The effects of insulin and glucagon on ketone-body turnover

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A double-isotope procedure was used to measure the effects of insulin and glucagon on ketone-body production and utilization (i.e. turnover) in the starved rat. Somatostatin was infused during the experiment to suppress the pancreatic release of either hormone. The immediate action of insulin in terms of ketone-body turnover that was most evident was a decreased production of 3-hydroxybutyrate, with no significant change in the turnover of acetoacetate. Similarly, the significant effect of glucagon infusion was to increase the production of 3-hydroxybutyrate, with minimum increase in acetoacetate turnover. The data support a direct effect of both hormones on the distribution of acetyl units derived from fatty acid β -oxidation.

The anti-ketogenic action of insulin and the ketogenic effect of glucagon have been long recognized both *in vivo* and *in vitro* (cf. McGarry & Foster, 1981). Although there is virtually complete agreement with these respective actions by the two hormones, some discrepancies have arisen in studies with insulin where its addition to liver perfusates did not elicit a decrease in ketone-body production (Penhos *et al.*, 1968; Haft, 1968). This lack of anti-ketogenicity has been attributed to inadequate concentrations of glucose in the perfusion solution (Neufeld *et al.*, 1983). It was concluded in this latter work that a fasting concentration of glucose was required for insulin to exert its anti-ketogenic action. The actions of both insulin and glucagon have been ascribed generally to changes in the rates of ketogenesis (McGarry & Foster, 1981).

Studies with a labelled fatty acid precursor alone do not provide adequate information about the action of these hormones on rates of individual and simultaneous production and utilization of ketone bodies (i.e. turnover), since other sources of acetyl-CoA (e.g. pyruvate, branched-chain amino acids and lactate) contribute to the production of ketone bodies. To determine the influence that these hormones exert on ketone-body turnover, the present study utilizes the double-isotope application to our '*in vivo*' rat infusion model (Ozand *et al.*, 1977, 1978; Reed *et al.*, 1984). In addition, the simultaneous administration of somatostatin has been used to suppress the pancreatic release of insulin or glucagon when the concentration of the

other hormone is being manipulated by infusion (Alberti *et al.*, 1973; Koerber *et al.*, 1974). In this manner, the effect of increasing insulin, for example, may be assessed in terms of the effect of hypoglycaemia on ketone-body turnover in the absence of hypoglycaemia-induced catecholamine release, which, in turn, triggers glucagon release (cf. McGarry & Foster, 1981). Likewise, the effect of increased concentrations of glucagon may be examined in the absence of significant changes in insulin concentrations, in a normal, i.e. non-diabetic, model *in vivo*. This system allows the creation of a unique state of insulinaemia or glucagonaemia in the rat, with circulating hormone concentrations maintained within physiological limits. In this work we have used [^{14}C]octanoate to trace the fatty acid pool of ketone-body precursors. We have selected this medium-chain fatty acid because its oxidation is not mediated by regulation of the carnitine transferase system, nor is it eligible for esterification (cf. McGarry & Foster, 1980, 1981). We have additionally determined that octanoate supports ketogenesis in the starved rat at rates comparable with those for long-chain fatty acids (e.g. oleic acid; results not shown). Thus the rationale for the choice of octanoic acid as the ketone-body precursor is that, if either entry of the fatty acid into mitochondria or the hydrolysis of triacylglycerol were primarily involved in the action of either hormone, no effect on ketone-body turnover would be observed. Since both hormones were found to be effective in the present work, the breakdown of triacylglycerol and the entry of fatty

acids into the mitochondrial matrix should be eliminated as sites for the direct and immediate action of insulin or glucagon.

Experimental

Animals and reagents

Wistar rats of both sexes, aged 5–6 weeks, were used. They were fed on regular laboratory chow (Ralston Purina Co., St. Louis, MO, U.S.A.) *ad lib.* until they were placed in metabolic cages and starved for 48 h (72 h for kinetic studies) before experimentation. The animals had free access to water.

The source of all reagents, radioactive chemicals, biochemicals for radioimmunoassay, and enzymes for perfusion studies have been reported (Ozand *et al.*, 1977, 1978). The crystalline zinc insulin was purchased from E. Lilly (Indianapolis, IN, U.S.A.) and was free of glucagon. Crystalline glucagon and somatostatin were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Perfusion procedure

The general procedure for perfusion has been described (Ozand *et al.*, 1977, 1978). In the present experiments, the 48 h-starved rats were injected intraperitoneally with 100 µg of somatostatin/kg body wt. in saline (0.9% NaCl) 30 min before the experiment. In all experiments, at zero time the animals also received a solution (2 ml/kg body wt.) of non-esterified fatty acids and citrate-depleted bovine serum albumin [4.6% (w/v) in saline] that contained 50 µg of somatostatin/ml (prime solution). The ³H-labelled ketone body and ¹⁴C-labelled fatty acid were added when turnover measurements were performed. The rats were then continuously injected (12 ml/kg body wt. per h) with a solution of 5% (w/v) bovine serum albumin in saline that contained 16.7 µg of somatostatin/ml (perfusion solution). When indicated, ³H-labelled ketone bodies and ¹⁴C-labelled fatty acids were added. After measurements of baseline concentrations of blood intermediates or hormones and of the rate of ketone-body turnover, either insulin or glucagon was added to the perfusion solution at 29 or 30 min. The hormone doses administered were: insulin, 5.3 munits (0.24 µg) of crystalline zinc insulin/kg; glucagon, 6 µg/kg. The animals were then injected for the next 30 min at a rate of 12 ml/kg per h with the perfusion solution also containing these hormones. When the effect of insulin was studied, the dose of insulin was 57 munits (2.6 µg)/kg per h, and when glucagon was injected it was 45 µg/kg per h. The perfusion solution contained additionally ³H-labelled ketone bodies and ¹⁴C-labelled fatty acids, as indicated. At the indicated time intervals, arterial blood was removed for indicated measurements.

Analyses and calculations

The analyses of intermediates (Ozand *et al.*, 1975) and of hormones (Ozand *et al.*, 1978) have been described. We have reported the methods of calculating ketone-body turnover and substrate conversion into ketone bodies (Reed *et al.*, 1984).

Results

The data presented in Figs. 1 and 2 illustrate the changes in this rat model, on infusion of insulin or glucagon, in the concentrations of both hormones as well as blood concentrations of glucose and ketone bodies. The efficacy of somatostatin is evidenced by the maintenance of insulin or glucagon on administration of the other hormone (Figs. 1a and 1b). Thus an approx. 6-fold difference was created in the blood ratio of insulin/glucagon (Fig. 1c). In 20 rats, comparing fed and starved, this insulin/glucagon ratio varied over a range of approx. 4-fold during the infusion. Additionally, we have administered glucose or alanine to these rats which had been continuously infused with somatostatin. In neither case was there a significant increase in the baseline blood concentrations of insulin or glucagon (results not shown).

The data in Fig. 2 demonstrate the expected actions of these hormones. There was a dramatic increase in the concentrations of glucose and ketone bodies on glucagon infusion. Correspondingly, the hypoglycaemic–anti-ketogenic action accompanying the infusion of insulin was clearly evident.

In Fig. 3 the changes in the blood concentration of [3-¹⁴C]octanoate are shown. The mean concentration before the administration of hormones (i.e. from 12 to 28 min) was relatively constant and remained approximately the same (28 µM before, 22 µM after) in both groups of rats. It was necessary to include some octanoate in the loading solution at the time of hormone administration at 29 min, because, as shown below, the utilization of this fatty acid was substantial. Had octanoate been omitted, a significant decline would have occurred in its concentration in the blood (results not shown). In the present experiments, the blood octanoate concentration increased approx. 50% with either hormone (Fig. 3). Apparently, the rate of utilization in either case was not of sufficient magnitude to remove, in the time remaining, this additional dose of octanoate.

The comparative rates of overall ketone-body production and utilization, octanoate utilization and octanoate conversion into ketone bodies before and after the injection of insulin (Table 1a) or glucagon (Table 1b) are shown. In these experiments, the changes in blood glucose and ketone-

Fig. 1. *Effect of pancreatic hormones on the blood concentrations of hormones*

Twelve rats were treated with somatostatin and were then infused for 30 min continuously (○), at which time they were given either insulin (■) or glucagon (●). The doses of these hormones and procedures were as described in the Experimental section. The results are averages $\pm$ S.E.M. ($n = 6$). The average body weight ($\pm$ S.E.M.) of 48 h-starved rats was 103 ± 6 g. * $P < 0.01$ when results in the insulin group were compared statistically with those in animals that received the glucagon injection. [Insulin]/[glucagon] is the molar ratio.

Fig. 2. *Effect of pancreatic hormones on the blood concentration of intermediates*

The experimental conditions were the same as in Fig. 1. The results are averages $\pm$ S.E.M. of the blood concentrations of glucose, acetoacetate and 3-hydroxybutyrate. The symbols and statistical significance are as described for Fig. 1.

Table 1. *Effect of pancreatic hormones on ketone-body turnover*

The experimental animals were as described in the Experimental section. The weights of rats after 48 h starvation were (average $\pm$ S.E.M., $n = 8$): in insulin experiments (a) 101.4 ± 1.3 g and in glucagon experiments (b) 113.1 ± 2 g. At zero time all animals were given a 'prime solution' that contained additionally $10 \mu\text{Ci}$ of D-3-hydroxy[4- ^3H]butyrate and $35 \mu\text{Ci}$ of sodium [$1\text{-}^{14}\text{C}$]octanoate (sp. radioactivity of 5.5 Ci/mol)/kg. The 'perfusion solution' given from 0 to 30 min contained additionally $64 \mu\text{Ci}$ of D-3-hydroxy[4- ^3H]butyrate and $200 \mu\text{Ci}$ of [$1\text{-}^{14}\text{C}$]octanoate/kg per h. The baseline blood concentrations (means $\pm$ S.E.M.) of intermediates at 12 min were: in (a), insulin experiment, acetoacetate $408 \pm 27 \mu\text{M}$, 3-hydroxybutyrate $2.51 \pm 0.18 \text{ mM}$, glucose $6.67 \pm 1.2 \text{ mM}$; in (b), glucagon experiment, acetoacetate $548 \pm 74 \mu\text{M}$; 3-hydroxybutyrate $2.99 \pm 0.29 \text{ mM}$; glucose, $4.24 \pm 0.2 \text{ mM}$. The baseline ketone-body turnover, octanoate utilization and octanoate conversion were measured between 12 and 28 min. At 29 min insulin (a) or glucagon (b) was administered at the doses indicated in the Experimental section in 2 ml of 'perfusion solution'/kg. At the time of hormone injection (29 min) the blood values were: in (a), insulin experiment, acetoacetate $438 \pm 52 \mu\text{M}$, 3-hydroxybutyrate $2.68 \pm 0.26 \text{ mM}$, glucose $8.00 \pm 1.68 \text{ mM}$; in (b), glucagon experiment, acetoacetate $557 \pm 71 \mu\text{M}$, 3-hydroxybutyrate $3.37 \pm 0.32 \text{ mM}$, glucose $4.51 \pm 0.3 \text{ mM}$. The hormones were added to the perfusion solution at the doses described in the Experimental section. The ketone-body turnover, octanoate utilization and conversion into ketone bodies under the influence of hormones were measured from 36 to 56 min. Results are shown as averages $\pm$ S.E.M. for eight rats in each experiment. The P values reflect statistically significant differences between 'before' and 'after' hormone injection (N.S., $P > 0.05$). The percentage conversion of octanoate utilized into total ketone bodies is listed.

	Ketone-body turnover ($\mu\text{mol/min per kg}$)						Octanoate conversion into ketone bodies (nmol/min per kg)						Octanoate converted into ketone bodies (%)	
	Acetoacetate utilization		Acetoacetate production		3-Hydroxybutyrate utilization		3-Hydroxybutyrate production		Acetoacetate		3-Hydroxybutyrate		Before	After
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After		
	P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.	
(a) Insulin	11.3 $\pm$ 1.4	10.3 $\pm$ 1.4	13.3 $\pm$ 1.4	10.3 $\pm$ 2.0	99.7 $\pm$ 12.5	64.0 $\pm$ 9.8	116.4 $\pm$ 11.4	67.4 $\pm$ 1.0	40.8 $\pm$ 4.7	30.6 $\pm$ 8.0	116.1 $\pm$ 13.2	71.0 $\pm$ 16.0	40	21
	P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.	
(b) Glucagon	17.1 $\pm$ 3.8	15.1 $\pm$ 4.0	16.9 $\pm$ 3.9	21.0 $\pm$ 3.9	99.5 $\pm$ 25.4	91.4 $\pm$ 23.8	102.7 $\pm$ 18.8	163.6 $\pm$ 18.8	21.0 $\pm$ 4.3	34.4 $\pm$ 4.7	104.6 $\pm$ 20.4	252.7 $\pm$ 22.9	38	64
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	P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.	
	P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.	
	P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.	
	P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.	
	P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.	
	P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.	
	P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.	
	P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.	
	P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.		P N.S.	
	P N.S.		P N.S.		P N.S.									

Fig. 3. Changes in blood octanoate concentrations. The experimental conditions were as described in Table 1. The results are averages $\pm$ S.E.M. for eight rats: (○) before injection of insulin or glucagon, (■) after injection of insulin, and (●) after injection of glucagon.

body concentrations after the injection of hormones at 29 min (results not shown) were similar to those of Fig. 2.

The rate of 3-hydroxybutyrate turnover, but not that of acetoacetate, was significantly decreased after insulin administration (Table 1a). The decrement in 3-hydroxybutyrate utilization was less than that in its production (36 and 49 μ mol/kg per min respectively); hence the anti-ketogenic action of insulin. Paralleling these changes was the decreased conversion of octanoate into 3-hydroxybutyrate, but not into acetoacetate. The overall rate of octanoate utilization was 24% higher after the hormone addition, an expected finding considering the 65% increase in circulating octanoate concentration (cf. Fig. 3). The conversion of octanoate into ketone bodies represented 46% of its utilization before insulin and 21% after the hormone injection.

Table 1(b) presents the same parameters after glucagon injection. Once again, the predominant effect was in the turnover of 3-hydroxybutyrate; its

production increased 35%. The utilization of acetoacetate decreased and its production increased after glucagon injection, but neither was significantly different. The conversion of octanoate into acetoacetate was increased by 64% and into 3-hydroxybutyrate by 142%. These results are consistent with a marginal increase in blood concentration of acetoacetate, compared with the significant increase in 3-hydroxybutyrate (Fig. 2). The rate of octanoate utilization was 36% higher after glucagon injection, again, a finding consistent with a 45% increase in circulating octanoate concentration (cf. Fig. 3). In this experiment the conversion of octanoate into ketone bodies represented 38% of its utilization before the glucagon infusion and 64% after.

Discussion

The present model demonstrates the immediate effects of insulin or glucagon. The data in Figs. 1 and 2 provide evidence that the infusion of somatostatin allows either insulin or glucagon concentration to be raised without response from the pancreas in terms of the other hormone. The concentrations of these hormones infused were adequate to elicit the ketogenic (glucagon) or anti-ketogenic (insulin) effects expected. Thus this model has allowed manipulation, within physiological limits, of the more important ratio insulin/glucagon, as opposed to their absolute concentrations. These data are consistent with the hypothesized direct and immediate effects of glucagon and insulin (Neufeld *et al.*, 1983).

The nature of the model (i.e. starved rat) is conducive to investigations of ketone-body turnover; the increased 'reduced' state of the liver suggests that major effects on ketone-body metabolism might be observed more readily in the turnover of 3-hydroxybutyrate rather than that of acetoacetate. In fact, the effects of both hormones were seen in terms of overall production of 3-hydroxybutyrate and not of acetoacetate (Table 1). The increased 'reduced' state of liver is evidenced by the approx. 5-fold excess of 3-hydroxybutyrate over acetoacetate (Fig. 2).

Since octanoate encounters no barrier to its entry into the mitochondrial matrix, any immediate effect of insulin or glucagon on ketogenesis from this substrate precludes entry as a site of action (Table 1). Likewise, since octanoate is not esterified as triacylglycerol, the observed effects of insulin and glucagon cannot be attributed to triacylglycerol hydrolysis. However, undoubtedly significant changes in lipolysis after insulin or glucagon have occurred, but, owing to the time of the experiment, should have been independent of the present results.

The utilization of octanoate for ketone-body synthesis decreased by 50% after insulin, and increased by 50% after glucagon injection. In both instances, this occurred while the overall utilization of octanoate increased. An immediate influence of each hormone on the distribution of acetyl-CoA units derived from β -oxidation is concluded.

The decreased 3-hydroxybutyrate utilization after insulin injection was coincidental with a decreased blood glucose concentration, i.e. increased utilization of glucose in peripheral tissues. This could not be explained by a sudden decrease in the rate of 3-hydroxybutyrate production, since no increased 3-hydroxybutyrate utilization occurred when its production was increased after glucagon injection (Table 1).

In summary, immediate effects of insulin and glucagon have been shown on ketone-body turnover. These effects appear to be directly involved with the disposition of acetyl-CoA units derived from the β -oxidation of fatty acids, and are not due to secondary actions of these hormones on lipolysis and triacylglycerol synthesis or breakdown. The predominance of effects on 3-hydroxybutyrate, reflecting significant changes in either its production or its utilization, probably reflects the 'reduced' redox state of the starved liver.

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