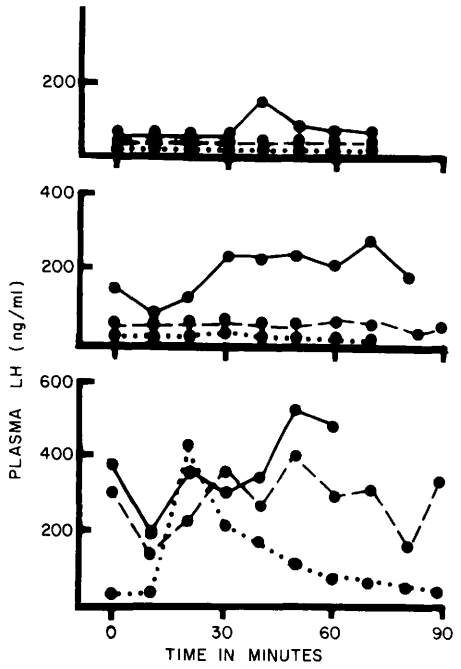

FIG. 2. Plasma LH concentrations are plotted at 10-min intervals for nine serially bled adult rats ovariectomized for 2 weeks and treated with 4 mg/g L-monosodium glutamate (MSG) as neonates.

release are functional in rats with extensive arcuate nuclei lesions. However, treatment with MSG resulted in a subnormal post-ovariectomy rise in plasma LH levels and abnormal pulsatile LH release.

Mean serum LH concentrations rise for a period of at least 90 days after ovariectomy of adult rats (19). In the present study, the plasma LH concentration rose between 2 weeks and a 4- to 8-week period after ovariectomy in both control rats and rats in the 4 mg/g MSG group. However, the MSG treatment interfered with ovarian negative feedback as evidenced by lower plasma LH levels in the MSG-treated rats than in controls at both time periods after ovariectomy. Others have previously reported mean serum LH concentrations to be suppressed in rats treated with MSG as neonates and ovariectomized for 10 days or 3 months as adults (20). The lower plasma LH levels in the MSG-treated rats are due to impairment to the pulsatile LH release mechanism.

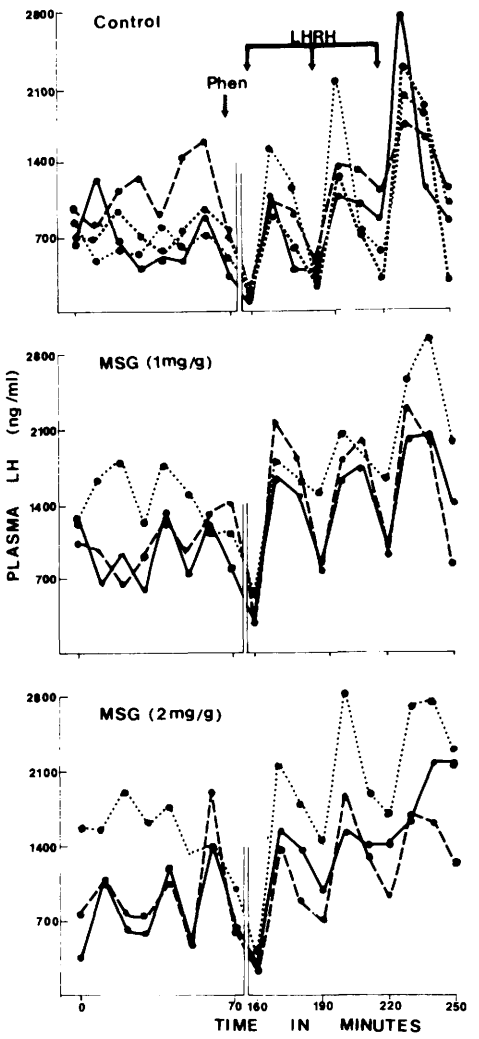

FIG. 3. Plasma LH concentrations are plotted for 10 serially bled adult rats ovariectomized for 4–8 weeks. Six of the rats were treated with 1 or 2 mg/g L-monosodium glutamate (MSG) as neonates. All rats were administered sodium phenobarbital (Phen; 75 mg/kg body wt, ip) and three sequential iv injections of LHRH (10 ng) at 30-min intervals. Note the break on the time scale.

Pulsatile increases in plasma LH concentration were observed in five of nine MSG (4 mg/g)-treated rats bled at 2 weeks after ovariectomy and in a 6/6 MSG (4 mg/g)-treated rats bled at 4–8 weeks after ovariectomy. However, the magnitude and/or frequency of the pulses were less than in controls, contributing overall to lower plasma LH concentrations.

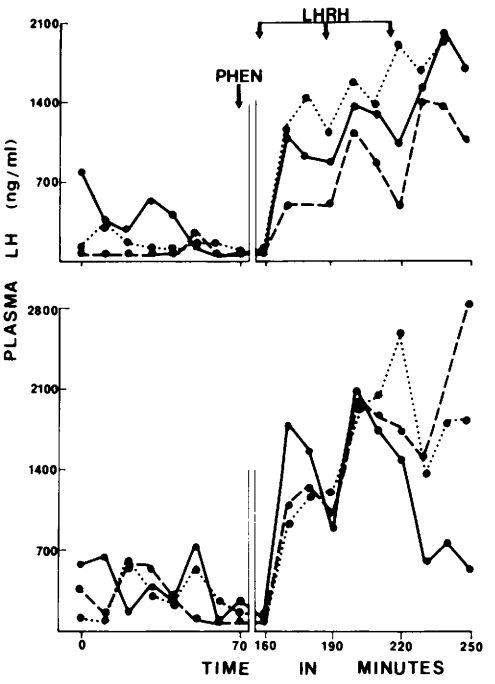

FIG. 4. Plasma LH concentrations are plotted for six serially bled adult rats ovariectomized for 4–8 weeks. All rats were treated with 4 mg/g L-monosodium glutamate (MSG) as neonates. See Fig. 3 for details.

The suppressions in plasma LH concentrations in MSG-treated rats are likely due to reduction in hypothalamic LHRH release rather than to a reduced responsiveness of the anterior pituitary gland to LHRH. It was possible to restore pulses in plasma LH concentration which resulted in markedly elevated plasma LH levels in phenobarbital-blocked rats by sequential injections of LHRH in all groups including rats in the 4 mg/g group which had low plasma LH concentrations and small pulsatile increases in plasma LH levels prior to the injection of phenobarbital.

It is not clear how MSG acts within the brain to suppress LH release. There is considerable evidence to indicate that LHRH is released in a pulsatile fashion to cause pulsatile LH release (21–23). Recent studies have shown that the majority of LHRH cell bodies in the rat are located in the rostral preoptic–hypothalamic area (24–27), but a smaller number of cell bodies are found more caudally in the medial forebrain bundle and medial basal hypothalamus (26, 27). These cell bodies are best visualized in a horizontal plane where they form an inverted U-shaped configuration running from an area close to the midline near the organum vasculosum of the lamina terminalis through the medial forebrain bundle to the caudal–basal hypothalamus lateral to the arcuate nuclei (26, 27). The LHRH cell bodies send axons to the median eminence via two major pathways (24–27). One pathway runs caudally in the periventricular region. The other pathway runs caudally through the lateral hypothalamus and then medially to the median eminence. The MSG likely does not lesion LHRH cell bodies or their axons. We observed no evidence for destruction of cell bodies outside of the arcuate nucleus (18) and others have shown MSG to have no effect on the LHRH content within the basal hypothalamus (20, 28).

One can conceive either that the more rostral LHRH cell bodies are not involved in pulsatile LH release or that only a portion of the LHRH neurons which innervate the median eminence are needed to elicit normal pulsatile LH release. Complete deafferentation of the medial basal hypothalamus in ovariectomized rats with knives whose radii (1.4 mm) were sufficiently great to include LHRH cell bodies in the lateral or basal hypothalamus (<1.4 mm lateral; (26, 27) within the area of the cut was often associated with normal or only slightly suppressed pulsatile LH release (4). Complete hypothalamic cuts made similarly in other rats have shown the LHRH content in the basal hypothalamus to decrease by 75% (29). When frontal cuts were placed more caudal to transect the anterior portion of the arcuate nucleus, the magnitude of LH pulses and the plasma LH levels were reduced (4). These suppressions were likely due to destruction of more LHRH neurons than that which occurred with more rostrally placed frontal cuts rather than to destruction of the arcuate nuclei per se as electrolytic lesions of the arcuate nuclei did not alter pulsatile LH release (7). Although we cannot completely rule out the possibility that MSG suppressed pulsatile LH release by lesioning arcuate nuclei cell bodies which

might exert a stimulatory influence on LHRH release, we consider this unlikely since electrolytic lesions of the arcuate nuclei did not suppress the plasma LH rhythm (7).

It is possible that MSG acted on monaminergic or LHRH neuron terminals in the median eminence to suppress plasma LH levels. Monamines have been shown to alter pulsatile LH release (6). However, we consider such effects to be modulatory to pulsatile LH release rather than the cause of pulsatile LH release. Epinephrine, norepinephrine, and serotonin-containing neurons which innervate the basal hypothalamus are located in the midbrain and brainstem (30–32) and their denervation (33, 34) does not interfere with pulsatile LH release (4–7). Dopamine also does not appear necessary to maintain pulsatile LH release. Studies utilizing dopaminergic agonists or antagonists have indicated that dopamine inhibits rather than stimulates pulsatile LH release (35–37), and electrolytic destruction of the arcuate nuclei and presumably destruction of the tuberoinfundibular dopamine pathway had no effect on pulsatile LH release (7). Moreover, treatment with MSG leaves the arcuate nuclei virtually devoid of dopamine perikarya (38) but pulsatile LH release persists. However, these data do not rule ou the possibility that MSG may have acted within the median eminence to interfere with LHRH release by exerting an effect either on the LHRH terminals themselves or on monaminergic terminals that can exert modulatory effects on the LHRH release process.

In the absence of any convincing evidence that would explain how MSG acts within the brain to interfere with LHRH release, we suggest that MSG suppression of pulsatile LH release may be more likely be a reflection of the general state of the animal than to a direct effect of MSG on neurons controlling the release of LHRH. The endocrinopathies present in MSG-treated rats (28) may simply result in a slowing down of the intact LHRH release process.

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Received February 6, 1981. P.S.E.B.M. 1981, Vol. 168.