Journal of Reproduction and Development, Vol. 52, No. 6, 2006

—Full Paper—

Acute Lipoprivation Suppresses Pulsatile Luteinizing Hormone Secretion without Affecting Food Intake in Female Rats

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Abstract. The present study examined the effect of acute lipoprivation on pulsatile luteinizing hormone (LH) secretion in both normal-fat diet, *ad libitum*-fed and fasted female rats. To produce an acute lipoprivic condition, mercaptoacetate (MA), an inhibitor of fatty acid oxidation, was administered intraperitoneally to *ad libitum*-fed or 24-h fasted ovariectomized (OVX) rats with or without an estradiol (E2) implant, that produces a negative feedback effect on LH pulses. The steroid treatment was performed to determine the effect of estrogen on lipoprivic changes in LH release, because estrogen enhances fasting- or glucoprivation-induced suppression of LH pulses. Pulsatile LH secretion was suppressed by MA administration in a dose-dependent manner in the *ad libitum*-fed OVX and OVX+E2 rats. LH pulses were more severely suppressed in the 24-h-fasted OVX and OVX+E2 rats compared to the *ad libitum*-fed rats. Estrogen slightly enhanced lipoprivic suppression but the effect was not significant. In the present study, increased plasma glucose and free-fatty acid concentrations may indicate a blockade of fatty acid metabolism by the MA treatment, but food intake was not affected by any of the MA doses. Acute vagotomy did not block lipoprivic suppression of LH pulses. Thus, the present study indicates that lipid metabolism is important for maintenance of normal reproductive function even in rats fed a normal-fat diet and lipoprivation may be more critical in fasted animals that probably rely more heavily on fatty acid oxidation to maintain appropriate metabolic fuel levels. In addition, failure of blockade of lipoprivic LH inhibition by vagotomy suggests that lipoprivic information resulting in LH suppression is not transmitted to the brain via the vagus nerve.

Key words: Fatty acids, Mercaptoacetate, Gonadotropin, Vagotomy

(**J. Reprod. Dev. 52:** 763–772, 2006)

Energy availability has been considered to be a critical factor maintaining gonadal functions [1] at various reproductive stages in mammalian species, not only in the adult animal [2], but also at the onset of puberty [3, 4] and during lactation [5]. Experimentally, food restriction [3, 6] or acute food deprivation [7] inhibits pulsatile luteinizing hormone (LH) secretion in rats through central mechanisms [8] and causes suppression of gonadal function. Pulsatile LH release is sensitive to the

Accepted for publication: August 17, 2006
Published online: September 29, 2006
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availability of oxidizable fuels, such as glucose [2], because pharmacological glucoprivation and insulin-induced hypoglycemia are well established to inhibit LH release and then gonadal activity [9, 10]. Glucose availability can be monitored by specific sensor(s) in the brain [11–13], including the hindbrain, to induce feeding [14] and suppress LH release [15].

Pharmacological blockade of the oxidation of fatty acids, another major metabolic fuel, has been known to eliminate estrous cyclicity and suppress sexual behavior in hamsters [1, 2] when lipoprivation is combined with glucoprivation. In addition, lipoprivation alone suppresses estrous cycles when hamsters are fasted or fed a high-fat diet [1]. Taken together, fatty acids may provide an additional metabolic signal for control of reproductive function, but in the hamster at least, fatty acid metabolism only becomes an important regulator of reproductive function during situations when fatty acid metabolism is favored. However, little is known about the importance of fatty acid metabolism for maintenance of the reproductive function of animals kept on a normal fat diet.

In the present study, we tested whether lipoprivation blocks pulsatile LH secretion in female rats maintained on a normal-fat diet. The ovariectomized (OVX) and estradiol-primed OVX (OVX+E2) rat models were used to examine the effect of estrogen during MA-induced suppression of LH pulses because glucoprivic suppression of LH pulses is enhanced in the presence of estrogen [9]. Since the rate of fatty acid oxidation increases and energy metabolism is shifted from glucose utilization to fatty acid utilization during fasting [16], the effect of lipoprivation on LH secretion was also examined in acutely fasted rats. Lipoprivation inhibited pulsatile LH secretion in these first two experiments. Therefore, the third experiment investigated whether vagal afferents, which are critical for the feeding response of rats to lipoprivation when fed a fat-enriched diet [17], mediate the lipoprivic signal to the central mechanisms regulating pulsatile LH secretion.

Materials and Methods

Animals and treatments

Adult female Wistar-Imamichi strain rats (Imamichi Institute for Animal Reproduction, Ibaraki, Japan) weighing 189–273 g were housed under constant conditions for photoperiod and temperature (14L:10D; lights on 05:00 h; temperature 24 ± 2 C) and fed pelleted food containing 25.4% crude protein, 4.4% fat, and 50.3% soluble non-nitrogenous matter (CE-2; Clea Japan, Inc., Tokyo, Japan) *ad libitum* or as mentioned otherwise. Water was available throughout the experiment. All surgical procedures were performed under ether anesthesia and aseptic conditions. The present study was approved by the Committee on Animal Experiments of the Graduate School of Bioagricultural Sciences, Nagoya University.

Animals having shown at least two 4-day consecutive estrous cycles were bilaterally ovariectomized two weeks before blood sampling, some of which served as the OVX group with no ovarian steroid influence. Some OVX animals were immediately implanted subcutaneously with a Silastic tubing (id, 1.5 mm; od, 3.0 mm; length 25.0 mm; Dow Corning, Midland, MI, USA) containing E2 (Sigma Chemical Co., St. Louis, MO, USA) dissolved in peanut oil at 20 μg/ml. The E2 implant has been shown to produce the plasma E2 levels found at diestrus 2. This estrogen treatment was chosen because it caused a significant suppression of LH pulses when animals were subjected to 48-h fasting [18] and enhanced glucoprivic suppression of LH pulses [9].

Blood samples (100 μl) were obtained from free-moving conscious rats every 6 min for 3 h through an indwelling atrial cannula (silicon tubing: id, 0.5 mm; od, 1.0 mm; Shin-Etsu Polymer Co., Tokyo, Japan) that had been attached on the day before blood sampling. Each blood sample was replaced with an equivalent volume of washed red blood cells obtained from other individuals to keep the hematocrit constant. During the 3-h sampling period, the animals were provided powdered food for ease of monitoring food consumption. Plasma samples were obtained by immediate centrifugation and were stored at –20 C until assayed for LH in all experiments and for metabolic fuels in Experiment 1 [3-hydroxybutyrate (3HB) and free fatty acids (FFA)]. Plasma glucose levels were assayed immediately after blood sampling in Experiment 1. Food intake and general behavior were monitored during blood sampling in all experiments.

Experimental protocols

Experiment 1: OVX and OVX+E2 rats received a single i.p. injection of sodium 2-mercaptoacetate (MA; Sigma) after collection of the first blood sample. The drug, which blocks β-oxidation of fatty acids by inhibiting mitochondrial acyl-CoA dehydrogenase [19, 20], was freshly prepared in ultra-pure water and injected at doses of 400 or 800 μmol/kg BW. These doses were chosen because MA at doses of 400 to 800 μmol/kg induces food intake in a dose-dependent manner during the light phase in rats maintained on a fat-supplemented diet [14]. Control animals received 0.2 M saline (4 ml/kg BW), which is equimolar to 800 μmol/kg BW of MA. Additional blood (50 μl) was collected at 30-min intervals to measure the levels of glucose, FFA, and 3HB.

Experiment 2: OVX and OVX+E2 rats were deprived of food for 24 h and then received a single i.p. injection of MA immediately after the onset of blood sampling. Since we expected that fasted animals would have a greater sensitivity to lipoprivation, these animals received lower doses of MA (200 or 400 μmol/kg BW). Control animals received 0.2 M saline (4 ml/kg BW).

Experiment 3: To assess the involvement of vagally-mediated signals in lipoprivic LH suppression, subdiaphragmatic dorsal and ventral branches of the vagus nerve were secured with polyethylene threads at the time of OVX+E2 surgery for acute sectioning in free-moving rats during blood sampling using a procedure described previously [21]. The vagal trunks were visualized with the aid of an operating microscope, and the threads were loosely placed around the vagal nerve to form snares. The other ends of the threads were kept subcutaneous at the back of the animals until the day before blood sampling. The polyethelene threads securing the vagal trunks were exteriorized at the back of all animals 24-h before sampling at the time of atrial cannulation. All animals, including sham-vagotomized rats, showed some weight loss after the vagal surgery and were therefore given 5% glucose in drinking water for 1 week to assist their recovery. The animals regained the weight lost within two weeks after the vagus surgery. Acute vagotomy was accomplished just after the first blood sampling by rapidly pulling the threads in the conscious rats. In the sham-vagotomized animals, the threads were not pulled so that the vagal nerve remained intact. The animals received an intraperitoneal injection of MA (800 μmol/kg BW) or saline (0.2 M, 4 ml/kg BW) immediately after vagotomy. All vagotomized animals were sacrificed and completeness of the sectioning of the vagal trunks was confirmed visually at the end of the sampling.

Assays

Plasma LH concentrations were determined with an RIA kit provided by the National Hormone and Pituitary Program (Baltimore, MD, USA). The concentrations were expressed in terms of NIDDK rat LH RP-3. The least detectable concentration of LH in a 50-μl plasma sample was 0.156 ng/ml. The intra- and inter-assay CVs were 6.2% and 12.3%, respectively, at 0.74 ng/ml.

Plasma concentrations of glucose (Glucose B-Test, glucose oxidase method, Wako, Osaka, Japan), 3HB (3-hydroxybutyrate dehydrogenase procedure, Sigma), and FFA (NEFA-C Test, Wako) were determined by commercial kits in samples obtained at 30-min intervals during the 3-h period after MA administration in the *ad libitum*-fed OVX and OVX+E2 rats.

Data analyses

LH pulses were identified with the PULSAR computer program [22] as described previously [23]. Mean LH concentrations, frequency and amplitude of LH pulses, food intake and plasma fuel concentrations were compared between treatments by one-way ANOVA followed by the Bonferroni multiple comparison test.

Results

Effect of lipoprivation on LH pulses in ad libitum-fed rats

Plasma LH concentrations were rapidly suppressed after injection of MA at both 400 and 800 μmol/kg BW in *ad libitum*-fed animals (Fig. 1A). Mean LH concentrations and LH pulse frequency were significantly ($P<0.05$, Bonferroni multiple comparison test) suppressed in both OVX and OVX+E2 rats by 800 μmol/kg MA but not by the lower dose (400μmol/kg) (Fig. 1B). LH pulse amplitude was not significantly ($P>0.05$) affected by any dose of MA in OVX or OVX+E2 rats.

The animals did not exhibit any apparent changes in their behaviors and were conscious and

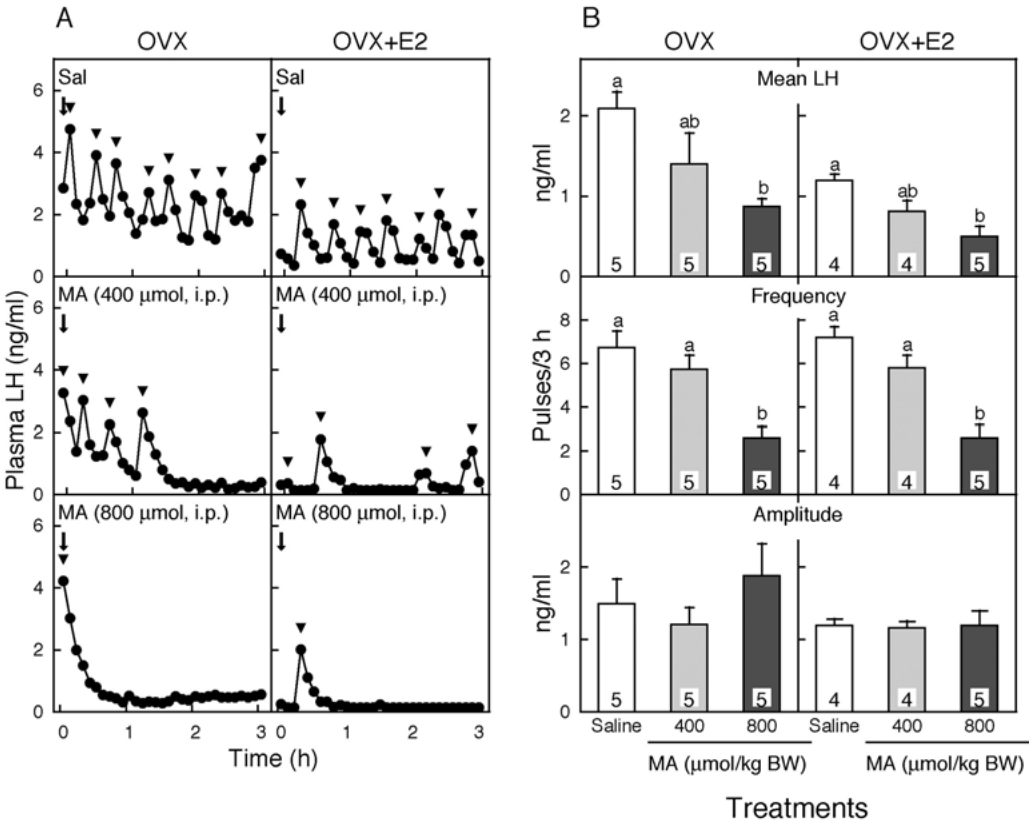

Fig. 1. A, Representative plasma LH profiles in OVX and OVX+E2 *ad libitum*-fed rats injected with saline (0.2 M, 4 ml/kg BW) or mercaptoacetate (MA) at 400 or 800 μmol/kg BW. Drugs were injected immediately after the onset of blood sampling (arrows). Arrowheads indicate the peaks of LH pulses identified by the Pulsar computer program. B, Mean plasma LH concentrations for 3 h and the frequency and amplitude of LH pulses in OVX and OVX+E2 rats injected with MA. Values are means ± SEM. Values with different letters are significantly (P<0.05) different from each other. The numbers in each column represent the number of animals used.

healthy after MA injection. MA administration had no significant (P>0.05) effect on the food intake of the OVX or OVX+E2 rats during the 3-h sampling period (Table 1).

Effect of lipoprivation on LH pulses in 24-h fasted rats

Twenty-four-h fasting did not have an apparent effect on the plasma LH concentrations of vehicle-treated animals (Fig. 2A). Administration of MA decreased plasma LH levels after 24-h of fasting at 400 μmol/kg, but the decrease was not apparent with the lower dose (200 μmol /kg).

In both OVX and OVX+E2 rats, the 400 μmol/kg dose of MA significantly (P<0.05) suppressed LH pulse frequency. The lower dose (200 μmol/kg) had no significant (P>0.05) effect on any of the parameters of LH pulses in either OVX or OVX+E2 rats (Fig. 2B).

MA administration did not cause behavioral changes and had no significant effect (P>0.05) on food consumption during the experimental period in the 24-h fasted OVX and OVX+E2 rats (Table 1).

Effect of acute vagotomy on lipoprivic LH suppression in ad libitum-fed rats

Acute vagotomy had no apparent effect on MA-induced suppression of LH pulses in OVX+E2 rats (Fig. 3A). In the vagotomized animals, MA suppressed LH pulses while saline treatment had no apparent effect. MA also suppressed LH pulses in the sham-vagotomized rats.

LH pulse frequency was significantly (P<0.05)

Table 1. Food intake in animals injected with vehicle or MA with various treatments

Ad libitum-fed rats (g/3 h)

	Vehicle	400 μmol MA	800 μmol MA
OVX (n=5)	0.52 ± 0.23	0.10 ± 0.06	0.52 ± 0.45
OVX+E2 (n=5)	1.30 ± 0.61	0.24 ± 0.17	0.78 ± 0.15
24-h-fasted rats (g/3 h)			
	Vehicle	200 μmol MA	400 μmol MA
OVX (n=5)	2.25 ± 0.57	2.02 ± 0.29	1.20 ± 0.11
OVX+E2 (n=5)	3.64 ± 1.04	3.16 ± 0.55	2.60 ± 0.83
Vagotomized rats with MA (g/3 h)			
	Vag+Sal	Vag+MA	Sham+MA
OVX+E2 (n=7)	0.40 ± 0.21	0.03 ± 0.03	0.38 ± 0.18

Values are means ± SEM. MA, mercaptoacetate; OVX, ovariectomized; E2, estradiol; Vag, vagotomized; Sal, saline; Sham, sham-vagotomized.

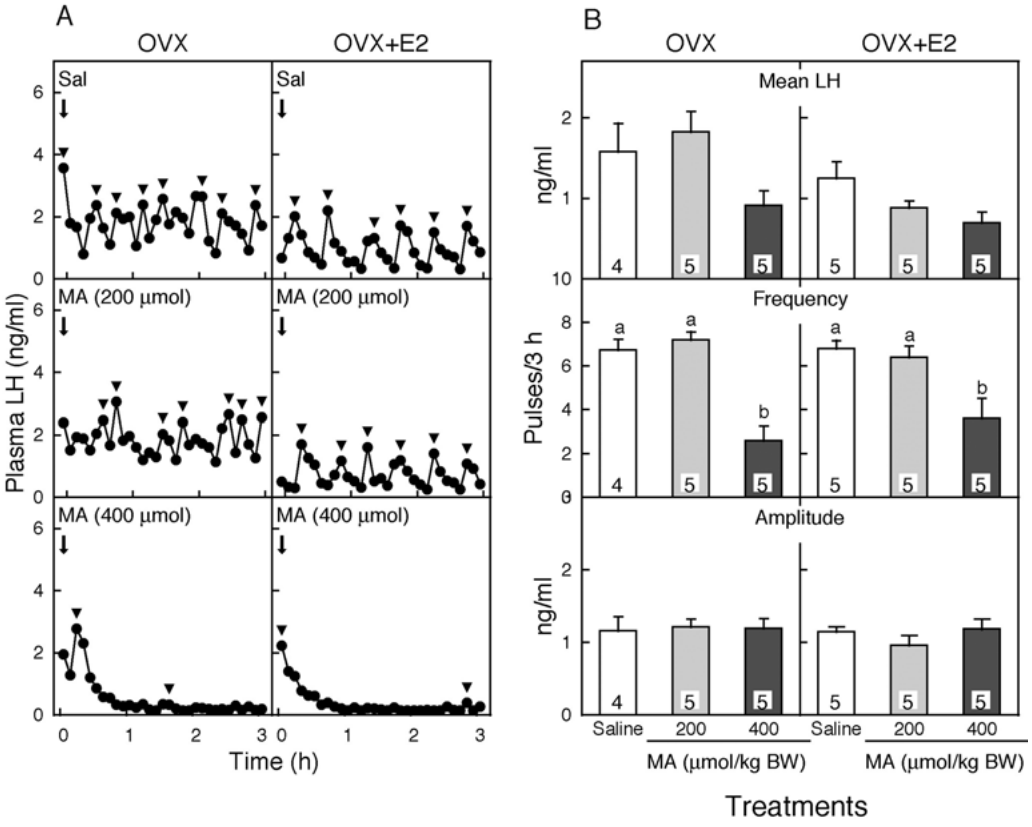

Fig. 2. A, Representative plasma LH profiles in 24-h-fasted OVX or OVX+E2 rats injected with saline (0.2 M, 4 ml/kg BW) or mercaptoacetate (MA) at 200 or 400 μmol/kg BW. Drugs were injected immediately after the onset of blood sampling (arrows). Arrowheads indicate the peaks of LH pulses identified by the Pulsar computer program. B, Mean plasma LH concentrations for 3 h and the frequency and amplitude of LH pulses in OVX and OVX+E2 rats injected with MA. Values are means ± SEM. Values with different letters are significantly ($P<0.05$) different from each other. The numbers in each column represent the number of animals used.

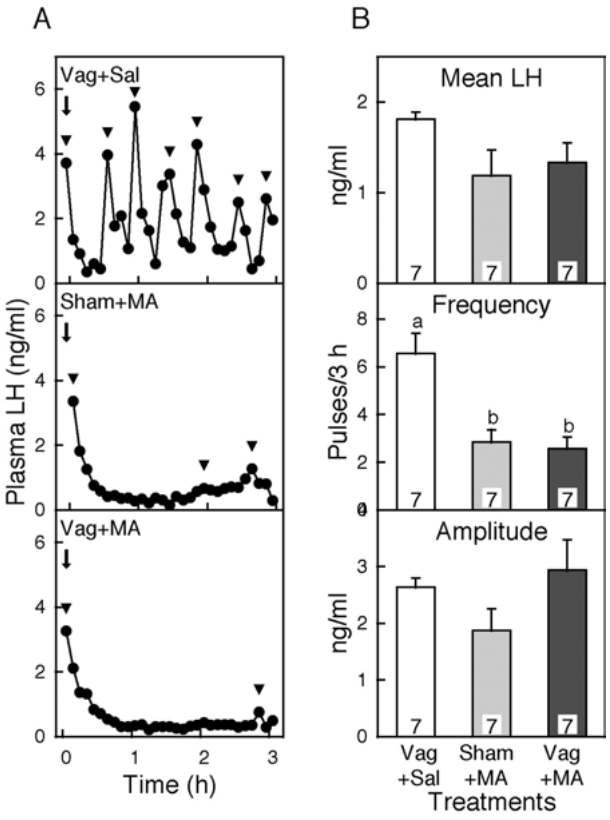

Fig. 3. A, Effect of acute subdiaphragmatic vagotomy on MA-induced suppression of LH secretion in OVX+E2 rats. Vag + Sal, vagotomized rats with saline injection (0.2 M, 4 ml/kg BW, ip); Vag + MA, vagotomized rats with MA injection (800 μmol/kg BW); Sham + MA, sham-vagotomized rats with MA injection. Acute vagotomy was performed after collection of the first blood sample and was followed immediately by bolus MA or saline injection (arrows). Arrowheads indicate the peaks of LH pulses identified by the Pulsar computer program. B, Effect of vagotomy on mean plasma LH concentrations for 3 h and the frequency and amplitude of LH pulses. Values are means ± SEM. Values with different letters are significantly (P<0.05) different from each other. The numbers in each column represent the number of animals used.

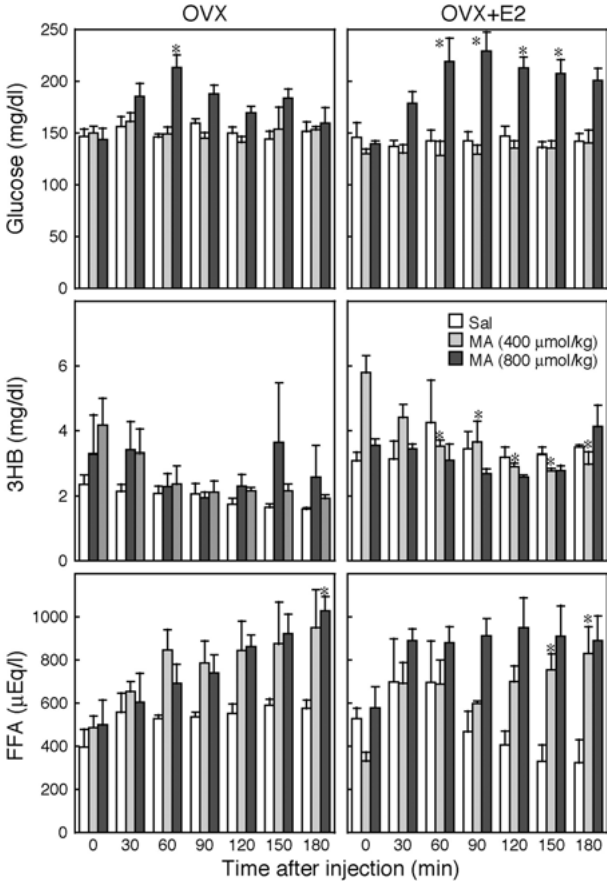

Fig. 4. Time-related changes in plasma fuels after administration of saline, or 400 μmol/kg BW MA, or 800 μmol/kg BW MA in *ad libitum*-fed OVX or OVX+E2 rats. Values are means ± SEM (n=4–5). Asterisks show significant differences (P<0.05) compared to the respective 0-min values.

lower in the vagotomized and sham-vagotomized animals treated with MA compared to the vagotomized rats treated with saline (Fig. 3B). MA administration did not cause any behavioral changes, and there was no difference in the mean 3-h food intake between groups (Table 1).

Effect of lipoprivation on plasma fuel concentrations in ad libitum-fed rats

Plasma glucose levels significantly increased at 60 min in OVX rats (P<0.05) and at 60–150 min in OVX+E2 rats (P<0.05) after MA injection at 800 μmol /kg BW compared to the pre-injection control values (Fig. 4).

Plasma levels of 3HB tended to decrease towards the end of the sampling period following injection of saline or either dose of MA in OVX rats, but the changes were not significant compared with pre-injection levels. In OVX+E2 rats, MA injection at 400 μmol/kg also induced a steady decrease in 3HB with a significantly smaller value (P<0.05) at 60–180 min post-treatment. Plasma 3HB in OVX+E2 rats did not show a significant change after saline or 800 μmol/kg MA injection.

MA adminitration caused an elevation in plasma FFA levels in both OVX and OVX+E2 rats. Plasma

FFA concentrations significantly increased ($P<0.05$) at 150–180 min (400 μmol/kg) in OVX+E2 rats and at 180 min (800 μmol/kg) in OVX rats after MA injection. FFA levels were not affected by saline administration in OVX and OVX+E2 rats.

Discussion

The present study indicates that lipoprivation acutely suppresses pulsatile LH release in the female rat maintained on a normal-fat diet. In addition, pulsatile LH secretion was more sensitive to lipoprivation in fasted animals that reportedly rely more heavily on fatty acid oxidation to maintain appropriate metabolic fuel levels [16]. Taken together, these data suggest that oxidation of fatty acids provides a metabolic fuel for maintenance of pulsatile LH secretion even in the female rat fed a normal-fat diet and that fatty acid oxidation may be more vital for maintenance of reproductive function particularly when fatty acids are a major fuel source during fasting periods [16]. Ohkura *et al*. [24] has reported that MA does not alter GnRH pulse generator activity in goats. The inconsistency between their study and ours could be due to a difference in experimental procedures, because they administered MA chronically and at a much lower dose (20 μmol/kg.h) than was used in the present study. It would be interesting to directly compare the role of fatty acids in the regulation of LH secretion between two such different animal models, ruminants and monogastrics, which have different pathways for energy metabolism.

The highest dose of MA (800 μmol/kg) was able to suppress LH secretion in both OVX and OVX+E2 rats with *ad libitum*-feeding. The LH suppression was less apparent at the lower dose (400 μmol/kg BW) because LH release was not significantly reduced in both the OVX+E2 and OVX animals at that dose. The highest dose may have produced a more severe lipoprivic condition in the *ad libitum*-fed rats, resulting in increased circulating glucose and FFA levels. On the other hand, the lower dose of MA (400 μmol/kg) was only able to suppress LH secretion in the 24-h fasted rats. It should be noted that inhibition of fatty acid oxidation suppressed pulsatile LH secretion more severely in the 24-h fasted animals than in the *ad libitum*-fed animals. An increased sensitivity to lipoprivic impairment of estrous cyclicity in fasted animals has also been observed in fasted-obese hamsters [25] and fasted-lean hamsters [26]. Together, these observations suggest that the availability of energy supplied by fatty acid oxidation is important for maintenance of normal LH pulses even in rats fed a normal-fat diet and that neuroendocrine pathways regulating LH secretion and subsequent reproductive functions are more sensitive to lipoprivation under concurrent energy deficient conditions.

The increased feeding response to blockade of fatty acid oxidation has been considered to be mediated by the vagus nerve [14, 17] because surgical [27, 28] and chemical [29] vagotomy blocked MA-induced feeding in high-fat diet fed rats and intraportal administration of MA increases hepatic vagus branch afferent activity [30]. In the current study, however, complete subdiaphragmatic vagotomy did not alter MA-induced LH suppression, suggesting that lipoprivic signals transmitted to the brain through the vagus nerve are not critical for lipoprivic LH suppression. This is consistent with the present results that food intake was not affected by any doses of MA at which LH pulses were suppressed in the *ad libitum*-fed or fasted rats. Vagotomy does not eliminate lipoprivic anestrus in fasted fat or lean hamsters or lipoprivic-induced deficits in estrous behavior [26, 31]. These reports also support our notion that lipoprivic suppression of LH secretion is mediated by a pathway(s) other than the vagus nerve. The present results raise a possibility that fatty acid availability is sensed by the brain but further studies are required to clarify this point.

The marked increases in plasma glucose levels after MA administration at the higher dose (800 μmol/kg) in the present study may indicate the activation of counterregulatory responses to the energy deficiency by lipoprivation as has been reported in glucoprivic animals. Administration of 2DG activates noradrenergic input from the solitary tract (NTS) to the PVN [32] and CRH neurons to suppress LH pulses [33] and increase the efferent discharge rate of sympathetic nerves [34], implying that the sympathetic nervous system and hypothalamo-pituitary-adrenal (HPA) axis is activated under glucoprivation. MA may also induce a similar sympathetic and/or HPA axis activation, resulting in blood glucose elevation because MA has been reported to induce c-Fos expression in the NTS and parabrachial nucleus

[35]. Further study is required to address this point.

In the present study, lipoprivic suppression of LH secretion was slightly but not significantly enhanced by estrogen in the *ad libitum*-fed rats. Our previous study showed that glucoprivic LH inhibition is enhanced by estrogen [9, 33] and that the enhanced suppression of LH secretion during glucoprivation is likely due to the increased estrogen receptor α in discrete brain areas, such as the PVN or A2 region of the medulla oblongata [36, 37]. These data suggest that lipoprivation suppresses LH pulses in the brain at least partly through a pathway different from glucoprivaion. In this context, Ritter and Dinh [35] have already reported that MA induced Fos-like immunoreactivity in some discrete brain areas that are different from those induced by 2DG administration.

A consistent observation of the present study was that MA did not influence food intake in any of the experiments. The majority of evidence indicates that the feeding response to MA treatment is dependent on the relative proportion of fat in the diet. Lipoprivation is able to induce food intake when rats consume a diet containing at least 18% fat [17]. A very low dose of MA increased food intake in rat pups that consumed a high-fat dam's milk [38]. Thus, the feeding response to lipoprivation occurs when fatty acid oxidation is the major source of metabolic energy. Absence of a feeding response in the present study is probably due to our rats eating a diet that had only 4.4% fat. A feeding response to MA was not observed even in the 24-h fasted rats, suggesting that our dose of MA did not induce enough of an energy deficit to stimulate lipoprivic feeding even under short-term fasting conditions. These observations, together with the lack of reversal of MA-induced LH suppression by vagotomy, indicate that the lipoprivic effects on food intake and LH secretion may be distinct from each other and may not be mutually inclusive.

In summary, the present results demonstrate that pharmacological blockade of fatty acid oxidation leads to suppression of pulsatile LH secretion even in normal-fat diet *ad-libitum* fed OVX and OVX+E2 rats. Such an action is magnified when fatty acids are the dominant energy substrates as shown in the fasted rats. In addition, peripheral lipoprivic information that results in LH suppression is not transmitted to the brain via the vagus nerve. The present findings demonstrate that the neuroendocrine mechanism controlling reproduction is sensitive to acute changes in fatty acid oxidation.

Acknowledgements

We are thankful to the National Hormone and Pituitary Program for the LH assay kit and Drs. G. R. Merriam and K. W. Watcher for the Pulsar computer program. The RIA and pulse analyses were performed at the Nagoya University Radioisotope and Computation Centers, respectively. M. S. was supported by a post-doctoral fellowship from the Japan Society for the Promotion of Science. This work was supported in part by Grants-in-Aid for Scientific Research 14360177 (to K.M.), 15380193 (to H.T.), and 15658082 (to H.T.) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

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