

# Prevention of Overt Hypoglycemia During Exercise Stimulation of Endogenous Glucose Production Independent of Hepatic Catecholamine Action and Changes in Pancreatic Hormone Concentration

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These studies were conducted to determine the magnitude and mechanism of compensation for impaired glucagon and insulin responses to exercise. For this purpose, dogs underwent surgery >16 days before experiments, at which time flow probes were implanted and silastic catheters were inserted. During experiments, glucagon and insulin were fixed at basal levels during rest and exercise using a pancreatic clamp with glucose clamped (PC/GC;  $n = 5$ ), a pancreatic clamp with glucose unclamped (PC;  $n = 7$ ), or a pancreatic clamp with glucose unclamped + intraportal propranolol and phentolamine hepatic  $\alpha$ - and  $\beta$ -adrenergic receptor blockade (PC/HAB;  $n = 6$ ). Glucose production ( $R_a$ ) was measured isotopically. Plasma glucose was constant in PC/GC, but fell from basal to exercise in PC and PC/HAB.  $R_a$  was unchanged with exercise in PC/GC, but was slightly increased during exercise in PC and PC/HAB. Despite minimal increases in epinephrine in PC/GC, epinephrine increased approximately sixfold in PC and PC/HAB during exercise. In summary, during moderate exercise, 1) the increase in  $R_a$  is absent in PC/GC; 2) only a moderate fall in arterial glucose occurs in PC, due to a compensatory increase in  $R_a$ ; and 3) the increase in  $R_a$  is preserved in PC/HAB. In conclusion, stimulation of  $R_a$  by a mechanism independent of pancreatic hormones and hepatic adrenergic stimulation is a primary defense against overt hypoglycemia. *Diabetes* 51:1310–1318, 2002

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CV, coefficient of variation; FFA, free fatty acids; NHAU, net hepatic alanine uptake; NHGlyU, net hepatic glycerol uptake; NHGO, net hepatic glucose output; NHLU, net hepatic lactate uptake; PC, pancreatic clamp with glucose unclamped; PC/GC, pancreatic clamp with glucose clamped; PC/HAB, pancreatic clamp with hepatic  $\alpha$ - and  $\beta$ -adrenergic receptor blockade.

Glucose utilization ( $R_a$ ) is increased during exercise, but hypoglycemia seldom occurs in individuals without diabetes because increases in glucagon and decreases in insulin stimulate endogenous glucose production ( $R_a$ ) (1–4). The serial arrangement of the pancreas and the liver allow marked pancreatic hormone changes in the portal vein, the conduit between these two organs, without dramatic changes in their peripheral concentrations (5,6). Pancreatic clamp studies (somatostatin and intraportal glucagon and insulin replacement) in the dog preserve the physiological entry site of glucagon and insulin into the blood and have shown that changes in glucagon and insulin during exercise control almost the entire increase in  $R_a$  with moderate exercise (2,3). In addition to the regulatory roles of glucagon and insulin, the catecholamines have also been proposed to mediate  $R_a$  during exercise, especially under conditions of particularly high metabolic stress (e.g., heavy exercise, hypoglycemia, and diabetes) (1,7). The onset of exercise causes an increase in sympathetic activity that correlates with an increase in  $R_a$ . However, studies designed to assess the role of sympathetic drive to the liver in the stimulation of  $R_a$ , using chronic denervation (8), local hepatic adrenergic blockade (9,10), and liver transplant patients (presumably free of sympathetic innervation to the liver) (11), have been uniformly negative. Nevertheless, studies performed in humans using the pancreatic clamp technique and combined  $\alpha$ - and  $\beta$ -adrenergic blockade suggest that epinephrine may prevent overt hypoglycemia during moderate exercise (12). A fall in the level of glycemia has also been suggested as a stimulus to  $R_a$  via autoregulation at the liver (13). The possibility that autoregulation may be involved in prevention of hypoglycemia during exercise has not been tested directly.

The present study was conducted to determine the mechanisms that control the stimulation of  $R_a$  during exercise in the absence of changes in glucagon and insulin. Understanding mechanisms that are effective in the absence of a fall in insulin is particularly relevant to people with diabetes treated with insulin because the physiolog-

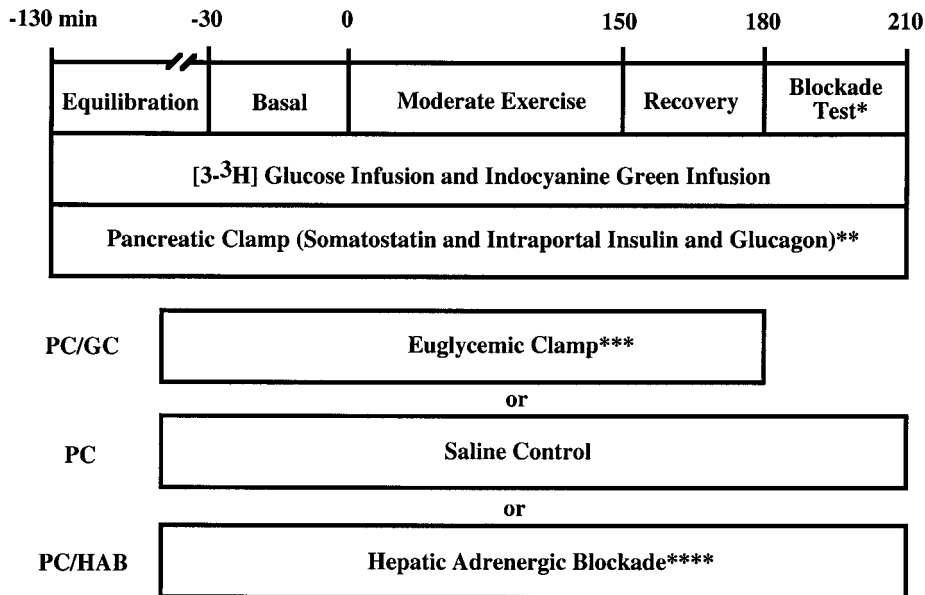


FIG. 1. Moderate exercise protocol in which a basal pancreatic clamp was performed with a euglycemic clamp (PC/GC), without glucose clamped (PC), or without glucose clamped and a selective hepatic adrenergic blockade (PC/HAB). \*Epinephrine and norepinephrine were infused into the portal vein at rates of 0.20 and 0.40  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively, in PC and PC/HAB. \*\*Somatostatin was infused into the peripheral circulation at 0.8  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . Intraportal glucagon was infused at 0.65  $\text{ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , and intraportal insulin was infused as needed to maintain euglycemia. \*\*\*Glucose was infused to maintain euglycemia. \*\*\*\*Phentolamine and propranolol were infused at rates of 2 and 1  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively, from  $t = -50$  to 210 min in PC/HAB.  $n = 5$  in PC/GC,  $n = 7$  in PC, and  $n = 6$  in PC/HAB.

ical decrease in insulin with exercise is lacking. In these studies, glucagon and insulin levels were fixed at basal using the pancreatic clamp technique with glucose clamped (PC/GC), the pancreatic clamp without glucose clamped (PC), or the pancreatic clamp without glucose clamped plus the blockade of hepatic  $\alpha$ - and  $\beta$ -adrenergic receptors (PC/HAB) in chronically catheterized and instrumented conscious dogs.

## RESEARCH DESIGN AND METHODS

**Animals and surgical procedures.** Experiments were performed on a total of 18 overnight fasted mongrel dogs (mean weight  $23.7 \pm 0.5$  kg) of either sex that had been fed a standard diet (Pedigree beef dinner and Wayne Lab Blox, with 51% carbohydrate, 31% protein, 11% fat, and 7% fiber based on dry weight). The dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the Vanderbilt University Animal Care Subcommittee approved the protocols. At least 16 days before each experiment, a laparotomy was performed under general anesthesia (0.04 mg/kg atropine and 15 mg/kg pentobarbital sodium presurgery and 1.0% isoflurane inhalation anesthetic during surgery). Catheters were inserted into the portal and hepatic veins for blood sampling purposes. An incision in the neck region allowed the isolation of the carotid artery, into which a silastic catheter (0.04 in internal diameter (ID)) was inserted and advanced to the aortic arch for sampling and hemodynamic measurements during experiments. Silastic catheters (0.03 in ID) were inserted into the vena cava for infusion purposes. Last, a silastic catheter (0.03 in ID) was inserted into the splenic vein and positioned so that the catheter tip rested just beyond the point where the splenic and portal veins coalesce. This catheter was used for the intraportal infusions of glucagon, insulin, phentolamine, propranolol, and catecholamines. Catheters were filled with heparinized saline, and the free ends were knotted. Ultrasonic transit time flow probes were fitted and secured to the portal vein and hepatic artery (Transonic Systems, Ithaca, NY). The knotted catheter ends and flow probe leads were stored in a subcutaneous pocket in the abdominal region (except for the carotid artery catheter, which was stored in a pocket under the skin of the neck), so that the complete closure of the skin incisions was possible.

Beginning 7 days after surgery, dogs were acclimatized to running on a motorized treadmill. Blood samples were drawn 3 days before the experiment to determine the leukocyte count and the hematocrit of the animals. Only animals with a leukocyte count  $<18,000/\text{mm}^3$ , a hematocrit  $>36\%$ , a good appetite (consumption of daily food ration), and normal stools were used.

All studies were conducted in dogs after an 18-h fast. The free catheter ends and flow probe leads were accessed through small skin incisions made under local anesthesia (2% lidocaine; Astra Pharmaceutical Products, Worcester, MA) in the abdominal and neck regions the morning of the experiment. The contents of the catheters were then aspirated and flushed with saline. The exposed catheters were connected to silastic tubing that was secured to the back of the dog with quick-drying glue.

**Experimental procedures.** Except where indicated, experiments consisted of a tracer equilibration period ( $-130$  to  $-30$  min), a basal period ( $-30$  to 0 min), a moderate exercise period (0–150 min), a recovery period (150–180 min), and a blockade test period (180–210 min) (Fig. 1). A primed (50 Ci) infusion (0.30 Ci/min) of [ $^3\text{H}$ ]glucose was initiated at  $t = -130$  min and continued throughout the study. A constant-rate indocyanine green ( $0.1 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ) was also started at  $t = -130$  min and continued throughout the study. Indocyanine green was used as a backup method of blood flow measurement if the Doppler probes did not provide a clear signal, and it served as confirmation of hepatic vein catheter placement. There was no Doppler flow probe failure in these studies. All dogs were studied during a pancreatic clamp that kept insulin and glucagon concentrations at basal levels, as described below. A peripheral infusion of somatostatin ( $0.8 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was started at  $t = -130$  min to inhibit endogenous insulin and glucagon secretion in all groups. Concurrent with the infusion of somatostatin was an intraportal glucagon replacement ( $0.65 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) designed to recreate basal levels in all groups. An intraportal insulin infusion was also initiated at this time. The insulin infusion rate was adjusted to maintain euglycemia throughout the basal period in all groups. In one group of dogs, glucose was clamped during exercise in conjunction with the pancreatic clamp (PC/GC;  $n = 5$ ). A second group of dogs was studied in an identical manner, but without a glucose clamp (PC;  $n = 7$ ). A third group of pancreatic-clamped dogs was studied with a hepatic  $\alpha$ - and  $\beta$ -adrenergic receptor blockade (PC/HAB;  $n = 6$ ). Selective hepatic adrenergic blockade was achieved by infusion of phentolamine and propranolol in a saline/ascorbate solution into the portal vein from  $t = -50$  to 210 min at rates of 2 and 1  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively. To test the effectiveness of the blockade, norepinephrine and epinephrine were infused at rates of 0.40 and 0.20  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively, from  $t = 180$ –210 min in PC/HAB and for comparison in PC. In PC/GC and PC, vehicle alone (a saline/ascorbate solution) was infused at a rate identical to that used in PC/HAB. A transducer connected to the carotid arterial catheter monitored heart rates. Portal vein and hepatic artery blood flows were monitored on-line throughout the experiments.

**Blood sample collection and processing.** Arterial and portal and hepatic vein blood samples were drawn at  $t = -30, -15, 0, 25, 50, 75, 100, 125, 150, 160, 170, 180$  in all groups. In addition, arterial blood samples were also drawn at  $t = 190, 200, 210$  in PC and PC/HAB. Plasma glucose concentrations were determined by the glucose oxidase method using a Beckman Instruments glucose analyzer (Fullerton, CA). For the determination of plasma glucose radioactivity, samples were deproteinized with barium hydroxide and zinc sulfate and then centrifuged. The supernatant was then evaporated to remove  $^3\text{H}_2\text{O}$  and reconstituted in 1 ml water and 10 ml scintillation fluid [Ecolite (+); ICN Biomedicals, Irvine, CA]. Radioactivity was determined on a Beckman liquid scintillation counter. Blood samples were deproteinized (0.5 ml blood in 1.5 ml of 4% perchloric acid), and then whole-blood lactate, alanine, and glycerol concentrations were determined on a Monarch 2000 centrifugal analyzer (Lexington, MA), using standard enzymatic methods (14). Free fatty acids (FFAs) were measured with the use of the Wako FFA C test kit (Richmond, VA). Immunoreactive insulin was

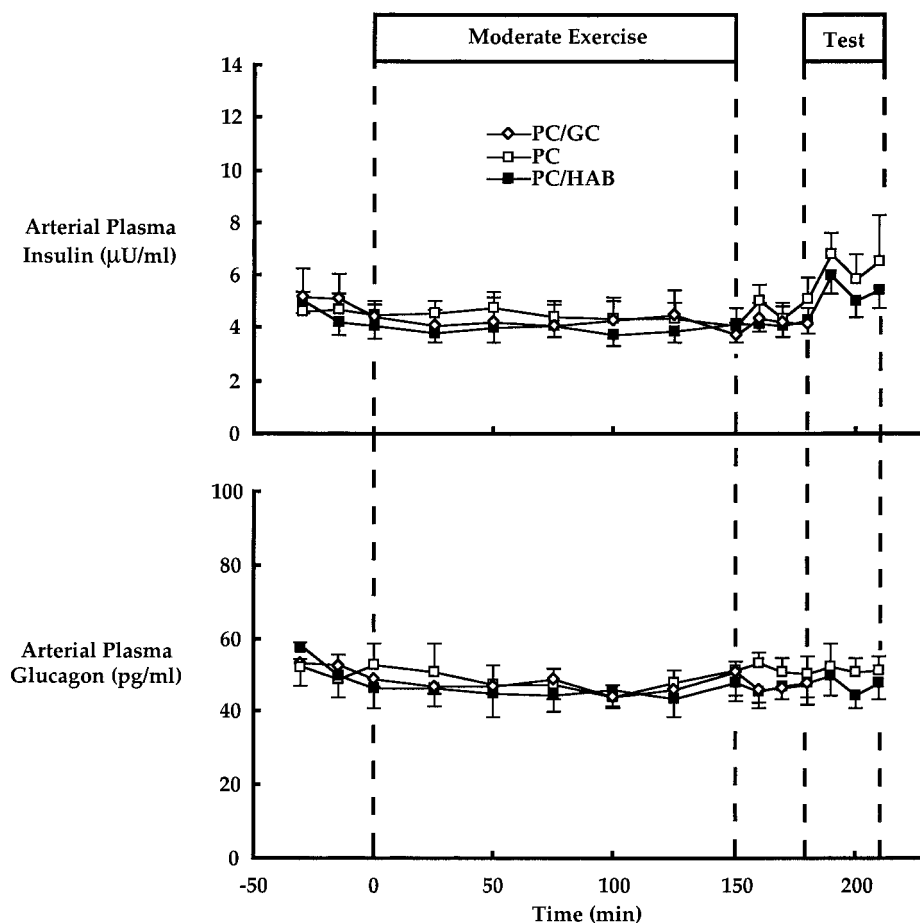


FIG. 2. Arterial insulin and glucagon concentrations during the basal, exercise, recovery, and blockade test periods in which a basal pancreatic clamp was performed with a euglycemic clamp (PC/GC), without glucose clamped (PC), or without glucose clamped and a selective hepatic adrenergic blockade (PC/HAB). Data are means  $\pm$  SE.  $n = 5$  in PC/GC,  $n = 7$  in PC, and  $n = 6$  in PC/HAB.

measured using a double-antibody procedure (interassay coefficient of variation [CV] of 16%) (15). Immunoreactive glucagon (3,500 molecular wt) was measured in plasma samples containing 500 kIU/ml Trasylol (FBA Pharmaceuticals, NY) using a double-antibody system modified from the method developed by Morgan and Lazarow for insulin (15). Blood samples for norepinephrine and epinephrine measurement were collected into tubes containing EGTA and glutathione and then centrifuged at 4°C, and plasma was stored at -70°C for subsequent high-performance liquid chromatography analysis. Catecholamine concentrations were calculated based on linear regression using dihydroxybenzylamine as an internal standard. The CVs using this method were 5 and 7% for norepinephrine and epinephrine, respectively. Plasma cortisol was measured with the Clinical Assays Gamma Coat radioimmunoassay kit (Travenol-Genetech Diagnostics, Cambridge, MA), with an interassay CV of 6%.

**Materials.** [ $^3\text{H}$ ]glucose was obtained from New England Nuclear (Boston, MA). Glucagon and insulin antisera were obtained from Dr. R.L. Gingerich (Washington University School of Medicine, St. Louis, MO), and the standard glucagon and [ $^{125}\text{I}$ ]glucagon were obtained from Linco Research (St. Louis, MO). Indocyanine green was purchased from Hynson, Westcott, and Dunning (Baltimore, MD). Enzymes and coenzymes for metabolite analyses were obtained from Boehringer Mannheim and Sigma.

**Calculations.** Net hepatic lactate uptake (NHLU), alanine uptake (NHAU), glycerol uptake (NHGlyU), and glucose output (NHGO) were determined according to the formula:  $\text{HAF} \times ([\text{H}] - [\text{A}]) + \text{PVF} \times ([\text{H}] - [\text{P}])$ , such that [A], [P], and [H] are the arterial, portal vein, and hepatic vein glucose concentrations, and HAF and PVF are the hepatic artery and portal vein blood flows, respectively. The sign was reversed for the calculation of NHLU, NHAU, and NHGlyU so that net uptake would be a positive number. Portal vein norepinephrine concentrations during the blockade test period were calculated according to the formula:  $(0.4 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}/\text{PVF}) + (\text{arterial norepinephrine concentration})$ . Because nonhepatic splanchnic tissue extracts ~50% of arterial epinephrine (16), the arterial contribution toward the portal vein concentration was divided by 2. Thus, portal vein epinephrine concentrations were calculated according to the following equation:  $(0.2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}/\text{PVF}) + (\text{arterial epinephrine concentration}/2)$ . Endogenous  $R_a$  and  $R_d$  were calculated using the two-compartment approach described by Mari (17).

**Statistical analysis.** Superanova (Abacus Concepts, Berkeley, CA) software installed on a Macintosh Power PC was used to perform statistical analyses. Statistical comparisons between groups and over time were made using ANOVA designed to account for repeated measures. Specific time points were examined for significance using contrasts solved by univariate repeated measures. Statistics are reported in the tables and figures. Data are presented as the means  $\pm$  SE. Statistical significance was defined as  $P < 0.05$ .

## RESULTS

**Arterial insulin and glucagon concentrations.** Plasma insulin and glucagon were unchanged from basal during exercise and recovery periods in PC/GC (basal concentrations of  $5 \pm 1 \mu\text{U/ml}$  and  $51 \pm 2 \text{ pg/ml}$ ), PC (basal concentrations of  $5 \pm 1 \mu\text{U/ml}$  and  $52 \pm 5 \text{ pg/ml}$ ), and PC/HAB (basal concentrations of  $5 \pm 1 \mu\text{U/ml}$  and  $53 \pm 5 \text{ pg/ml}$ ) (Fig. 2).

**Plasma epinephrine and norepinephrine concentrations.** Arterial plasma epinephrine rose from a basal level of  $104 \pm 25 \text{ pg/ml}$  to a level of  $170 \pm 45 \text{ pg/ml}$  at 150 min of exercise in PC/GC. Epinephrine increased considerably more when glucose levels were not clamped. Epinephrine rose from a basal level of  $116 \pm 21 \text{ pg/ml}$  to  $546 \pm 129 \text{ pg/ml}$  at 150 min of exercise in PC and from a basal level of  $86 \pm 17 \text{ pg/ml}$  to  $685 \pm 162 \text{ pg/ml}$  at 150 min of exercise in PC/HAB (Fig. 3). These data demonstrate the remarkable sensitivity of the exercise-induced epinephrine response to mild hypoglycemia. Plasma norepinephrine (Fig. 3) and cortisol (Fig. 4) were not different between groups during the basal period and responded similarly to exercise. Mean portal vein epinephrine and norepinephrine concentrations during the blockade test period were esti-

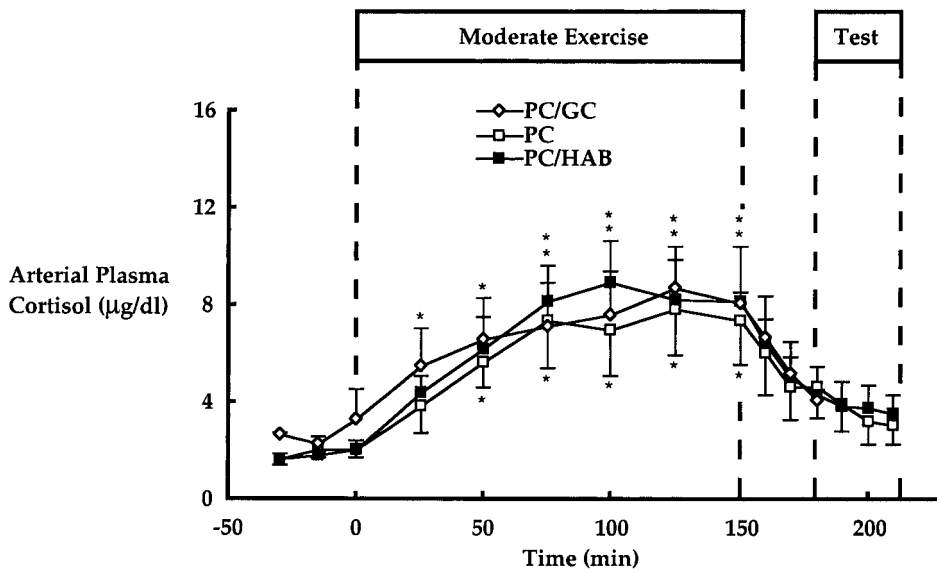


FIG. 3. Arterial cortisol concentration during the basal, exercise, recovery, and blockade test periods in which a basal pancreatic clamp was performed with a euglycemic clamp (PC/GC), without glucose clamped (PC), or without glucose clamped and a selective hepatic adrenergic blockade (PC/HAB). Data are means  $\pm$  SE.  $n = 5$  in PC/GC,  $n = 7$  in PC, and  $n = 6$  in PC/HAB. \*Significantly increased by exercise ( $P < 0.05$ ).

mated to be  $10,810 \pm 1,020$  pg/ml and  $21,980 \pm 2,070$  pg/ml, respectively, among PC and PC/HAB.

**Arterial plasma glucose concentration and kinetics.** Basal arterial plasma glucose was similar in PC/GC, PC, and PC/HAB. Plasma glucose remained similar to basal levels in PC/GC during exercise ( $\sim 2 \pm 1$  mg/dl change from basal) (Fig. 5). Plasma glucose fell similarly during exercise in PC ( $114 \pm 4$  to  $82 \pm 8$  mg/dl at  $t = 150$  min) and

PC/HAB ( $107 \pm 4$  to  $79 \pm 15$  mg/dl at  $t = 150$  min). Plasma glucose rose to  $212 \pm 9$  mg/dl in PC, compared with only  $139 \pm 16$  mg/dl in PC/HAB during the blockade test period (Fig. 5). To maintain euglycemia in PC/GC, the mean rate of exogenous glucose infusion was  $2.2 \pm 0.5$ ,  $3.3 \pm 0.8$ , and  $3.7 \pm 1.2$   $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  at 50, 100, 150 min of moderate exercise, respectively. Although NHGO increased from basal to exercise in PC/GC, PC, and PC/HAB, the onset of

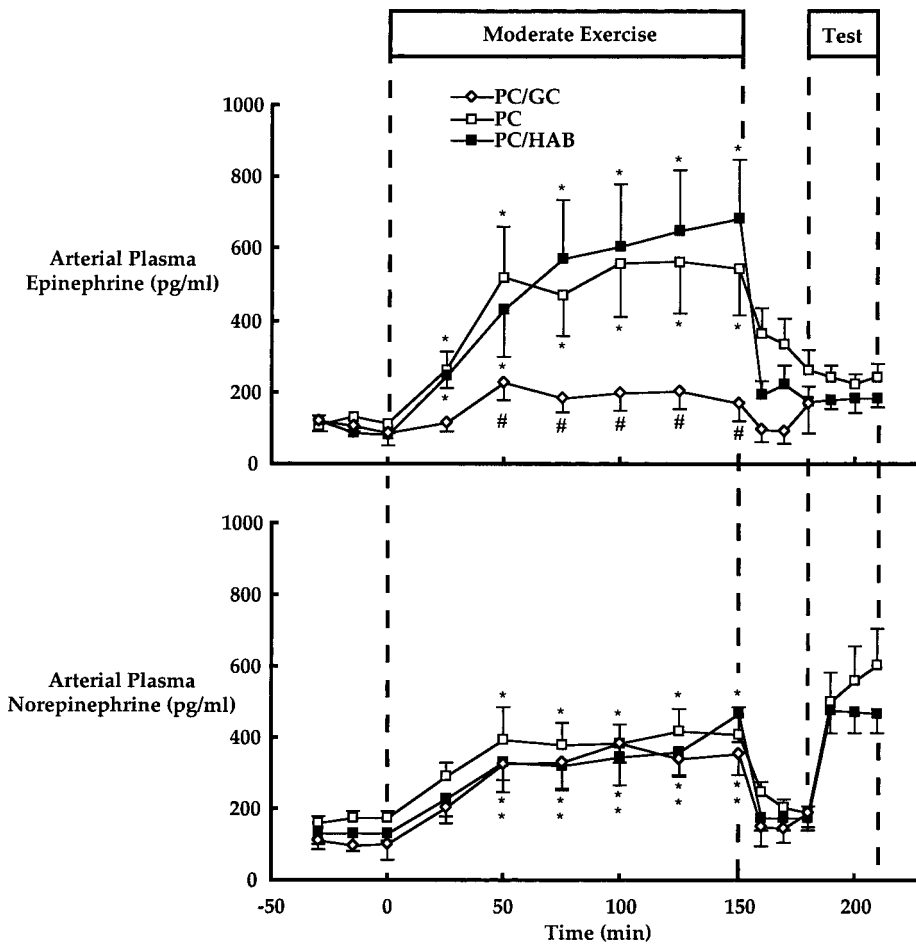


FIG. 4. Arterial epinephrine and norepinephrine concentrations during the basal, exercise, recovery, and blockade test periods in which a basal pancreatic clamp was performed with a euglycemic clamp (PC/GC), without glucose clamped (PC), or without glucose clamped and a selective hepatic adrenergic blockade (PC/HAB). Data are means  $\pm$  SE.  $n = 5$  in PC/GC,  $n = 7$  in PC, and  $n = 6$  in PC/HAB. #Significantly reduced compared with corresponding time points in PC and PC/HAB ( $P < 0.05$ ). \*Significantly increased by exercise ( $P < 0.05$ ).

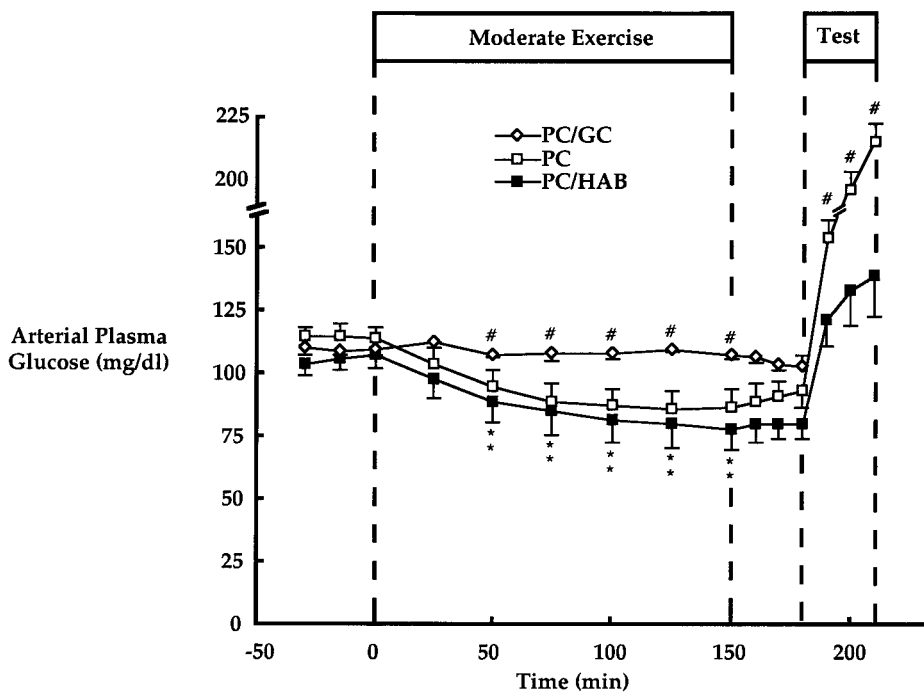


FIG. 5. Arterial glucose concentration during the basal, exercise, recovery, and blockade test periods in which a basal pancreatic clamp was performed with a euglycemic clamp (PC/GC), without glucose clamped (PC), or without glucose clamped and a selective hepatic adrenergic blockade (PC/HAB). Data are means  $\pm$  SE.  $n = 5$  in PC/GC,  $n = 7$  in PC, and  $n = 6$  in PC/HAB. #Significantly reduced compared with corresponding time points in PC and PC/HAB ( $P < 0.05$ ). \*Significant difference from basal values ( $P < 0.05$ ).

the exercise-induced NHGO response was delayed in PC/GC (Table 1).

$R_a$  was not significantly increased in PC/GC with exercise (from a basal rate of  $2.8 \pm 0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  to  $3.3 \pm 1.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  at 150 min of exercise) (Fig. 6).  $R_a$  increased ( $P < 0.05$ ) from a basal rate of  $3.0 \pm 0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  to  $5.4 \pm 0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  at 150 min of exercise in PC and from a basal rate of  $2.7 \pm 0.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  to  $5.1 \pm 0.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  at 150 min of exercise in PC/HAB. The increment with exercise was greater when glucose was allowed to fall in PC and PC/HAB, as compared with PC/GC.  $R_a$  was greater ( $P < 0.05$ ) in PC ( $15.4 \pm 1.6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) compared with PC/HAB ( $6.7 \pm 1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) during the blockade test period (Fig. 6). Despite lower arterial glucose levels in PC and PC/HAB compared with PC/GC during exercise, there were no significant differences in  $R_d$  between groups during the studies (Fig. 6).

**Arterial lactate and alanine concentrations and net hepatic balances.** Basal arterial lactate was not different between PC/GC, PC, and PC/HAB. Arterial lactate increased by twofold during exercise in PC/GC (Table 2). In comparison, the exercise-induced response was exagger-

TABLE 1

Net hepatic glucose output ( $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) during the basal and exercise periods during which a basal pancreatic clamp was performed with a euglycemic clamp (PC/GC), without glucose clamped (PC), or without glucose clamped and a selective hepatic adrenergic blockade (PC/HAB)

|        | Basal         | Moderate exercise |                 |                 |
|--------|---------------|-------------------|-----------------|-----------------|
|        |               | 50 min            | 100 min         | 150 min         |
| PC/GC  | $1.8 \pm 0.7$ | $2.7 \pm 0.6$     | $3.4 \pm 0.7^*$ | $3.2 \pm 0.6^*$ |
| PC     | $1.8 \pm 0.4$ | $3.4 \pm 0.6^*$   | $4.4 \pm 0.6^*$ | $4.0 \pm 0.5^*$ |
| PC/HAB | $1.9 \pm 0.8$ | $2.9 \pm 0.5^*$   | $4.8 \pm 1.2^*$ | $4.3 \pm 0.5^*$ |

Data are means  $\pm$  SE.  $n = 5$  for PC/GC,  $n = 6$  for PC, and  $n = 6$  for PC/HAB. \*Significant difference from basal values ( $P < 0.05$ ).

ated ( $P < 0.05$ ) in PC/HAB and PC, increasing by fourfold. Arterial alanine was similar in PC/GC, PC, and PC/HAB during the basal and exercise periods (Table 2). NHLU and NHAU were similar during the rest and exercise periods in PC/GC, PC, and PC/HAB (Table 2).

**Arterial FFA and glycerol concentrations and net hepatic balances.** Arterial FFA levels were similar in all groups in the basal state and during exercise (Table 3). Basal glycerol levels were not different between groups. Glycerol rose to  $\sim 250\%$  of basal levels with exercise in PC/GC. The increase was nearly twice as much when glucose was allowed to fall in PC and PC/HAB. NHGlyU was similar in PC/GC, PC, and PC/HAB during the basal period and in response to exercise (Table 2).

**Heart rate and hepatic blood flow.** Heart rates were similar in the basal period and increased approximately twofold during exercise in all groups (Table 4). Basal portal vein blood flow was  $\sim 20\%$  greater in PC/GC and PC/HAB compared with PC. Furthermore, portal vein blood flow remained greater in PC/HAB compared with PC during exercise (Table 4). Hepatic artery blood flow values were similar in all groups during the basal and exercise periods (Table 4). Despite differences in portal vein blood flow values, the total hepatic blood flow was similar in all groups throughout the study.

## DISCUSSION

The results of the present study demonstrate that exercise in the absence of changes in glucagon and insulin results in a moderate decrement in arterial plasma glucose, and not overt hypoglycemia. Resistance to the onset of overt hypoglycemia was caused by a compensatory increase in  $R_a$ . Remarkably, this compensatory increase in  $R_a$  occurred even in the presence of selective hepatic adrenergic blockade, indicating that this second tier of protection against overt hypoglycemia is not likely due to hepatic catecholamine action. The similar physiological response

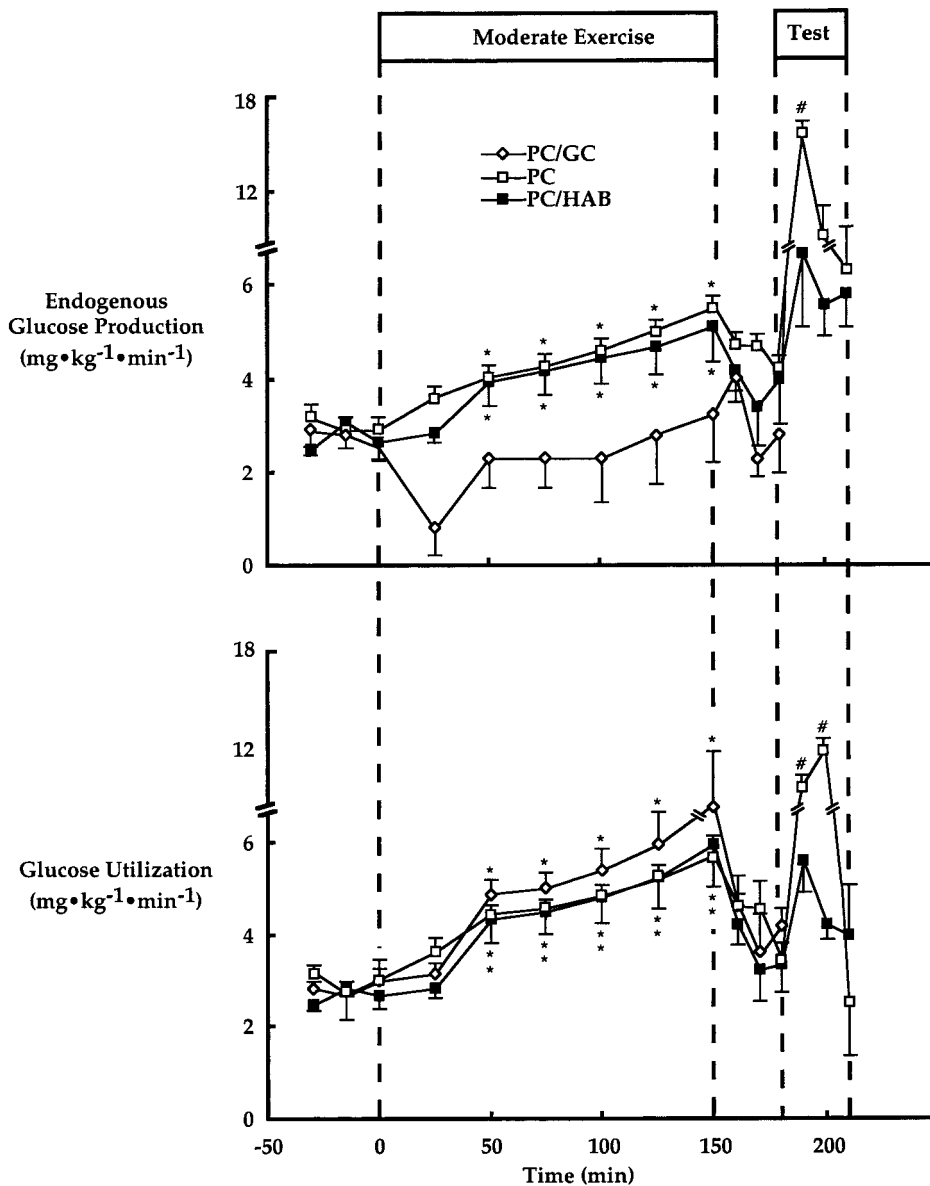


FIG. 6. Endogenous glucose production and glucose disappearance during the basal, exercise, recovery, and blockade test periods in which a basal pancreatic clamp was performed with a euglycemic clamp (PC/GC), without glucose clamped (PC), or without glucose clamped and a selective hepatic adrenergic blockade (PC/HAB). Data are means  $\pm$  SE.  $n = 5$  in PC/GC,  $n = 7$  in PC, and  $n = 6$  in PC/HAB. #Significantly reduced compared with corresponding time points in PC and PC/HAB ( $P < 0.05$ ). \*Significant difference from basal values ( $P < 0.05$ ).

in PC and PC/HAB implicates other mechanisms, such as glucose autoregulation of the liver (13).

Hepatic adrenergic receptors were selectively blocked by infusing propranolol and phentolamine into the portal vein (9,10). The local hepatic delivery of adrenergic blockers has a distinct advantage in that undesirable extrahepatic effects are greatly diminished. The specificity of the adrenergic blockade for the liver is documented by similar FFA, glycerol, lactate, and heart rate responses in PC and PC/HAB. These responses are consistent with the results of previous studies using this technique (9,10). It is important to point out that calculated portal vein epinephrine and norepinephrine concentrations were 17- and 55-fold higher, respectively, during the blockade test period compared with the exercise period. Despite the extremely high concentrations of catecholamines,  $\sim 70\%$  of the adrenergic stimulus was attenuated, supporting the contention that the existence of blockade breakthrough during the exercise period was probably minimal.

Marker et al. (12) proposed that epinephrine is a mediator of  $R_a$  under mildly hypoglycemic exercise conditions.

This contention was based on a greater fall in plasma glucose during exercise in the presence of PC/HAB compared with PC. However, it is important to mention that higher insulin levels in the blockade group may have contributed to the greater fall in plasma glucose in this previous study. In addition, the peripheral administration of  $\alpha$ - and  $\beta$ -adrenergic blockers in these studies (12) makes the evaluation of catecholamine action directly at the liver difficult because adrenergic blockers have broad peripheral effects (i.e., hemodynamic and metabolic). Therefore, the ability to selectively block hepatic adrenergic receptors is an important experimental advantage of our model.

The gut extracts  $\sim 50\%$  of the epinephrine delivered to it (18,19). This means that the arterial epinephrine concentration overestimates the concentration of catecholamine at the liver. Nonetheless, infusion of epinephrine, which resulted in arterial levels similar to those in the present study, has been shown to increase  $R_a$  by  $\sim 1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (20). However, there were numerous peripheral effects (e.g., elevated FFAs and lactate) that could have

TABLE 2

Arterial levels and net hepatic uptake of lactate, alanine, and glycerol during the basal and exercise periods during which a basal pancreatic clamp was performed with a euglycemic clamp (PC/GC), without glucose clamped (PC), or without glucose clamped and a selective hepatic adrenergic blockade (PC/HAB)

|  | Basal     | Moderate exercise |              |              |
|--|-----------|-------------------|--------------|--------------|
|  |           | 50 min            | 100 min      | 150 min      |
| Arterial blood lactate (mol/l)   |           |                   |              |              |
| PC/GC  | 655 ± 168 | 1,177 ± 238*      | 1,152 ± 205* | 975 ± 132*   |
| PC   | 533 ± 120 | 1,890 ± 463*      | 1,732 ± 352* | 1,586 ± 318* |
| PC/HAB   | 634 ± 85  | 2,153 ± 558*      | 1,896 ± 497* | 1,823 ± 444* |
| Net hepatic lactate uptake<br>(mol · kg <sup>-1</sup> · min <sup>-1</sup> )  |           |                   |              |              |
| PC/GC  | 0.8 ± 0.7 | 3.5 ± 3.9*        | 2.1 ± 3.6*   | 1.1 ± 4.3    |
| PC   | 0.8 ± 0.4 | 2.5 ± 4.1*        | 5.9 ± 5.1    | 3.2 ± 6.1    |
| PC/HAB   | 1.7 ± 1.5 | 0.6 ± 4.0         | 1.0 ± 3.1    | 1.0 ± 4.8    |
| Arterial blood alanine (mol/l)   |           |                   |              |              |
| PC/GC  | 350 ± 60  | 400 ± 33          | 372 ± 63     | 348 ± 37     |
| PC   | 324 ± 65  | 367 ± 62          | 340 ± 65     | 296 ± 44     |
| PC/HAB   | 333 ± 51  | 320 ± 37          | 317 ± 39     | 306 ± 48     |
| Net hepatic alanine uptake<br>(mol · kg <sup>-1</sup> · min <sup>-1</sup> )  |           |                   |              |              |
| PC/GC  | 2.5 ± 0.6 | 2.9 ± 1.1         | 3.3 ± 1.0    | 1.9 ± 0.6    |
| PC   | 1.9 ± 0.6 | 3.0 ± 1.0         | 3.7 ± 0.9    | 2.6 ± 0.5    |
| PC/HAB   | 1.6 ± 0.6 | 2.7 ± 0.5         | 2.5 ± 0.3    | 3.0 ± 0.6    |
| Arterial blood glycerol (mol/l)  |           |                   |              |              |
| PC/GC  | 63 ± 7    | 172 ± 20*         | 188 ± 17*    | 173 ± 23*    |
| PC   | 101 ± 16  | 237 ± 36*         | 280 ± 38*    | 263 ± 15*    |
| PC/HAB   | 81 ± 13   | 215 ± 37*         | 261 ± 19*    | 262 ± 53*    |
| Net hepatic glycerol uptake<br>(mol · kg <sup>-1</sup> · min <sup>-1</sup> ) |           |                   |              |              |
| PC/GC  | 1.3 ± 0.2 | 3.3 ± 0.2*        | 4.3 ± 0.3*   | 3.9 ± 0.5*   |
| PC   | 1.7 ± 0.4 | 3.9 ± 0.8*        | 5.8 ± 1.1*   | 2.6 ± 0.5*   |
| PC/HAB   | 1.3 ± 0.2 | 4.4 ± 0.9*        | 4.5 ± 0.7*   | 4.7 ± 0.7*   |

Data are means ± SE. *n* = 5 for PC/GC, *n* = 6 for PC, and *n* = 6 for PC/HAB. \*Significant difference from basal values (*P* < 0.05).

indirectly caused the liver to release more glucose. Furthermore, experiments conducted in adrenalectomized humans (free of epinephrine) showed that exercise-induced increases in *R<sub>a</sub>* were largely intact (21). With respect to the potency of norepinephrine, a 10-fold increase in epinephrine has been shown to cause stimulation of *R<sub>a</sub>* (1.5–1.9 mg · kg<sup>-1</sup> · min<sup>-1</sup>), similar to a 30-fold increase in norepinephrine (22). It is important to mention that this experimentally induced increment in norepinephrine was far beyond the exercise-induced increments in circulating norepinephrine measured in the present study.

A fall in arterial glucose of <25 mg/dl corresponded to a >2 mg · kg<sup>-1</sup> · min<sup>-1</sup> increase in *R<sub>a</sub>* during exercise in the presence of fixed pancreatic hormones and hepatic adrenergic blockade. Therefore, it is reasonable to propose that

TABLE 3

Arterial plasma FFAs (μEq/l) during the basal and exercise periods was performed with a euglycemic clamp (PC/GC), without glucose clamped (PC), or without glucose clamped and a selective hepatic adrenergic blockade (PC/HAB)

|        | Basal     | Moderate exercise |              |              |
|--------|-----------|-------------------|--------------|--------------|
|        |           | 50 min            | 100 min      | 150 min      |
| PC/GC  | 912 ± 137 | 964 ± 148         | 1,256 ± 210* | 1,270 ± 207* |
| PC     | 780 ± 60  | 1,051 ± 165*      | 1,218 ± 154* | 1,263 ± 187* |
| PC/HAB | 634 ± 95  | 758 ± 71          | 1,187 ± 118  | 1,221 ± 148* |

Data are means ± SE. *n* = 5 for PC/GC, *n* = 6 for PC, and *n* = 6 for PC/HAB. \*Significant difference from basal values (*P* < 0.05).

autoregulation at the liver may be an important factor in the avoidance of overt hypoglycemia during exercise. This is supported by investigations that have demonstrated the exquisite sensitivity of the liver to circulating glucose concentrations with respect to the modulation of *R<sub>a</sub>* during exercise, even when endocrine changes were undetectable (23–25). Moreover, recent studies have illustrated the importance of hormone-independent stimulation of *R<sub>a</sub>* during mild hypoglycemia in human subjects (26). The mechanism of hormone-independent stimulation of *R<sub>a</sub>* may be of pathological significance because it appears to be deficient in individuals with type 1 diabetes (26). In the present studies, there was a slight trend toward a greater *R<sub>d</sub>* in PC/GC compared with PC and PC/HAB, presumably due to the increased effect of glucose mass action on *R<sub>d</sub>*. However, significant differences in *R<sub>d</sub>* were not detectable. This is not altogether surprising because changes in the level of glycemia will predictably be of greater proportion than changes in *R<sub>d</sub>*, particularly within the range of glucose levels in these studies (27,28).

Dramatic exercise-induced changes in epinephrine occurred in the presence of the pancreatic clamp due to the modest decrement in glycemia that occurred, and a euglycemic clamp normalized the exaggerated increase. Remarkably, the exaggerated epinephrine increase in PC and PC/HAB occurred with a decrement in arterial glucose of only ~10 mg/dl. These results provide further evidence for the extraordinary sensitivity of glucoreceptor mechanisms

TABLE 4

Heart rates and portal vein and hepatic artery blood flows during the basal and exercise periods was performed with a euglycemic clamp (PC/GC), without glucose clamped (PC), or without glucose clamped and a selective hepatic adrenergic blockade (PC/HAB)

|   | Basal   | Moderate exercise |          |          |
|---|---------|-------------------|----------|----------|
|   |         | 50 min            | 100 min  | 150 min  |
| Heart rates (beats/min)   |         |                   |          |          |
| PC/GC   | 105 ± 1 | 147 ± 12          | 172 ± 21 | 163 ± 22 |
| PC  | 96 ± 6  | 180 ± 14          | 173 ± 12 | 177 ± 12 |
| PC/HAB  | 88 ± 6  | 177 ± 11          | 187 ± 11 | 189 ± 7  |
| Portal vein blood flow<br>(ml · kg <sup>-1</sup> · min <sup>-1</sup> )    |         |                   |          |          |
| PC/GC   | 24 ± 3  | 21 ± 3            | 21 ± 1   | 21 ± 3   |
| PC  | 19 ± 2  | 19 ± 2            | 20 ± 1   | 19 ± 1   |
| PC/HAB  | 24 ± 2  | 23 ± 2            | 23 ± 1   | 24 ± 1   |
| Hepatic artery blood<br>flow (ml · kg <sup>-1</sup> · min <sup>-1</sup> ) |         |                   |          |          |
| PC/GC   | 7 ± 1   | 6 ± 1             | 7 ± 1    | 6 ± 1    |
| PC  | 6 ± 1   | 7 ± 1             | 7 ± 1    | 7 ± 1    |
| PC/HAB  | 6 ± 1   | 6 ± 1             | 6 ± 1    | 7 ± 1    |

Data are means ± SE. *n* = 5 for PC/GC, *n* = 6 for PC, and *n* = 6 for PC/HAB. \*Significant difference from basal values (*P* < 0.05).

during exercise (29,30). The gluco-regulatory mechanism that is sensitized by exercise has not been ascertained. Studies have shown that resection of the carotid bodies slightly reduced the epinephrine response and significantly attenuated the  $R_a$  response to modest hypoglycemia (31). Other studies from our laboratory suggest that these receptors may play a role in neuroendocrine regulation during exercise (32). Therefore, regulatory mechanisms associated with the carotid bodies may be a factor. The modulation of counterregulatory hormone levels may also be mediated by hepatic and hypothalamic glucoreceptors. Although vagal blockade had no effect on the counterregulatory response to insulin-induced hypoglycemia in dogs (33), denervation of portal vein afferents diminished the epinephrine response to hypoglycemia in rodents (34). The reason for the discrepancy between these studies may be that the nonvagal afferents in the dog could relay information regarding the glucose level. With respect to the importance of hypothalamic glucoreceptors, maintenance of brain euglycemia during peripheral hypoglycemic conditions obliterates the counterregulatory hormone response (35,36). Therefore, hepatic and hypothalamic glucoreceptors may both act to stimulate the increase in epinephrine during hypoglycemia during exercise.

The exaggerated counterregulatory hormone responses that ensued with a decrement in glucose resulted in greater increases in lactate and glycerol compared with euglycemia (29). It is likely that the increased catecholamine concentrations in PC and PC/HAB increased muscle glycogenolysis (37) and adipose tissue lipolysis (38). It is important to note that although there were differences in the arterial levels of these gluconeogenic precursors in PC and PC/HAB compared with PC/GC, their hepatic uptakes were not significantly different. Therefore, it is unlikely that these extrahepatic differences in precursor mobilization were a significant cause of the differences in  $R_a$ .

The similar  $R_a$  responses in PC and PC/HAB suggest that hepatic adrenergic mechanisms do not have an important gluco-regulatory role during moderate exercise. It is possible, however, that the adrenal cortex may stimulate intrahepatic gluconeogenic efficiency or facilitate the

stimulatory effect of another factor (1). This is supported by studies in corticosterone-replaced adrenalectomized rats, which demonstrated an increased gluconeogenic capacity compared with those that were corticosterone deficient (39). In general, however, the regulatory role of the glucocorticoids is considered to be minimal within a single bout of exercise and usually requires several hours to produce a stimulatory effect on gluconeogenic parameters (1,40).

The present study incorporated the use of the pancreatic clamp, selective hepatic adrenergic blockade, and glucose clamp procedures to more closely delineate the gluco-regulatory mechanisms that prevent hypoglycemia during exercise. Maintenance of euglycemia (PC/GC) in the presence of a pancreatic clamp abolished the increase in  $R_a$  normally observed during exercise. Allowing glucose to fall by <25 mg/dl resulted in a compensatory increase in  $R_a$  in PC and PC/HAB. Thus, the selective blockade of hepatic adrenergic receptors did not have any effect on  $R_a$  during exercise when glucagon and insulin were fixed at basal. The effectiveness of the blockade was demonstrated by the attenuation of  $R_a$  during the combined norepinephrine and epinephrine challenge. The selectivity of the blockade was illustrated by similar FFA, glycerol, lactate, and heart rate responses to exercise in PC and PC/HAB. Based on the results of this study, a decrement in glucose per se or a signal elicited by a moderate decrement in glucose, but largely independent of pancreatic hormone and hepatic catecholamine action, stimulates glucose release from the liver during exercise.

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