Neuropeptides xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Effects of estradiol on lactoprivic signaling of the hindbrain upon the contraregulatory hormonal response and metabolic neuropeptide synthesis in hypoglycemic female rats

Santosh K. Mandal, Prem K. Shrestha, Fahaad S.H. Alenazi, Manita Shakya, Hussain N. Alhamami, Karen P. Briski*

Department of Basic Pharmaceutical Sciences, School of Pharmacy, College of Health and Pharmaceutical Sciences, University of Louisiana at Monroe, Monroe, LA 71201, United States

ARTICLE INFO

Keywords:
Estradiol
L-lactate
Norepinephrine
RFamide-related peptide-1/-3, neuropeptide Y
Pre-pro-kisspeptin
Nitric oxide synthase

ABSTRACT

Background: Caudal dorsomedial hindbrain detection of hypoglycemia-associated lactoprivation regulates glucose counter-regulation in male rats. In females, estradiol (E) determines hypothalamic neuroanatomical and molecular foci of hindbrain energy sensor activation. This study investigated the hypothesis that E signal strength governs metabolic neuropeptide and counter-regulatory hormone responses to hindbrain lactoprivic stimuli in hypoglycemic female rats.

Methods: Ovariectomized animals were implanted with E-filled silastic capsules [30 (E-30) or 300 μg (E-300)/mL] to replicate plasma concentrations at estrous cycle nadir versus peak levels. E-30 and E-300 rats were injected with insulin or vehicle following initiation of continuous caudal fourth ventricular L-lactate infusion.

Results: Hypoglycemic hypercorticosteronemia was greater in E-30 versus E-300 animals. Glucagon and corticosterone outflow was correspondingly fully or partially reversed by hindbrain lactate infusion. Insulin-injected rats exhibited lactate-reversible augmentation of norepinephrine (NE) accumulation in all preoptic/hypothalamic structures examined, excluding the dorsomedial hypothalamic nucleus (DMH) where hindbrain lactate infusion either suppressed (E-30) or enhanced (E-300) NE content. Expression profiles of hypoglycemia-reactive metabolic neuropeptides were normalized (with greater efficacy in E-300 animals) by lactate infusion. DMH RFamide-related peptide-1 and -3, arcuate neuropeptide Y and kisspeptin, and ventromedial nucleus nitric oxide synthase protein responses to hypoglycemia were E dosage-dependent.

Conclusions: Distinct physiological patterns of E secretion characteristic of the female rat estrous cycle elicit differential corticosterone outflow during hypoglycemia, and establish both common and different hypothalamic metabolic neurotransmitter targets of hindbrain lactate deficit signaling. Outcomes emphasize a need for insight on systems-level organization, interaction, and involvement of E signal strength-sensitive neuropeptides in counter-regulatory functions.

1. Introduction

Insulin-induced hypoglycemia (IIH) is a recurring complication of rigorous pharmacotherapeutic management of type I diabetes mellitus that deprives the brain of sufficient energy fuel provision. The nervous system responds to hypoglycemia by hypothalamic coordination of counter-balancing autonomic, neuroendocrine, and behavioral outflow that alleviates neuro-glucopenia. The hypothalamus is informed of neuro-metabolic instability by specialized intra- and extra-hypothalamic neuron populations that adjust synaptic firing in reaction to

Abbreviations: aCSF, artificial cerebrospinal fluid; AICAR, 5-aminoimidazole-4-carboxamide-riboside; AMPK, 5′ adenosine monophosphate-activated protein kinase; ARH, arcuate hypothalamic nucleus; AVPV, anteroventral periventricular nucleus; CRH, corticotropin-releasing hormone; CV4, caudal fourth ventricle; DMH, dorsomedial hypothalamic nucleus; E, estradiol; $GAD_{65/67}$, glutamate decarboxylase$_{65/67}$; GnRH, gonadotropin releasing hormone; IIH, insulin-induced hypoglycemia; INS, insulin; LH, luteinizing hormone; LHA, lateral hypothalamic area; MCH, melanin-concentrating hormone; MPN, medial preoptic nucleus; NE, norepinephrine; nNOS, neuronal nitric oxide synthase; NPH, neutral protamine Hagedorn insulin; NPY, neuropeptide Y; ORX-A, orexin A; OVX, ovariectomy; pAMPK, phosphoAMPK; PVH, paraventricular hypothalamic nucleus; POMC, pro-opiomelanocortin; RFRP-1/-3, RFamide-related peptide-1/-3; RIIH, recurrent insulin-induced hypoglycemia; rPO, rostral preoptic area; *sc*, subcutaneous; SDS, sodium dodecyl sulfate; TBS, tris-buffered saline; VMH, ventromedial hypothalamic nucleus

* Corresponding author at: Department of Basic Pharmaceutical Sciences, School of Pharmacy, College of Health and Pharmaceutical Sciences, University of Louisiana at Monroe, 356 Pharmacy Bienville Building, 1800 Bienville Drive, Monroe, LA 71201, United States.

E-mail address: briski@ulm.edu (K.P. Briski).

https://doi.org/10.1016/j.npep.2018.05.004
Received 6 December 2017; Received in revised form 27 March 2018; Accepted 9 May 2018

diminished energy substrate availability. The caudal dorsomedial hindbrain is a vital source of metabolic sensory input as local deficits of the oxidizable glycolytic end-product L-lactate trigger neural mechanisms that elevate blood glucose (Patil and Briski, 2005). Lactate delivery to this area during hypoglycemia intensifies glucose decrements while normalizing hypothalamic hypoglycemia-sensitive metabolic neuropeptide expression, which denotes influence of local energy status on downstream hypothalamic elements of the brain gluco-regulatory network (Gujar et al., 2014). Caudal hindbrain A2 noradrenergic neurons likely detect energetic sequelae of hypoglycemia as 5′ adenosine monophosphate-activated protein kinase (AMPK) is activated in a lactate-reversible manner in this (but not other) hindbrain catecholamine cell group, alongside augmented hypothalamic norepinephrine (NE) levels (Shrestha et al., 2014).

Estradiol (E) controls metabolic status in female mammals through regulation of energy procurement, ingestion, metabolism, partitioning, storage, and expenditure (Wade and Schneider, 1992). A2 cells express estrogen receptor-alpha and -beta proteins (Ibrahim et al., 2013) and metabolo-sensory biomarkers, e.g. glucokinase, K_{ATP}, and AMPK (Briski et al., 2009; Cherian and Briski, 2011; Ibrahim et al., 2013), which supports a likely function to input E influence on the gluco-regulatory network. E governs A2 neuron and hypothalamic nucleus AMPK, hypothalamic metabolic neurotransmitter, and counter-regulatory hormone responses to caudal hindbrain delivery of the AMP mimic 5-aminoimidazole-4-carboxamide-riboside (AICAR) in ovariectomized (OVX) female rats (Ibrahim et al., 2013; Alenazi et al., 2014; Ibrahim and Briski, 2014). Endogenous E secretion fluctuates over the female rat estrous cycle; a 4- to 5-fold mid-cycle rise in plasma hormone levels from baseline (metestrus-/diestrus I-stage) to peak concentrations (proestrus-stage) (Butcher et al., 1974) transforms E feedback to the hypothalamic-pituitary-gonadal (HPG) axis from positive to negative. This project addressed the premise that opposite extremes of estrous cycle E secretion establish unique forebrain neuroanatomical and molecular foci of IIH-associated hindbrain lactoprivic signaling in female rats, and that disparities variation may coincide with differential counter-regulatory hormone outflow. OVX animals were implanted with subcutaneous (*sc*) E-filled silastic capsules designed to replicate circulating E levels at estrous cycle baseline versus maximal concentrations (Goodman, 1978; Briski et al., 2001) in advance of *sc* insulin injection and concurrent caudal fourth ventricular infusion of artificial cerebrospinal fluid with or without L-lactate. Hypothalamic gluco-regulatory structures characterized by hindbrain lactoprivic-driven augmentation of NE accretion in hypoglycemic male rats (Shrestha et al., 2014) were micropunch-dissected for norepinephrine (NE) ELISA and Western blot analyses of relevant gluco-regulatory neurotransmitter protein expression, including glutamate decarboxylate$_{65/67}$ ($GAD_{65/67}$) [ventromedial nucleus (VMH)], corticotropin-releasing hormone (CRH) [paraventricular nucleus (PVH)], neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) [arcuate nucleus (ARH)] and orexin-A (ORX-A) [lateral hypothalamic area (LHA)]. Reproduction is tightly coupled to metabolic status, as gonadotropin-releasing hormone (GnRH) release is diminished by energy shortage (Clarke et al., 1990; Chen et al., 1992a; Chen et al., 1992b; Heisler et al., 1993; Singh and Briski, 2005). IIH inhibits gonadotropin secretion in female mammals (Clarke et al., 1990; Goubillon and Thalabard, 1996; Cagampang et al., 1997; He et al., 1999; Lado-Abeal et al., 2002). A corollary aim of this study was to investigate the impact of E signal strength on lactoprivic regulation of NE activity and reproduction-relevant neurotransmitter protein expression in preoptic loci involved in female reproduction, e.g. rostral preoptic area (rPO) [gonadotropin-releasing hormone (GnRH)], anteroventral periventricular nucleus (AVPV) [prepro-kisspeptin], and medial preoptic nucleus (MPN), [$GAD_{65/67}$].

2. Methods and materials

2.1. Animals

Adult female Sprague-Dawley rats (3–4 months of age) were maintained in groups (2–3 animals per cage) under a 14-h light/10-h dark lighting schedule (light on at 05:00 h), while allowed free access to standard laboratory rat chow (Harlan Teklad LM-485; Harlan Industries, Madison, WI, USA) and tap water. Animals were accustomed to daily handling for a minimum of one week before surgery. All protocols were conducted in accordance with NIH guidelines for care and use of laboratory animals, under ULM Institutional Animal Care and Use Committee approval.

2.2. Experimental design

On day 1, rats were bilaterally OVX and implanted with a 26-gauge stainless-steel cannula guide (Prod no. C315G/SPC; Plastic One, Inc., Roanoke, VA) aimed at the caudal fourth ventricle (CV4) [Coordinates: 0 mm lateral to midline; 13.3 mm posterior to *bregma*; 6.1 mm ventral to skull surface] under ketamine/xylazine anesthesia (0.1 mL/100 g *bw ip*, 90 mg ketamine: 10 mg xylazine/mL; Henry Schein, Melville, NY). After surgeries, animals were treated by intramuscular injection of enrofloxacin (baytril 2.27%; 10 mg/kg) and subcutaneous (*sc*) injection of ketoprofen (3 mg/kg), then transferred to individual cages. On day 7, rats received a *sc* silastic capsule (10 mm/100 g *bw*, 0.062 in. i.d, 0.125 in. o.d.) filled with 30 [E-30] or 300 [E-300] μg estradiol benzoate/mL safflower oil under isoflurane anesthesia. Our studies (Briski et al., 2001) show that these disparate E doses replicate circulating physiological hormone levels measured on metestrus versus proestrus, respectively, in 4-day cycling rats (Butcher et al., 1974). On day 11, E-30 and E-300 animals were each divided into 3 treatment groups ($n = 5$/group). Continuous intra-CV4 infusion of artificial cerebrospinal fluid (aCSF; groups 1 and 2) or aCSF containing L-lactate (L; 25 μM/2.0 μL/h (Patil and Briski, 2005); group 3) was performed between 08.50 and 11.00 h, using 33-gauge 0.5 mm-projecting internal injection cannulas (prod. no. C315I/SPC; Plastics One). At 09.00 h, rats in group 1 were injection *sc* with sterile vehicle (V; Eli Lilly & Co., Indianapolis, IN), while animals in groups 2 and 3 were treated by injection of neutral protamine Hagedorn insulin (INS; 12.5 U/kg *bw* (Paranjape and Briski, 2005); Henry Schein). Animals were sacrificed by decapitation at 11.00 h for brain and blood collection. Dissected brains were immediately snap-frozen in liquid nitrogen-cooled isopentane and stored at −80 °C. Plasma was obtained by immediate centrifugation and stored at −20 °C.

2.3. Western blot analysis of AMPK, phosphoAMPK (pAMPK), and metabolic neurotransmitter protein expression in hypothalamic gluco-regulatory loci

Forebrains were cut into serial 100 μm-thick frozen sections. The rPO (+0.48 to 0.00 mm), AVPV (0.00 to −0.30 mm), MPN (−0.20 to −0.06 mm), ARH (−2.00 to −3.20 mm), VMH (−2.00 to −3.20 mm), DMH (−2.40 to −3.20 mm), and LHA (−2.40 to −3.60 mm) were separately micro-punch dissected from the right hemi-forebrain, over pre-determined distances relative to *bregma*, and collected into separate 20 μL volumes of lysis buffer [2.0% sodium dodecyl sulfate (SDS), 0.05 M dithiothreitol, 10.0% glycerol, 1.0 mM EDTA, 60 mM Tris-HCl, pH 7.2]. Tissue samples were obtained using Stoelting (Kiel, WI) calibrated hollow punch tools of 0.50 mm (rPO, AVPV, MPN, ARH, VMH, DMH) or 0.76 mm (LHA) diameter. For each treatment group, heat-denatured tissue aliquots from individual subjects were combined and separated on 10–15% gradient Tris-glycine gels (90 V, 105 min; Tris-glycine SDS running buffer) (Cherian and Briski, 2011, 2012). Proteins were transblotted (30 V, overnight at 4 °C; Towbin buffer) to 0.45-μm PVDF membranes (Osmonics, Gloucester, MA). Membranes were