Fourth Ventricular Alloxan Injection Suppresses Pulsatile Luteinizing Hormone Release in Female Rats

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Abstract. Previous studies have suggested the presence of a glucose-sensing mechanism in the hindbrain that appears to regulate reproductive function as well as feeding behavior. The ependymocytes lining the ventricular wall of the hindbrain showed immunoreactivities to pancreatic glucokinase (GK), a key enzyme for glucose sensing in pancreatic B cells. Our goal in the present study was to test whether the GK-immunopositive ependymocytes in the wall of the fourth cerebroventricle (4V) play a role in regulating gonadal activity. Our approach was to determine the effect of injecting alloxan, a GK inhibitor, into the 4V on pulsatile luteinizing hormone (LH) secretion. Estrogen-primed ovariectomized rats received an injection of alloxan (10 or 20 µg/animal) into the 4V and blood samples were collected every 6 min for 3 h for measurement of blood LH, corticosterone and glucose levels. Pulsatile LH secretion was suppressed after alloxan injection and all pulse parameters were significantly (P<0.05) inhibited by 20 µg alloxan. Plasma corticosterone levels were increased significantly (P<0.05) by 20 µg alloxan, suggesting that LH pulse suppression by alloxan may be at least partly mediated by activation of the hypothalamo-pituitary-adrenal axis. The present results suggest that acute suppression of GK activity in the hindbrain inhibits pulsatile LH secretion in female rats, and supports the idea that GK-immunopositive ependymocytes may sense glucose levels in the cerebrospinal fluid and play a role in regulation of LH secretion.

Key words: Alloxan, LH, Glucokinase, Hindbrain, Ependymocytes

It has been well established that reduction of glucose availability inhibits the activity of the hypothalamo-pituitary-gonadal axis in mammalian species [1–3]. Pharmacological glucoprivation induced by systemic injection of a glucose antagonist, 2-deoxy-D-glucose (2DG), inhibits estrous cyclicity in female hamsters [1] and rats [4], and pulsatile luteinizing hormone (LH) release in female rats [5]. Glucose availability might monitored by a glucose-sensing mechanism either in the peripheral organs and/or brain to regulate gonadotropin secretion and then reproductive functions.

It is now well accepted that glucose-sensing regions are at least partly located in the brain. In addition to several hypothalamic areas, such as the hypothalamic ventromedial, paraventricular and arcuate nuclei and lateral hypothalamic area [6–8], the hindbrain has also been suggested as a glucose-sensing region, because food intake is increased by 5-thio-D-glucose (5TG) infusion into the fourth cerebroventricle (4V) but not into the third cerebroventricle (3V) in rats with an obstruction of the connection between the 3V and 4V [9]. Feeding is also induced by local application of 5TG into the
nuclei in the medulla oblongata but not in the hypothalamus [10]. In addition, a previous study demonstrated that 2DG injection into the 4V suppresses pulsatile LH secretion and increases food intake in male rats [11]. These results suggest that a part of the glucose-sensing mechanism is located in the hindbrain which regulate both food intake and reproduction.

We have previously demonstrated that pancreatic glucokinase (GK)-like immunoreactivity is present in specific brain regions including the ependymocytes of the rat forebrain and hindbrain with glucose transporter (GLUT) 2-like immunoreactivity on their cilia [12]. Pancreatic-type GK mRNA has also been found in the ependymocytes lining the 4V with RT-PCR (Moriyama et al. submitted for publication). Since both GK and GLUT2 play key roles in sensing blood glucose levels for release of insulin in pancreatic B cells [13, 14], the ependymocytes of the hindbrain could perform a similar function, sensing glucose levels in the cerebrospinal fluid.

Alloxan inhibits GK enzymatic activity [15], but also destroys glucose-sensitive pancreatic B cells at a higher dose [16]. The impairment of glucoprivic feeding by administration of 40 µg of alloxan into the lateral cerebral ventricle [17] suggests that this substance caused chronic damage to the brain glucose-sensing mechanism which mediates glucoprivic feeding. On the other hand, a lower dose (20 µg) of alloxan injected into the 4V acutely induced feeding and did not impair glucoprivic feeding in rats [18], suggesting that alloxan injection at the dose of 20 µg did not cause permanent damage on putative glucose-sensing cells in the brain but acutely inhibited GK enzymatic activity. Therefore, alloxan could be a useful tool to induce a transient suppression of GK activity and to then localize and characterize brain glucose-sensing cells, when administered at an appropriate dose.

The aim of the present study was to test whether the GK-containing ependymocytes on the wall of the 4V are involved in glucose sensing for regulation of pulsatile LH release in female rats. For this purpose, the effects of alloxan injection into the 4V at subtoxic doses on pulsatile LH secretion were examined in estrogen-primed ovariectomized (OVX) rats. The effects on plasma glucose and corticosterone levels were also determined.

Materials and Methods

Animals and treatments

Female Wistar-Imamichi strain rats were housed in a controlled environment (14L:10D, lights-on at 0500 h; 23 ± 3 °C) with food (CE-2, Clea Japan Inc., Japan) and water ad libitum. Animals having shown at least two consecutive 4-day estrous cycles were OVX and immediately implanted with subcutaneous Silastic tubing (1.5 mm i.d.; 3.0 mm o.d.; 25 mm in length; Dow Corning, Midland, MI) containing estradiol-17β (E2, Sigma, St. Louis, MO) dissolved in peanut oil at 20 µg/ml one week before the day of the sampling. The Silastic implant has previously been shown to produce a physiological plasma level of E2 at diestrus in female rats [19]. The present study was approved by the Committee on Animal Experiments of the Graduate School of Bioagricultural Sciences, Nagoya University.

Brain surgery

The animals were stereotaxically implanted with a stainless-steel guide cannula (23 gauge, Plastics One, Roanoke, VA) in the 4V (8.3 mm posterior and 12.5 mm ventral to bregma at midline) according to a rat brain atlas [20]. The animals were allowed one-week recovery prior to blood sampling. At the end of the experiment, the placement of the cannula tip was verified by infusing brilliant blue at the same flow rate as the alloxan infusion. Only the data from animals with correct cannula placement were used.

Experimental protocols

One week after the brain surgery, blood samples were collected from freely-moving conscious animals at 6-min intervals for 3 h beginning at 13:30 h through an indwelling atrial cannula that was inserted through the right jugular vein on the day prior to blood sampling. Sample volume was 100 µl for LH, except that an additional 50µl of blood samples were collected every 12 min for the first 1 h and every 30 min for the last 2 h to determine plasma corticosterone and glucose concentrations. Two microliters of alloxan solution or equal volume of the vehicle (citrate-buffered saline; CBS, pH4.3) was infused at 1 µl/min into the 4V immediately before the onset of blood sampling with a microinjection pump (EP-60, EICOM, Kyoto, Japan) through an internal brain cannula (26G,
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Plastics One) that was inserted into the guide cannula. An equivalent volume of rat red blood cells taken from donor rats and diluted with heparinized saline was replaced through the atrial cannula after collecting each sample.

Alloxan (Sigma) was dissolved in ice-cold CBS (at 10 or 20 µg/2 µl immediately before use (within 1 min) to avoid the degradation of the drug [21]. The doses were chosen because a previous study reported that 15 or 20 µg but not 10 µg of alloxan increased food intake [22]. In addition, the administration of 40 µg of alloxan into the lateral cerebral ventricle [17] impairs glucoprivic feeding, but 20 µg of alloxan did not [22].

Food was deprived during the blood sampling period, because food intake may affect LH release as well as blood glucose and corticosterone levels. Animals had free access to water throughout the experiment.

Measurement of daily and glucoprivic food intake after alloxan treatment

To determine if our dose of alloxan impairs normal feeding, daily food intake was determined at 13:30 h in some animals for a week after 20 µg of alloxan or vehicle injection into the 4V. In addition, one week after the 20 µg alloxan of injection, food consumption was measured for 3 h (13:30–16:30 h) after peripheral 2DG treatment (400 mg/kg i.p.).

Assays

Plasma LH concentrations were determined in 50-µl plasma samples by a double-antibody radioimmunoassay (RIA) with a rat LH RIA kit provided by the National Hormone and Pituitary Program (Baltimore, MD). Values were expressed in terms of NIDDK rat LH PR-3. The least detectable LH level was 0.156 ng/ml and the intra- and inter-assay coefficients of variation were 12.2% at 0.188 ng/ml and 9.1% at 0.345 ng/ml, respectively.

Glucose concentrations in plasma samples (1.5 µl) were determined by the glucose oxidase method with a commercial kit (Glucose B-Test, Wako, Tokyo, Japan).

Data analysis

LH pulses were identified by the PULSAR computer program [23] using previously described pulse detection criteria [24]. Statistical differences in LH pulse parameters and mean plasma glucose and corticosterone concentrations between groups were determined by the Fisher’s PLSD test following one-way ANOVA. Statistical differences in food intake between groups was determined by Student’s t-test.

Results

Frequent LH pulses were observed in the rats with vehicle injection into the 4V (Fig. 1A). Alloxan had no apparent effect on pulsatile LH secretion at 10 µg and all LH pulse parameters in this group were not significantly (P>0.05) different from vehicle-treated controls (Fig. 1B). Injection of the higher dose (20 µg) of alloxan into the 4V immediately suppressed pulsatile LH release and the inhibition lasted throughout the 3-h sampling period in some animals (Fig. 1A). All LH pulse parameters in animals treated with this higher dose (20 µg) of alloxan were significantly (P<0.05) lower than those treated with 10 µg of alloxan or vehicle (Fig. 1B).

Mean plasma glucose concentrations tended to increase after 20 µg of alloxan injection when compared with the vehicle-treated groups, though no significant (P>0.05) difference was found between these groups (Fig. 2A).

Mean plasma corticosterone levels increased after the injection of 20 µg of alloxan (Fig. 2B). The values were significantly (P<0.05) higher in animals treated with 20 µg of alloxan compared with those treated with 10 µg of alloxan or vehicle for the last 2 h of the sampling period (Fig. 2B). In the 10-µg alloxan-treated animals, plasma corticosterone concentrations showed no significant (P>0.05) difference compared with vehicle-treated animals.

Daily food intake in rats injected with 20 µg of alloxan into the 4V was not significantly (P>0.05)
different from those in vehicle-treated controls (Fig. 3). Likewise, there was no significant (P>0.05) difference in 2DG-induced 3-h food intake at one week after the 20 µg of alloxan injection between alloxan (2.50 ± 0.38 g, mean ± SEM, n=6) and vehicle-injected (2.52 ± 0.60 g, n=6) animals.

Discussion

The present study demonstrated that injection of 20 µg of alloxan into the 4V strongly suppressed pulsatile LH secretion in E2-primed OVX rats. This alloxan-induced suppression of pulsatile LH secretion could be due to inactivation of GK, located in some cells around the 4V or central canal, because the injection was limited within the 4V or posterior to the 4V, as confirmed by dye injection after the experiment. Previously, we showed that ependymocytes lining the walls of the cerebral ventricles and central canal had pancreatic-type GK-like immunoreactivity [12], and that these ependymocytes also express pancreatic-type GK mRNA (Moriyama et al. submitted for publication).
It is, therefore, likely that alloxan injected into the 4V inhibited GK activity in these ependymocytes and thereby caused suppression of LH release. One hypothesis accounting for our finding is that GK activity in hindbrain ependymocytes might be kept at a high level during euglycemia at which time a normal LH pulse frequency is observed. During hypoglycemia, however, a decrease in GK activity in these cells leads to inhibition of LH pulses, and a suppression of reproductive activity. Such a reproductive response is observed in animals during periods of energy deficiency.

The dose of alloxan used in the present study does not seem to induce damage to the putative glucose-sensing system, probably consisting of GK-containing cells, because injection of 20 µg of alloxan did not impair daily food intake for a week after injection (Fig. 3). In addition, glucoprivic feeding in response to injection of 2DG was similar to that observed in vehicle-treated rats when measured one week after injection of the 20-µg alloxan dose. Ritter et al. [18] previously reported that glucoprivic feeding was impaired by fourth ventricular alloxan injection using a larger dose (40 µg) in male rats, and concluded that cells involved in the glucoprivic control of feeding can be selectively and permanently damaged by intracerebroventricular alloxan administration. The same group reported that 20 µg of alloxan injected acutely into the 4V induced feeding behavior but did not change the glucoprivic feeding response in rats [22]. Thus, alloxan injection at the dose of 20 µg in the present study probably did not cause any permanent damage to the putative glucose-sensing cells in the 4V, but acutely inhibited GK activity in those cells, thereby mimicking a hypoglycemic condition.

The inhibition of LH pulses in the present study was accompanied by increased plasma corticosterone levels suggesting that inhibition of 4V GK activity by alloxan activated the hypothalamo-pituitary-adrenal (HPA) axis. Thus,
the suppression of pulsatile LH secretion by alloxan could be due to activation of the HPA axis. Our previous results suggest that fasting and acute glucoprivation both suppress LH secretion through increased noradrenaline (NA) release in the hypothalamic paraventricular nucleus (PVN) [25, 26] and hypothalamic corticotropin-releasing hormone (CRH) release in female rats [25, 27]. Local NA administration into the PVN strongly suppresses LH pulses via endogenous CRH release in female rats [25]. Therefore, we hypothesize that 4V alloxan injections acutely inhibit GK activity in the ependymocytes lining the ventricle and thereby mimic an energy deficient condition that stimulates noradrenergic neurons located in the hindbrain. Activation of NA neurons in such brainstem regions as the solitary tract nucleus (NTS) causes NA release in the PVN and subsequent CRH release, followed by suppression of LH secretion during the period of energy deficiency.

In the present experiment, 4V injection of alloxan at any dose did not cause hyperglycemia (Fig. 2A). These results are consistent with a previous study [22] showing that 20 µg alloxan injection into the 4V did not alter blood glucose levels. Taken together, these findings suggest that glucose-sensing cells involved in glucoprivic suppression of pulsatile LH secretion and the acute feeding response to glucoprivation may be different from those responsible for glucose homeostasis.

In conclusion, acute suppression of GK activity pulsatile LH secretion in female rats, supports the notion that GK-immunopositive ependymocytes play a role in regulation of LH secretion, probably by sensing glucose levels within the cerebrospinal fluid.

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