Effects of Glucagon and Insulin on Lipolysis and Ketogenesis in Sheep

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ABSTRACT

The hepatic and portal productions of acetoacetate and β-hydroxybutyrate and lipolysis were studied in normal and insulin-controlled alloxan-diabetic sheep. Since hyperinsulinemia is associated with glucagon administration, the latter group of sheep were used to maintain constant plasma insulin levels. After control values were obtained glucagon was infused intraportally at 90 μg/hr for two hours. The ketone body production by portal drained viscera was not significantly affected by glucagon. In alloxanized sheep, glucagon significantly ($P < 0.01$) increased net hepatic production of acetoacetate (from -0.54 ± 0.08 to 0.46 ± 0.07 g/hr). Lipolysis also increased. However, in the normal sheep, hyperinsulinemia prevented any stimulatory effect of glucagon on hepatic ketogenesis and lipolysis. Therefore, while glucagon appears capable of stimulating ketogenesis and lipolysis, these effects are readily suppressed by insulin.

RÉSUMÉ

Cette expérience visait à étudier la production hépatique et portale d'acéto-acétate et de β-hydroxybutyrate, ainsi que la lipolyse, chez des moutons sains et chez d'autres atteints de diabète provoqué avec de l'alloxane et contrôlé au moyen d'insuline. Comme l'administration de glucagon provoque une hyperinsulinémie, on utilisa le dernier groupe de moutons pour maintenir un taux constant d'insuline plasmatique. Après avoir déterminé les valeurs témoins, on infusa du glucagon dans la veine porte, au rythme de 90 μg/hr, durant deux heures. La production de corps cétoniques dans les viscères drainés par cette veine n'en subit pas une influence appréciable. Chez les moutons injectés avec de l'alloxane, le glucagon augmenta de façon appréciable la production hépatique nette d'acéto-acétate (de -0.54 ± 0.08 à 0.46 ± 0.07 g/hr). Il se produisit aussi une augmentation de la lipolyse. Chez les moutons sains, l'hyperinsulinémie empêcha cependant tout effet stimulant du glucagon sur la cétogénèse et la lipolyse hépatiques. Par conséquent, tandis que le glucagon semble capable de stimuler la cétogénèse et la lipolyse, l'insuline supprime rapidement ces effets.

INTRODUCTION

Glucagon is a potent hormone which affects hepatic carbohydrate, protein and lipid metabolism. It has been shown to stimulate both gluconeogenesis and glycogenolysis in sheep (6). In addition, there is evidence which suggests that glucagon can stimulate lipolysis in nonhepatic tissues (2, 8, 13). Furthermore, studies in other species indicate that glucagon also is capable of stimulating hepatic ketogenesis *in vivo* (12) and *in vitro* (17). The present study, therefore, was undertaken to examine the role of glucagon in influencing hepatic ketogenesis in sheep.

MATERIALS AND METHODS

Animals and Experimental Procedures

The animals used in this study were adult, nonpregnant, nonlactating crossbred

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ewes weighing 50-60 kg. They were housed in individual pens in a temperature controlled room. They received, prior to and during experiments, a maintenance diet of alfalfa pellets (800 g/day) delivered in 24 equal aliquots. Water and a salt lick were provided *ad libitum*. The sheep were accustomed to laboratory personnel and it was not necessary to restrain the animals during experiments.

The general features of the experiments were as previously published (6). At least ten days prior to experimentation, catheters were implanted into the portal and hepatic veins, a mesenteric vein tributary and aorta (9). In each experiment hepatic and portal blood flows were determined by infusing para-aminohippurate (PAH) into a mesenteric tributary (10). Three sets (a set consists of simultaneous samples from aorta and hepatic and portal veins) of control samples at one-half hr intervals were taken. Then the two hr glucagon infusion (at 90 μg/hr) via the mesenteric catheter was initiated and three sets of blood samples were taken one-half, one and two hr after this. Net productions were determined from means of the three samples during control and infusion periods respectively.

Four experiments were conducted using normal sheep and four with alloxanized sheep. The diabetes in alloxanized sheep was controlled by daily injections of insulin (Lente Insulin)[1]. This animal model permitted the infusion of glucagon into sheep normal with respect to glucose parameters but which were unable to compensate for glucagon infusion by insulin secretion. Thus, the effects of glucagon and insulin could be differentiated (6).

Analytical Methods

Neutral filtrates were prepared (5) for blood glucose and ketone analyses. Plasma filtrates for glycerol analyses were prepared as previously described (11).

Blood glucose concentrations were measured with glucose oxidase (Glucostat, Worthington Biochemicals). Acetoacetate and β-hydroxybutyrate were measured immediately after the completion of the experiment by fluorimetric assay (15) with reagents obtained from Sigma Chemical Co. Glycerol was determined by fluorimetric assay using a commercially prepared enzymatic kit (Boehringer-Mannheim Corp.) (8).

PAH was determined as previously described (10). Insulin was assayed by radioimmunoassay (7). Bovine insulin was used for the standard. However, ovine and bovine insulin standards give similar values in sheep plasma (7).

Calculations

Blood flows in the portal and hepatic veins were calculated as described elsewhere (10).

Net organ productions of ketones were calculated as the product of venoarterial blood concentration differences and the blood flows and therefore:

$$\text{Net Hepatic Production} = [F_{hv}\,(C_{hv} - C_a)] - [F_{pv}\,(C_{pv} - C_a)] \quad (1)$$

$$\text{Net Portal Production} = F_{pv}\,(C_{pv} - C_a) \quad (2)$$

where F_{hv} and F_{pv} are hepatic and portal venous blood flows (l/hr), respectively, and C_{hv}, C_{pv} and C_a are the concentrations (g/l) of acetoacetate or β-hydroxybutyrate in hepatic and portal venous and arterial blood. Negative values indicate net uptake or removal.

RESULTS

Table I summarizes the portal and hepatic blood flows and glucose and insulin concentrations obtained before and during the glucagon infusions. During the control periods the insulin and glucose concentrations and blood flows did not differ significantly between groups. However, during glucagon infusion the insulin concentration increased in normal sheep only. Glucose concentrations were significantly elevated in both groups but to a greater extent in the insulin-controlled alloxan-diabetic sheep.

The changes in glycerol concentration

[1]Connaught Laboratories Ltd., Toronto, Ontario.

TABLE I. Arterial Glucose and Insulin Concentrations and Hepatic and Portal Venous Blood Flows before and during Glucagon Infusions in Normal and Insulin-controlled Alloxan-diabetic Sheep[a]

	Concentration		Blood Flow	
	Glucose	Insulin	Hepatic	Portal
	mg/100 ml	μU/ml	l/min	l/min
Normal				
Control	46 ± 2[b]	20 ± 3	2.06 ± 0.14	1.45 ± 0.15
Infusion	65 ± 6[c]	50 ± 5[c]	2.30 ± 0.11	1.60 ± 0.12
Alloxanized				
Control	43 ± 3	16 ± 4	2.13 ± 0.06	1.53 ± 0.05
Infusion	88 ± 12[c,d]	14 ± 4[d]	2.56 ± 0.09	1.83 ± 0.13

[a]Glucagon was infused at 90 μg/hr for two hours
[b]Values are means ± SE of four experiments. For each experiment control and infusion values are means of three sampling times
[c]Significantly different from corresponding control value ($P < 0.01$, paired t-test)
[d]Significantly different from corresponding value in normal sheep ($P < 0.05$)

during glucagon infusion are illustrated in Table II. In normal sheep during glucagon infusion glycerol concentrations did not change whereas in alloxanized sheep glycerol concentrations increased.

The data on ketone body metabolism is summarized in Table III. In both groups of sheep during control periods there was a net production of acetoacetate and β-hydroxybutyrate by the portal drained viscera. This did not change significantly during glucagon infusion. The liver exhibited a net uptake of acetoacetate and a tendency toward an output of β-hydroxybutyrate during the control period. During glucagon infusion in normal sheep, net hepatic uptake of acetoacetate decreased whereas β-hydroxybutyrate uptake did not change significantly. The arterial concentration of ketone bodies decreased. In the alloxanized sheep the liver metabolism changed to a net production of acetoacetate. The arterial concentration of ketone bodies increased.

DISCUSSION

During the control periods the normal and alloxanized sheep were similar with respect to the blood levels of insulin, glucose and glycerol. During glucagon infusion in normal sheep insulin concentrations increased but they did not in insulin-controlled alloxan-diabetic sheep. Thus, the infusion of glucagon into the latter group permitted an evaluation of effects of glucagon without a concomitant hyperinsulinemia whereas infusion in the former group facilitated studying glucagon-insulin interactions. This model has been used previously to study the effects of hormones *in vivo* in sheep (6).

Hyperglycemia is known to affect glycerol and ketone body metabolism. However, since hyperglycemia occurred during glucagon infusion in both groups (Table I) it should not account for any of the differences observed. Furthermore, since hyperglycemia was greater in the insulin-controlled alloxan-diabetic sheep it should tend to depress the glucagon effect and thereby tend to reduce any differences between the two groups of sheep. Thus, it would not alter any interpretations with respect to the effects of either glucagon or insulin.

There is little or no glycerol kinase activity in adipose tissue (16). Furthermore, Bergman (3) has shown glycerol concentrations are linearly related to the rate of glycerol production. Thus, in this study arterial glycerol concentrations were taken to reflect changes in the rate of lipolysis.

The net hepatic removal of ketone bodies decreased in both groups of sheep during glucagon infusion. Whereas in normal sheep the decreased hepatic removal was associated with decreased blood concentrations, in alloxanized sheep the change to net hepatic production of ketones was associated with an increase in ketone concentration. In the former case, the decrease in hepatic removal seems to be secondary to insulin reducing the ketone concentration in blood by enhancing peripheral re-

TABLE II. Plasma Glycerol Concentrations in Normal and Insulin-controlled Alloxan-diabetic Sheep before and during Glucagon Infusion[a]

Time (Min)	−60	−30	0	30	60	120
	Glycerol (μM)					
Normal	29 ± 3[b]	29 ± 3	29 ± 4	32 ± 9	26 ± 1	32 ± 10
Alloxanized	28 ± 6	24 ± 7	27 ± 7	40 ± 9[c]	35 ± 7[c]	32 ± 5

[a]Glucagon infusion (90 μg/hr for two hours) immediately after 0 min sample
[b]Values are mean ± SE of four experiments
[c]Significantly different from corresponding control (−60, −30 and 0 min) values ($P < 0.05$, paired t-test)
[d]Significantly different from corresponding value in normal sheep ($P < 0.05$)

TABLE III. Arterial Concentrations and Net Hepatic and Portal Productions of Acetoacetate (AAA) and β-hydroxybutyrate (HBA) in Normal and Insulin-controlled Alloxan-diabetic Sheep before and during Glucagon Infusion[a]

	Arterial Conc.		Hepatic Production		Portal Production	
	AAA	HBA	AAA	HBA	AAA	HBA
	mg/100 ml		g/hr		g/hr	
Normal						
Control	0.52 ± 0.05[b]	2.83 ± 0.39	−0.27 ± 0.06	0.22 ± 0.12	0.23 ± 0.10	0.95 ± 0.28
Infusion	0.36 ± 0.02[c]	2.32 ± 0.39	−0.10 ± 0.11[c]	−0.07 ± 0.19	0.31 ± 0.09	0.71 ± 0.20
Alloxanized						
Control	0.90 ± 0.25	2.19 ± 0.14	−0.54 ± 0.08	0.05 ± 0.07	0.33 ± 0.06	0.96 ± 0.06
Infusion	1.05 ± 0.32[e]	2.94 ± 0.17[d]	0.46 ± 0.07[d,e]	0.14 ± 0.06	0.26 ± 0.03	0.60 ± 0.11

[a]Glucagon was infused at 90 μg/hr for two hours
[b]Values are means ± SE of four experiments. For each experiment control and infusion values are the means of three sampling times. Negative values indicate net removal
[c]Values are significantly different from corresponding control values ($P < 0.05$, paired t-test)
[d]Values are significantly different from corresponding control value ($P < 0.01$, paired t-test)
[e]Significantly different from corresponding value in normal sheep ($P < 0.05$)

moval (1). In the latter case, the change from net hepatic removal to net hepatic release of ketone bodies, particularly acetoacetate, was a direct effect of glucagon on the liver. Thus, the rise in blood concentrations was secondary to hepatic events. This is consistent with observations reported for other species (12).

It is concluded that in sheep, glucagon can stimulate hepatic ketogenesis directly. This may be facilitated by mobilization of lipid. Free fatty acids (FFA) are known precursors of ketone bodies (4, 14).

Infusion of glucagon at 90 μg/hr probably would elevate plasma glucagon concentrations above 1 ng/ml, well beyond physiological levels. Plasma levels in sheep normally are 150-250 pg/ml. Nevertheless, this study shows that insulin can suppress the lipolytic and ketogenic effects of high doses of glucagon. The data further suggest that hyperglucagonemia in conjunction with hypoinsulinemia may play an important role in the development of hyperketonemia by promoting lipolysis and hepatic ketogenesis.

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BOOK REVIEW

VETERINARY REPRODUCTION AND OBSTETRICS — FOURTH EDITION. *G. H. Arthur. Published by Baillière Tindall, London. 1975. 616 pages. Price $20.00 approx.*

Veterinary Obstetrics by F. Benesch was first published in 1938. The second edition by F. Benesch and J. G. Wright appeared in 1951 and the third edition, Wright's Veterinary Obstetrics by G. H. Arthur, in 1964. The format of this edition is similar to the previous ones but as stated by the author in the preface, the changed title indicates "the importance of satisfactory reproduction in the economy of livestock enterprises . . .".

The book is in five parts and deals with the female and male of six species of domestic animals. Part one describes the estrous cycle and its control, the development of the conceptus, pregnancy, parturition and the puerperium. In parts two and three dystocia an dthe caesarean operation are described, and in part four infertility in the cow, mare, ewe, sow, bitch and cat. The final section is on normal and abnormal reproduction of male animals and includes a chapter on artificial insemination.

The range of the book is extensive, covering several disciplines including physiology, endocrinology, bacteriology, virology, surgery and therapeutics and, as a result, the material is of a general nature.

The descriptions of estrus and parturition and of procedures such as the caesarean section are good, as are those dealing with situations which require judgement and decisions, obstetrical cases for example. The information is of a practical nature, it is well written and the illustrations are effective, though not always accurate, e.g. a mid-line incision is recommended for the caesarean operation in the mare but a low flank incision is shown in the accompanying photograph.

However, there are sections in the book where the descriptions are too brief: for example, oogenesis and spermatogenesis are not explained and only limited information is provided on the morphology of spermatozoa and tests for the evaluation of semen. The same criticism applies to new knowledge on animal reproduction, e.g. the detail on the various uses of prostaglandins. Furthermore, reference to prostaglandins and certain other topics are listed as personal communications. In a book which provides only key references, personal communications are of no value to the reader who is seeking in-depth knowledge.

The basic material is good, but there is insufficient information on modern knowledge of animal reproduction and, as a result, the book will be of limited usefulness to the practising veterinarian. — *M. D. Eaglesome.*