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Effects of Somatostatin and Adrenergic Blockade on Glucagon, Insulin and Glucose in Exercising Sheep

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Abstract

This study was conducted to characterize the mechanisms of hyperglycaemia in exercising sheep. Sheep were run on a treadmill for 45 min (5·5 km h^{-1}, 8% incline) during adrenergic blockade (propranolol or phentolamine mesylate infusions) and during suppression of the rise in glucagon by infusion of somatostatin (SRIF). Propranolol did not alter the glucagon, insulin or glucose responses, except it tended to increase the metabolic clearance of glucose, presumably as a result of blocking the β-adrenergic inhibition of glucose uptake. Phentolamine mesylate administration was associated with a suppression of the rise in glucagon concentrations, a reversal of α-adrenergic inhibition of insulin release and a reduction in glucose appearance during exercise. SRIF prevented the rise in glucagon and reduced insulin concentrations to below resting values. Propranolol and phentolamine mesylate did not alter the glucagon, insulin or glucose responses to SRIF. However, SRIF prevented the insulin rise that occurred during phentolamine administration. The increment in glucose appearance produced in response to exercise was the same for SRIF, SRIF plus phentolamine mesylate and phentolamine mesylate in the first 25 min of exercise, but was significantly less than in the controls. During the last 20 min of exercise, glucose appearance was not significantly different from the control for any of the groups. The depression by SRIF and α-adrenergic blockade of the increment in glucose appearance due to exercise was associated with an impairment of the glucagon response. It appears, therefore, that glucagon may stimulate glucose production early in exercise in sheep directly, as well as by having a permissive effect.

Introduction

Increased glucose production and hyperglycaemia are characteristic responses to exercise in sheep (Brockman 1979*a*, 1979*b*). The mechanism of the increase in glucose production is not clear. It appears that in sheep (Brockman 1979*b*) and dogs (Issekutz and Vranic 1980) glucagon may be responsible for about half the increase in glucose production early in exercise. The studies in dogs (Kawamori and Vranic 1977; Issekutz and Vranic 1980) indicate that low levels of insulin are necessary for this increase in glucose production. Moreover, studies in man (Wahren *et al.* 1971) suggest that lowered insulin may produce the increase in hepatic glucose output.

The purpose of this study was to quantitate the contributions of glucagon and adrenergic mechanisms to the increased glucose production during exercise. The exercise-induced rise in glucagon was inhibited by infusion of somatostatin. Adrenergic stimuli were inhibited by the administration of propranolol or phentolamine mesylate before and during exercise. Somatostatin administration and α-blockade were associated with equal suppression of the glucose response to exercise. β-Blockade had no effect on the hormonal and metabolic adjustments to exercise.

Materials and Methods

Animals

Six yearling crossbred (predominantly Columbia–Suffolk) lambs weighing 28-31 kg were used in this study. They received 400 g of lucerne pellets twice daily, and water and salt licks were provided *ad libitum*. However, the sheep had not been fed for 24 h at the time of an experiment. The sheep were accustomed to running on the treadmill: each was run at least twice weekly for 4 weeks before being used for any experiment.

The day before a series of experiments was begun on a sheep, polyvinyl catheters were fitted in each jugular vein. The positioning was such that the tip of the left was about 15 cm deep and the tip of the right was at least 20 cm deeper than the left. The right catheter was used for administering solutions. However, the somatostatin (SRIF) priming dose was injected through the left catheter which was also used for taking blood samples. The catheters remained in place for the entire series of experiments on any given sheep. Between experiments, patency was maintained by filling the catheters with heparin (500 U ml^{-1}).

Experimental Procedure

All experiments were conducted on a treadmill (Model 18-72D, Quinton Instruments, Seattle, Wa.). At the beginning of an experiment, a sheep was placed on the stationary treadmill. A priming dose of 50 μCi of [2-^{3}H]glucose (Amersham Corp., Oakville, Ontario) was administered. The isotope was then infused at 0·6 μCi min^{-1} (White *et al.* 1969), 3 h being allowed for isotopic equilibration and obtaining pre-exercise samples. The sheep was then exercised for 45 min at 5·5 km h^{-1} with the treadmill at an 8% incline. The isotope infusion was continued for 45 min after termination of exercise.

In those experiments where phentolamine mesylate (PM, Rogitine, Ciba-Geigy Canada Ltd, Dorval, Quebec) or propranolol (Inderal, Ayerst, Dorval, Quebec) was infused, the drug was administered throughout the entire isotope-infusion period. The priming doses, 20 and 30 mg, respectively, were administered just after the isotope infusion was started. The infusion rates were 0·10 and 0·38 mg min^{-1}, respectively. In experiments in which SRIF (Lot # AY-24,910, Ayerst Research Lab., Dorval, Quebec) was used, 160 μg was injected intravenously immediately before exercise was begun. This was followed by infusion at 2 μg min^{-1} throughout the exercise period.

All solutions were made up in pyrogen-free sterile saline (154 mM NaCl). SRIF solutions also contained as carrier 0·2 mg lactose per microgram of SRIF. The carrier of the isotope was 100 mg dextrose per millicurie of [2-^{3}H]glucose.

The controlled experiments were conducted in two groups. In group 1 experiments, saline, PM, PM plus SRIF and SRIF were infused. In group 2 experiments, saline, propranolol, propranolol plus SRIF and SRIF were infused. In each group, six sheep were paired for experiments. For one sheep of a pair, the experimental order was control, adrenergic blockade, adrenergic blockade plus SRIF and SRIF. For the other, the order of experiments was reversed. This was done to minimize effects of conditioning on the interpretation. Experiments on each sheep were conducted at 3-day intervals.

Blood samples were taken in heparinized syringes at 120, 90, 60, 30, 15 min and just before exercise was initiated, at 5, 15, 25, 35 and 45 min of exercise, and at 15 and 45 min after exercise. They were immediately placed into tubes chilled on ice and the plasma was harvested after centrifugation in a refrigerated centrifuge.

Dipotassium ethylenediamine tetraacetate was added to the plasma at 4 mg ml^{-1} of plasma. A proteinase inhibitor, aprotinin [Trasylol, Boehringer–Ingleheim (Canada) Ltd, Dorval, Quebec], was added to aliquots of plasma (500 kallikrein inactivating units per millilitre of plasma) to be used in glucagon determinations.

Analytical Methods

Glucose concentrations and radioactivities were determined for all samples, the former by a commercial kit (Statzyme, Worthington Diagnostics, Freehold, New Jersey) and the latter by counting reconstituted aliquots directly after freeze-drying to remove the labelled water. After the aliquots were reconstituted with 1 ml of water, 4·5 ml of phase-combining solution (Amersham Corp.) were added. Quench correction was by the external standard ratios method. Blood samples taken prior to administration of the primer dose of isotope were used to determine the background counts for

each experiment. Radioactivity determinations by this procedure gave values $101 \cdot 8 \pm 1 \cdot 1\%$ (mean $\pm$ s.e., $N = 10$) of the values obtained by isolation as potassium gluconate (Blair and Segal 1960).

Glucagon and insulin concentrations were determined in plasma by double antibody radioimmunoassays (Brockman 1979*a*) using antibodies developed in guinea pigs. The radioiodinated glucagon and insulin were purchased from New England Nuclear (Canada) Ltd, Lachine, Quebec and Amersham Corp., respectively. Hormone standards were porcine glucagon and bovine insulin (Lilly Research Laboratories, Indianapolis, Indiana). The sensitivity of the glucagon assay was < 20 pg ml^{-1}. Its intra- and interassay repeatabilities were 10 and 18%, respectively. The sensitivity of the insulin assay was $< 0 \cdot 5$ μU ml^{-1}. Intra- and interassay repeatabilities were both 6%.

Calculations

Glucose appearance rates (RA) were calculated by the non-steady-state equation of Steele (1959) using a pool fraction of 0·65 (Cowan and Hetenyi 1971) (eqn 1). Pool sizes were estimated by curve fitting (Steele *et al.* 1956). The metabolic clearance rates (MCR) of glucose were calculated as the ratio of glucose removal and mean glucose concentration for each interval (eqn 2).

$$\text{Rate of appearance, } A, = [F - (0 \cdot 65 \cdot V \cdot \overline{C} \cdot \Delta S \cdot \Delta t^{-1})]\, \overline{S}^{-1} \quad (1)$$

$$\text{Metabolic clearance rate} = (A - 0 \cdot 65 \cdot V \cdot \Delta C \cdot \Delta t^{-1})\, \overline{C}^{-1} \quad (2)$$

F and V are the infusion rate of isotope (μCi min^{-1}) and distribution volume of glucose (litres). $\overline{C}$ and $\overline{S}$ are mean concentration and specific radioactivity, respectively, and ΔC and ΔS the changes in concentration and specific radioactivity, respectively, of glucose for the interval, Δt, during which the rates of glucose appearance and clearance were calculated.

Statistical Analyses

For each treatment group, significance during exercise was assessed by analysis of variance in a randomized block design. Each value during and after exercise was compared to the combined pre-exercise values using the individual degree of freedom analysis (Li 1964). Significant differences among treatments at each sampling time or interval were determined by comparing the respective increments (values minus the mean of the three pre-exercise values) due to exercise. All treatments were compared to the control experiments. In addition, the SRIF experiments were compared to SRIF plus adrenergic blockade experiments. The level of significance was 0·05.

Results

β-Adrenergic blockade was not associated with any significant effect on glucose concentrations (Fig. 1). SRIF, with and without propanolol, resulted in similar inhibition of the hyperglycaemia of exercise. Glucose appearance was also significantly depressed by SRIF during the first 15 min of exercise (Fig. 2). This response to SRIF was not altered by propranolol. While propranolol did not affect glucose production, it increased the MCR of glucose significantly over two intervals during exercise.

Propranolol administration was not associated with any significant alteration of the glucagon or insulin responses to exercise (Fig. 3). SRIF, with and without propranolol, prevented the rise in glucagon during exercise. Insulin concentrations were significantly lower during exercise than before in both SRIF-treated groups, and significantly lower than in the respective non-SRIF group during exercise.

α-Adrenergic blockade prevented the hyperglycaemic response to exercise (Fig. 4). In fact, after 25 min, the concentration of glucose was significantly lower than pre-exercise values. During phentolamine treatment glucose concentrations during exercise were significantly lower than for phentolamine plus SRIF. During SRIF treatment alone, the plasma glucose concentrations were significantly greater than the pre-exercise values for the last 10 min of exercise, but were significantly less than in the control experiments.

Phentolamine and SRIF equally suppressed the increment in glucose production due to exercise, but these effects were not additive (Fig. 5). At no time during exercise was the glucose production following PM treatment significantly different than during PM plus SRIF treatment. The MCR, on the other hand, was significantly greater during 25–45 min of exercise for the PM group than for the other groups.

PM significantly reduced the glucagon response early in exercise (Fig 6), but only for 5 min. SRIF plus PM and SRIF alone both prevented the rise in glucagon. The insulin concentration, on the other hand, was significantly elevated by exercise with PM. This rise was prevented by SRIF. Insulin concentrations were the same during SRIF administration with and without PM.

Discussion

The calculation of RA during non-steady state has limitations. Norwich *et al.* (1974) using inulin in dogs estimated that this approach has an error of 11 $\pm$ 3%. The error may be greater when $\Delta S/\Delta t$ is larger, as in the early intervals of exercise. In studies on sheep (R. P. Brockman, unpublished observations), the error in estimate of RA over 10 min when RA increased three to fourfold was 9 $\pm$ 2%. The error was the same when the interval was increased to 15 min, but was larger, although not markedly so, when the interval was decreased to 5 min. The largest error in estimating RA was obtained when RA was decreasing and then it tended to be underestimated. This situation occurred at the end of exercise.

SRIF was used in this study to inhibit the secretion of glucagon and insulin during exercise. The purpose of this was to develop a model to examine the direct effects of sympathetic stimulation on glucose appearance during exercise versus those effects mediated indirectly by pancreatic hormones. Adrenergic blockade did not alter the inhibitory effect of SRIF on glucagon and insulin secretion (Brockman 1979*b*). SRIF, in fact, prevented the rise in insulin due to exercise during α-adrenergic blockade.

Studies with human subjects suggest that SRIF may impair the rise in growth hormone due to exercise (Chalmers *et al.* 1977). This has not been reported in sheep.

Fig. 1. Concentration of glucose before, during and after exercise in control (——) experiments and during infusions of propranolol (- - -), propranolol plus SRIF (–·–·–)(and SRIF (·····). Open symbols denote significant differences ($P < 0{\cdot}05$) from corresponding pre-exercise values. The values for SRIF and propranolol plus SRIF during and after exercise are significantly different from corresponding values for control and propranolol experiments, respectively. Values are means $\pm$ s.e., $N = 6$.

Fig. 2. Rates of appearance (RA) and metabolic clearance rates (MCR) of glucose before, during and after exercise. Groups are control, propranolol (PR), propranolol plus SRIF and SRIF infusions. D denotes significant differences ($P < 0{\cdot}05$) from corresponding pre-exercise values. An asterisk within the bars indicates that values are significantly different from the corresponding control values. An asterisk above a bar indicates that the values are significantly different from the corresponding propranolol plus SRIF values. Values are mean + s.e., $N = 6$.

Fig. 3. Insulin and glucagon concentrations in plasma before, during and after exercise. Experimental groups are control (——), propranolol (- - -), propranolol plus SRIF (–·–·–) and SRIF (····) infusions. Open symbols denote significant differences ($P < 0{\cdot}05$) from corresponding pre-exercise values. Glucagon concentrations during SRIF infusion and during propranolol plus SRIF infusion are significantly different from control and propranolol values during exercise, respectively. Insulin values for both SRIF groups are significantly lower than corresponding values during exercise in control experiments. Values are means $\pm$ s.e., $N = 6$.

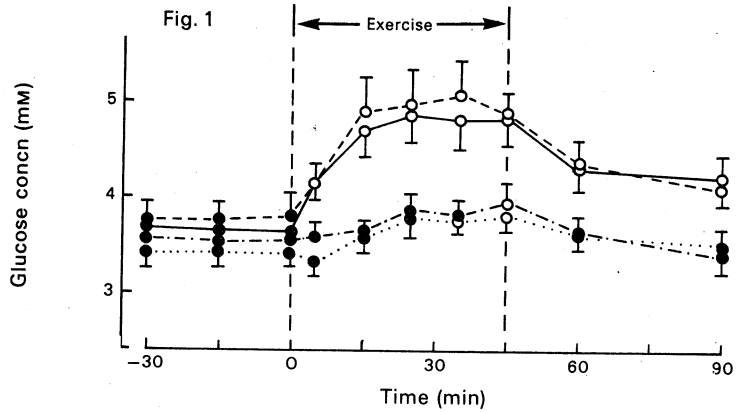


Fig. 1
Exercise
Glucose concn (mM)
5
4
3
−30
0
30
60
90
Time (min)

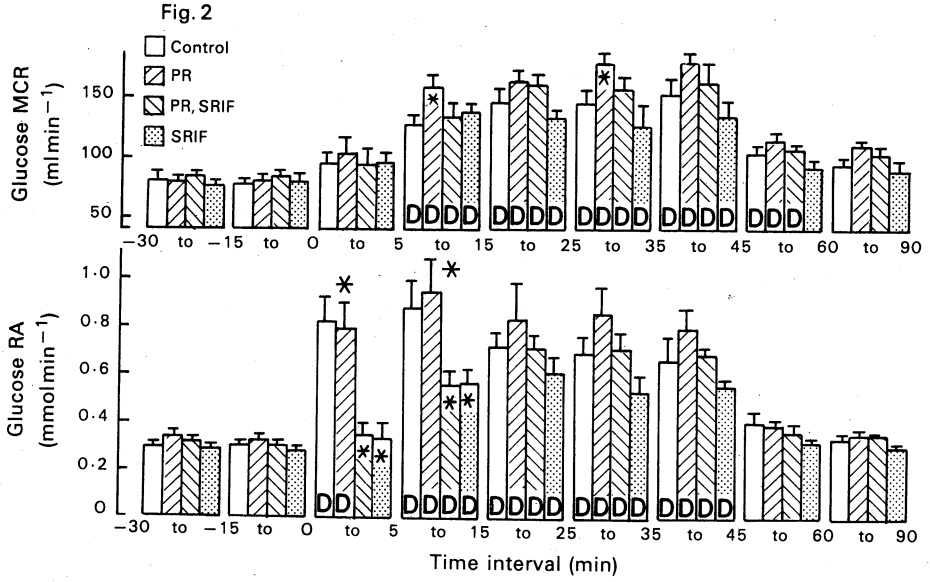


Fig. 2
Control
PR
PR, SRIF
SRIF
Glucose MCR (ml min−1)
150
100
50
Glucose RA (mmol min−1)
1·0
0·8
0·6
0·4
0·2
0
−30 to −15 to 0 to 5 to 15 to 25 to 35 to 45 to 60 to 90
Time interval (min)

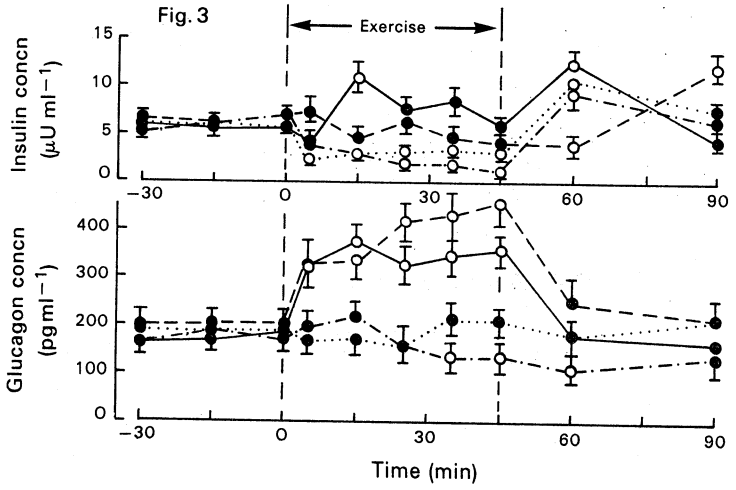


Fig. 3
Exercise
Insulin concn (μU ml−1)
15
10
5
0
Glucagon concn (pg ml−1)
400
300
200
100
0
−30
0
30
60
90
Time (min)

Fig. 4

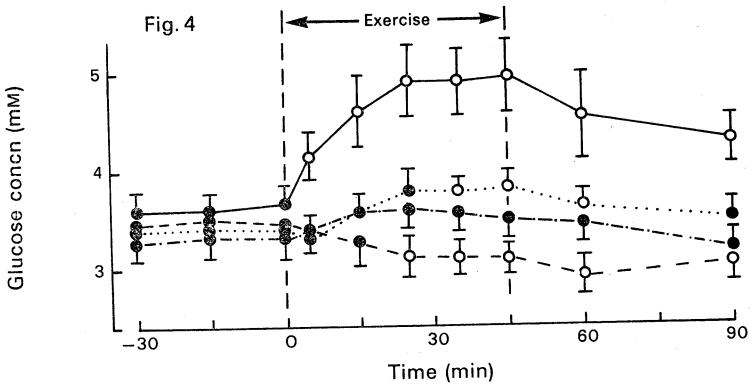


Fig. 5

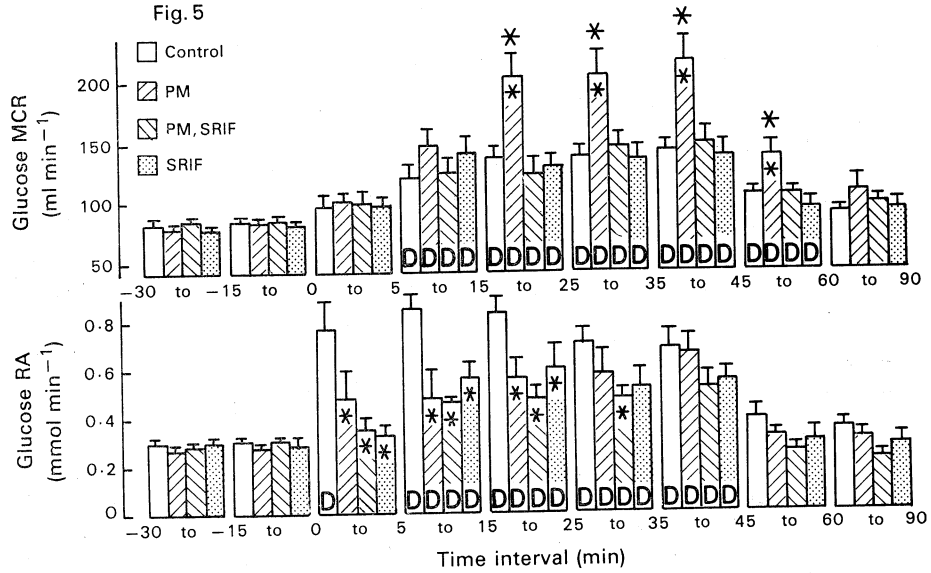
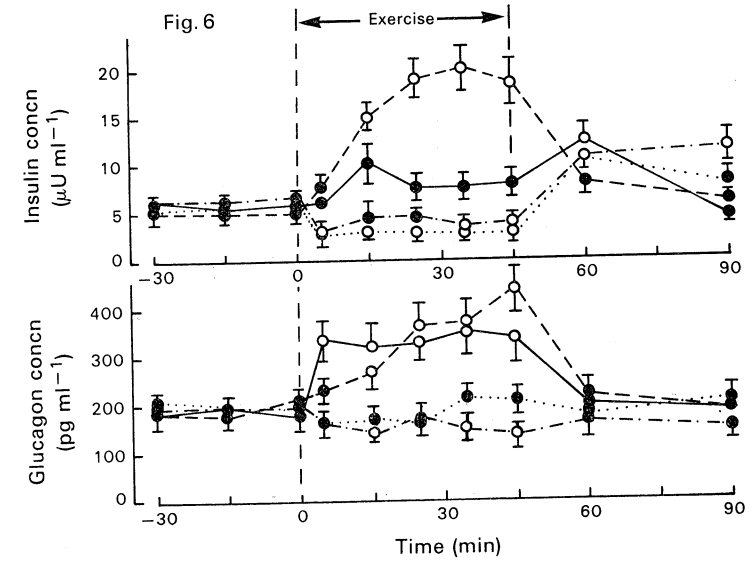


Fig. 6



However, since infusions of less than 2 h do not appear to have any significant effect on glucose metabolism in sheep (Bassett and Wallace 1965; Manns and Boda 1965), it is unlikely that any suppression of growth hormone by SRIF would have any significant effect over 45 min. This is supported by *in vivo* studies in dogs (Cherrington *et al.* 1976) and human subjects (Rizza *et al.* 1979) where SRIF depression of growth hormone was without effect on glucose metabolism. Furthermore, SRIF seems to have no direct influence on glucose metabolism, its effects being due to alterations in the secretion of glucagon and insulin.

The lack of an effect of propranolol on glucose appearance suggests that β-adrenergic stimulation is without direct effects on hepatic glucose metabolism. However, the higher MCR during two intervals of exercise is consistent with inhibition of glucose uptake by tissues, an effect which may be due to increased cellular glucose-6-phosphate levels from β-adrenergically mediated glycogenolysis (Issekutz and Vranic 1980). These conclusions are supported by studies in resting sheep where propranolol administration did not alter epinephrine-induced hyperglycaemia, but did prevent the rise in lactate (Bassett 1970). Similarly, the lack of effect of propranolol on glucagon and insulin concentrations during exercise is in agreement with studies where β-adrenergic stimulation did not alter glucagon secretion (Bassett 1972) and β-blockade had no effect on epinephrine inhibition of insulin secretion (Bassett 1970) in resting sheep. Stimulatory β-adrenergic effects on insulin secretion are evident only during blockade of the more potent inhibitory α-adrenergic influences (Hertelendy *et al.* 1969; Bassett 1970).

Administration of PM, in contrast, was associated with prevention of hyperglycaemia during exercise (Fig. 4). Its inhibitory effects on the increase in the glucose appearance during exercise (Fig. 5) can be accounted for in three ways. Firstly, PM may directly inhibit the hepatic α-adrenergic receptors (Phillips *et al.* 1969; Sherline *et al.* 1972); secondly, it may indirectly impair the rise in plasma glucagon (Bloom *et al.* 1973; Bloom and Edwards 1975; Brockman 1979*b*); and, thirdly, it may indirectly elevate insulin concentrations (Wahren *et al.* 1971; Kawamori and Vranic 1977).

Fig. 4. Concentration of glucose before, during and after exercise in control (——) experiments and during infusions of PM (- - -), PM plus SRIF (–·–·), and SRIF (· · · ·). Open symbols denote significant differences ($P < 0{\cdot}05$) from corresponding pre-exercise values. Values for SRIF are significantly different from corresponding control values throughout exercise and from corresponding PM values after 5 min of exercise. PM plus SRIF values are significantly different from PM values during 15–45 min of exercise. Values are means ± s.e., $N = 6$.

Fig. 5. Rates of appearance (RA) and metabolic clearance rates (MCR) of glucose before, during and after exercise. Experimental groups are control, PM, PM plus SRIF and SRIF infusions. D denotes significant differences ($P < 0{\cdot}05$) from corresponding pre-exercise values. An asterisk within the bars indicates that the value is significantly different from corresponding control value. An asterisk above a bar indicates that the value is significantly different from the corresponding PM plus SRIF value. Values are means + s.e., $N = 6$.

Fig. 6. Insulin and glucagon concentrations in plasma before, during and after exercise. Experimental groups are control (——), PM (- - -), PM plus SRIF (–·–·), and SRIF (· · · ·) infusions. Open symbols denote significant differences ($P < 0{\cdot}05$) from corresponding pre-exercise values. Glucagon concentrations during SRIF and PM plus SRIF infusions are significantly different from corresponding control and PM values, respectively, during exercise except in the latter case at 5 min. Insulin concentrations are significantly higher during exercise of the PM group. In both SRIF groups, insulin values are significantly lower than the control exercise values. Values are means ± s.e., $N = 6$.

No direct effects of PM on the liver are apparent. There were no significant differences in the increase in RA or MCR of glucose due to exercise between the SRIF and SRIF plus PM treatments. The concentrations of insulin and glucagon were the same, thus, the α-adrenergic effects of circulating catecholamines or hepatic nerves on glucose metabolism should have been evident. However, if glucagon has a permissive effect, as was concluded from studies with exercising dogs (Vranic and Kawamori 1979), the inhibition of the rise in glucagon may have masked any direct effect of sympathetic stimulation on the liver. Therefore, a direct stimulatory effect of circulating catecholamines on the increase in glucose appearance during exercise cannot be ruled out.

The delay by PM of the rise in glucagon due to exercise (Fig. 6) suggests that glucagon mediates the stimulatory sympathetic effects on glucose appearance. However, the concentration of glucagon in the PM treatment was significantly lower than in the control for 5 min of exercise only, whereas the RA of glucose was suppressed for 25 min (Fig. 5). Therefore, the effect on glucagon cannot in itself account for the inhibitory effects of PM on the exercise-induced increment in RA. The higher levels of insulin (Fig. 6) must also contribute to this (Wahren *et al.* 1971; Kawamori and Vranic 1977). While basal or pre-exercise levels of insulin appear to permit an increase in RA during exercise, studies with dogs suggested that higher levels are inhibitory (Kawamori and Vranic 1977). The lack of significant differences in RA during exercise between PM and PM plus SRIF treatments is consistent with the removal of stimulatory effects of glucagon and inhibitory effects of insulin on RA having balanced each other. Therefore, it seems that during exercise α-adrenergic stimuli prevent a rise in insulin, thereby permitting the increase in glucose appearance early in exercise.

It seems clear that the increase in RA of glucose early in exercise is due to hormonal influences. This is not the case later in exercise. It is possible that hypoxia associated with exercise provides a stimulus for release of glucose by the liver. Baum *et al.* (1979) reported that hypoxic dogs developed hyperglycaemia during α-adrenergic blockade despite no change in glucagon concentrations in plasma. Such an effect of hypoxia is consistent with acutely hypoxic human subjects also becoming hyperglycaemic (Sutton 1977) during exercise compared with no change or a slight decline in glucose ordinarily.

In addition to influencing glucose appearance, insulin appears to enhance the utilization of glucose (Vranic *et al.* 1976; Brockman 1979*b*). The increased MCR during exercise with PM treatment can be accounted for by elevated insulin levels. However, since insulin concentrations usually do not rise during exercise (Brockman 1979*a*) it is likely that a change in sensitivity to insulin during exercise (Vranic and Kawamori 1979) is of more significance with respect to glucose utilization than are any changes in insulin concentrations. This contention is supported by the observation that in sheep during greater work loads, insulin concentrations decreased (Brockman 1979*a*) but MCR increased.

As discussed above, insulin secretion during exercise seems to be regulated by inhibitory α-adrenergic influences. On the other hand, the mechanism of the glucagon responses is less clear. The delay in the rise of glucagon during PM treatment is consistent with a report that PM administration to rats prevented the glucagon rise during swimming (Harvey *et al.* 1974), but is not very convincing evidence of a sympathetic effect. Bloom and Edwards (1978) similarly failed to observe the effect of PM on glucagon secretion. Glucagon secretion due to stimulation of the sympathetic innervation to the pancreas in young valves was not altered by PM. Furthermore, other studies with young calves (Bloom *et al.* 1974, 1978) indicated that parasympathetic

activity may have a stimulatory effect on glucagon secretion. This seems to be particularly important in the absence of sympathetic influences. It is also possible that relative local hypoxia and hypercapnia may enhance the secretion of glucagon (Bloom *et al.* 1977).

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