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Role of dorsal vagal motor nucleus orexin-receptor-1 in glycemic responses to acute versus repeated insulin administration

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Abstract

The potent orexigenic neuropeptide, orexin-A (ORX-A), acts at multiple sites within the central neuroaxis to control autonomic responses to energy imbalance, including the dorsal vagal motor nucleus (DMV), where it regulates pancreatic efferent nerve firing. Recent evidence that recurrent insulin-induced hypoglycemia (RIIH) attenuates lateral hypothalamic ORX-A-ergic neuronal transcriptional activation and prepro-orexin gene expression suggests that this phenotype undergoes functional adaptation to repeated glucoprivation. We examined the hypothesis that RIIH-associated patterns of ORX-A neurotransmission and/or orexin-receptor-1 (OR-1) expression within the DMV may be correlated with exacerbated hypoglycemic and impaired pancreatic counterregulatory responses to repeated insulin administration. Male rats were pretreated by bilateral intra-DMV infusion of the OR-1 antagonist, SB-334867, or vehicle prior to *s.c.* injection of Humulin NPH (NPH), or diluent alone. Other animals were injected with one or four doses of NPH, on as many days, or diluent alone, and pretreated by bilateral intra-DMV administration of graded doses of ORX-A or vehicle on the final day of the study. Effects of acute versus repeated insulin administration on ORX-A and OR-1 protein levels in the microdissected dorsal vagal complex (DVC) were evaluated by radioimmunoassay and Western blot analyses, respectively. SB-334867 treatment prior to acute NPH administration decreased plasma glucose and suppressed peak glucagon secretion, whereas exogenous ORX-A administration prior to RIIH did not reverse amplified patterns of hypoglycemia. RIIH did not alter intra-DVC ORX-A tissue concentrations, but diminished OR-1 levels in that site. These results show that DMV OR-1 function is critical for optimal glucagon secretory responsiveness to acute hypoglycemia, and that RIIH-associated downregulation of receptor expression in that brain site may contribute to impaired restoration of euglycemia. The current data provide unique evidence that ORX-A acts via OR-1-dependent mechanisms within DMV to regulate glucagon counterregulatory function during hypoglycemia, and that decreased receptor-mediated signaling during RIIH may underlie characteristic intensification of hypoglycemia.

Keywords: Dorsal vagal motor nucleus; Orexin-A; OR-1; Recurrent insulin-induced hypoglycemia; Glucagon; Humulin NPH

1. Introduction

Hypoglycemia is a potent threat to neurological function since glucose is the sole substrate fuel that can adequately support vital nerve cell activities. Conventional therapeutic management of insulin-dependent diabetes mellitus (IDDM) is highly correlated with iatrogenic insulin-induced hypoglycemia (IIH) (Cryer and Polonsky, 1997). Diabetic patients utilizing such treatment regimens experience an average of one or two episodes of symptomatic hypoglycemia weekly. Antecedent hypoglycemia is a primary factor in the development of hypoglycemia-associated autonomic failure in IDDM, a pathophysiological condition characterized

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by diminished hypoglycemic awareness and impaired glucose counterregulation (Cryer, 2001). By attenuating compensatory mechanisms for relief of neuroglucopenia, recurring IIH (RIIH) amplifies the potential for hypoglycemic neural dysfunction and injury.

A central component of counterregulatory collapse is supported by evidence for RIIH-associated reductions in neuronal transcriptional activation in key CNS metabolic loci, including the lateral hypothalamic area (LHA). Involvement of the LHA in the regulation of food intake and body weight is well established (Bernardis and Bellinger, 1996). Neurons that synthesize the potent orexigenic neuropeptide, orexin-A (ORX-A), are confined to the LHA and adjacent hypothalamic loci (Sakurai et al., 1998; Broberger et al., 1998; Nambu et al., 1999), and project to numerous structures that control autonomic and/or behavioral responses to energy imbalance, including parasympathetic preganglionic neurons in the dorsal vagal motor nucleus (DMV), the motor component of the dorsal vagal complex (DVC) (Peyron et al., 1998; Date et al., 1999). ORX-A action within the DMV increases pancreatic nerve firing and blood glucose levels, while maximal hypoglycemic induction of vagal pancreatic nerve firing requires DMV orexin-receptor-1 (OR-1) stimulation (Wu et al., 2004). LHA ORX-A neurons exhibit enhanced c*fos* and preproorexin gene expression following IIH (Cai et al., 1999; Griffond et al., 1999; Moriguchi et al., 1999), responses that are diminished by repeated administration (Paranjape et al., in press). At present, there is little information on the involvement of DMV OR-1 in patterns of circulating levels of glucose and the pancreatic counterregulatory hormone, glucagon, during acute hypoglycemia, as well as its role in exacerbated glycemic responses to RIIH. The current studies examined the hypothesis that alterations in DVC OR-1 signaling, due to modified ORX-A neurotransmission and/or OR-1 expression, underlie RIIH-associated reduction in pancreatic glucagon secretion and coincident exacerbation of hypoglycemia.

2. Methods and materials

2.1. Experiment 1: effects of pharmacological blockade of DMV OR-1 on plasma glucose and glucagon profiles following acute insulin administration

Ten days before the experiment, adult male rats (250–300 g bw) were implanted with 26-gauge double-guide cannulas (cat no. C235G-1.0 SPC; Plastics One, Inc., Roanoke, VA) aimed dorsal to DMV [coordinates: AP: −14.0 mm posterior to bregma; L: 0.5 mm; DV: 7.3 mm ventral to skull surface] under ketamine/xylazine anesthesia (0.1 mL/100 g bw, 100 mg ketamine/10 mg xylazine/mL; Henry Schein, Inc., Melville, NY). The animals were implanted with silastic intracardiac venous catheters 48 h before use. At 9 h, the rats were pretreated by bilateral administration of the selective OR-1 antagonist, SB-334867 (Cat. No. 1960; Tocris Bioscience, Ellisville, MO), at a dose of either 2 or 5 μg/500 nL, or the vehicle, propylene glycol (PG) via 33-gauge double internal cannulas (Cat. No. C235I; Plastics One, Inc.) projecting 1.0 mm beyond the cannula guides. At 11 h (time zero), the animals were injected *s.c.* with Humulin Neutral Protamine Hagedorn (NPH, 12.5 U/kg bw; Henry Schein, Inc.) or diluent (Eli Lilly, Inc., Indianapolis, IN). Serial blood samples were collected over a 12 h period for analysis of glucose and glucagon profiles, as previously described (Paranjape and Briski, 2005).

2.2. Experiment 2: effects of intra-DMV administration of exogenous ORX-A on blood glucose profiles during RIIH

Double-guide cannulas were implanted dorsal to the DMV and silastic catheters were inserted into the right jugular vein, 10 and 2 days before the experiment, respectively, as described above. Male rats were divided into six treatment groups, and treated as follows: *group 1*: *s.c.* diluent injection, plus bilateral intra-DMV administration of 500 nL artificial cerebrospinal fluid (aCSF) on day 1–4; *group 2*: *s.c.* diluent injection, plus bilateral intra-DMV administration of 100 nM ORX-A (Cat. No. 06012; Sigma Aldrich, St. Louis, MO) on day 4; *group 3*: *s.c.* injection of diluent on days 1–3 and NPH (12.5U/kg bw) on day 4, plus bilateral intra-DMV administration of a CSF on day 4; *group 4*: *s.c.* NPH injection, plus bilateral intra-DMV administration of aCSF on day 4; *group 5*: *s.c.* NPH injection, plus bilateral intra-DMV administration of 20 pM ORX-A on day 4; and *group 6*: *s.c.* NPH injection, plus bilateral intra-DMV administration of 100 nM ORX-A on day 4. Serial blood samples were obtained over a 12 h period following treatments on day 4, and analyzed for glucose.

2.3. Experiment 3: radioimmunoassay of ORX-A concentrations in microdissected dorsal vagal complex (DVC) tissue

Adult male rats were divided into three treatment groups: *group 1*: *s.c.* diluent injection on days 1–4 (VVVV); *group 2*: *s.c.* diluent on days 1–3 and *s.c.* NPH (12.5 U/kg bw) on day 4 (VVVI); and *group 3*: *s.c.* NPH on days 1–4 (IIII). The animals were sacrificed by decapitation 25 min after injections (11 h) on day 4, and the brains were rapidly dissected and snap frozen in isopentane cooled in dry ice. Each brain was stored at − 80 °C and subsequently cut into serial 300 μM sections in a cryostat. The DVC was microdissected by the Palkovits's punch technique, utilizing neuroanatomical landmarks from *Brain Maps: Structure of the Rat Brain*