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Distribution of glucagon-like peptide-1 immunoreactivity in the hypothalamic paraventricular and supraoptic nuclei

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ABSTRACT

Glucagon-like peptide-1 (GLP-1) plays a role in modulating neuroendocrine and autonomic function. The hypothalamic paraventricular nucleus (PVN) contains aggregations of GLP-1 fibers and expresses GLP-1 receptors, making it a likely site of action for GLP-1 signaling. The current study was designed to establish domains of GLP-1 action, focusing on axosomatic appositions on different neuroendocrine and autonomic cell populations in the PVN. The data indicate abundant GLP-1-immunoreactive terminal appositions on corticotropin-releasing hormone neurons in the medial parvocellular PVN. GLP-1 positive boutons can also be observed in apposition to oxytocinergic neurons and on retrogradely labeled pre-autonomic neurons projecting to the region of the nucleus of the solitary tract. In contrast, there were very few vasopressinergic neurons with GLP-1 appositions. Overall, the data indicate that the central GLP-1 system preferentially targets neurons in hypophysiotrophic zones of the PVN, consistent with excitatory actions of GLP-1 on adrenocorticotropin release. GLP-1 is also in position to influence oxytocin secretion and control outflow to brainstem cardiovascular relays.

1. Introduction

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted from the intestine by nutrient signals, in particular, glucose ingestion (Kieffer and Habener, 1999). Like many 'gut' peptides, GLP-1 is also expressed in brain. Expression of GLP-1 is largely confined to groups of cells in the nucleus of solitary tract (NTS) and ventrolateral medulla of the brainstem (Larsen et al., 1997a,b). These neurons send GLP-1-immunoreactive axons and terminals to numerous regions of the brain, including regions responsible for integration of neuroendocrine stress responses (paraventricular nucleus) and energy balance (arcuate nucleus) (Drucker, 1990; Larsen et al., 1997a,b; Sarkar et al., 2003). The GLP-1 receptor (GLP-1R) is expressed in regions in receipt of GLP-1 fibers, including the PVN, the periventricular hypothalamus, the dorsomedial hypothalamus (DMH), and the arcuate nucleus (Arc) (Merchenthaler et al., 1999; Shughrue et al., 1996; Tang-Christensen et al., 2001).

Taken together, the distribution of GLP-1 and its receptor in brain suggests that central GLP-1 is functioning as a neurotransmitter/neuromodulator in neuroendocrine regulatory systems. This hypothesis is supported by functional data indicating a role for GLP-1 in ingestion and hypothalamo-pituitary-adrenocortical (HPA) axis function. Centrally infused GLP-1 elicits dose dependent suppression of food intake (Navarro et al., 1996; Tang-Christensen et al., 1996; Turton et al., 1996) that may be related to visceral signaling associated with food consumption (Vrang et al., 2003). In addition to its anorectic effects, GLP-1 also activates the hypothalamo-pituitary-adrenal (HPA) stress axis. Intracerebroventricular infusion of GLP-1 stimulates ACTH and/or corticosterone release (Kinzig et al., 2003; Larsen et al., 1997a,b). Induction of visceral illness by peripheral injections of lithium chloride (LiCl) activates GLP-1 expressing neurons in the NTS and the dorsomedial parvocellular region of the PVN (presumably CRH expressing neurons) (Rinaman, 1999). Importantly, the HPA response to LiCl is blocked by pre-administration of the GLP-1 antagonist des-His_1, Glu_8-exendin-4 (dHG-exendin) (Kinzig et al., 2003), suggesting that NTS GLP-1 is responsible for HPA activation induced by this stimulus. Anatomical data indicate that GLP-1 neurons from the NTS innervate the PVN (Larsen et al., 1997a,b; Rinaman, 1999), and form synaptic contacts with CRH-immunoreactive neurons (Sarkar et al., 2003), further consistent with a direct role for GLP-1 in HPA axis signaling. Finally, central GLP-1 increases blood pressure and heart rate, and activates brainstem neurons projecting to sympathetic preganglionic neurons, indicative of stimulatory effects of GLP-1 on the sympathetic nervous system (Yamamoto et al., 2002).

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The data to date imply an important connection between medullary GLP-1 neurons and CRH neurons in the PVN. However, the PVN is an anatomically heterogeneous region, including several subtypes of neurons. In addition to parvocellular CRH neurons, this nucleus contains (1) magnocellular arginine vasopressin neurons projecting to the posterior pituitary; (2) magnocellular oxytocin neurons projecting to the posterior pituitary; and (3) parvocellular pre-autonomic neurons projecting to brainstem and spinal cord sites controlling cardiovascular function and sympathetic/parasympathetic activation (Swanson and Kuypers, 1980). Given diverse physiological actions of central GLP-1, it is important to determine the anatomical relationships between GLP-1 projections and the various functional components of PVN. Therefore, the current study was designed to assess anatomical interactions between GLP-1-immunoreactive fibers and various neural subtypes in the PVN and SON.

2. Materials and methods

Rats (Sprague–Dawley or Long-Evans) were acquired from Harlan Labs (Indianapolis, IN). All animals were triply housed in a temperature- and humidity-controlled facility at the University of Cincinnati, on a 6 a.m. to 6 p.m. 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.

2.1. Antibodies

Immunostaining protocols were performed using previously characterized primary antibodies/antisera. A monoclonal mouse anti-GLP-1 antibody was acquired from Dr. D'Alessio, used at a 1:10000 dilution, using biotinylated tyramide amplification (see below). Rabbit anti-AVP neurophysin (1:30000) and rabbit anti-oxytocin neurophysin (1:10000) antisera were gifts from Dr. Alan Robinson (Roberts et al., 1993). Rabbit anti-CRF (RC70) antiserum (1:10000) was a gift from Dr. Wylie Vale, Salk Institute. Rabbit anti-Fluorogold antiserum (1:10000) was acquired from Chemicon (Catalog# AB153).

2.2. Retrograde tracing

To determine whether GLP-1 axons innervate brainstem-projecting neurons in the PVN, the retrograde tracer Fluorogold (FG; Fluorochrome Inc., Denver, CO) was stereotaxically injected into the region of the NTS (Stern, 2001; Stern and Zhang, 2003). Male Long-Evans rats were anesthetized by intraperitoneal injection of a ketamine–xylazine cocktail (85–95 and 10–15 mg/kg, respectively). FG (2% in saline; 200 nl) was pressure-injected unilaterally into the dorsal vagal complex at the level of the obex. The injection point was 4.8 mm posterior to the interaural line, 1.0 mm lateral to the midline, and 8.0 mm below the dorsal surface of the brain, using the coordinate system of Paxinos and Watson (1998). After 5–7 days of FG injection, animals were sacrificed by overdose of Pentobarbital and perfused with 150 ml phosphate buffered saline (PBS), followed by 200 ml of 4% paraformaldehyde (generated from powdered paraformaldehyde). Brains were removed from the skull, post-fixed in 4% paraformaldehyde overnight, and cryoprotected by immersion in 30% sucrose in PBS. Brains were quickly frozen in powdered dry ice, and sectioned at 25 μm on a sliding microtome (Leica Microsystems Inc., Bannockburn, IL). Slices were placed into cryoprotectant solution (0.1 M phosphate buffer, 30% sucrose, 1% polyvinylpyrrolidone, and 30% ehtyleneglycol) and stored at −20 °C until processed for immunohistochemistry.

2.3. Adrenalectomy

CRH is difficult to visualize by standard immunostaining procedures, since CRH is quickly transported to terminals after synthesis and does not accumulate in perikarya under unstimulated conditions. Therefore, we examined PVN CRH expression following adrenalectomy, a treatment that blocks feedback inhibition of the HPA axis. Adrenalectomy reliably increases CRH immunoreactivity in PVN perikarya (Sawchenko et al., 1984; Wolfson et al., 1985).

Male Sprague–Dawley rats were anesthetized by intraperitoneal injection of an anesthesia cocktail (ketamine, 85–95 mg/kg; xylazine, 10–15 mg/kg). Incisions were made bilaterally and both adrenals were removed. Drinking water was replaced by saline after surgery. After 7 days of recovery, animals were overdosed with Pentobarbital and perfused with 4% paraformaldehyde as noted above.

2.4. Immunostaining

GLP-1, arginine vasopressin (AVP), oxytocin (OT), CRH, and FG were visualized using a standard dual immunofluorescence labeling protocol on floating sections from non-treated animals (GLP-1 and AVP, GLP-1 and OT), FG injected animals (GLP-1 and FG), and adrenalectomized animals (GLP-1 and CRH) (Mueller et al., 2005). Briefly, free floating brain sections were incubated with blocking solution (0.1% bovine serum albumin and 0.2% Triton X-100 in 50 mM KPBS), and incubated in primary antibody solution (GLP-1) overnight. Sections used to visualize FG sites were treated with 0.3% H_2O_2 in 50 mM potassium PBS (KPBS; 40 mM potassium phosphate dibasic, 10 mM potassium phosphate monobasic, and 0.9% sodium chloride) for 10 min to eliminate endogenous peroxidase activities prior to incubation in primary antiserum solution. Sections were then incubated with

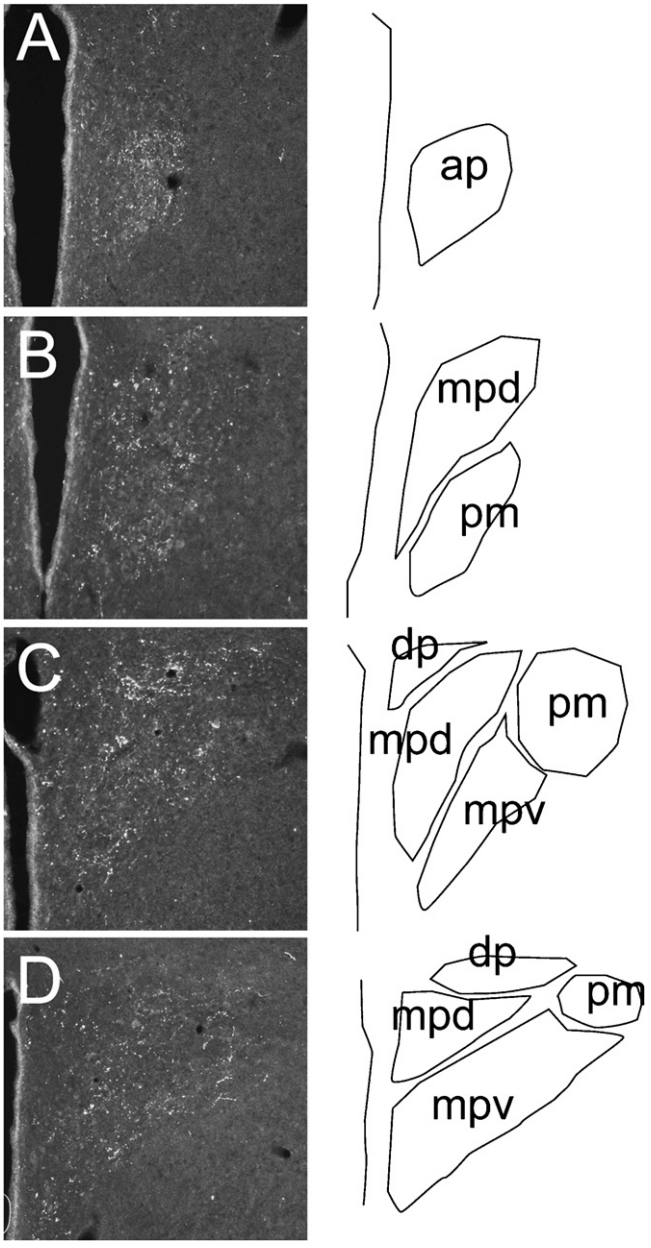

Fig. 1. Distribution of GLP-1 fibers at various rostrocaudal levels of the PVN. Sections in (A–D) were taken at 180 um intervals through the PVN of a single animal, beginning at the level of the anterior parvocellular subdivision (approx. 1.32 mm posterior to Bregma, using the coordinate system of Paxinos and Watson (1998). Schematic delineations of PVN subdivisions are on the right of each image. Note rich fiber plexi in regions rich in hypophysiotrophic neurons, including the anterior parvocellular subdivision (ap) and dorsal region of the medial parvocellular subdivision (mpd). Innervation was also present in pre-autonomic subregions of the PVN (dorsal parvocellular (dp) cell group, ventral division of the medial parvocellular zone (mpv)). Note that innervation of the posterior magnocellular division (pm) was documented in the rostral component of this cell group (B) and at the periphery of this cell group at mid-PVN level (C), corresponding to regions rich in oxytocin neurons.