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Characterization of the hypotensive effects of glucagon-like peptide-2 in anesthetized rats

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HIGHLIGHTS

- We characterized the mechanisms of GLP-2-induced hypotension.
- The GLP-2-induced maximum ΔMAP was reduced by vagotomy or prazosin, but not propranolol.
- The effects of the i.c.v. but not i.v. injection of GLP-2 were reduced by atropine.
- GLP-2 centrally modulates vagal afferent inputs and inhibits the sympathetic nervous system.

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ABSTRACT

Glucagon-like peptide-2 (GLP-2) is a proglucagon-derived peptide released from enteroendocrine cells and neurons. We recently reported that GLP-2 induced hypotension. In the present study, we characterized the mechanisms of GLP-2-induced hypotension. GLP-2 was administered peripherally or centrally to male Wistar rats anesthetized with urethane and α-chloralose. The rats were vagotomized or systemically pretreated with atropine, prazosin, or propranolol before the GLP-2 administration. The central and peripheral administration of GLP-2 reduced mean arterial blood pressure (MAP). The maximum change of MAP (maximum ΔMAP) was reduced by vagotomy or prazosin, but not propranolol. The effects of the central but not peripheral administration of GLP-2 were reduced by atropine. These results suggest that GLP-2 modulates vagal afferent inputs and inhibits the sympathetic nervous system in the brain to induce hypotension.

1. Introduction

Glucagon-like peptide-2 (GLP-2) is derived from a proglucagon precursor and liberated via tissue-specific post-translational processing in the gut and central nervous system [11]. GLP-2 was identified as a potent intestinotrophic hormone in rodents [20], and enhanced nutrient absorption in rodents and in patients with short bowel syndrome [7]. On the other hand, intracerebroventricular (i.c.v) injection of GLP-2 inhibited food intake in rats [18], and had antidepressant-like effects in the forced swim test in mice [6].

GLP-2 binds to a specific Gs-coupled receptor closely related to the super-family of glucagon/secretin/gip receptors [12]. The GLP-2 receptor is predominantly expressed in the gastrointestinal tract and the central nervous system (CNS), with limited expression in the lungs, the cervix, vagal afferents, and other peripheral organs [4]. GLP-2 activated vagal afferent neurons, the nucleus solitary tract (NTS), and the dorsomedial hypothalamus (DMH) as detected by c-Fos immunoreactivity (Fos-IR) [13,16,18]. These neurons regulate cardiovascular systems [1,10,21]. Indeed, we recently demonstrated that peripherally and centrally administered GLP-2 reduced mean arterial blood pressure (MAP) in Wistar rats in an anesthetized state [16]. GLP-2 administration increased Fos-IR in the NTS and the caudal ventrolateral medulla (CVLM), and decreased it in brainstem catecholamine neurons [16]. We hence hypothesized that GLP-2 acted on a specific brain nucleus to inhibit sympathetic nerve activity. Although we have shown histological evidence of the role of the sympathetic nervous system in GLP-2-induced hypotension, we have not yet shown functional evidence.

In the present study, we evaluated the influences of pharmacological and surgical blockades of the autonomic system on the hypotensive effects of GLP-2 in rats to confirm our hypothesis. The afferent parasympathetic inputs and efferent outputs were blocked with vagotomy. The sympathetic and parasympathetic transmissions to effector organs were inhibited with antagonists of adrenoceptors or muscarinic receptors.

Abbreviations: CNS, central nervous system; CVLM, caudal ventrolateral medulla; DMH, dorsomedial hypothalamus; Fos-IR, c-Fos immunoreativity; GLP-2, glucagon-like peptide-2; i.c.v., intracerebroventricular; IML, intermediolateral nucleus; i.v., intravenous; LC, locus coeruleus; MAP, mean blood pressure; NTS, nucleus solitary tract; PBS, phosphate-buffered saline; RVLM, rostral ventrolateral medulla.

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2. Materials and methods

All experimental protocols were approved by the Institutional Animal Care and Use Committee at Tokyo University of Science, and were conducted according to the guidelines of the National Institute of Health and the Japan Neuroscience Society.

2.1. Animals

Male Wistar rats (8–10 weeks, Japan SLC Inc., Shizuoka, Japan) were used for experiments. They were kept in a controlled environment, with controlled lighting (12 h light/dark cycle, lights on from 8:00 to 20:00 h), temperature (23 ± 1 °C) and relative humidity (55 ± 5%) for at least 5 days before the experiments, and given free access to food and water.

2.2. Surgical procedures and measurements of MAP and heart rate

Surgical procedures and measurements of MAP and heart rate were essentially the same as described previously [16]. For the intravenous (i.v.) administration of PBS (phosphate-buffered saline) or GLP-2 (30 μg/kg), a catheter was inserted into a femoral vein. For the intracerebroventricular (i.c.v.) administration of PBS or GLP-2 (6 μg per rat), a cannula was inserted into a lateral cerebral ventricle (0.8 mm posterior and 1.4 mm lateral to the bregma, 3.5 mm ventral to the surface of the skull) according to the atlas of Paxinos and Watson (1986) [14]. The infusion cannula, which extended 0.5 mm below the distal end of the guide cannula, was connected with a 10-μl Hamilton syringe (model 1801 RN; Hamilton Company, NV, USA).

Rats were anesthetized with an intraperitoneal injection of a mixture of urethane (1 g/kg) and α-chloralose (25 mg/kg), and a tracheal tube was inserted. Arterial pressure was measured with a pressure transducer (TP-200T, Nihon Koden, Tokyo, Japan) through a transducer amplifier (AP-601G; Nihon Koden), and was monitored by a PowerLab data acquisition system (AD Instruments, Castle Hill, Australia).

2.3. Vagotomy

On the day of MAP measurements, vagotomy was performed to investigate the role of parasympathetic innervation in cardiovascular responses elicited by the injection of GLP-2 into the femoral vein or i.c.v. For these experiments, silk sutures were placed loosely around the vagal nerves bilaterally for subsequent identification and sectioning of the nerves. The cervical vagal nerve was

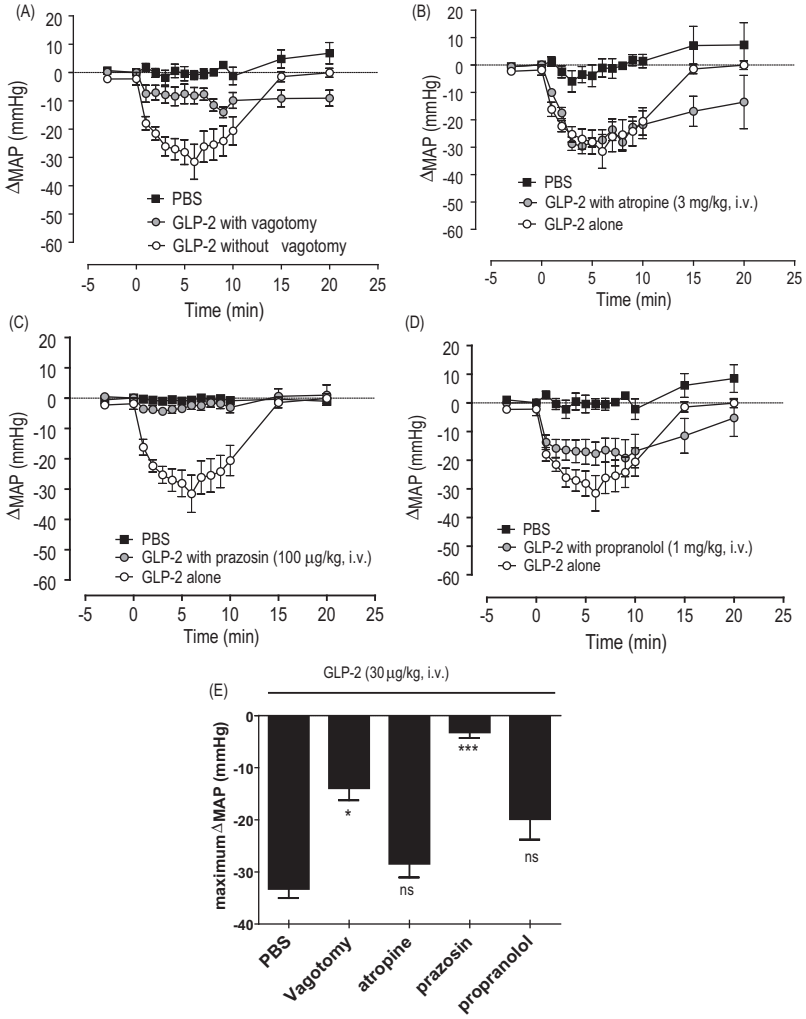

Fig. 1. Effects of i.v. administration of GLP-2 on MAP in anesthetized rats. The rats were vagotomized (A) or pretreated with atropine (B), prazosin (C), or propranolol (D). GLP-2 or PBS was administered at 0 min. Bar graphs show maximum change of MAP (E). $^{*}p < 0.05$, $^{***}p < 0.001$, ns: non-significant, One-way ANOVA with Dunnett's multiple comparison test. Data are expressed as the mean ± SEM; $n = 4$.