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Short Communication

Conjoint regulation of glucagon concentrations via plasma insulin and glucose in dairy cows

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ABSTRACT

Insulin and glucagon are glucoregulatory hormones that contribute to glucose homeostasis. Plasma insulin is elevated during normoglycemia or hyperglycemia and acts as a suppressor of glucagon secretion. We have investigated if and how insulin and glucose contribute to the regulation of glucagon secretion through long term (48 h) elevated insulin concentrations during simultaneous hypoglycemia or euglycemia in mid-lactating dairy cows. Nineteen Holstein dairy cows were randomly assigned to 3 treatment groups: an intravenous insulin infusion (HypoG, n = 5) to decrease plasma glucose concentrations (2.5 mmol/L), a hyperinsulinemic-euglycemic clamp to study effects of insulin at simultaneously normal glucose concentrations (EuG, n = 6) and a 0.9% saline infusion (NaCl, n = 8). Plasma glucose was measured at 5-min intervals, and insulin and glucose infusion rates were adjusted accordingly. Area under the curve of hourly glucose, insulin, and glucagon concentrations on day 2 of infusion was evaluated by analysis of variance with treatments as fixed effect. Insulin infusion caused an increase of plasma insulin area under the curve (AUC)/h in HypoG (41.9 ± 8.1 mU/L) and EuG (57.8 ± 7.8 mU/L) compared with NaCl (13.9 ± 1.1 mU/L; $P < 0.01$). Induced hyperinsulinemia caused a decline of plasma glucose AUC/h to 2.3 ± 0.1 mmol/L in HypoG ($P < 0.01$), whereas plasma glucose AUC/h remained unchanged in EuG (3.8 ± 0.2 mmol/L) and NaCl (4.1 ± 0.1 mmol/L). Plasma glucagon AUC/h was lower in EuG (84.0 ± 6.3 pg/mL; $P < 0.05$) and elevated in HypoG (129.0 ± 7.0 pg/mL; $P < 0.01$) as compared with NaCl (106.1 ± 5.4 pg/mL). The results show that intravenous insulin infusion induces elevated glucagon concentrations during hypoglycemia, although the same insulin infusion reduces glucagon concentrations at simultaneously normal glucose concentrations. Thus, insulin does not generally have an inhibitory effect on glucagon concentrations. If simultaneously glucose is low and insulin is high, glucagon is upregulated to increase glucose availability. Therefore, insulin and glucose are conjoint regulatory factors of glucagon concentrations in dairy cows, and the plasma glucose status is the key factor to decide if its concentrations are increased or decreased. This regulatory effect can be important for the maintenance of glucose homeostasis if insulin secretion is upregulated by other factors than high glucose such as high plasma lipid and protein concentrations at simultaneously low glucose.

1. Introduction

Glucose is an essential substrate for mammary lactose synthesis and its requirement increase significantly after parturition in dairy cows [1]. In early lactation, most of the available glucose is used for lactose synthesis in the

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mammary gland [2]. Because plasma glucose can be a limiting factor for milk synthesis, the milk production level depends on plasma glucose concentrations [3]. In ruminants, most of the available glucose is derived from hepatic gluconeogenesis [3]. The regulation of glucose homeostasis is mediated by both insulin and glucagon [4]. During hyperglycemia, insulin secretion is increased to maintain plasma glucose concentrations at a physiological level. Insulin regulates plasma glucose homeostasis through the facilitation of glucose transport to skeletal muscles and adipose tissue and increases hepatic glycogen synthesis while the gluconeogenesis is simultaneously inhibited [5,6]. Glucagon regulation of glucose homeostasis is mediated via increased gluconeogenesis, glycogenolysis, and decreased glycogenesis [7]. Research in humans indicated that insulin is generally accepted as a suppressor of glucagon secretion that induces a decreased glucagon secretion through the inhibition of glucagon gene transcription, activation of the gamma amino butyric acid (GABA) and $GABA_A$ receptor, and modulation of K-ATP channel activity [8]. Effects of insulin in interaction with glucose concentrations on glucagon concentrations have, to our knowledge, not been shown in dairy cows. Thus, the objective of the present study was to investigate effects of hyperinsulinemic hypoglycemic, and hyperinsulinemic-euglycemic clamps lasting for 48 h on glucagon concentrations in mid-lactating dairy cows.

2. Material and methods

The details of animals and treatments of this study were described elsewhere [9]. In brief, the study was carried out in 19 non pregnant Holstein dairy cows at a parity of 3.0 ± 0.1 and at 28.0 ± 0.2 (mean $\pm$ SD) wk after parturition. Cows in later lactation were selected to allow the investigation of the direct effects of treatment infusion without interference with a negative energy balance and the characteristic endocrine and metabolic changes during the transition period. Starting at 2 wk before and throughout the experiment, animals were fed with hay ad libitum and with a concentrate rich in protein and energy twice daily according to the individual milk production. Cows were randomly assigned to 3 treatment groups: an adjusted intravenous insulin infusion to induce hypoglycemia at a plasma glucose concentrations of 2.5 mmol/L (HypoG, n = 5), a hyperinsulinemic-euglycemic clamp to maintain plasma glucose concentrations at the preinfusion concentrations to study effects of insulin at simultaneously normal glucose concentrations (EuG, n = 6), and a 0.9% saline infusion (NaCl, n = 8) as a control with normal concentrations of insulin and glucose. Infusions and/or clamps started at 9 AM and were continued until 9 AM 2 d later through one of the indwelling intravenous catheters (Cavafix Certo Splittocan, B. Braun Melsungen AG, Germany), whereas the second catheter was used for blood sampling.

Hyperinsulinemic hypoglycemia was induced by infusion of bovine insulin (Sigma-Aldrich, Saint Louis, MO, #I4011, USA). Insulin infusion rates were adjusted according to plasma glucose concentrations, which was measured every 5-min in the first 2 h and hourly during the further course of infusion. Hyperinsulinemic-euglycemic clamp was performed by insulin infusion at a constant infusion rate of 0.6 mU/kg BW/min (according to the average infusion rate in HypoG) and an additional continuously regulated glucose infusion (Dr G. Bichsel AG, Interlaken, Switzerland) to maintain plasma glucose at the preinfusion concentrations. Control (NaCl) cows were continuously infused with a 0.9% saline solution at an infusion rate of 20 mL/h administered through an automatic pump (Perfuser, B. Braun Melsungen AG).

The reference samples were taken 1 wk and immediately before the start of infusions. Plasma glucose concentrations were measured enzymatically in duplicate with an automated analyzer (Cobas Mira 2, Hoffmann-La Roche, Basel, Switzerland) by commercial kit (BioMerieux no. 61270, Marcy l'Etoile, France, intra- and interassay coefficient of variation (CV) both <4%). Plasma insulin was measured in duplicate by radioimmunoassay as described previously [10] with intra- and interassay CV 8.2% and 15.5%, respectively, and plasma glucagon concentrations were measured in duplicate by using a commercial radioimmunoassay kit (cat. # GL-32K, MILLIPORE, Zug, Switzerland, intra- and interassay CV 4.0% and 12.5%, respectively).

The area under the curve (AUC) of the measured variables during day 2 of the experiment was calculated by the trapezoidal rule (combination of rectangular and triangular area compartments). AUC was calculated of the whole time span and then divided by the number of hours to achieve values which are similar to a single measurement concentration (AUC/h). Data were evaluated by using a general linear models procedure of SAS (SAS Institute Inc, Cary, NC, USA, 2002–2008, Release 9.2), including treatments (HypoG, EuG, and NaCl) as fixed effect. Data obtained from the reference samples were included as covariates into the model to compensate for initial differences between individuals. Differences between means were determined by the Tukey test. Data are presented as means $\pm$ standard error of the mean, and differences were considered significant if $P < 0.05$.

3. Results

During infusions (48 h), the mean insulin infusion rate after the increase of insulin concentrations by administration of a bolus of 4 mU/kg BW insulin solution was 0.6 mU/kg BW/min in HypoG and EuG. In EuG, the glucose infusion rate to keep plasma glucose concentrations at a preinfusion level was 2.20 ± 0.04 mmol/kg/min (mean $\pm$ standard error of the mean). Insulin infusion caused an increased plasma insulin AUC/h (Table 1; $P < 0.01$) at simultaneously decreased plasma glucose AUC/h ($P < 0.01$), in HypoG as compared with plasma insulin and glucose AUC in NaCl. In HypoG, plasma glucagon AUC/h increased markedly during insulin infusions as compared with preinfusion levels and compared with NaCl (Fig. 1; Table 1; $P < 0.01$). In EuG, the intravenous insulin administration combined with glucose infusion caused an increase of plasma insulin AUC/h ($P < 0.01$) at simultaneously unchanged plasma glucose concentrations. In contrast to HypoG, the simultaneous infusion of insulin and glucose in EuG caused a decline of plasma glucagon concentrations compared with the preinfusion level and compared with HypoG and NaCl ($P < 0.01$).