Fasting-induced suppression of LH secretion does not require activation of ATP-sensitive potassium channels

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Huang W, Acosta-Martínez M, Horton TH, Levine JE. Fasting-induced suppression of LH secretion does not require activation of ATP-sensitive potassium channels. Am J Physiol Endocrinol Metab 295: E1439–E1446, 2008. First published October 7, 2008; doi:10.1152/ajpendo.90615.2008.—Reproductive hormone secretions are inhibited by fasting and restored by feeding. Metabolic signals mediating these effects include fluctuations in serum glucose, insulin, and leptin. Because ATP-sensitive potassium (KATP) channels mediate glucose sensing and many actions of insulin and leptin in neurons, we assessed their role in suppressing LH secretion during food restriction. Vehicle or a KATP channel blocker, tolbutamide, was infused into the lateral cerebroventricle in ovariec-
tomized mice that were either fasted or fasted for 48 h. Tolbutamide infusion resulted in a twofold increase in LH concentrations in both fasted and fasted mice compared with both fed and fasted vehicle-treated mice. However, tolbutamide did not reverse the suppression of LH in the majority of fasted animals. In sulfonfonyurea (SUR)-null mutant (SUR1−/−) mice, which are deficient in KATP channels, and their wild-type (WT) littermates, a 48-h fast was found to reduce serum LH concentrations in both WT and SUR−/− mice. The present study demonstrates that 1) blockade of KATP channels elevates LH secretion regardless of energy balance and 2) acute fasting suppresses LH secretion in both SUR1−/− and WT mice. These findings support the hypothesis that KATP channels are linked to the regulation of gonadotropin-releasing hormone (GnRH) release but are not obligatory for mediating the effects of fasting on GnRH/LH secretion. Thus it is unlikely that the modulation of KATP channels either as part of the classical glucose-sensing mechanism or as a component of insulin or leptin signaling plays a major role in the suppression of GnRH and LH secretion during food restriction.

sulfonfonyurea; luteinizing hormone; gonadotropin-releasing hormone; metabolic signals

IN MOST MAMMALIAN SPECIES, reproductive capacity is closely linked to the availability of oxidizable metabolic fuels (57, 73). A short-term fast or prolonged reduction in caloric intake suppresses the hypothalamic-pituitary-gonadal axis, as reflected by reduced LH secretion or disrupted ovulatory cycling in mice (2, 28, 68), hamsters (5), rats (7, 8, 46), sheep (23, 36), monkeys (16, 38), and humans (39, 44). Refeeding can rapidly reverse these effects (9, 16, 80). It is generally held that these reproductive responses to reduced energy intake are mediated by a deceleration or complete arrest of the pulsatile neurosecretion of gonadotropin-releasing hormone (GnRH) (30). It remains unclear how neural, endocrine, and/or metabolic signals may be conveyed from peripheral tissues to the hypothalamic “GnRH pulse generator” to regulate reproductive activity in response to negative energy balance. Among the most likely candidates for these somatic signals are circulating oxidizable metabolites, such as glucose (12, 51, 59) and fatty acids (57, 63), as well as serum leptin (2, 27, 52, 58) and insulin (10, 13, 21, 37, 69) concentrations, which reflect prevailing levels of adiposity. Thus GnRH pulsatility may be sustained in positive energy balance by any one or all of these peripheral signals, while, conversely, a deceleration of GnRH pulse generator activity may be precipitated by fasting-induced decreases in one or more of them.

ATP-sensitive potassium (KATP) channels have been implicated as downstream cellular effectors of several of the foregoing metabolic signals, including glucose (3, 41, 47), leptin (48, 66), and insulin (48, 67). The channels comprise a complex of four each of two protein subunits, the pore-forming Kir6.x subunit and the sulfonfonyurea (SUR) regulatory subunit. There are two isoforms of the SUR subunit, SUR1 and SUR2; subunits Kir6.2 and SUR1 make up channels found in both pancreas and brain (32, 33, 62, 64). These channels are particularly prevalent in neuropeptide Y (NPY)/agouti-related peptide (AgRP) (22, 45, 72) and proopiomelanocortin (POMC) (31, 54) neurons of the arcuate and ventromedial nuclei, where they have been shown to mediate a glucose-sensing function (31, 72), as well as responses to insulin and leptin (41, 48, 72). Notably, subsets of these same neuronal groups project to GnRH neurons and regulate their secretory activity (40, 42). The KATP channels that could mediate metabolic inhibition of GnRH secretion may also be those that are expressed in GnRH neurons themselves, as our collaborators and we have recently characterized (79). Similarly, the Kir6.2/SUR1-containing KATP channels are expressed in glucose-sensing brain stem neurons (20, 22) that may mediate glucoprivic suppression of GnRH release (17, 50) and estrous cyclicity (60), as well as glucoprivic feeding and homeostatic control of blood glucose (56). Neuroanatomic studies also provide relevant evidence that neurons in the hindbrain send projections to the GnRH neurons (71) and affect GnRH/LH secretion (15, 18). The KATP channels are thus positioned to mediate glucoprivic regulation of GnRH release at both neural loci.

The foregoing observations suggest that KATP channel modulation in glucose-sensing hypothalamic or brain stem neurons may mediate the suppressive effects of negative energy balance on GnRH and LH secretions. According to this hypothesis, prolonged reduction in circulating glucose concentrations would lead to activation of KATP channels and reduced excitability in the neuronal circuitries that govern GnRH neurose-
cretion. A variant of this hypothesis holds that fasting-induced reductions in leptin or insulin lead to reduced KATP channel activation, presumably heightening excitability in POMC neu-
rons or other cell populations that exert inhibitory control over GnRH neurons. In the present studies, we tested the general hypothesis that K_{ATP} channel modulation mediates fasting-induced GnRH suppression by either or both of these mechanisms. To do this, we assessed the effects of pharmacological blockade of K_{ATP} channels, as well as deletion of the gene for the SUR1 K_{ATP} channel subunit, on fasting-induced suppression of LH secretion. We predicted that the blockade of K_{ATP} channels would reverse, while K_{ATP} channel elimination would prevent, any fasting-induced decline in LH concentrations. Surprisingly, our results clearly demonstrate that fasting can suppress LH, and presumably GnRH secretion, independently of K_{ATP} channel activation. These observations effectively rule out an obligatory role for the classic glucose-sensing mechanism, as well as K_{ATP} channel-mediated insulin or leptin signaling, in the inhibitory effects of negative energy balance on GnRH and LH secretion.

MATERIALS AND METHODS

Animals

Female C57BL/6 mice (Charles River Laboratories, Wilmington, MA) were used in experiment 1, while age-matched isogenic adult wild-type (WT) and SUR1^{−/−} female mice were used in experiment 2. SUR1^{−/−} male and female mice (C57BL/6 × 129 SvJ) were generously provided by Dr. Mark A. Magnuson (Vanderbilt University, Nashville, TN) (65). Matings of male SUR1^{−/−} or C57BL/6 mice with SUR1^{−/−} female mice failed to produce viable offspring. Although vaginal plugs were observed after pairing, indicating that mating had occurred, few pups were born, and in the litters born the number of pups per litter was small and the pups died shortly after birth. Therefore male SUR1^{−/−} mice were mated to female C57BL/6 mice to generate heterozygous SUR1-knockout mice. Subsequent matings of the heterozygous SUR1-knockout mice were then used to produce both WT and SUR1^{−/−} mice. All animals were housed in temperature-controlled facilities (23–25°C) with a 12:12-h light-dark cycle (0500–1700). The animals were fed standard laboratory chow and had access to water ad libitum. All surgical and experimental procedures were performed in strict accordance with protocols approved by the Institutional Animal Care and Use Committee of Northwestern University.

Experiment 1: Effects of Intracerebroventricular Tolbutamide Infusion on LH Secretion in Fed and Fasted Female Mice

On day 0, the animals were anesthetized with ketamine (80 mg/kg ip; Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (32 mg/kg ip; Burns Veterinart Supply, Rockville Center, NY) and bilaterally ovariec-tomized (OVX). At the same time, all animals received guide cannulas fitted with obdurators (CMA/7 guide cannulas; CMA/Microdialysis, North Chelmsford, MA); stereotactic surgical techniques were used to target the guide cannulas to the right lateral ventricle (coordinates 0.2 mm caudal to bregma, 2.0 mm ventral to the skull, 1.0 mm lateral) (53). Five days later, a subset of the mice were fasted, while the rest received food ad libitum. All animals had free access to water. Forty-eight hours after fasting, all animals were briefly anesthetized by isoflurane inhalation and an infusion cannula was fitted into the guide cannula. After insertion, either vehicle [0.1% DMSO in artificial cerebrospinal fluid (aCSF)] or tolbutamide (500 μM in 0.1% DMSO) was infused through the cannula at a rate of 1 μl/min for 2 min (equal to total dose of 270 ng tolbutamide). The components of aCSF were (in mM) 124 NaCl, 5 KCl, 26 NaHCO3, 2.6 NaH2PO4, 10 dextrose, 10 HEPES, 2 MgSO4, and 2 CaCl2. After infusion, the probe was maintained in place for one additional minute and then replaced with the obdurator. Four minutes later, the animals were anesthetized by CO2 inhalation, and terminal blood samples were collected by cardiac puncture and centrifuged at 13,000 rpm at 4°C for 10 min. Blood samples also were collected from an additional group of fasted animals 25 min after tolbutamide infusion. This time course was selected because bolus injection of tolbutamide induces a transient elevation in insulin secretion that peaks within 4 min and returns to baseline within 20 min (11). Plasma was then harvested and stored at −20°C for subsequent LH radioimmunoassays (RIAs). All animals were weighed on day 5 immediately before being fasted, on day 6 during fasting, and on day 7 before they were euthanized.

Experiment 2: Effects of 48 h of Fasting on LH Secretion in Female SUR1-Knockout Mice

On day 0, both WT and SUR1^{−/−} mice were anesthetized by isoflurane inhalation (Baxter, Deerfield, IL) and underwent bilateral OVX. Ovaries were weighed, and initial body weight was recorded. Five days later, all animals were again briefly anesthetized by isoflu-rane inhalation and 80–100 μl of blood was collected by tail vein puncture. Blood glucose concentrations were measured with a Prestige Smart System Glucose Monitor (Home Diagnostics, Fort Lauderdale, FL). Blood samples were centrifuged, and plasma was stored at −20°C for subsequent LH RIAs. On completion of tail blood collection, food was removed from the SUR1^{−/−} (n = 10) and WT (n = 11) animals to initiate the fast. During fasting, the animals received water ad libitum. An additional group of nine WT animals continued to receive food during this time. After 48 h of fasting, the animals were anesthetized by CO2 inhalation and killed by cervical dislocation. Blood samples were collected by cardiac puncture, and again blood glucose concentrations were determined. Plasma was obtained from this second set of samples and stored at −20°C for LH, insulin, and leptin RIAs. Bilateral uterine tissues were removed and weighed. All the animals were weighed on day 5 immediately before being fasted, on day 6 during fasting, and on day 7 before they were killed.

Hormone Assays

Plasma LH concentrations were determined by RIA using reagents obtained from the National Institute of Diabetes and Digestive and Kidney Diseases, including LH reference (RP-3) and anti-rat LH antibody (S-11). The assay had a lower limit of detection of 0.2 ng/ml. The intra-assay and interassay coefficients of variation (CVs) for LH assays were 7.6% and 11.5%, respectively. Leptin was measured with the Rat Leptin RIA Kit from Linco Research (catalog no. RL-83K, St. Charles, MO). This kit shows 100% cross-reactivity with mouse leptin; serial dilution curves of rat and mouse sera run in our laboratory are parallel. The sensitivity of the leptin assay is 0.5 ng/ml. Insulin assays were conducted with the Sensitive Rat Insulin RIA from Linco Research (catalog no. SRI-13K); this kit has a sensitivity of 0.2 ng/ml. This kit shows 100% cross-reactivity with mouse insulin; serial dilution curves of rat and mouse sera run in our laboratory are parallel. The intra-assay CVs for leptin and insulin assays were 1.48% and 2.7%, respectively.

Statistical Analysis

All summary data are presented as means ± SE. The effects of intracerebroventricular (ICV) vehicle or tolbutamide infusion on LH concentrations in fed and fasted mice were assessed by two-way ANOVA followed by Bonferroni’s multiple-comparison post hoc tests (GraphPad Software, San Diego, CA).

Comparisons of body weight and LH and glucose concentrations before and after 48 h of fasting in WT and SUR1^{−/−} mice were performed by two-way ANOVA with repeated measures followed by Bonferroni’s multiple-comparison post hoc test (GraphPad Software). Ovary weight before fasting, and uterine weight, leptin concentra-tions, insulin concentrations, and glucose-to-insulin ratio after
fasting were compared between WT and SUR1−/− mice by unpaired t-tests. For all statistical analysis, significant differences were reported at P ≤ 0.05.

RESULTS

Experiment 1: Effect of ICV Tolbutamide on LH Secretion in Fed and Fasted Female Mice

LH levels were dramatically decreased in fasted animals (ANOVA: fed vs. fasted, P < 0.001) (Fig. 1). Infusion of tolbutamide into the lateral ventricle stimulated similar fold increases in serum LH concentrations in the fed and fasted groups (ANOVA: vehicle vs. tolbutamide, P = 0.01). Although infusion of tolbutamide stimulated a nearly twofold increase in LH secretion in fasted animals (0.23 ± 0.03 ng/ml for vehicle vs. 0.45 ± 0.08 ng/ml for tolbutamide, P < 0.05) (Fig. 1), it did not elevate the LH concentrations of fasted mice to those of fed mice treated with vehicle. Similarly, tolbutamide infusion also stimulated a twofold increase in plasma LH concentrations in the fed mice (1.43 ± 0.16 ng/ml for vehicle vs. 2.68 ± 0.64 ng/ml for tolbutamide, P < 0.05). The release of LH in response to tolbutamide in both the fed and fasted groups was rapid; the increases occurred within 4 min after infusion of the drug. There did not appear to be any additional increase in LH secretion after tolbutamide infusion because serum concentrations collected from a separate group of fasted animals 25 min after tolbutamide infusion (mean ± SE: 0.32 ± 0.05 ng/ml, n = 11) were not significantly different from concentrations observed at 4 min (mean ± SE: 0.45 ± 0.08, n = 15) (P = 0.27).

Experiment 2: Effect of 48 h of Fasting in WT and SUR1−/− Female Mice

Body and tissue weights. There was no significant difference in initial body weight between WT and SUR1−/− female mice. The 48-h fast caused similar and significant weight loss in both WT and SUR1−/− mice (20.96 ± 0.88 g before fasting vs. 15.93 ± 0.68 g after fasting in WT mice; 21.94 ± 0.92 g before fasting vs. 16.69 ± 0.82 g after fasting in SUR1−/− mice) (Fig. 2A) (ANOVA: before vs. after, P < 0.001). The weights of ovaries before fasting and the weights of uteri postmortem did not differ between the WT and SUR1−/− mice (Table 1).

Glucose concentrations. Fasting resulted in a significant decline in serum glucose (ANOVA: fed vs. fasted, P < 0.001) (Fig. 2B). There was no significant effect of genotype on serum glucose concentrations (ANOVA: genotype, P = 0.57).

Insulin concentrations. glucose-to-insulin ratio, and leptin concentrations after fasting. After 48 h of fasting, insulin concentrations were significantly higher in SUR1−/− mice than in WT mice (0.37 ± 0.04 ng/ml and 0.59 ± 0.07 ng/ml in WT and SUR1−/−, respectively, P = 0.04; Fig. 2C), while the glucose-to-insulin ratio in SUR1−/− mice was significantly lower than that in WT mice (16.7 ± 1.88 and 7.46 ± 1.81 of WT and SUR1−/−, respectively, P = 0.02; 2D). However, leptin concentrations were not significantly different between the two genotypes (P = 0.80; Fig. 2E).

LH concentrations. LH concentrations before and after fasting in each individual animal are depicted in Fig. 3A. Basal LH concentrations before fasting did not differ between WT and SUR1−/− mice. Fasting significantly reduced serum LH levels (ANOVA, P < 0.001) independently of genotype (ANOVA, P = 0.54). The reduction of LH in both genotypes after 48 h of fasting compared with the prefab levels was significant by post hoc multiple comparisons (1.85 ± 0.37 ng/ml before fasting vs. 0.55 ± 0.13 ng/ml after fasting in WT mice, P < 0.01; 1.27 ± 0.30 ng/ml before fasting vs. 0.27 ± 0.05 ng/ml after fasting in SUR1−/− mice, P < 0.05; Fig. 3B).

DISCUSSION

It is generally held that inhibition of gonadotropin secretion in response to negative energy balance is a consequence of the suppression of pulsatile GnRH release (30), because food deprivation and refeeding have been shown to cause deceleration and reacceleration of LH pulsatility, respectively (8, 69). Because pulsatile LH secretion can be modulated within hours of food restriction or refeeding, it has been hypothesized that the GnRH pulse generator is sensitive to circulating metabolic signals that directly or indirectly reflect the availability of oxidizable metabolic fuels (57, 73). Among the most likely of these metabolic cues are serum glucose, leptin, and insulin, which are all reduced during food restriction and restored to original levels after refeeding. All three of these factors, glucose, leptin, and insulin, modulate KATP channel activity in neurons that are known to regulate GnRH pulsatility; therefore, we sought to determine whether KATP channel modulation mediates the effects of food restriction on GnRH release, as reflected by surrogate measurements of serum LH. Although we have recently confirmed (79) that KATP Channel modulation can regulate LH secretion and presumably GnRH release in the OVX mouse, our findings reveal that the suppressive effects of food restriction on LH release do not require the expression of SUR1, the isoform of regulatory protein that is expressed in glucose-sensing neurons of the hypothalamus and brain stem (4, 43, 47) and required for central glucoregulatory responses to insulin (55). By subjecting the mice to a 48-h fast, we induced an ~25% reduction in body weight and a 20–38% reduction in blood glucose levels. Although these reductions in blood glucose levels are not as great as those reported in some
studies (24, 25), they were associated with a significant loss of body weight and reduction in LH secretion. Contrary to our hypothesis, treatment with tolbutamide did not override the fasting-induced suppression of the hypothalamic-pituitary-gonadal axis and restore LH secretion to the levels found in fed animals. In contrast, we determined that blockade of KATP channels with tolbutamide produces similar percentage increases in serum LH concentrations regardless of whether animals are fed or fasted, arguing against the idea that activated KATP channels maintain the fasting-induced suppression of GnRH/LH. Thus, if fasting-induced reductions in glucose, insulin, and/or leptin do lead to reduced GnRH pulsatility, then the mechanisms responsible for any such effects appear not to involve the activation of KATP channel signaling pathways.

Table 1. Ovarian weights before fasting and uterine weights after fasting in wild-type and SUR1−/− female mice

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>SUR1−/−</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary weight before fasting, mg</td>
<td>12.18±0.65</td>
<td>12.10±1.03</td>
<td>NS</td>
</tr>
<tr>
<td>Uterine weight after fasting, mg</td>
<td>36.64±3.79</td>
<td>32.85±2.40</td>
<td>NS</td>
</tr>
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</table>

Values are means ± SE. NS, not significant.

Previous studies in sheep (12) and rats (51) showed that central glucoprivation, induced by ICV infusion of the glucose antimitabolite 2-deoxyglucose, can rapidly reduce GnRH pulsatility. These findings suggested that the hypothalamic GnRH pulse generator is sensitive to inhibition during hypoglycemia and raised the possibility that diminished extracellular glucose concentrations during food restriction may be transduced as a metabolic cue for suppression of GnRH pulsatility. Fluctuations in extracellular glucose concentrations have been known for many years to be registered by glucose-responsive neurons (41). In many glucose-sensing neurons, particularly those that are excited when exposed to higher levels of glucose, the uptake and oxidation of glucose produces an increase in the intracellular ATP-to-ADP ratio, which in turn prompts binding of ATP to KATP channels in the plasma membrane and blockade of the channel pore. The KATP channels thereby link the cellular metabolism of glucose to alterations in neuronal excitability in glucose-responsive neurons (1), and this may serve as one mechanism by which fasting-associated reductions in serum glucose may be registered and transduced into reduced neurosecretion of GnRH. In support of this idea, it was recently reported that the presumed reduction in glucose oxidation in
concentrations in WT and SUR1

It remains possible that KATP channel-mediated glucose fasting-induced suppression of reproductive hormone secre-
is not an obligatory component of the mechanisms that mediate modulation.

manner to suppress GnRH release. It is also possible that KATP metabolism are registered and transduced in a KATP-independent manner. Fluctuations in glucose availability and me-
sensing operates as one of many redundant mechanisms, or alternatively that fluctuations in glucose availability and metabolism are registered and transduced in a KATP-independent manner to suppress GnRH release. It is also possible that KATP channels can mediate hypoglycemic suppression of GnRH pulsatility but that this mechanism is only triggered in response to more severe hypoglycemic stress than that produced by our food restriction protocol. A neuroprotective function has been ascribed to KATP channels in other neuronal populations that are not known to be glucose responsive under euglycemic conditions.

The KATP channels also mediate some of the effects of insulin and leptin in hypothalamic and arcuate neurons. Both hormones, in part, modulate phosphatidylinositol 3-kinase (74) to prompt changes in KATP channel activity, with the direction of these effects depending upon the cell context. Reproductive hormone secretions and ovulatory cyclicity are impaired in mice deficient in leptin (19), leptin receptor (68), brain insulin receptors (10), and insulin receptor substrate (IRS)-2 (14). In metabolically challenged rodents, a drop in peripheral leptin concentrations occurs that is accompanied by a deceleration of LH pulsatility, and administration of leptin can stimulate LH pulsatility in these animals (2, 58) and sheep (26, 27). Similarly, insulin concentrations fall during food restriction (52), and in at least one study insulin treatment was found to stimulate pulsatile LH secretion (70). Moreover, insulin concentrations rise in refed animals before the restoration of pulsatile LH secretion (69), implicating endogenous insulin as a permissive signal for the maintenance of basal GnRH pulsatility.

Consistent with previous observations, we observed that leptin concentrations tended to be reduced in fasted animals of both genotypes. We measured leptin and insulin at the termination of the experiment to determine the status of the hormones as a result of the 48-h fast but were unable to obtain sufficient serum to measure leptin and insulin from the same animals before and after fasting. At the time the fasted animals were killed, blood was collected from a separate group of age-matched WT mice that had undergone similar surgical procedures to provide baseline data from fed animals from our colony, but additional SUR−/− animals were not available. Leptin levels appeared to decline slightly in the fasted WT animals compared with the fed WT animals, although the effect was not significant (fed 3.4 ± 0.4 ng/ml vs. fasted 2.6 ± 0.9 ng/ml; \( P = 0.07 \), 1-tailed \( t \)-test). Because the leptin levels of the fasted WT and SUR−/− mice did not differ, it is plausible that the leptin levels of the SUR−/− mice had also declined. Insulin concentrations similarly trended downward in fasted WT animals (fed 0.51 ± 0.31 ng/ml vs. fasted 0.41 ± 0.11 ng/ml; \( P = 0.21 \), 1-tailed \( t \)-test). Forty-eight hours after fasting, insulin concentrations in SUR1−/− mice were significantly higher than in the fast WT mice; this was expected because this genotype was previously shown to be mildly hyperinsulinemic under fasting conditions (61). Unfortunately, insufficient numbers of SUR−/− mice were available to provide an equivalent fed group. These data indicate that, irrespective of the presence or absence of alterations in leptin or insulin, fasting produced significant declines in serum LH concentrations in both genotypes. Because LH concentrations fell to an equal extent in both WT and SUR−/− animals compared with the fed WT animals, although the effect was not significant (fed 3.4 ± 0.4 ng/ml vs. fasted 2.6 ± 0.9 ng/ml; \( P = 0.07 \), 1-tailed \( t \)-test). Because the leptin levels of the fasted WT and SUR−/− mice did not differ, it is plausible that the leptin levels of the SUR−/− mice had also declined. Insulin concentrations similarly trended downward in fasted WT animals (fed 0.51 ± 0.31 ng/ml vs. fasted 0.41 ± 0.11 ng/ml; \( P = 0.21 \), 1-tailed \( t \)-test). Forty-eight hours after fasting, insulin concentrations in SUR1−/− mice were significantly higher than in the fasted WT mice; this was expected because this genotype was previously shown to be mildly hyperinsulinemic under fasting conditions (61). Unfortunately, insufficient numbers of SUR−/− mice were available to provide an equivalent fed group. These data indicate that, irrespective of the presence or absence of alterations in leptin or insulin, fasting produced significant declines in serum LH concentrations in both genotypes. Because LH concentrations fell to an equal extent in both WT and SUR−/− mice, it is reasonable to conclude that KATP channels are not necessary to communicate the diminishment of insulin or leptin concentra-
tions to neurons governing the neurosecretion of GnRH. It is possible that the absence throughout development of the SUR1 subunit in the SUR−/− mice from the numerous neuronal phenotypes involved in nutrient sensing resulted in compensatory adaptations leading to these observations.
We observed that ICV infusion of tolbutamide produced a doubling of LH concentrations over baseline concentrations in fed mice, as well as a twofold increase over the diminished LH concentrations produced by the 48-h fast. The increase in circulating LH concentrations occurred by the time the blood samples were collected 4 min after infusion of tolbutamide. It could be argued that the fasted animals simply required additional time to respond to tolbutamide and release LH. This appears not to be the case. Additional data were collected from a separate set of fasted animals 25 min after infusion of tolbutamide. The LH concentrations of fasted animals did not continue to increase and appeared to have begun to wane by this time. In this respect, the pharmacokinetics of tolbutamide are similar to those of K\textsubscript{ATP} channel-associated insulin secretion (11). These findings suggest that fasting is not associated with any overt change in the number or functional state of K\textsubscript{ATP} channels that directly or indirectly modulate GnRH release.

Although these results are not consistent with a role for K\textsubscript{ATP} channel modulation in the suppression of LH by fasting, they do nevertheless confirm that K\textsubscript{ATP} channels are functionally linked to the GnRH neurosecretory process. Similarly, we and our collaborators have recently demonstrated (79) that GnRH neurons themselves express K\textsubscript{ATP} channels that are responsive to metabolic inhibition and fluctuations in extracellular glucose; in that study, the ionic currents observed during K\textsubscript{ATP} channel activation were greater in tissues harvested from estrogen-treated mice. We have also observed that estrogen and progesterone treatments enhance the stimulatory actions of ICV tolbutamide on LH pulsatility in OVX rats (29), while the combined treatment with these steroids maximally stimulates \(K_{\text{ATP}} \) mRNA expression in the preoptic area. It is thus possible that K\textsubscript{ATP} channels may play a more important physiological role in mediating steroid hormone feedback actions on GnRH neurons or their afferents than in signaling low energy availability to the reproductive axis.

The modulation of GnRH secretion by K\textsubscript{ATP} channels, as demonstrated previously (79) and inferred from the present work, may also serve a neuroprotective role. In other neuronal populations, such as K\textsubscript{ATP}-expressing neurons in the substantia nigra, K\textsubscript{ATP} channels appear to restrain hypoxia-induced seizure propagation (77). The substantia nigra is critical in controlling the propagation of generalized seizures, and opening of K\textsubscript{ATP} channels in nigral neurons strongly suppresses neuronal activity during hypoxic challenge. Findings in K\textsubscript{ATP}-null mutant mice (77) as well as in SUR1-null mutants (49) also clearly demonstrate that the β-cell type K\textsubscript{ATP} channels play critically important roles in raising the thresholds for seizure initiation and propagation induced by metabolic insufficiency or excitotoxic insult (76). It is not clear whether GnRH neurons, or the neurons that comprise their afferent circuitries, are especially vulnerable to hypoxic, hypoglycemic, and/or excitotoxic stress, and thus the importance of any such neuroprotective mechanism remains to be clarified.

The present observations suggest that the suppression of LH secretion during fasting does not depend on K\textsubscript{ATP} channel-mediated glucose sensing or the modulation of K\textsubscript{ATP} channel activity by insulin, leptin, or other metabolic cues. The physiological and cellular mechanisms that mediate the reproductive hormonal response to food restriction thus remain unknown. All of the foregoing cues, glucose, leptin, and insulin, remain viable candidates as signals for suppression of LH secretion; however, their actions would appear to be mediated by K\textsubscript{ATP} channel-independent pathways. It also remains possible, if not likely, that food restriction simultaneously prompts fluctuations in several of these and other circulating metabolic cues, as well as alterations in ascending neural signals, and that this convergent information is transduced and summed within the neural systems that govern GnRH pulsatility. Thus summation of these signals may then collectively surpass some critical cellular threshold for the suppression of the GnRH pulse-generating mechanism. Nevertheless, our findings reveal that K\textsubscript{ATP} channel modulation is not an obligatory component of this integrative response to a metabolic challenge.

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GRANTS

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