Effects of Heat Exposure on Adrenergic Modulation of Insulin and Glucagon Secretion in Sheep

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Abstract. The effects of heat exposure on the adrenergic modulation of pancreatic secretion were investigated. Five ewes fed at maintenance level (ME base) were housed in thermoneutral (TN; 20°C) and hot (30°C) environments. Heat exposure caused an increase in respiration rate and a slightly higher rectal temperature, and decreases in basal insulin and glucose concentrations. Infusions of saline plus epinephrine caused increases in glucagon and glucose concentrations, and no significant change in insulin secretion. Phentolamine (an adrenergic a-antagonist) plus epinephrine augmented insulin secretion; however, this insulin secretory response was inhibited by heat exposure. Propranolol (a n-antagonist) plus epinephrine produced a slight decrease in insulin secretion in the TN environment, whereas no effect was observed during heat exposure. While glucagon secretion through a-adrenergic stimulation was not affected by heat exposure, homeostatic signals controlling insulin release seemed to be affected during heat exposure. We thus hypothesised that insulin concentration is decreased in sheep fed at maintenance level in hot environments, and that this response is mediated in part by a modulation of β-adrenergic function.

Key words: Insulin, Glucagon, Heat exposure, Adrenergic modulation, Sheep

IN high ambient environments, various adaptation responses are observed in domestic animals to maintain body homeostasis. Alterations in metabolic and endocrine mechanisms, which control the distribution and utilization of nutrients in the body, play an essential role in the adaptation to a hot environment. These responses control heat production. Lower plasma levels of triiodothyronine (T3), which has a catabolic effect, have been noted in cattle exposed to heat [1, 2]. In our previous report, the basal- and secretagogue-induced concentrations of insulin, which has the anabolic effect of directing nutrients toward tissues, were lower in nonlactating cows during heat exposure [3]. However, it is still unclear what mechanisms are responsible for this response.

It is well known that the sympathoadrenomedullary system plays an important role in controlling the pancreatic islets. Insulin secretion is facilitated through β-adrenergic stimulation and inhibited by α-adrenergic stimulation in sheep and other species in a normal ambient (TN) temperature [4–7]. Glucagon secretion is facilitated through the activation of the α-adrenergic system in sheep [7].

We hypothesized that homeostatic signals, such as catecholamines, control pancreatic secretion change in heat-exposed animals. Therefore, the major objective of this study was to assess the effects of heat exposure on insulin and glucagon secretion in sheep fed at maintenance level, and to establish the involvement of adrenergic modulation on pancreatic hor-
mone secretions during heat exposure. Alterations in glucose concentration were also monitored during the experiment.

**Materials and Methods**

**Animals**

The study design was approved by the Animal Ethics Committee of National Institute of Animal Industry. Five mature nonpregnant and nonlactating Suffolk ewes (33.5–45.0 kg) were employed. The ewes were fed alfalfa hay cubes once daily at 1600 h at a level which was sufficient for maintenance (100% of ME requirements) using Japanese feeding standards [8]. The feed allowance (dry matter) employed in the present study was restricted to avoid feed refusals. In fact, the ewes did not refuse any of the diet offered in either environment. Water and salt blocks were continuously available. The ewes were housed in metabolic cages in the Zootron artificial climatic facility (National Institute of Animal Industry), and were exposed first to a thermoneutral environment (TN; 20 ± 0.5 °C, relative humidity (RH) 70 ± 5.0%, 32 d), and then to a hot environment (30 ± 0.5°C, RH 70 ± 5.0%, 24 d). They were allowed to adjust to the hot environment for 9 d before the experimental treatments resumed. Silicone catheters were inserted into both jugular veins (one side used for all infusions and the other side used for blood sampling) 6 days prior to the first experiment, and maintained to the end of the experiment.

**Experimental procedures**

Experimental design is represented in Fig. 1. All ewes were subjected to the following treatments: Experiment 1, Saline (Control); Experiment 2, the α-adrenergic antagonist phentolamine (Sigma; 10 nmol/kg LW/min); Experiment 3, β-antagonist propranolol (Sigma; 20 nmol/kg LW/min) were intravenously infused over 75 min. Epinephrine (Sigma; 1.0 nmol/kg LW/min) was infused 15 min after the onset of the α-, β-antagonist or saline infusion and was continued for 60 min. A previous report [7] demonstrated that the dose of adrenergic antagonists (phenolamine and propranolol) used in this study has near maximal effect in sheep. All reagents were given at a constant rate of 0.5 ml/min via the one venous catheter using an infusion pump.

Venous blood samples were collected through the catheter at 15 min intervals for 120 min after the infusion began. Blood samples for basal values were also taken at 15 min and immediately before each administration. All three treatments were assigned to all of the five sheep in a random order in both the TN and hot environments. Intervals between each infusion experiment were 4 or 5 d. Rectal temperature, respiration rate, heart rate and body weight were monitored at 10 d intervals in each environment.

**Analysis**

Each blood sample was placed into a heparinized tube containing the proteolytic inhibitor aprotinin (500 IU/ml blood; Trasylo1, Bayer Leverkusen, Germany). Plasma was harvested from the blood samples and stored at −20°C for later determinations of insulin, glucagon and glucose. The plasma insulin and glucagon concentrations were measured using commercially available RIA kits (Insulin Eiken, Eiken Chemical, Tokyo, Japan; and Glucagon Daiichi, Daiichi Radioisotope Kenkyujo, Tokyo, Japan, respectively). Plasma glucose was assayed using a commercially available colorimetric kit (Glucose C-II Test-Wako, Wako Pure Chemical, Tokyo, Japan). All hormone and metabolite concentrations were determined in the same assay, thus removing interassay variation. The intraassay coefficients of variation of the insulin, glucagon and glucose were 3.0, 3.4 and 4.8%, respectively.

**Statistics**

The effects of heat exposure on basal levels of insulin, glucagon and glucose were analyzed by ANOVA using the GLM procedure of SAS [9]. The statistical model used was described in our previous report [10]. The analysis, which was a mixed model with a random effect (animals) and a fixed effect (temperature), was tested against the interaction as error. The animal vs. temperature interaction was initially analyzed, but no significant interaction was found. Therefore, in this model, the interactions described above were included in the error term. The basal hormone and metabolite values obtained in
all three experiments in each environment were included in this analysis (n = 30; five animals, three treatments and two environments). Basal hormone and metabolite concentrations were calculated from values obtained at 15 min and immediately before each administration.

The area encompassed by the basal level and the concentration curve (area under curve; AUC) was calculated during the epinephrine infusion period (60 min) for plasma insulin, glucagon and glucose concentrations. The AUC value is an index showing the magnitude of a response following a treatment [3]. The difference between the TN and hot environments for the AUC and physiological responses were evaluated using a randomized block design (animals were blocks). Significance was declared when the probability of no difference between the means was less than 0.05.

**Results**

The ewes did not refuse any of the diet offered in either environment. Body weight was maintained at a constant level throughout the experiments in the TN and hot environments. Heat exposure resulted in a marked increase (P < 0.001) in respiration rate, and resulted in a decrease in heart rate (P < 0.05) (Table 1). Rectal temperature was slightly, but significantly higher (P < 0.001) in the hot compared with the TN environment (Table 1).

Basal insulin and glucose concentrations were decreased by the heat exposure, whereas basal glucagon was not significantly affected (Table 1).
Infusions of saline plus epinephrine (control experiment) caused increases in glucagon and glucose concentrations, and no change in insulin secretion in both the TN and hot environments (Fig. 2). Insulin AUC during the simultaneous infusion of epinephrine and α-antagonist, phentolamine (effectively adrenergic β-stimulation) were positive; that is, insulin secretion was stimulated in the TN and hot environments, but the heat exposure significantly inhibited (P <0.01) the insulin AUC (Fig. 2). During the propranolol plus epinephrine infusion (effectively α-stimulation), the mean values for the insulin AUC were numerically negative in each environment, and did not differ compared with the control experiment (saline plus epinephrine) (Fig. 2). No effect of heat exposure was found on insulin AUC with respect to the α-stimulation. There was a positive glucagon AUC response during the control and α-stimulation, but the response to β-stimulation was negative. The glucagon AUC was not affected by the heat exposure in any of the treatments. The β-stimulation inhibited (P <0.01) glucose AUC in both environments, whereas the glucose AUC was larger (P <0.01) during the α-stimulation, again in both environments, compared with the control experiment (Fig. 2). The glucose AUC were not affected by the heat exposure.

Discussion

We found a decrease in basal insulin concentration in sheep during heat exposure in this study. This result agreed with our previous study using nonlactating dairy cows [3]. In a previous report looking at insulin secretion in heat-exposed sheep [11], no significantly affected levels of basal insulin were reported. This may have been because energy levels were different between the present and previous experiments. The level of energy intake was adjusted to the requirements for maintenance (100% of maintenance ME requirement) in the present study, whereas in the study conducted by Achmadi et al. [11], it was adjusted to meet 120% of the ME requirement for maintenance. Because our sheep were fed at just maintenance requirements, however, energy balance may have tended to be negative, because the maintenance requirements increase during heat exposure [8]. In sheep fed above the maintenance level, an increase in insulin in the hot environment may play an important role to reduce gluconeogenesis and to direct nutrients toward storage organs, as discussed by Achmadi et al. [11]. The energy intakes of the animals were adjusted to similar levels in both the TN and hot environments in both experiments, so that the effects of energy status and environmental temperature were not confounded. As for our previous study, we concluded that an inhibition in insulin secretion could be involved in the adaptation mechanisms in heat-exposed sheep fed at maintenance level.

In the present study, insulin secretion was augmented by adrenergic β-stimulation (simultaneous phentolamine and epinephrine infusion), and was inhibited by α-stimulation (simultaneous propranolol and epinephrine infusion), which agrees with a number of earlier studies [4-7]. The principal result
of the present study is the inhibition of the adrenergic β-system in facilitating insulin secretion during heat exposure. To our knowledge, this is the first time this relationship has been shown. Stimulation of the adrenergic α-system to decrease insulin secretion was not affected by heat exposure.

In sheep, whole body heat production is lower following adaptation to high ambient temperatures [12]. Thermogenesis by the sympathetic nervous system is associated with adrenergic β activity [13]. Therefore, lower adrenergic β-function, including the regulation of insulin secretion, may be integratively associated with adaptation to a hot environment. In a hot environment, alterations in each organ response to homeostatic signals seem to play important roles to prevent an excessive rise in body temperature, enabling the organism/animal to adapt to the environment. Changes in the adrenergic modulation of pancreatic β-cells to alter the rate of insulin secretion may be a part of process of acclimatization of an animal’s energy metabolism during heat exposure.

The exact mechanism underlying this finding will demand further research, however, several possibilities exist. Adrenergic β-stimulation promotes insulin release by increasing the cAMP concentration in the pancreatic β-cells. During heat exposure, glucose turnover rate decreases in sheep [12]. Although glucose kinetics was not measured in this study, basal glucose concentrations were significantly lower in the heat-exposed sheep. Therefore, it is possible that decreased β-function stimulating insulin release may be related to decreased ATP generation, which led to impaired cAMP generation, caused by the decreased availability of glucose in the hot environment.

The pancreatic β-cells of sheep, like other species, appear to have a predominance of α-adrenergic receptors. Therefore, in the control experiment (saline plus epinephrine), insulin secretion had been expected to be suppressed. However, plasma insulin did not change significantly during saline and epinephrine infusion in this study. Because of the small number of animals, the large variance in the individual responses, and the small inhibition of insulin secretion in the control treatment, we considered that the insulin responses may have been masked.

Glucagon secretion is stimulated by adrenergic α-activity in sheep [6, 7]. An increase in glucagon
secretion in response to an arginine injection was shown in dairy cattle in different physiological states during heat exposure [3, 10, 14]. Although basal glucagon was not significantly changed during heat exposure (P < 0.1), it was numerically higher in this study. No effect of either α- or β-blockades on glucagon secretion in the hot environment was observed. Therefore, the adrenergic nervous system probably has no important role in modifying glucagon secretion during heat exposure.

In conclusion, heat exposure caused a decrease in the circulating level of insulin in sheep fed at a maintenance level for requirement. The ability of the adrenergic β-function to stimulate insulin secretion was impaired by the heat exposure, whereas the inhibitory effect of the α-system was unaffected.

Therefore, it is likely that adrenergic β-dysfunction in the pancreas is a part of the mechanism by which heat exposure affects insulin secretion. Adrenergic modulation seems to play no important role in glucagon secretion in sheep exposed to heat.

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