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Oxyntomodulin analog and exendin-4 derivative lower plasma glucose in cattle

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ABSTRACT

The present study was undertaken with the aim of examining whether and how exendin-4 (1–3) fragment, ie, Ex-4 (1–3) fragment, contributes to the regulation of glucose. An analog of oxyntomodulin (OXM) ([Gly^2, Glu^3]-OXM), a glucagon analog ([Gly^2, Glu^3]-glucagon), and two derivatives of Ex-4 (glucandin and [Gly^2, Glu^3]-glucandin) were synthesized by substituting with Gly^2, Glu^3 at the N-terminuses of OXM and glucagon and/or by attaching Ex-4 (30–39) amide at the C-terminus of glucagon. Effects of these peptides on plasma insulin and glucose concentrations were investigated in cattle by conducting 3 in vivo experiments. In all 3 experiments, 0.1% BSA saline was injected as a control. In experiment 1, glucandin (amino acid sequence was glucagon [1–29]-Ex-4 [30–39] amide) and [Gly^2, Glu^3]-glucandin were injected at the dose rates of 5 μg/kg BW in 4-mo-old Holstein steers. Results showed that glucoregulatory effects of glucandin were similar to those of glucagon. [Gly^2, Glu^3]-glucandin stimulated insulin secretion at 2 to 10 min and lowered glucose concentrations at 15 to 75 min. Experiment 2 was carried out to better understand the glucose-lowering potency of [Gly^2, Glu^3]-glucandin, in comparison with Ex-4 and glucagon-like peptide-1 (GLP-1), using 4.5-mo-old Holstein steers. [Gly^2, Glu^3]-glucandin was injected at dose rates of 0.3 μg/kg BW, 1.0 μg/kg BW, 3.2 μg/kg BW, and 6.4 μg/kg BW. Ex-4 and GLP-1 were injected at dose rates of 0.3 μg/kg BW. Results showed that the insulinotropic and glucose-lowering effects of [Gly^2, Glu^3]-glucandin were not as potent as for Ex-4 and GLP-1, and the minimum effective dose of [Gly^2, Glu^3]-glucandin to regulate plasma glucose concentrations was 3.2 μg/kg BW. In experiment 3, [Gly^2, Glu^3]-OXM and [Gly^2, Glu^3]-glucagon were injected at dose rates of 5 μg/kg BW in 5-mo-old Holstein steers. Both [Gly^2, Glu^3]-OXM and [Gly^2, Glu^3]-glucagon increased insulin concentration. [Gly^2, Glu^3]-OXM potently lowered plasma glucose, but [Gly^2, Glu^3]-glucagon did not change it. In summary, our findings clearly demonstrate that Ex-4 (1–3) fragment contributes to the regulation of glucose. [Gly^2, Glu^3]-OXM and [Gly^2, Glu^3]-glucandin are insulinotropic and glucose-lowering peptides. It was of interest that the substitution of the first 3 amino acids of OXM with Ex-4 (1–3) could reverse the upregulation of glucose by OXM into downregulation of glucose. In lowering glycemia, [Gly^2, Glu^3]-OXM seemed almost as effective as Ex-4, and [Gly^2, Glu^3]-glucandin was less profound than Ex-4. These findings contributed new insights into the hormonal regulation of glucose in ruminants. The action of [Gly^2, Glu^3]-OXM and [Gly^2, Glu^3]-glucandin might provide an advantage in glycemic control of insulin resistance in cattle and humans.

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1. Introduction

Oxyntomodulin (OXM), glucagon, glucagon-like peptide-1 (GLP-1), and exendin-4 (Ex-4) are peptide hormones playing important roles in glucose regulation in both humans and animals [1–7, reviewed in 8]. These peptides have a high level of sequence homology, and their insulinotropic actions are mediated through the GLP-1 receptor (GLP-1R) [6–10]. OXM and glucagon stimulate insulin secretion directly by binding to the GLP-1R and indirectly by promoting glucose production from hepatocytes where glucagon receptors (GCGR) are abundant [1,2,5–7,11,12]. GLP-1 and Ex-4 stimulate insulin secretion and lower blood glucose; Ex-4 is more potent than GLP-1 [3,4,6,7,13]. Because of the glucose-lowering actions of these peptides which are naturally synthesized and secreted in vivo in the control of type II diabetes (reviewed in [14,15]), many pharmacologic efforts have focused on making use of their preserved ability.

Research on peptide regulation of insulin secretion and glucose homeostasis in cattle is limited, although many reports on dietary regulation of insulin, especially in dairy cows, are available [16–21, reviewed in 22]. In this regard, we have reported the insulinotropic and glucoregulatory actions of OXM, glucagon, GLP-1 (7–36) amide, and Ex-4, as well as interrelationships among their effects in male Holstein cattle [5–7]. OXM, glucagon, and GLP-1 have insulinotropic actions in cattle under normoglycemic conditions. Insulin secretion induced by OXM in cattle is not only because of increased hepatic glucose production but is also a direct effect of OXM on the insulin-secreting islet beta-cells, consistent with reports in monogastric animals [2,8]. Moreover, OXM attenuates the potent glucose-lowering action of Ex-4 [7]. We proposed that the glucose-lowering action of Ex-4 might include the insulin-independent pathway, in addition to the well-characterized mechanisms for the action of Ex-4 reported in monogastric animals [14,15] and that the first 3 amino acids of Ex-4, ie, Ex-4 (1–3), might be important for its glucoregulatory action [7].

As a follow-up on our previous reports, the present study was undertaken with the aim to examine whether and how Ex-4 (1–3) fragment contributes to the regulation of glucose. In this study, 4 compounds (2 analogs of OXM and glucagon and 2 derivatives of Ex-4) were chemically synthesized by substituting with Ex-4 (1–3) at the first 3 amino acids of native OXM and glucagon and/or by attaching Ex-4 (30–39) amide at the C-terminus of glucagon. The effects of these compounds on plasma insulin and glucose concentrations were investigated with the hypothesis that these exogenous compounds may play a role in the regulation of glucose, particularly by Ex-4.

2. Materials and methods

2.1. Peptides

Peptides used in this study were synthesized in our laboratory by 9-fluorenylmethyloxycarbonyl solid-phase peptide synthesis procedures and purified by reverse-phase high-performance liquid chromatography (TSK gel ODS-120 A column; TOSOH, linear gradient of 0%–60% CH_3CN). As shown in Table 1, [Gly^2, Glu^3]-OXM was synthesized by substitution of the first 3 amino acids of OXM with Ex-4 (1–3). Therefore, the second and the third amino acids were Gly and Glu in the sequence of [Gly^2, Glu^3]-OXM, and Ser and Gln in the sequence of native OXM. The peptide sequence of [Gly^2, Glu^3]-glucagon was Ex-4 (1–3)-glucagon (4–29). The amino acid sequence of glucandin consisted of glucagon (1–29) elongated at its C-terminus by Ex-4 (30–39) amide. [Gly^2, Glu^3]-glucandin was synthesized as Ex-4 (1–3)-glucagon (4–29)-Ex-4 (30–39) amide. Glucandin and [Gly^2, Glu^3]-glucandin were considered as Ex-4 derivatives.

2.2. Experiments

A total of three experiments were carried out in Holstein steers raised under natural light–dark conditions at the beef cattle farm of Obihiro University of Agriculture and Veterinary Medicine, Japan. Throughout the experiments, steers were fed mixed rations for growing cattle (crude protein 14%, total digestible nutrient 72%, crude fat 2%, crude fiber 10%, crude ash 9%; Marubeni Nisshin, Tokyo, Japan) twice a day (at 9:00 AM and 4:00 PM). Water, Timothy hay, and mineral block were supplied ad libitum. Steers ($n = 8$) were randomly assigned to the experimental groups using incomplete Latin square design with 1-d

Table 1
Amino acid sequences of analogs and Ex-4 derivatives in comparison with the native peptides.

Peptide name	Amino acid sequence/sequence modification	
Native peptides		
Ex-4	Ex-4 (1–39) amide	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2 [10]
OXM	OXM (1–37)	HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNKNNIA (accession P01272)
Glucagon	Glucagon (1–29)	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT (accession P01272)
GLP-1	GLP-1 (7–36) amide	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-NH_2 (accession P01272)
Analogs/Ex-4 derivatives		
[Gly^2, Glu^3]-OXM	Ex-4 (1–3)-OXM (4–37)	HGEGTFTSDYSKYLDSRRAQDFVQWLMNTKRNKNNIA
[Gly^2, Glu^3]-glucagon	Ex-4 (1–3)-glucagon (4–29)	HGEGTFTSDYSKYLDSRRAQDFVQWLMNT
Glucandin	Glucagon (1–29)-Ex-4 (30–39) amide	HSQGTFTSDYSKYLDSRRAQDFVQWLMNTGPSSGAPPPS-NH_2
[Gly^2, Glu^3]-glucandin	Ex-4 (1–3)-glucagon (4–29)-Ex-4 (30–39) amide	HGEGTFTSDYSKYLDSRRAQDFVQWLMNTGPSSGAPPPS-NH_2

Abbreviations: Ex-4, exendin-4; GLP-1, glucagon-like peptide-1; OXM, oxyntomodulin.