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Role of central glucagon-like peptide-1 in hypothalamo-pituitary-adrenocortical facilitation following chronic stress

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Abstract

Central glucagon-like peptide-1 (GLP-1) regulates food intake, glucose homeostasis, and behavioral and neuroendocrine responses to acute stress. Given its pronounced role in acute stress regulation, the GLP-1 system is a prime candidate for mediating the prolonged drive of the hypothalamo-pituitary-adrenocortical axis by chronic stress. To test this hypothesis, we evaluated the necessity and sufficiency of GLP-1 for production of chronic stress-induced changes in HPA axis function. Exogenous GLP-1 or the GLP-1 receptor antagonist, dHG-exendin, were delivered into the 3rd ventricle of control animals or animals exposed to chronic variable stress (CVS) for 7 days. Animals in the CVS groups received GLP-1 or dHG-exendin immediately prior to each stress exposure. Prior to and at the end of the 7-day trial, chronically-stressed animals were subjected to a novel stressor to test for HPA axis facilitation. Neither GLP-1 nor dHG-exendin affected CVS-associated increases in adrenal weight or decreases in basal plasma glucose levels. In addition, neither exogenous GLP-1 nor dHG-exendin altered any index of HPA axis activity in unstressed rats. However, GLP-1 enhanced CVS-induced facilitation of corticosterone (but not ACTH) response to an acute stress, whereas dHG-exendin inhibited facilitation. In addition, GLP-1 decreased body weight in chronically-stressed animals. dHG-exendin increased food intake and body weight in unstressed animals, consistent with a tonic role for GLP-1 in body weight regulation. Overall, our data suggest that brain GLP-1 modulates HPA axis activity within the context of chronic stress, perhaps at the level of the adrenal gland.

Keywords: ACTH; Chronic variable stress; Corticosterone; Glucocorticoids; Exendin; Intracerebroventricular injection; Body weight; Food intake

Introduction

The hypothalamo-pituitary-adrenocortical (HPA) axis coordinates the release of glucocorticoids in response to stress. The HPA axis responds to real or anticipated homeostatic disruption by stimulating release of ACTH secretagogs (such as corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP)) from hypophysiotrophic neurons in the medial parvocellular region of paraventricular nucleus of hypothalamus (PVN) (Antoni, 1986; Whitnall, 1993). This neurohemal signal promotes release of adrenocorticotropic hormone (ACTH) by pituitary corticotrophs, which consequently causes a release of adrenal corticosteroids.

Ascending brainstem systems play a major role in excitation of HPA axis stress responses (c.f., (Herman et al., 2003; Sawchenko et al., 2000)). Recent studies from our group suggest that glucagon-like peptide-1 (GLP-1), a neuropeptide that is selectively expressed in the nucleus of solitary tract (NTS) and the ventrolateral medulla (Drucker, 1990; Kinzig et al., 2003; Merchenthaler et al., 1999), plays a major role in HPA activation (Kinzig et al., 2003). The PVN is heavily innervated by GLP-1 fibers that form direct synaptic contacts with CRH immunoreactive cell bodies (Larsen et al., 1997; Rinaman, 1999; Sarkar et al., 2003; Shughrue et al., 1996), confirming that GLP-1 is in position to directly modulate activity of CRH neurons. Intracerebroventricular infusions of GLP-1 increase plasma ACTH and/or corticosterone (Kinzig et al., 2003; Larsen et al., 1997), indicating that GLP-1 receptor binding is sufficient to trigger HPA activation. Furthermore, pretreatment of GLP-1

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antagonist attenuated ACTH and corticosterone responses induced by systemic lithium chloride (LiCl) injection and elevated platform exposure (Kinzig et al., 2003), indicating that GLP-1 signaling is also necessary for acute HPA axis stress responses. GLP-1 containing neurons in the NTS are activated by visceral stress (LiCl injection) (Rinaman, 1999), consistent with a role in central stress integration. Overall, these data support the hypothesis that GLP-1 regulates HPA axis responses to a variety of stressors by stimulating CRH release from PVN neurons.

To date, the GLP-1 system has been studied exclusively in the realm of acute stress. While activation of the HPA axis is typically an adaptive response to an acute stress, chronic activation of the HPA axis can be deleterious and has been linked to a number of different pathologies, including metabolic disease and depression (McEwen and Stellar, 1993). Recent data strongly suggest that different brain circuitries are involved in acute and chronic stress responses. For example, regions such as the paraventricular thalamus regulate HPA axis responses to chronic, but not acute stressors (Bhatnagar and Dallman, 1998a). In addition, chronic stress produces a well-documented enhancement of HPA axis responses to new stressors, a process that may involve regions such as the paraventricular thalamus(Bhatnagar and Dallman, 1998a) and perhaps central amygdaloid nucleus (Dallman et al., 2003). Consequently, it is critical to determine whether the endogenous GLP-1 system is responsible for deleterious changes in HPA axis function seen following chronic stress. Therefore, the current study was designed to test the role of GLP-1 in the establishment and maintenance of chronic stress-induced HPA hyperactivity, as produced by a well-characterized chronic stress model (chronic variable stress (CVS)).

Materials and methods

Animals

Male Sprague–Dawley rats (275–300 g) were acquired from Harlan Labs (Indianapolis, IN). All animals were individually housed in a temperature- and humidity-controlled facility at the University of Cincinnati, on a 6am to 6pm light-dark cycle with free access to standard chow and water. All experimental procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Surgery

All rats were anesthetized by an intra-peritoneal injection with a cocktail of ketamine (85–95 mg/kg) and xylazine (10–15 mg/kg). Guide cannulas (22 gauge; Plastics One, Inc., Roanoke, VA) were stereotaxically implanted into the 3rd ventricle, in accordance with the atlas of Paxinos and Watson (1998) (−2.2 mm anteroposterior (AP) and −7.5 mm dorsoventral (DV) with respect to bregma). Animals were allowed to recover for 7 days following surgery, at which point cannula placement was verified by administering 20 ng of angiotensin II (American Peptide Company, Inc., Sunnyvale, CA). Rats that failed to show a dipsogenic response to angiotensin II (>10 ml water consumed in 60 min) were removed from the study.

Drug infusion, restraint challenge, and chronic variable stress (CVS)

After surgery, dummy cannulas were removed and replaced every 3rd day for at least two weeks to acclimate animals to handling and maintain cannula patency. In preparation for the study, the frequency of training was increased to twice/daily (9–11am and 3–6pm) for 4 days, followed by vehicle intra 3rd ventricular (i3vt) injection twice/daily for 5 days.

Experimental procedures were initiated following the 5th day of vehicle injection. Animals were weight-matched and divided into 6 groups (6 rats in each group): vehicle-handled, GLP-1-handled, dHG-exendin-handled, vehicle-chronic variable stress (CVS), GLP-1-CVS, and dHG-exendin-CVS. On the morning on day 1, prior to any drug treatment or the initiation of CVS, rats in the CVS group received an initial restraint stress test (pre-CVS restraint) (Fig. 1). An additional group of animals received restraint testing prior to beginning a regimen of vehicle or drug injections in the absence of CVS, to test the ability of GLP-1 or dHG-exedin to alter HPA axis stress responses upon prolonged delivery. Animals were restrained in plastic tubes for 30 min and subsequently returned to their home cages. Blood samples were collected by tail clip (Vahl et al., 2005) at the beginning of the restraint ($t=0$), at the end of the restraint ($t=30$), and at 60 and 120 min after the initiation of restraint ($t=60$, 120, respectively). Plasma was collected and kept frozen at −20 °C until analyzed.

From the afternoon of day 1, all animals received infusion of either vehicle (sterile-filtered artificial cerebrospinal fluid (aCSF)) (2 μl), GLP-1 ((Ser8)-GLP-1 (7–36) amide, 2 μg/2 μl; American Peptide Company, Inc., Sunnyvale, CA), or the GLP-1 receptor antagonist dHG-exendin (des-His_1, Glu_9-exendin-4, 100 μg/2 μl; American Peptide Company, Inc.). GLP-1 and dHG-exendin were dissolved in aCSF and the aliquots were kept frozen at −20 °C until injected.

After injection, animals were kept undisturbed in their home cages for 15–30 min to ensure drug action. Thereafter, CVS groups were subjected to one of five stressors that were selected randomly. Stressors used were warm swim (31–33 °C) for 20 min, cold swim (16–18 °C) for 5 min, hypoxia (8% oxygen) for 30 min, cold exposure (4 °C) for 1 h, or shaking (100 rpm) for 1 h. Control animals did not experience any additional

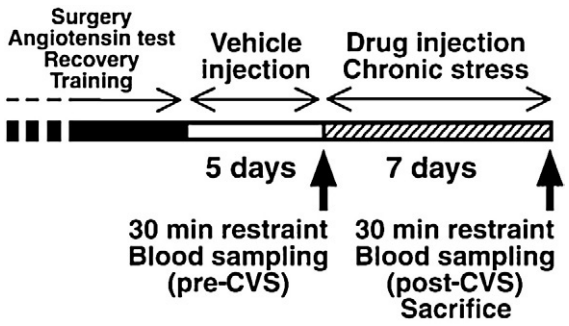

Fig. 1. Experimental design. Pre- and post-CVS acute restraint tests were carried out in the morning of on day 1 (prior to the first stress) and day 8 of CVS/handling, respectively.