Effect of Trauma on Plasma Glucagon and Insulin Concentrations in Sheep

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ABSTRACT

Portal plasma glucagon and insulin concentrations were measured before and after acute trauma (liver biopsy). The trauma was sufficient to increase glucagon concentrations and depress insulin concentrations. These changes were associated with a marked hyperglycemia. Infusion of glucagon was insufficient to prevent stress inhibition of insulin secretion. The stimulation of glucagon secretion and inhibition of insulin secretion were of about one hour duration. These findings indicate that glucagon and insulin in conjunction with the nervous system may play an important role in the development of stress related hyperglycemia.

RÉSUMÉ

Cette expérience visait à mesurer la quantité de glucagon et d'insuline plasmatiques, dans la veine porte, avant et après le prélèvement d'une biopsie hépatique. Un tel traumatisme s'avéra suffisant pour faire augmenter la quantité du glucagon et diminuer celle de l'insuline. Ces changements s'accompagnaient d'une hyperglycémie marquée. L'infusion de glucagon ne réussit pas à prévenir l'inhibition de la sécrétion d'insuline attribuable à l'agression. La stimulation de la sécrétion de glucagon et l'inhibition de celle d'insuline durèrent environ une heure. Ces observations révèlent que le glucagon et l'insuline, de concert avec le système nerveux, peuvent jouer un rôle important dans le développement de l'hyperglycémie reliée à l'agression.

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Submitted May 26, 1975.

INTRODUCTION

Previous reports have indicated that trauma (1, 10) and stress (4) can impair carbohydrate metabolism and alter the hormonal status in anesthetized animals. Increased concentrations of glucagon (4), a potent glycogenolytic and gluconeogenic agent in sheep (6), may account in part for the hyperglycemia associated with stress. In addition, reduced insulin concentrations would facilitate hyperglycemia (13).

In this report the effects of acute trauma on plasma glucagon and insulin concentrations were evaluated in conscious sheep in experiments conducted for other purposes as well. The trauma was induced by removing a small piece of liver, later used in analyses not reported here.

MATERIALS AND METHODS

Adult nonpregnant, nonlactating crossbred sheep weighing 50-60 kg were used in this study. They received one kg of alfalfa pellets daily divided into hourly aliquots to maintain steady state rumen fermentation. Water was available *ad libitum*. All animals were accustomed to the experimental room and handling, e.g. blood sampling. At least ten days prior to experimentation polyvinyl catheters were placed into the portal vein (1.10 x 1.36 mm) for blood sampling and a mesenteric vein tributary (0.84 x 1.10 mm) for infusing of experimental solutions (12).

During the experiments 12 ml aliquots of blood were taken at the times indicated

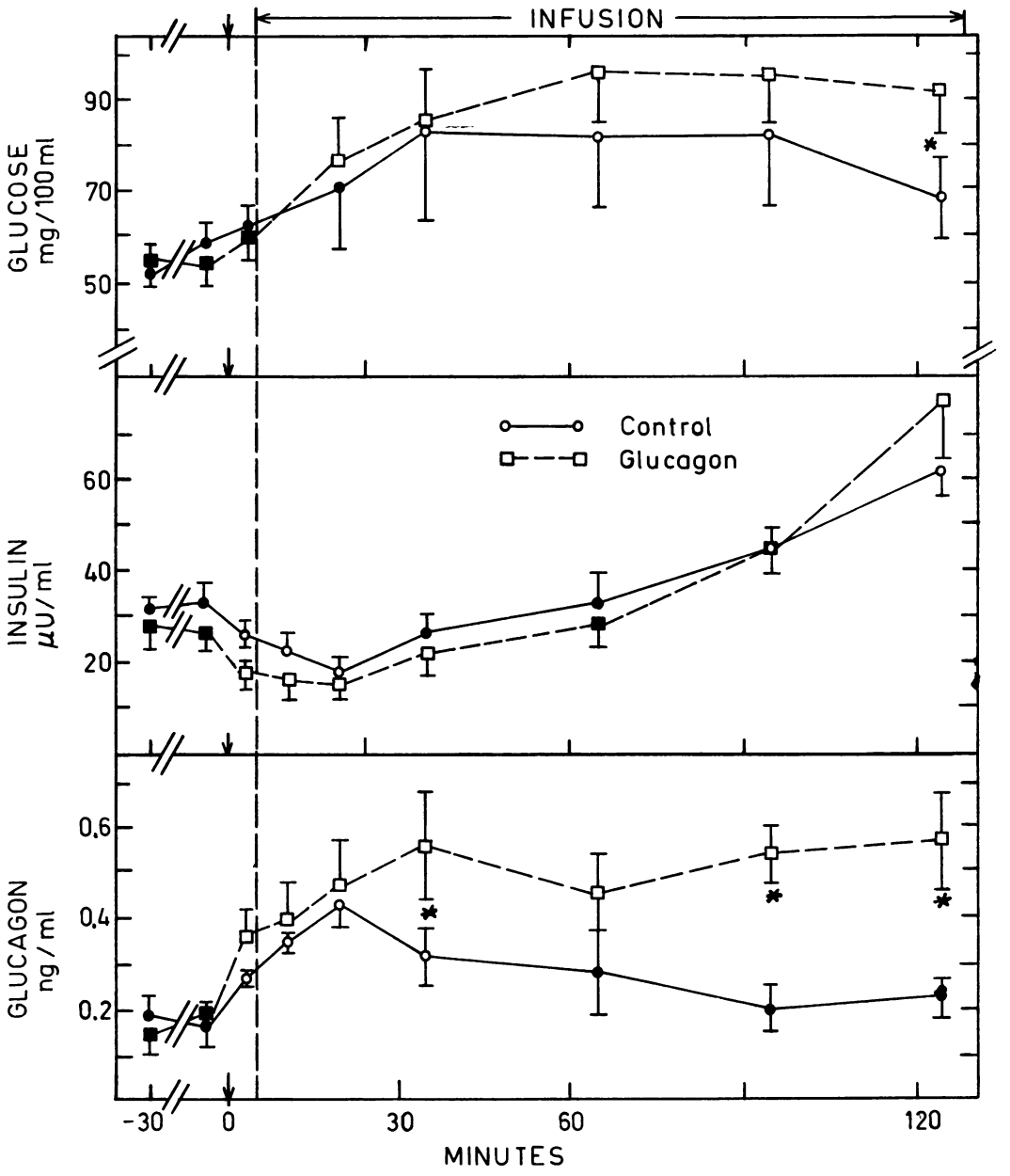

Fig. 1. Changes in portal vein concentrations of glucagon, insulin and glucose following liver biopsy in four control sheep and six sheep infused with glucagon. The vertical arrow indicates the time of biopsy. Local anesthesia was accomplished in the -30 to -5 minute interval. Values are means ± SE. Open symbols represent significant difference from -5 and -30 minute values ($P<0.05$, paired t-test). Asterisks represent significant differences between corresponding control and glucagon ($P<0.05$).

in Figs. 1 and 2. They were placed in chilled tubes containing 2500 K.I.U. of the proteinase inhibitor Trasylol[1] to reduce destruction of glucagon in blood and 7.5 mg of disodium ethylenediamine-tetraacetic acid as the anticoagulant.

In the experiments shown in Fig. 1 between the -30 and -5 min blood sampling interval the right flank was prepared for aseptic surgery. Local anesthesia was accomplished with an inverted L-block infiltration of 2% lidocaine hydrochloride[2]. A 15 cm flank incision was made starting 4 cm below the lumbar vertebral processes. A

[1]FBA Pharmaceuticals, New York, New York.

[2]Xylocaine, Astra Chemicals, Mississauga, Ontario.

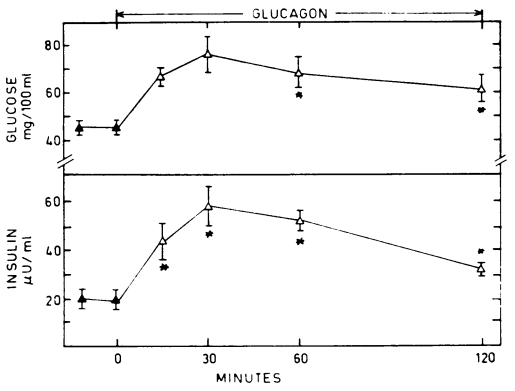

Fig. 2. Changes in portal vein insulin and glucose during glucagon infusion in five sheep. No surgery was performed in this experiment. Values are means ± SE. Open symbols represent significant differences from -15 and 0 minute values ($P<0.05$, paired t-test). Asterisks represent significant differences between corresponding values of Figs. 1 and 2 in glucagon infused sheep ($P<0.05$).

2-3 g liver sample was then removed at 0 min. After taking the first postbiopsy blood sample carrier (0.9% NaCl containing 0.2% bovine serum albumin) alone or carrier with glucagon was infused into the mesenteric vein at 0.386 ml/min. Glucagon was infused at 20 μg/hr to determine whether trauma could alter the glucagon induced hyperinsulinemia.

In a third set of experiments, glucagon was infused but no biopsy was performed. This was done to show the hyperinsulinemic effect of glucagon (Fig. 2).

Blood glucose concentrations were determined by the glucose oxidase method[3]. Glucagon and insulin concentrations were determined by radioimmunoassays using the assay systems reported previously (9). The glucagon antibody used in this study cross-reacts to some extent with gut glucagon-like immunoreactivity but nevertheless, the assay was capable of reflecting the relative changes in concentration of pancreatic glucagon in the plasma.

RESULTS

Figure 1 illustrates that trauma associated with the liver biopsy was sufficient to depress the plasma insulin concentrations and to elevate the plasma glucagon concentrations in sheep. These changes were transient, of less than 60 min duration. The insulin concentrations were depressed despite the marked hyperglycemia which was evident after the biopsy was performed in both control and glucagon infusion groups. However, after 60 minutes the insulin concentrations increased. The glucose and insulin concentrations were similar in both groups with glucose concentrations tending to be greater in the glucagon infused group. Figure 2 shows the normal insulin and glucose responses to glucagon infusions, indicating that trauma depressed the insulinogenic response of glucagon.

DISCUSSION

This report indicates that glucagon and insulin secretion can be increased and decreased, respectively, by trauma. These hormonal changes which promote hyperglycemia are probably sympathetic responses. Epinephrine stimulates glucagon secretion (3). Other studies in sheep (2, 8) show that epinephrine can inhibit while α-adrenergic blockade can enhance insulin secretion. Furthermore, studies with adrenalectomized calves indicate that direct stimulation of the splanchnic nerve enhances glucagon secretion and depresses insulin secretion (5).

There is the possibility that lidocaine (the local anesthetic) may be responsible for the hormonal changes. However, this seems unlikely. First of all, the changes were induced immediately after the liver biopsy was taken. This was a half hour after lidocaine was administered. Secondly, by 60 min the concentrations were not significantly different from -30 min values (except, of course, glucagon concentration during glucagon infusion) whereas the anesthetic effect lasted at least another two hours. Therefore, any systemic effect of lidocaine should have been apparent for that time rather than the transient effect which was observed. Thus, the rapid hormonal changes appear to be a physiological response to acute trauma.

Glucagon is known to enhance hepatic

[3]Glucostat, Worthington Biochemicals, Freehold, New Jersey.

gluconeogenesis (6) and insulin has been shown to inhibit glucose production and enhance the rate of removal of glucose (13). Thus, the hormonal changes following trauma (or stress) promote hyperglycemia. In this study, the blood glucose elevations are associated with increased glucagon and depressed insulin concentrations.

These findings suggest that the pancreatic hormones in conjunction with the nervous system play an important role in the minute to minute regulation of glucose metabolism including promotion of hyperglycemia in times of stress. This is consistent with the report by Phillips *et al* (11), that catecholamines require the pancreas to promote hyperglycemia. Glucagon, indeed, appears to be a stress hormone in sheep (7).

ACKNOWLEDGMENTS

This work was supported in part by the Medical Research Council of Canada. R. P. Brockman was an MRC Fellow when this work was carried out.

The authors wish to thank Mr. D. Geary for his help in drafting the figures.

REFERENCES

1. **ASHBY, M. M., D. F. HEATH and H. B. STONER.** A quantitative study of carbohydrate metabolism in the normal and injured rat. J. Physiol., Lond. **179**: 193-237. 1965.
2. **BASSETT, J. M.** The effects of glucagon on plasma concentrations of insulin, growth hormone, glucose, and free fatty acids in sheep: comparisons with the effects of catecholamines. Aust. J. biol. Sci. **24**: 311-320. 1971.
3. **BASSETT, J. M.** Plasma glucagon concentrations in sheep: their regulation and relation to concentrations of insulin and growth hormone. Aust. J. biol. Sci. **25**: 1277-1287. 1972.
4. **BLOOM, S. R., P. M. DANIEL, D. I. JOHNSTON, O. OGAWA and O. E. PRATT.** Release of glucagon, induced by stress. Q. J. exp. Physiol. **58**: 99-108. 1973.
5. **BLOOM, S. R., A. V. EDWARDS and N. J. A. VAUGHAN.** The role of sympathetic innervation in the control of plasma glucagon concentrations in the calf. J. Physiol., Lond. **233**: 357-366. 1973.
6. **BROCKMAN, R. P. and E. N. BERGMAN.** Effect of glucagon on plasma alanine and glutamine metabolism and hepatic gluconeogenesis in sheep. Am. J. Physiol. **228**: 1627-1633. 1975.
7. **BROCKMAN, R. P., E. N. BERGMAN, P. K. JOO and J. G. MANNS.** Effects of glucagon and insulin on net hepatic metabolism of glucose precursors in sheep. Am. J. Physiol. (in press). 1976.
8. **HERTELENDY, F., L. MACHLIN and D. M. KIPNIS.** Further studies on the regulation of insulin and growth hormone secretion in the sheep. Endocrinology **84**: 192-199. 1969.
9. **MANNS, J. G.** Separation of pancreatic and gut glucagon-like-immunoreactivity with observations on plasma concentrations of these hormones during lactation. Can. J. Physiol. Pharmac. **50**: 554-560. 1972.
10. **NIJJAR, M. S. and W. F. PERRY.** Effect of trauma on serum insulin levels in rabbits. Diabetologia **7**: 357-359. 1971.
11. **PHILLIPS, R. W., W. A. HOUSE, R. A. MILLER, J. L. MOTT and D. L. SOOBY.** Fatty acid, epinephrine and glucagon hyperglycemia in normal and depancreatized sheep. Am. J. Physiol. **217**: 1265-1268. 1969.
12. **ROE, W. E., E. N. BERGMAN and K. KON.** Absorption of ketone bodies and other metabolites via the portal blood of sheep. Am. J. vet. Res. **27**: 729-736. 1966.
13. **WEST, C. E. and R. F. PASSEY.** Effect of glucose load and of insulin on the metabolism of glucose and of palmitate in sheep. Biochem. J. **102**: 58-64. **1967.**