Inhibition of LH secretion by localized administration of estrogen is enhanced in the ventromedial hypothalamus during feed restriction in the young weather

McManus, C. J., Goodman, R. L., Llanza, N. V., Valent, M., Dobbins, A. B., Connors, J. M., Hileman, S. M.
Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV 26506-9229 USA

Introduction

Inadequate energy availability results in a reversible suppression of the reproductive axis with inhibition of GnRH release the primary mechanism [1-4]. One of the ways by which reproductive function is compromised during undernutrition is through enhanced negative feedback potency of gonadal steroids. For example, estradiol is extremely effective in suppressing LH secretion in undernourished ewes [5] or feed-deprived castrated male sheep (wethers) [6]. Similarly, feed restriction in wethers causes a small suppression of LH secretion that is enhanced by estrogen infusion [7]. The locus for enhanced responsiveness to steroid negative feedback is probably not the GnRH neurons themselves since few GnRH neurons contain estrogen receptors of the α-subtype (ERα) [8-9] or androgen receptors (AR) [10] and although ER of the β-subtype and estrogen-related receptors have been found to be expressed in some GnRH neurons [11-13], there is no evidence implicating these receptors in the control of GnRH release. Thus, steroid negative feedback probably is conveyed to the GnRH neurons via afferents [14].
Two hypothalamic areas in which changes in responsiveness to steroid negative feedback may occur are the preoptic area (POA) and the ventromedial hypothalamus (VMH). These regions have been identified as areas in which significant changes in the expression of ER occur in response to feed restriction [15-18]. In the sheep, the POA contains the majority of GnRH cell bodies [19]. The VMH has a possible involvement in the regulation of feed intake, control of tonic LH release [20], and control of some reproductive behaviors [21]. Thus, the POA and VMH may play an important role in the enhancement of steroid negative feedback during negative energy balance.
In order to determine if the POA and/or VMH are sites at which enhancement of the responsiveness to steroid negative feedback occurs during feed restriction, we administered estradiol-17β (E) locally through microimplantation via chronic guide tubes directed to the POA or VMH. Recent data suggest that the suppression of the reproductive axis occurs through the central inhibition of GnRH neurons [22] and that feed-restriction increases dopamine receptor function [23]. Therefore, in order to examine the role of dopamine in the response to localized steroid administration to the POA and VMH, we administered the dopamine-D_2 receptor antagonist sulpiride to see if the expected suppression of LH in steroid-treated, feed-restricted wethers involved dopaminergic input.

Materials and Methods

All procedures were approved by the West Virginia University Animal Care and Use Committee and follow NIH guidelines for use of animals in research. Long-term castrated male sheep of predominantly Suffolk breeding and approximately 20 wks of age were used. They were maintained in an indoor facility with daylength adjusted to approximate natural daylength, temperature maintained between 15-23°C, and access to water and a daily alfalfa pellet and corn ration. Bilateral 18-G stainless steel guide tubes (GT) were stereotaxically placed as previously described [24-26] 2 mm dorsal to the target sites for microimplants (POA: 1.5 mm lateral to midline, 3 mm dorsal to supraoptic recess of the third ventricle, at the rostral point of this recess in the AP plane, n=13; VMH: 2 mm lateral to midline, 4 mm dorsal to floor of the third ventricle, 1 - 2 mm anterior to the most anterior portion of the infundibular recess in the AP plane, n=13). GT were blocked with 22-G wire stylets and dental acrylic was applied for anchorage and protected with a plastic cap [24].

After at least 12 d of recovery from surgery, during which time animals were fed according to NRC requirements for maintenance [27], wethers were assigned within hypothalamic area to be restricted (R) or fed (F) with the mean weight of all groups approximately equal. Thus, four treatments were used: POA-R (n=7), POA-F (n=6), VMH-R (n=7), and VMH-F (n=6). R animals were fed to lose 15% of initial body weight over 8 wks. Animals were weighed weekly and diets adjusted accordingly. We previously demonstrated that a similar feeding regimen resulted in a steroid-independent suppression of LH pulse frequency after 7 wks and body weight had decreased over 15% from initial body weight in young wethers [28]. Therefore, steroid treatment began on d 42 of feed restriction to correspond to a time when the wethers should exhibit enhanced sensitivity to steroid negative feedback, but before animals lost enough weight to cause steroid-independent suppression of the reproductive axis. Peripheral blood samples were collected via jugular venipuncture at 12-minute intervals, a frequency of blood collection based on previous experience to allow for easy identification of LH pulses [29-30]. Blood samples were collected for 4 hrs on d 42 as a pre-treatment control period. Immediately after this blood sample collection, animals received microimplants consisting of sterile 22-G blunt-ended stainless steel tubes that extended 1 mm beyond the GT and into which had been tamped crystalline E (Sigma, St. Louis, MO) or cholesterol (Sigma, St. Louis, MO; control; C). Microimplants were tamped in steroid 50 times and their exterior was wiped clean with sterile gauze [24]. The implants were placed into neural tissue for 3 d with blood samples collected on the last day (d 45; 6 hrs), and then implants were removed and replaced with sterilized 22-gauge wire stylets. Blood samples were collected after 3 d of no treatment (d 48; 4 hrs). Animals then received E or C (depending on their prior treatment) for 3 d until sample collection on d 51 (6 hrs) was completed. Implants were then removed, replaced with wire stylets, and samples collected for 4 hrs on d 54. On d 55, animals were sacrificed for histological verification of GT implantation sites.

In order to determine if dopamine was involved in the steroid-induced suppression of LH during feed restriction, the dopamine-D2 receptor antagonist sulpiride was administered (1.2 mg/kg, i.m.) to all wethers after 4 hrs of frequent blood sample collection on d 45 and 51 of feed restriction [24]. Frequent sample collection continued for an additional 2 hrs after the administration of sulpiride (for a total of 6 hrs of blood collection).

Histological verification of the implantation sites was performed as described previously [24]. Histological analysis indicated that 5 of the 6 POA-F wethers, 7 of the 7 POA-R wethers, 5 of 6 VMH-F wethers, and 5 of 7 VMH-R wethers had correct placements of the GT. One VMH-R wether had an incorrect placement; histological preparations from the remaining animals were not available. Data from these animals were not included in the analyses.

Concentration of LH was determined by radioimmunoassay [24-25] and a pulse of LH was defined as previously described [24]. Significant effects of hormone treatment and feeding regimen on LH and body weight were identified using two-way ANOVA for repeated measures and paired Student's t-tests. Analysis of the effects of sulpiride was conducted via two-way ANOVA and Student's t-test for the 2 hrs periods prior to and after administration of sulpiride. Results are presented as mean ± SEM.

Results and Discussion

Mean body weight was lower in POA-R than POA-F by week 5 of restriction ($P = 0.041$) and lower in VMH-R than VMH-F by week 3 ($P = 0.009$). Differences in mean body weight between the F and R groups for each placement remained significant ($P < 0.05$) for the remainder of the experiment.

Localized administration of E to the POA of R wethers was associated with a decrease in the mean number of LH pulses/4 hrs as compared to F wethers during treatment with E-containing microimplants ($P = 0.049$). However, an overall difference between POA-F and POA-R groups ($P = 0.031$) without a group by treatment interaction ($P = 0.718$) was also observed, indicating that LH pulsatility was reduced by feed restriction per se in addition to steroid treatment. Localized administration of E to the VMH caused a decrease in the number of LH pulses/4 hrs in R, but not F, wethers ($P = 0.020$). A significant decrease in LH pulse frequency was also observed in R wethers during E treatment as compared to the respective untreated sampling period ($P = 0.039$). No difference in the number of LH pulses/4 hrs was found between VMH-F and VMH-R in response to C treatment or during any of the control blood collections ($P > 0.05$).

Sulpiride treatment did not stimulate LH secretion in R-, E-implanted wethers. The difference in pulse frequency in response to sulpiride administration was similar between C + Sulpiride and E + Sulpiride for all groups ($P > 0.05$) indicating that the suppression of mean LH in response to localized E administration in R animals was not increased by the administration of the dopamine antagonist. Sulpiride treatment also did not reverse the steroid-independent suppression of LH in the POA-R wethers.

The results of this experiment indicate that the VMH is a site in which the responsiveness to E-negative feedback is enhanced during feed restriction in young castrate male sheep. LH pulse frequency was clearly decreased in response to localized administration of E to the VMH in R, but not F, wethers. This enhanced responsiveness was specific to E since no change in LH pulse frequency occurred in either VMH-R or VMH-F wethers in response to localized administration of C. The mechanism by which this enhancement of responsiveness to E-negative feedback occurs is currently unknown. One potential mechanism is that the number of cells expressing ERα may be altered in response to

changes in energy balance. We are currently investigating the possibility that a change in the expression of ERα also occurs within the VMH of the R wether.
We observed a significant decrease in LH pulse frequency in POA-R wethers compared to POA-F wethers in response to localized administration of E. Because an overall decrease in the LH pulse frequency was detected in POA-R as compared to POA-F wethers during control treatment periods, it is difficult to separate steroid-dependent effects from steroid-independent suppression of the reproductive axis. Both E-treated and untreated ovariectomized, growth restricted lambs show low levels of serum LH [5] and inhibition of GnRH pulsatility by undernutrition is evident in the absence of ovarian steroids [31]. This has made the assessment of the steroid-dependent effects of nutrient restriction more difficult [7]. Whether steroid-dependent and steroid-independent suppression occurs via similar, separate, or overlapping mechanisms remains to be determined.
The chemical identity of the estrogen-responsive neurons in the POA and VMH which change responsiveness to steroid negative feedback in response to changes in energy balance have yet to be identified. We tested if the suppressive effect of E in feed-restricted wethers could be overcome by an injection of the dopamine-D2 receptor antagonist sulpiride. In the current experiment, no increase in LH pulse frequency was observed in response to sulpiride administration, indicating that the system involved in the enhanced responsiveness to E-negative feedback during negative energy balance does not involve the D2 receptor, which has been implicated in the inhibition of LH during seasonal anestrus in the ewe [32]. Double-labeling immunocytochemistry has shown that substantial numbers of ERα-immunoreactive cells within the POA of ewes contain the inhibitory neurotransmitter gamma amino-butyric acid (GABA) while the ERα-immunoreactive cells in the ventromedial nucleus of the ewe and the AR-immunoreactive cells in ventromedial nucleus of the ram contain the inhibitory neuropeptide somatostatin [33]. The role of these neurochemicals, along with others, in the suppression of the reproductive axis in conditions of negative energy balance has yet to be fully elucidated.
In conclusion, E acts locally within the VMH to cause a suppression of LH (and presumably GnRH) during feed restriction but not under conditions of adequate feed intake in the wether. E may act locally in the POA to cause a suppression of LH under conditions of feed restriction, but definite conclusions cannot be drawn because differences in LH pulse frequency were observed which may represent steroid-independent effects of feed restriction. Further studies are required to identify the neurochemical nature of the inhibitory signal(s) that suppress GnRH secretion during negative energy balance.

References

Booth PJ. J Reprod Fert 1990; Suppl 40: 89-100.
Bronson FH. Mammalian Reproductive Biology. Chicago: The University of Chicago Press; 1989.
I'Anson H, Foster DL, Foxcroft GR, Booth PR. Oxford Rev Reprod Biol 1991; 13: 239-311.
Schillo KK. J Anim Sci 1992; 70: 1271-1282.
Foster DL, Olster DH. Endocrinol 1985; 116: 375-381.

Hileman SM, McManus CJ, Kang L, Llanza NV, Dobbins A, Valent M. In: Program for the 37th annual meeting of the Society for the Study of Reproduction; 2004; Vancouver, BC. Abstract 64.
Beckett JL, Sakurai H, Famula TR, Adams TE. Biol Reprod 1997; 57: 408-414.
Herbison AE, Robinson JE, Skinner DC. Neuroendocrinol 1993; 57: 751-759.
Lehman MN, Karsch FJ. Endocrinol 1993; 133: 4443-4450.
Herbison AE, Skinner DC, Robinson JE, King IS. Neuroendocrinol 1996; 63: 120-131.
Hrabovszky EL, Steinhauser A, Barabas K, Shughrue PJ, Petersen SL, Merchenthaler I, Liposits Z. Endocrinol 2001; 142: 3261-3264
Herbison AE, Skynner MJ, Sim JA. Endocrinology 2001; 142: 492-493.
Legan SJ, Tsai H-W. J Neuroendocrinol 2003; 15: 1164-1170.
Lehman MN, Goodman, RL, Karsch FJ, Jackson GL, Berriman SJ, Jansen, HT. Brain Res Bull 1997; 44: 445-457.
Hileman SM, Lubbers LS, Jansen HT, Lehman MN. Neuroendocrinol 1999; 69: 430-437.
Li H-Y, Wade GN, Blaustein JD. Endocrinol 1994; 135: 240-247.
Roemmich JN, Li X, Rogol AD, Rissman EF. Endocrinol 1997; 138: 5366-5373.
Estachio MAC, Tsukamura H, Yamada S, Tsukahara S, Hirunagi K, Maeda K-I. Neurosci Letters 1996; 208: 25-28.
Lehman MN, Robinson JE, Karsch FJ, Silverman AJ. J Comp Neurol 1986; 244: 19-35.
Scott CJ; Kuehl DE, Ferreira SA, Jackson GL. Endocrinol 1997; 138: 3686-3694.
Blache D, Fabre-Nys CJ, Venier G. Brain Res 1991; 546: 241-249.
I'Anson H, Manning JM, Herbosa CG, Pelt J, Friedman CR, Wood RI, Bucholtz DC, Foster DL. Endocrinol 2000; 141: 520-527.
Carr KD, Tsimberg Y, Berman Y, Yamamoto N. Neurosci 2003; 119: 1157-1167.
Anderson GM, Connors JM, Hardy SL, Valent M, Goodman RL. J Neuroendocrinol 2001; 13: 1051-1058.
Havern RL, Whisnant CS, Goodman RL. Endocrinol 1994; 134: 1905-1914.
Coolen LM, Jansen HT, Goodman RL, Wood RI, Lehman MN. J Neurosci Meth 1999; 91: 1-8.
National Research Council. Nutrient requirements for sheep. Washington, DC: National Academy Press; 1985.
McManus CJ, Valent M, Llanza NV, Goodman RL, Hileman SM. In: Program for the 36th annual meeting of the Society for the Study of Reproduction; 2003; Cincinnati, OH. Abstract 430.
Goodman RL, Karsch FJ. Endocrinol 1980; 107: 1286-1290.
Goodman RL, Parfitt DB, Evans NP, Dahl GE, Karsch FJ. Endocrinol 1995; 136: 2412-2420.
Foster DL, Ebling FJP, Micka AF, Vannerson LA, Bucholtz DC, Wood RI, Suttie JM, Fenner DE. Endocrinol 1989; 125: 342-350.
Bertrand F, Thiery J-C, Picard S, Malpaux B. J Endocrinol 1999; 163: 243-254.
Herbison AE. J Reprod Fert 1995; Suppl 49: 271-283.