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SHORT COMMUNICATION

Effect of Short-Term Administration of Glucagon on Gene Expression of the Insulin Receptor in Primary Cultured Calf Hepatocytes

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ABSTRACT

This study investigated whether increased glucagon levels, caused by the short-term administration of glucagon, lead to an increase in gene expression of the insulin receptor (InsR) in calf hepatocytes cultured *in vitro*. After 72 hrs of culturing, glucagon was added to calf hepatocytes at a five different concentrations of 0, 1, 10, 100 and 1000 nM. InsR mRNA expression was determined by internally controlled reverse transcriptase polymerase chain reaction. No changes in InsR mRNA expression (InsR/β-actin gray scale) were detected in hepatocytes treated with glucagon compared with the control group and there were no significant differences between the different concentrations. In conclusion, short-term administration of glucagon did not directly influence the gene expression of InsR in primary cultured calf hepatocytes.

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INTRODUCTION

Dairy cows experience an increased demand for glucose to support milk production during early lactation. Increased glucose demand can be met through increased gluconeogenesis, an increased supply of glucose precursors, or a combination of both of these processes (Williams *et al*., 2006). Hormonal regulation of nutrient partitioning is of critical importance in maintaining the metabolic equilibrium of lactating dairy cows. The production of glucose by the liver and the utilization of glucose by extrahepatic tissues are vital steps in the conversion of feed nutrients to milk. The roles of glucagon and insulin in the regulation of energy metabolism in ruminants are crucial. Insulin is an anabolic hormone that decreases the output of glucose by the liver and has been related inversely to milk yield. It binds to a glycoprotein receptor (InsR) in the cell membrane that is a member of the tyrosine kinase receptor family. Binding of insulin to InsR is crucial in maintaining blood glucose homeostasis by promoting glycogen synthesis (Liu *et al*., 2010).

Glucagon is a peptide comprising of 29 amino acids that is released from pancreatic α-cells and acts against insulin. Glucagon promotes gluconeogenesis and stimulates glycogenolysis leading to glucose output from hepatocytes when blood glucose levels are low (Longuet *et al*., 2008). These actions are transduced via a G protein-coupled receptor. In recent years, glucagon has gained much interest in the field of ruminant research, in particular with regard to its role in energy metabolism and as a feed supplement (Bobe *et al*., 2009). In the liver, glucagon binds to its receptor and activates signal transduction networks and transcription factors, playing an important physiological role (Berglund *et al*., 2010).

At the onset of lactation, the levels of plasma insulin and hepatic glycogen decrease with a simultaneous increase in the levels of plasma glucagon and hepatic triacylglycerols. In addition, the glucagon concentration has been reported to increase under various conditions such as dexamethasone treatment increases the glucagon concentration in the plasma of young calves (Hammon *et al*., 2005). Similarly, the glucagon concentration also increases with weaning in dairy calves (Klotz and Heitmann, 2006). Further, both glucagon concentration and the glucagon-to-insulin ratio have been reported to be augmented by rumen-protected fat feeding (Hammon *et al*., 2008). The plasma glucagon concentration in postpartum dairy cows quadrupled after the administration

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of increasing amounts of chromium-L-methionine (Smith *et al*., 2008). In hot environments, glucagon responses following arginine and butyrate injections were augmented significantly in heifers (Itoh *et al*., 1998). Daily subcutaneous injections of 15 mg of glucagon during the first 14 days postpartum with or without co-administration of 400 ml of pure glycerol orally was reported to alleviate some of the symptoms of fatty liver, such as the increase in plasma non-esterified fatty acids and decrease in plasma glucose and insulin levels, in Holstein dairy cows after parturition (Osman *et al*., 2010).

Hepatocytes in primary monolayer culture are a well-established experimental model. In this study, the *in vitro* effect of glucagon on InsR mRNA expression in cultured calf hepatocytes was investigated.

MATERIALS AND METHODS

A liver (caudate process) was obtained from a thiamylal sodium-anesthetized neonatal Holstein calf (male) under sterile conditions. Hepatocytes were cultured using the collagenase perfusion method as decribed previously (Zhang *et al*., 2011). Glucagon was added at five different concentrations of 0, 1, 10, 100, and 1000 nM to pre-cultured hepatocytes in five different groups). Each concentration group comprised six repeats.

Primer design and DNA amplification were performed as described previously (Liu *et al*., 2010). After incubation for 12 hrs, total RNA was isolated from hepatocytes by using the TRIzol RNA purification kit (Invitrogen, Carlsbad, California, USA) according to the supplier's protocol. The RNA extract was diluted (1:40) and analyzed at 260 and 280 nm by spectrophotometry. Samples with an optical density ratio at 260/280 of >1.9 were retained for further analysis (Zhang *et al*., 2011). Complementary DNA synthesis and InsR mRNA internally controlled reverse transcriptase polymerase chain reaction (RT-PCR) were performed according to Liu *et al*. (2010). Image processing and grey-scale analysis was performed with the TANLON gel image analysis system (Shanghai, China). Based on the grey-scale analysis, the ratio of the grey-level between the PCR products of InsR and the β-actin genes indicated the relative changes in expression of the InsR gene in the test samples.

Data analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was determined by one-way ANOVA, followed by Duncan's least significant difference test. $P<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

As shown in Fig.1 and Table 1, with continuously increasing glucagon concentrations, there were no significant differences in the InsR mRNA expression levels of hepatocytes (InsR/β-actin gray scale) between the different concentrations compared with the control group ($P>0.05$).

Dairy cows in early lactation exhibit a negative energy balance as the glucose supply is insufficient. One of the physiological functions of glucagon is to counter hypoglycemia. It is stimulated during hypoglycemia and promotes hepatic glucose production, ultimately raising blood glucose levels.

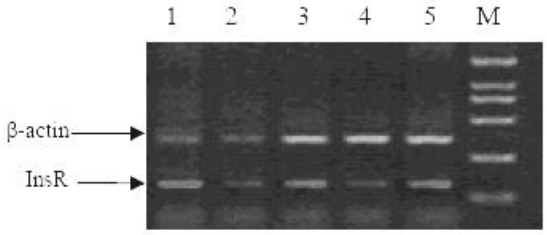

Fig. 1: PCR products of the *insR* gene in hepatocytes treated with different concentrations of glucagons. M: Marker DL 2000; lanes 1–5: Glucagons concentrations of 0, 1, 10, 100, and 1000 nM, respectively.

Table 1: Abundance of InsR mRNA in primary hepatocytes treated with different concentrations of glucagons (n=6)

Glucagon (nM)	InsR mRNA (μg; mean±SD)
0	0.58±0.06
1	0.54±0.09
10	0.53±0.10
100	0.52±0.04
1000	0.55±0.03

All values are statistically similar ($P>0.05$).

Glucagon infusion had a prominent hyperglycemic–glycogenolytic stimulatory effect in calves, accompanied by a marked increase in plasma insulin (Madsen *et al*., 1976). Administration of glucagon over 24 hours did not alter cytosolic phosphoenolpyruvate carboxykinase mRNA expression or result in immediate alterations in total phosphoenolpyruvate carboxykinase activity or gluconeogenic capacity in the liver of dairy cows (Williams *et al*., 2006). Fatty liver is a metabolic disease that affects up to 50% of dairy cows in early lactation. Fatty liver and ketosis can be treated by consecutive intravenous injections of glucagons (10 mg/day) for 14 days (Bobe *et al*., 2003).

InsR and glucagons have been shown to interact. The appropriate expression of InsR in mouse pancreatic α-cells is required for glucose-dependent glucagon secretion (Diao *et al*., 2005). Incubation of rat liver cells with glucagon led to an increase in the phosphorylation of specific serine residues within insulin receptors, particularly in the presence of insulin (Issad *et al*., 1992).

The results of this study suggested that short-term administration of glucagon did not directly influence the gene expression of InsR in primary cultured calf hepatocytes. This indirectly suggested that the short-term administration of glucagon to regulate glucose levels in dairy cows with fatty liver disease or dairy cows during early lactation might not be related to InsR gene expression. Determining InsR gene expression in the liver of cows which have been injected glucagons can be favorable to support the results. Further studies using extended incubation times (e.g., 72, 144, or 196 hrs) may be helpful in elucidating the effect of long-term administration of glucagon on the gene expression of InsR in cultured calf hepatocytes.

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