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Sympathoadrenal influence on glucose, FFA, and insulin levels in exercising rats

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Published in:

The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

1989

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Scheurink, A. J. W., Steffens, A. B., Bouritius, H., Dreteler, G. H., Bruntink, R., Remie, R., & Zaagsma, J. (1989). Sympathoadrenal influence on glucose, FFA, and insulin levels in exercising rats. *The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 256, R161-R168.

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Sympathoadrenal influence on glucose, FFA, and insulin levels in exercising rats

A. J. W. SCHEURINK, A. B. STEFFENS, H. BOURITIUS, G. H. DRETELER, R. BRUNTINK, R. REMIE, AND J. ZAAGSMA

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SCHEURINK, A. J. W., A. B. STEFFENS, H. BOURITIUS, G. H. DRETELER, R. BRUNTINK, R. REMIE, AND J. ZAAGSMA. *Sympathoadrenal influence on glucose, FFA, and insulin levels in exercising rats*. *Am. J. Physiol.* 256 (Regulatory Integrative Comp. Physiol. 25): R161–R168, 1989.—The effects of sympathoadrenal manipulations on the exercise-induced alterations in blood glucose, plasma free fatty acids (FFA), and insulin were investigated in intact and adrenalectomized rats. Exercise consisted of strenuous swimming against a countercurrent for 15 min. Before, during, and after swimming, blood samples were taken through a permanent heart catheter. Adrenalectomy (Adm) markedly reduced the exercise-induced increase in both glucose and FFA. This effect was counteracted by intravenous infusion of epinephrine (E, 20 ng/min). Intravenous infusion of 50 ng E/min into Adm rats caused an exaggerated increase in glucose. In two additional experiments 1) specific adrenoceptor agonists and antagonists were administered to exercising intact and Adm rats, and 2) E or norepinephrine (NE; 20 ng/min) was infused into intact resting rats. The results suggest that E from the adrenal medulla directly affects glucose and insulin but not FFA concentrations in the blood. NE released from peripheral sympathetic nerve endings probably acts in two different ways: as neurotransmitter on liver and pancreas and as a hormone on adipose tissue.

exercise; adrenal medulla, sympathetic nerve endings; adrenergic agonists and antagonists

DURING EXERCISE the sympathoadrenal system is activated to increase the supply of glucose and free fatty acids (FFA) to the working muscle (4, 6). Recent findings in our laboratory demonstrated that in rats a functional dissociation appears between the release of epinephrine (E) by the adrenal medulla and the release of norepinephrine (NE) by the peripheral nerve endings of the sympathetic nervous system (13). However, a complete dissociation does not occur in vivo, since the release of NE by the peripheral nerve endings is influenced by adrenal E via adrenergic presynaptic mechanisms (13).

At present, data available regarding the role of the two branches of the sympathoadrenal system on glucose and FFA metabolism during exercise are conflicting. According to Winder et al. (2, 3, 24), adrenal E is not essential for hepatic glycogenolysis. They concluded that release of glucose by the liver is due to the activation of hepatic sympathetic nerves (22, 23). In contrast, Galbo and co-workers (16) failed to find any effect of hepatic denervation

on hepatic glucose metabolism during exercise. They showed in their experiments that adrenal medullary hormones enhance net glycogen breakdown in liver (11, 12, 16). With regard to FFA metabolism, it is generally accepted that during exercise enhanced sympathoadrenal activity is responsible for the increase in lipolysis. However, almost no information is available with respect to the relative contribution of adrenal E and/or sympathetic NE in this process. The adrenal medulla seems to be involved, since infusion of E into the blood circulation resulted in enhanced plasma FFA levels in resting rats (19). But also NE, released after activation of the sympathetic nervous system, seems to play an important role in the release of FFA from adipose tissue (19, 25). However, it should be noted that white adipose tissue is not directly innervated by sympathetic nerve endings (14).

Indirect actions of the sympathoadrenal system, for example on insulin release by the pancreatic β -cell, may also interfere with the regulation of glucose and FFA levels during exercise. According to Richter et al. (11, 12), hormones from the adrenal medulla initiate the reduction in plasma insulin concentrations during exercise. In addition, infusion of E into the blood circulation of resting rats reduced plasma insulin concentrations (19). On the other hand, activation of the sympathetic nervous system may exert a reduction in plasma insulin levels. It is well known that stimulation of the pancreatic splanchnic nerves inhibits the release of insulin from the β -cell of the endocrine pancreas (25). However, infusion of NE into the blood circulation of resting rats did not affect plasma insulin concentrations (19).

In summary, during exercise catecholamines are released from the sympathoadrenal system, and this is followed by an increase in glucose and FFA release. The aim of the present study was to distinguish the adrenal medullary and sympathetic influences on glucose and FFA metabolism during exercise. Recently we showed that adrenal and sympathetic nerve activity can be dissociated by means of adrenalectomy and/or administration of adrenoceptor agonists and antagonists (13). Therefore, in the present study, exercise-induced alterations in blood glucose, plasma FFA, and insulin concentrations were measured in adrenalectomized and control rats, with and without administration of adrenoceptor agonists and antagonists.

MATERIALS AND METHODS

Animals and housing. Male Wistar rats weighing 300–350 g at the beginning of the experiments were used. They were housed individually in Plexiglas cages (25 × 25 × 30 cm) on a 12-h light-dark cycle (0700–1900 h light on) at a room temperature of 20 ± 2°C. The rats had continuous access to food (Muracon laboratory chow) and water unless otherwise stated.

Surgery. All surgery was performed under ether anesthesia. All animals were provided with a silicon heart catheter through the right jugular vein externalized on the top of the skull according to the techniques described earlier (18). This method allows frequent repeated blood sampling in unanesthetized undisturbed freely moving rats (17, 21). Whenever intravenous infusions were to be performed, the rats were provided with a smaller second silicon heart catheter implanted in the contralateral (left) jugular vein (19). Because of its small size, this second catheter did not prevent venous return from the head of the animals. Experiments started 1 wk after insertion of the heart catheter(s) when the rats were above preoperative weight. In certain experiments the rats were surgically adrenalectomized 2 wk before the insertion of the heart catheter(s) to enable adrenocortical regeneration. During the recovery period the rats did not require much saline as drinking water. Adrenodemedullation was verified histologically after termination of the experiments. Medullary tissue was completely absent in the recovered rat after correct adrenalectomy. Only the results of completely adrenalectomized rats were presented.

Exercise. Exercise was performed in a pool made of stainless steel (length, 3.00 m; width, 0.40 m; and depth, 0.90 m) filled 70% with water of 33 ± 2°C. At one end the pool was equipped with a starting platform (33 × 37 cm) placed ~2 cm above the water surface. This starting platform could be lowered into the water down to the bottom of the swimming pool. A water pump (Loewe, Silenta) provided a marked countercurrent (0.22 m/s) that forced the animal to swim continuously. At the end of the exercise period, a removable resting platform (20 × 37 cm) was offered to the swimming rat at the upstream side of the swimming pool. The rats readily learned to climb on this lighted and warmed platform within 2 min after it was presented. The rats were accustomed to the experimental exercise conditions six to seven times before the onset of the actual experiments to eliminate emotional stress of novelty.

Blood-sampling procedure. Forty minutes before the start of an experiment, the animals were connected with a polyethylene blood-sampling tube (0.4 m length; 1.45 mm OD, and 0.75 mm ID) through which blood was sampled as described earlier (17). Blood samples of 0.35 ml were withdrawn for determination of blood glucose, plasma FFA, and insulin concentrations. After each blood sample, a transfusion of 0.35 ml of citrated donor blood was given to avoid diminution of the blood volume with related changes in hemodynamics. Donor blood was obtained from unstressed rats with permanent heart catheters. Between the withdrawal of blood samples, the tip of the heart catheter was filled with 6% citrate

solution as an anticoagulant. Citrate was used to avoid activation of endothelial lipase.

Experimental procedure. All experiments were performed in the light period between 1000 and 1300 h. On the experimental day, food was removed 1.5 h before the onset of the experiment. To measure basal levels of the blood components, two blood samples in a 10-min interval (at $t = -11$ and -1 min) were taken in the home cage of the undisturbed rat. Subsequently, the rat was placed for 20 min on the starting platform of the swimming pool. Blood samples were taken at $t = 1.5$, 10, and 20 min after the transfer from home cage to starting platform. Immediately after the $t = 20$ -min blood sample, the starting platform was slowly lowered to the bottom of the swimming pool. This lasted for ~5 min, and after ~2 min, one blood sample was taken. The moment the rat started to swim was defined as $t = 0$ min. Then the animal had to swim vigorously against the countercurrent for 15 min. Blood samples were taken at $t = 1$, 5, 10, and 15 min. At the end of the exercise period, the resting platform was lowered. Postexercise blood samples were taken at $t = 19$, 24, 29, and 39 min.

Administration of pharmacological compounds. In *experiment III*, adrenoceptor agonists and antagonists were intravenously administered before and during exercise. Administration of the adrenoceptor agonists E and fenoterol was performed by infusion of the drug through the small second heart catheter. The infusion was started immediately after the last blood sample on the starting platform ($t = 20$ min), and the infusion was continued for 20 min, i.e., during the lowering of the starting platform and the exercise period. E infusion rates were 20 and 50 ng in 0.1 ml saline/min. Ascorbic acid (0.1%) was added to prevent oxidation of E. The β_2 -selective adrenoceptor agonist fenoterol (Boehringer-Ingelheim) was infused at a rate of 7 μ g in 0.1 ml saline/min. The adrenoceptor antagonists ICI 118551 (β_2 -selective, ICI Pharma) and yohimbine (α_2 -selective, Sigma) were given in a single dose (0.1 and 0.13 mg/rat, respectively) before exercise. They were dissolved in 0.1 ml saline and injected intravenously together with the donor blood after the blood sample at $t = 10$ min on the starting platform.

Chemical determinations. Blood samples were immediately transferred to chilled (0°C) centrifuge tubes containing 0.01% EDTA and 10 μ l heparin solution (500 U/ml) as an anticoagulant. Blood glucose was measured by the ferricyanide method of Hoffman (Technicon AutoAnalyzer TM II) with 0.05 ml blood taken from the 0.35-ml sample. The remaining 0.3 ml blood was centrifuged for 15 min at 5,000 rpm at 4°C. A part of the supernatant (100 μ l) was used for the FFA assay, and 100 μ l plasma were stored at -30°C for the insulin assay. Plasma FFA were determined in 100 μ l of supernatant according to the method of Antonis (1) by adapting it for a small volume. The plasma was immediately extracted, and the evaporated extracts were stored at -30°C until determination. Rat-specific plasma immunoreactive insulin was determined by means of a radioimmunoassay kit (NOVO, Denmark). Guinea pig serum M8309 served as antiserum. Duplicate assays were performed on 25- μ l samples. The bound and free 125 I-labeled

insulin was separated by means of a polyethylene glycol solution (23.75% wt/wt) as suggested by Henquin et al. (8).

Statistics. Data are expressed as average change \pm SE from the basal value at $t = -1$ min in the home cage. Wilcoxon matched-pairs signed-rank test was used when the levels of the blood components at a certain time during the experiment were compared with the basal value in the home cage. Analysis of variance and Mann-Whitney U test were applied to determine for each sample point the significant differences between a definitive experiment and the control experiment. The criterion of significance was set at $P < 0.05$.

EXPERIMENTS AND RESULTS

Experiment I. Control experiment. The aim of the control experiment was to study the effect of exercise on blood glucose, plasma FFA, and insulin concentrations under the given experimental conditions. Twelve rats, well accustomed to the whole experimental procedure, participated in this study. The results are presented in Table 1 and Fig. 1. Table 1 shows the absolute basal levels at $t = -1$ min, in the home cage, of blood glucose, plasma FFA, and insulin in all experiments, and Fig. 1 presents the changes toward basal values in blood glucose, plasma FFA, and insulin in the control experiment. Ten minutes after transfer of the rat to the starting platform, blood glucose concentrations were significantly augmented above basal levels. A further increase in blood glucose occurred after 15 min of exercise. Blood glucose levels were maximal at $t = 19$ and 24 min; thereafter a rapid decrease occurred. Plasma FFA levels increased during exercise with maximal levels in the first two blood samples after swimming. The increase in plasma FFA above basal levels was significant at the time points $t = 10, 15, 19, 24, 29,$ and 39 min during and after exercise. Plasma insulin concentrations were slightly but not significantly reduced in the period on the starting platform. During exercise a further significant decline in insulin levels occurred. After exercise, plasma insulin returned

TABLE 1. Basal values of plasma FFA, blood glucose, and plasma insulin

	<i>n</i>	Plasma FFA, $\mu\text{eq/ml}$	Blood Glucose, mg/dl	Plasma Insulin, $\mu\text{U/ml}$
Control (expt I)	12	0.12 ± 0.016	105.5 ± 2.4	43.7 ± 4.3
Adm (expt II)	9	0.13 ± 0.028	103.3 ± 2.4	30.3 ± 3.0
ICI 118551 (expt IIIa)	9	0.17 ± 0.032	108.2 ± 1.9	41.7 ± 5.9
Adm + fenoterol (expt IIIb)	4	0.13 ± 0.020	110.3 ± 3.5	23.4 ± 5.4
Adm + 20E ng E (expt IIIc)	6	0.14 ± 0.023	108.7 ± 2.3	26.3 ± 2.6
Adm + 50E ng E (expt III d)	5	0.15 ± 0.032	105.2 ± 2.1	22.0 ± 2.0
Adm + yohimbine + fenoterol (expt III e)	4	0.10 ± 0.019	111.0 ± 3.9	28.3 ± 11.8
E infusion (expt IVa)	7	0.16 ± 0.027	106.7 ± 3.6	37.0 ± 6.3
NE infusion (expt IVb)	7	0.12 ± 0.016	105.6 ± 1.5	43.3 ± 5.5

Values are means \pm SE; *n*, no. of rats. FFA, free fatty acids; Adm, adrenalectomized; E, epinephrine; NE, norepinephrine.

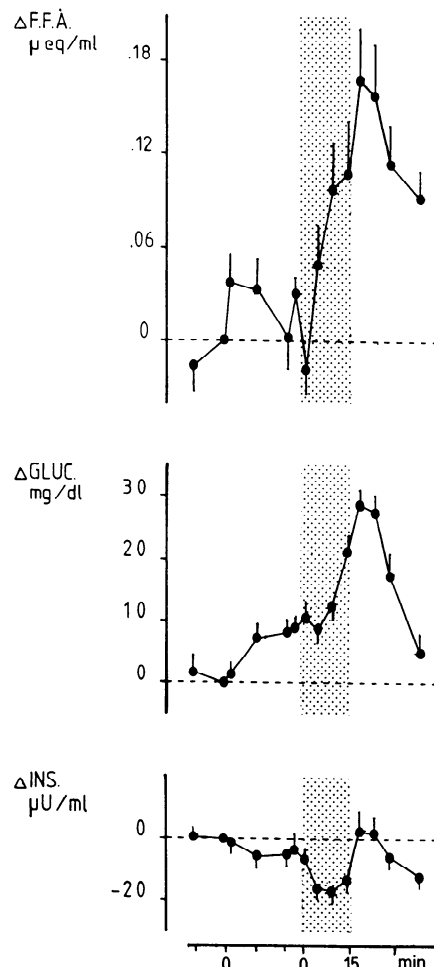


FIG. 1. Plasma free fatty acids (FFA), blood glucose (gluc), and plasma insulin (ins) concentrations before, during, and after exercise (●—●). Data for FFA, glucose, and insulin are expressed as average changes \pm SE from basal values. (Basal value was measured at $t = -1$ min in home cage, immediately before rat was transferred to starting platform in swimming pool.) Swimming period is indicated by dotted area.

to basal levels with a slight decline in the last samples at $t = 29$ and 39 min. Plasma insulin levels were significantly changed from basal levels at $t = 5, 10, 15,$ and 39 min during and after exercise.

Experiment II. Adrenodemedullation. Nine bilaterally adrenalectomized (Adm) rats were submitted to exercise to investigate the role of the adrenal medulla on blood glucose, plasma FFA, and insulin concentrations during exercise. The results are presented in Table 1 and Fig. 2. Basal levels of plasma insulin in Adm rats were significantly lower than the control values in intact rats. The exercise-induced increase in blood glucose and FFA was reduced in Adm rats (significant at $t = 1, 5, 10, 15, 19, 24,$ and 29 min for glucose and $t = 19, 24,$ and 29 min for FFA). The alternations in plasma insulin concentrations during exercise in Adm rats were not different from those in the control experiments. During recovery on the resting platform, a significant reduction in plasma insulin concentrations in Adm rats in comparison with the control values occurred at $t = 24$ and 29 min postexercise.

Experiment III. Presynaptic regulatory mechanisms. Pharmacological modulations of the presynaptic regulation of NE release led to considerable changes in plasma

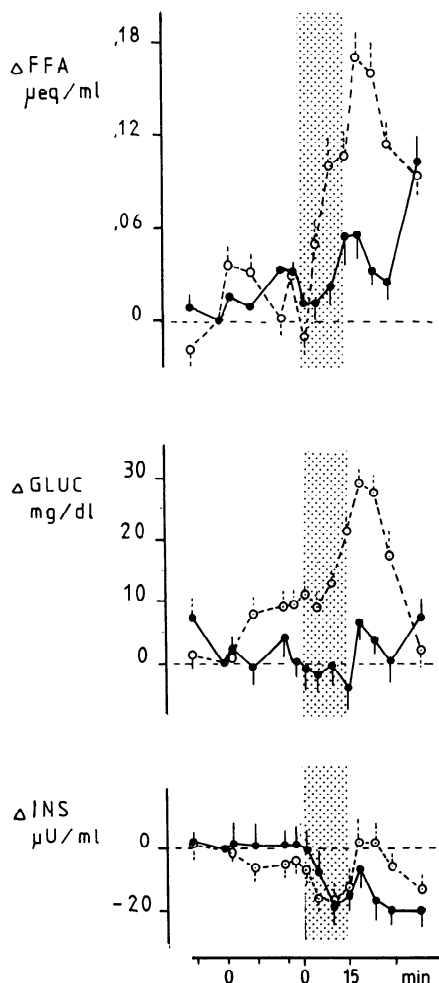


FIG. 2. Effect of adrenalectomy on plasma free fatty acids (FFA), blood glucose (gluc), and plasma insulin (ins) concentrations before, during, and after exercise (●—●). Control experiment with intact rats is depicted (○---○). Data are expressed as average changes \pm SE from basal value. Swimming period is indicated by dotted area.

catecholamine levels in exercising intact and Adm rats (13). The effect of these pharmacological interventions on blood glucose, plasma FFA, and insulin were investigated in this composed experiment.

In *Experiment IIIa*, the β_2 -selective adrenoceptor antagonist ICI 118551 was administered intravenously into six intact rats to suppress NE release from the peripheral nerve endings of the sympathetic nervous system (13). The effects on blood glucose, plasma FFA, and insulin are presented in Table 1 and Fig. 3A. The injection of the β_2 -antagonist reduced blood glucose for the rest of the experiment in comparison with the control experiment (significant at all sample times except for $t = 39$ min). Plasma FFA levels were slightly reduced during exercise (significant during lowering of the starting platform), and plasma insulin concentrations were not significantly changed in comparison with the control experiment. In *experiment IIIb*, the β_2 -selective adrenoceptor agonist fenoterol was infused intravenously during exercise into five Adm rats. This led to high plasma NE concentrations (13). The results are presented in Table 1 and Fig. 3B. Blood glucose, plasma FFA, and insulin were increased in comparison with Adm rats (significant at $t = 5, 10, 15, 19, 24, 29,$ and 39 min for glucose, at $t =$

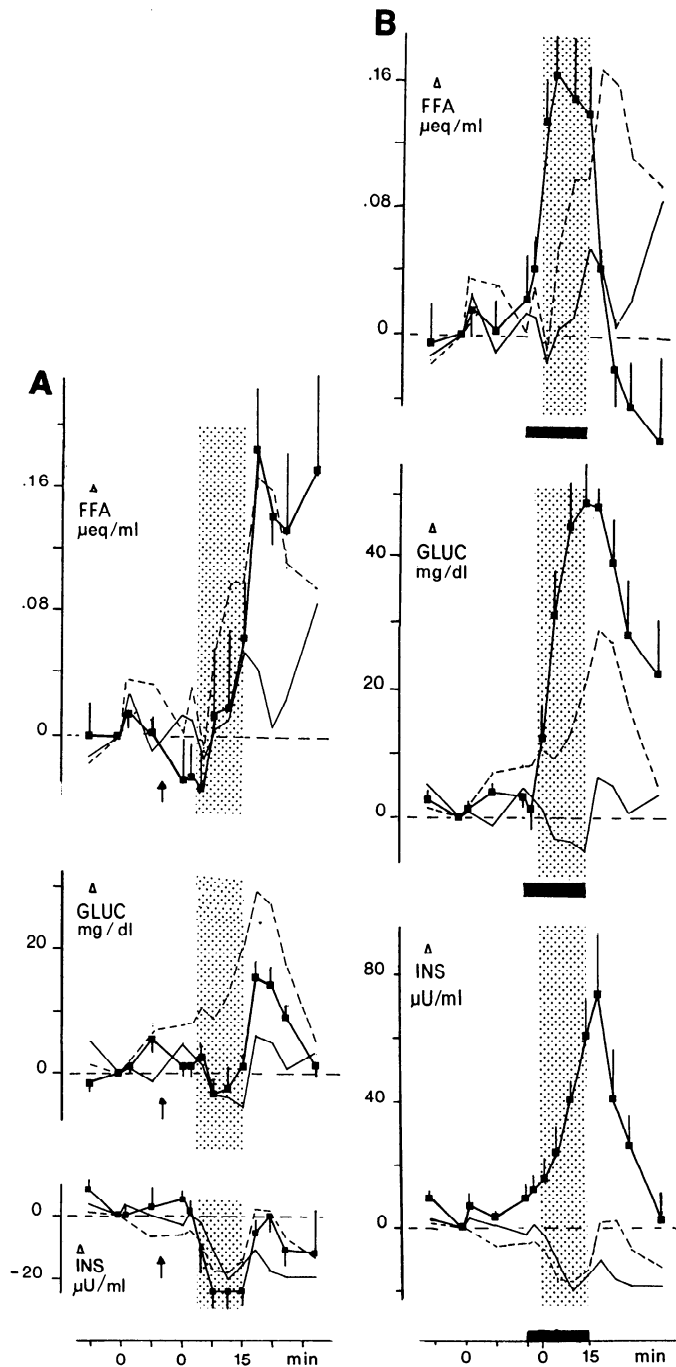


FIG. 3. A: effect of intravenous administration of β_2 -selective adrenoceptor antagonist ICI 118551 on plasma free fatty acids (FFA), blood glucose (gluc), and plasma insulin (ins) concentrations in exercising adrenalectomized (Adm) rats (●—●). Control experiments with intact (---) and Adm (—) rats are depicted. Data are expressed as average changes \pm SE from basal values. Swimming period is indicated by dotted area. Time point of injection is indicated by an arrow. B: effect of an intravenous infusion of the β_2 -selective adrenoceptor agonist fenoterol on plasma FFA, blood glucose, and plasma insulin concentrations in exercising Adm rats (●—●). Control experiments with intact and Adm rats are depicted (--- and —), respectively. Period in which infusion was given is indicated by a horizontal line at bottom of each graph. Data are expressed as in A. See facing page for C-E.

1, 5, and 10 min for FFA, and at all time points during and after infusion for insulin).

In *experiment IIIc*, a physiological dose (20 ng/min) of E was infused intravenously during exercise into six Adm

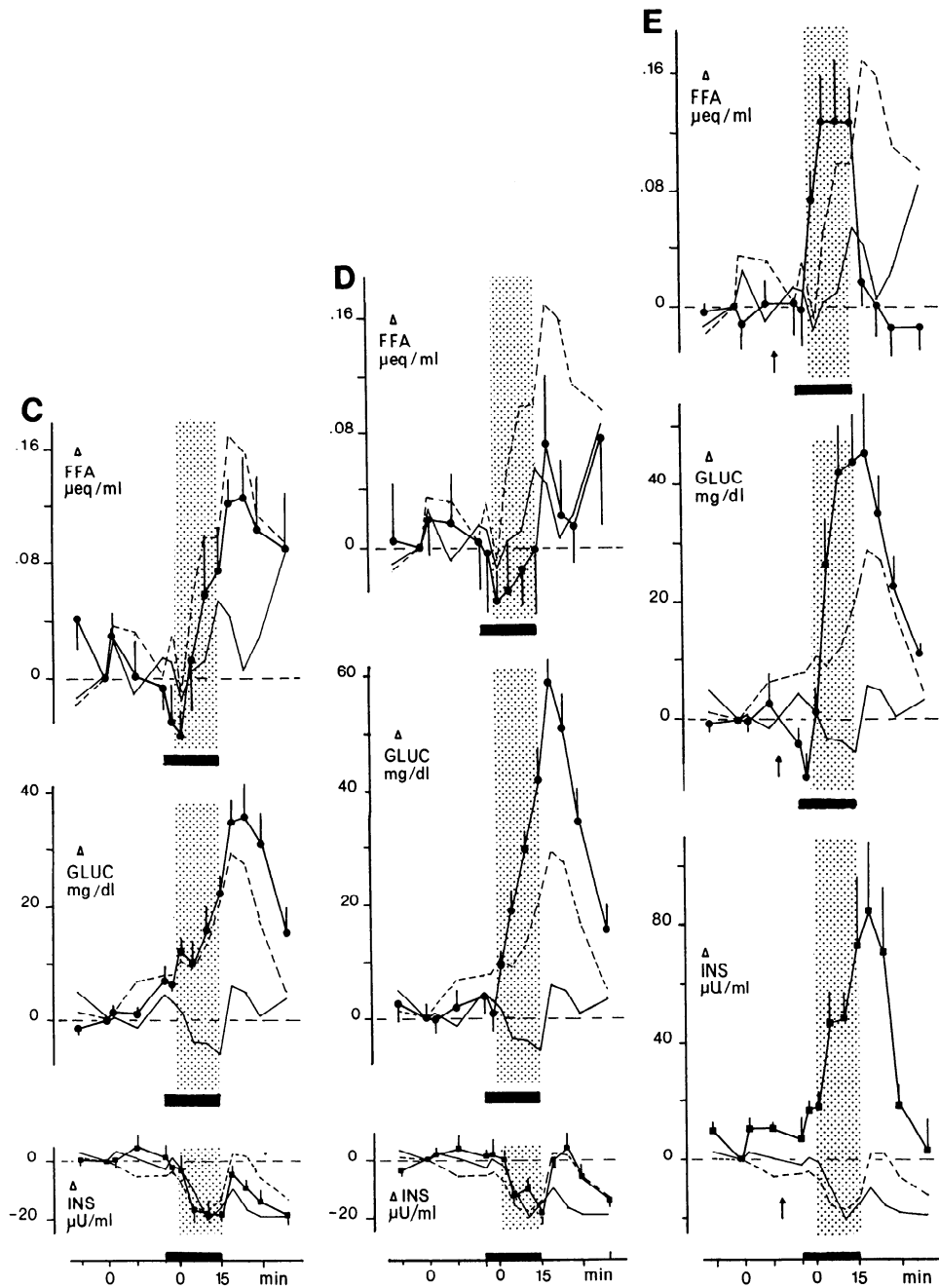


FIG. 3 continued. C: effect of an intravenous infusion of 20 ng epinephrine (E)/min on plasma FFA, blood glucose, and plasma insulin concentrations in exercising Adm rats (●—●). Control experiments with intact (---) and Adm (—) rats are depicted. Data are expressed as in B. D: effect of an intravenous infusion of 50 ng E/min on plasma FFA, blood glucose, and plasma insulin concentrations in exercising Adm rats (●—●). Control experiments with intact (---) and Adm (—) rats are depicted. Data are expressed as in B. E: effect of intravenous injection of the α_2 -selective adrenoceptor antagonist yohimbine in combination with an intravenous infusion of the β_2 -selective adrenoceptor agonist fenoterol on plasma FFA, blood glucose, and plasma insulin concentrations in exercising Adm rats (●—●). Control experiments with intact (---) and Adm (—) rats are depicted. Data are expressed as in A and B.

rats to restore the normal NE and probably also E pattern (13). Blood glucose, plasma FFA, and insulin alterations are presented in Table 1 and Fig. 3C. Blood glucose and plasma FFA were significantly enhanced above Adm levels at $t = 1, 5, 10, 15, 19, 29,$ and 39 min for glucose and at $t = 19$ and 24 min for FFA. Blood glucose and plasma FFA levels showed a remarkable similarity with the values in normal exercising rats in the control experiment. Plasma insulin levels were not changed during infusion in comparison with Adm or normal exercising rats. Infusion of a high dose (50 ng/min) of E into five Adm rats (*expt IIIId*, results in Table 1 and Fig. 3D) caused a steep increase in blood glucose. Blood glucose levels were significantly increased above Adm values at $t = 5$ until 29 min and above control values at $t = 10$ until 29 min. Plasma FFA levels did not differ from Adm values. Plasma insulin levels in this

experiment were not different from Adm and/or normal values. *Experiment IIIe* consisted of a single intravenous injection of the α_2 -selective adrenoceptor antagonist yohimbine at $t = 10$ min on the starting platform combined with an intravenous infusion of the β_2 -selective adrenoceptor agonist fenoterol during exercise in four Adm rats. This leads to extremely high plasma NE concentrations (13). The results are presented in Table 1 and Fig. 3E. Injection of the α_2 -adrenoceptor blocker caused an increase in plasma insulin concentration and a decline in blood glucose. Plasma FFA seemed not affected by α_2 -blockade. An increase in blood glucose levels occurred during infusion of the β_2 -antagonist fenoterol and exercise. The increase in insulin was enhanced by infusion of fenoterol during exercise. Plasma levels of insulin after combined α_2 -blockade and β_2 -stimulation were higher than when only fenoterol was infused without injection

of yohimbine. Plasma FFA were increased during fenoterol infusion in this combined experiment. Plasma concentrations were significantly changed in comparison with Adm values at $t = 20$, during lowering, 5, 10, 15, 19, 24, and 29 min for glucose, $t = 1, 5, 10,$ and 15 min for

FFA, and $t = 1, 5, 10, 15, 19, 24, 29,$ and 39 min for insulin.

Experiment IV. Intravenous infusion of E and NE. To investigate the hormonal effects of E and NE on blood glucose, plasma FFA, and insulin concentrations, either E (20 ng in 0.1 ml saline/min) or NE (20 ng in 0.1 ml saline/min) was infused for 15 min into the blood circulation of seven resting rats. The infusion was administered through the small heart catheter between $t = 0$ and 15 min, and blood samples were withdrawn at $t = -11, -1, 1, 5, 10, 15, 17, 22, 27,$ and 37 min for determination of blood glucose, plasma FFA, and insulin. The results are presented in Table 1 and Fig. 4, A and B. Infusion of 20 ng E into the blood circulation caused a significant increase in blood glucose within 10 min after start of the infusion. Plasma insulin levels were reduced during E infusion and increased immediately after termination of the infusion. Plasma FFA levels were not changed during infusion of E but a reduction in plasma FFA concentrations occurred after termination of the infusion. The changes in blood glucose, plasma FFA, and insulin were significant at $t = 10, 15, 17, 22, 27$ min, $t = 27$ min, and $t = 1$ min, respectively. Infusion of 20 ng NE/min had only an effect on plasma FFA concentrations. Plasma FFA levels were significantly increased at $t = 22$ min.

DISCUSSION

Blood glucose and plasma FFA concentrations increased during exercise in control rats, suggesting that the production and release of glucose and FFA highly exceeded the expenditure of these energy substrates. Plasma insulin levels declined, probably due to a diminished release of insulin by the pancreatic β -cell (6). These changes in glucose, FFA, and insulin concentrations are the result of an activation of the sympathoadrenal system during exercise, as reflected by increased concentrations of E and NE in the blood circulation (4, 6, 13). Accordingly, blood glucose and plasma FFA concentrations are affected by alterations in sympathoadrenal activity. Recently (13) we showed that Adm caused a 60% reduction in the exercise-induced increase in plasma NE concentrations. Plasma E was not detectable in Adm rats. In the present study, Adm led to a marked reduction in the exercise-induced increase in both blood glucose and plasma FFA concentrations. The reduced catecholamine levels in the Adm rats had only a minor effect on plasma insulin alterations during exercise. This seemingly suggests that the markedly reduced increase in sympathetic activity as indicated by low plasma NE levels in Adm rats is still sufficient to inhibit insulin release during exercise. However, it should be noted that different blood glucose levels in Adm and control rats may interfere with

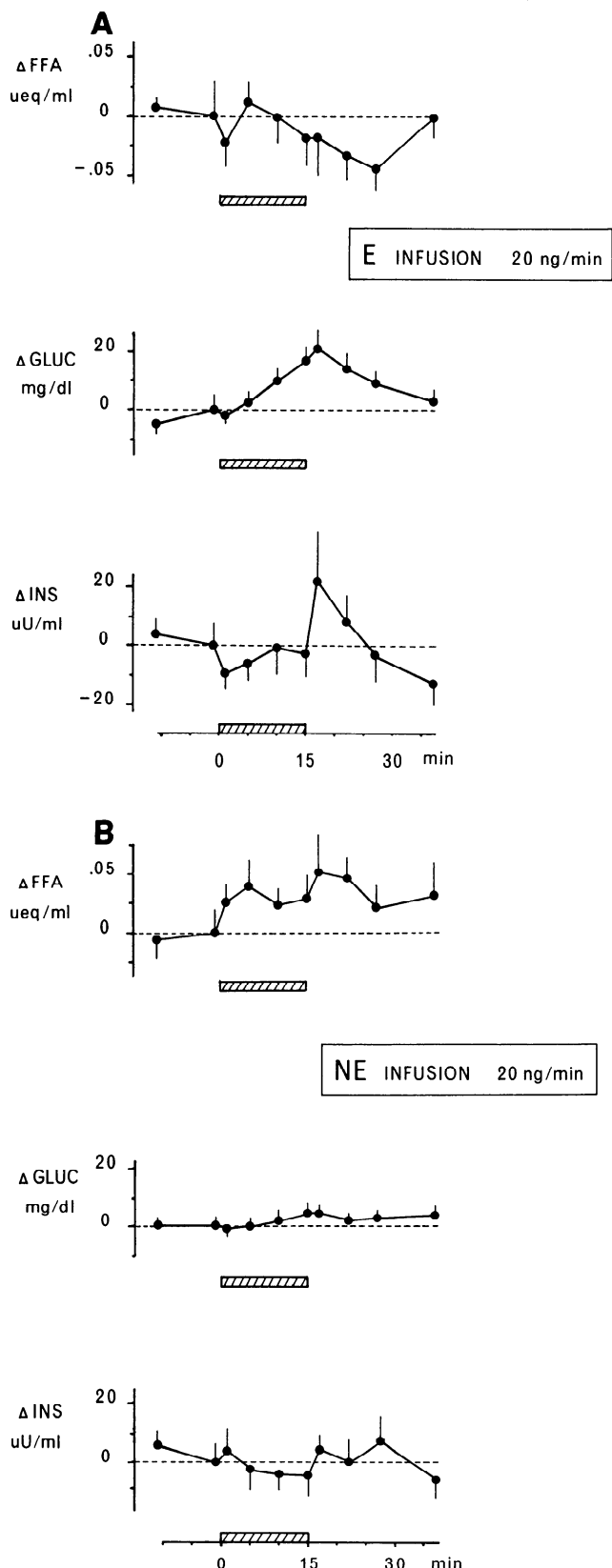


FIG. 4. A: effect of an intravenous infusion of 20 ng epinephrine (E)/min (●—●) on plasma free fatty acids (FFA), blood glucose (gluc), and plasma insulin (ins) concentrations in nonexercising rats. Data are expressed as average changes \pm SE from basal values at $t = -1$ min before start of infusion. Period in which infusion was given is indicated by a horizontal line at bottom of each graph. B: effect of an intravenous infusion of 20 ng NE/min (●—●) on plasma FFA, blood glucose, and plasma insulin concentrations in nonexercising rats. Data are expressed as in A.

the regulation of insulin release by the sympathoadrenal system.

Infusion of 20 ng E/min into Adm rats completely restored the normal increase in plasma NE levels during exercise (13). In the present study the infusion of 20 ng E/min into Adm rats also restored blood glucose and plasma FFA patterns toward control values (see Fig. 3C). Infusion of a high dose of E (50 ng/min) into exercising Adm rats caused an exaggerated increase in blood glucose compared with control animals and Adm rats infused with 20 ng E/min (see Fig. 3D). Plasma FFA concentrations were lower than control values after infusion of 50 ng E/min and did not differ from those of exercising Adm rats. These results suggest a relation between plasma E and blood glucose concentrations. In a previous study with nonexercising rats (19), however, we showed that intravenous infusion of 50 ng E/min increased plasma FFA concentrations besides an increase in blood glucose and a decrease in plasma insulin. This seems in contrast to the results of the present study. It might be that the increase in plasma FFA after infusion of 50 ng E in the nonexercising animals may be due to a change in the rate of reesterification of FFA. Because insulin is required for this process, reduced plasma insulin concentrations during 50-ng E infusion in the previous study may have led to reduced reesterification and then to an increase in plasma FFA concentrations. In the present study a near-physiological dose of 20 ng E/min was infused into nonexercising rats. This still resulted in an increase in blood glucose and a reduction in plasma insulin during infusion (see Fig. 4A). Plasma FFA concentrations were not affected. Accordingly, E in a physiological range does not directly influence lipolysis. After termination of the E infusion plasma insulin concentrations were increased, and blood glucose returned to preinfusion levels. The facilitated entry of glucose into adipocytes, affected by insulin, probably caused an increase in the reesterification of FFA, resulting in a decline in plasma FFA concentrations immediately after termination of the infusion.

In conclusion, the results of the present study with exercising and nonexercising rats suggest that E, originating from the adrenal medulla (13), directly affects blood glucose and plasma insulin concentrations. The effects of E on plasma FFA concentrations seems secondary to the alterations in blood glucose and in particular plasma insulin concentrations.

Circulating NE appeared to act exactly in the opposite way. In exercising Adm rats, infusion of 20 ng E restored both plasma NE (13) as well as plasma FFA concentrations toward control values (see Fig. 3C). After infusion of the high dose of 50 ng E in exercising Adm rats, both plasma NE (13) and FFA levels were relatively reduced (see Fig. 3D). In nonexercising rats, infusion of 20 ng NE/min increased plasma FFA concentrations (see Fig. 4B). Blood glucose and plasma insulin concentrations were not influenced by NE infusion. The same results were also obtained with a higher dose (50 ng/min) of NE in a previous study (19). These findings suggest a relation between plasma NE and plasma FFA concentrations. However, it has to be borne in mind that the present

results regarding NE are only appropriate for a hormonal function of sympathetic NE. We recently proposed that all NE in plasma originates from the peripheral nerve endings of the sympathetic nervous system (13). After sympathetic activation only a small part of the released neurotransmitter NE leaks to the blood circulation. Accordingly, intravenous infusion of 20 ng NE/min into the general blood circulation seems to be insufficient to mimic local sympathetic activity in peripheral organs such as liver or endocrine pancreas. This implies that the increase in plasma FFA concentrations during intravenous infusion of 20 ng NE/min may be considered as a hormonal effect of NE on adipose tissue. This hormonal effect of NE on FFA release seems very functional, since white adipose tissue is not directly innervated by the sympathetic nervous system (14).

Infusion of the β_2 -selective adrenoceptor agonist fenoterol in exercising Adm rats immediately enhanced blood glucose, plasma FFA, and plasma insulin concentrations (see Fig. 3B). The effects on blood glucose and plasma insulin were seemingly caused by a direct stimulation of β_2 -adrenoceptors on liver, muscle, and pancreatic β -cells (9, 11, 12, 25). The decrease in blood glucose and plasma insulin concentrations after administration of the β_2 -selective adrenoceptor antagonist ICI 118551 to intact exercising rats in the present study may indicate the involvement of β_2 -adrenoceptors in the regulation of blood glucose and plasma insulin concentrations. However, it has to be remarked that according to literature, catecholaminergic activation of hepatic glycogenolysis is mediated by α -adrenoceptors rather than by β -adrenoceptors in adult male rats (5, 20). Alternatively, the increase of blood glucose might be explained by increased muscle glycogenolysis resulting in decreased uptake of glucose from the blood. Injection of the α_2 -adrenoceptor antagonist yohimbine reduced blood glucose (see Fig. 3E), suggesting that also α_2 -adrenoceptors are involved in the regulation of blood glucose levels. These α_2 -adrenoceptors are located in liver where they stimulate glycogenolysis (5, 22). Because the present study was performed in Adm rats, the decline in blood glucose after α_2 -blockade suggests that sympathetic NE may have activated the α_2 -adrenoceptors in liver. This is in accordance with findings of Shimazu (14) and Yamaguchi (26). The results of the experiments in which α_2 - and β_2 -selective adrenoceptor agonists and antagonists were administered have to be interpreted cautiously because of abnormal blood glucose, plasma FFA, and insulin profiles leading to deviating interactions in glycogenolysis, glycogenesis, lipolysis, and lipogenesis. In addition, interference with intermediary metabolism, e.g., lactate and glycerol production and changed hormone release, e.g., adrenocorticotrophic hormone may have occurred. For example, plasma FFA concentrations increased during fenoterol infusion, which then should be due to a direct action of fenoterol on β_2 -adrenoceptors in adipose tissue. However, according to literature (10, 15), lipolysis is preferentially mediated via a β_1 -adrenoceptor mechanism. An alternative explanation might be that infusion of fenoterol in exercising Adm rats immediately enhanced plasma NE concentrations via stimulation of

presynaptic β_2 -adrenoceptors on the sympathetic nerve endings (13). Then the high NE levels might have increased lipolysis by β_1 -adrenoceptor stimulation. Further studies have to be performed to unravel the specific α - and β -adrenergic effects on blood glucose, plasma FFA, and insulin and in addition on metabolism.

In summary, the results of the present study suggest that E from the adrenal medulla directly affects glucose and insulin concentrations in the blood. E probably has no direct effect on FFA release. NE released by the peripheral nerve endings of the sympathetic nervous system acts in two different ways: a neurotransmitter function on liver and pancreas β -cell and a hormone function on FFA release on adipose tissue.

The authors thank G. Bouws for technical assistance and J. Poelstra for typing the manuscript.

This research was supported by a grant from the Foundation of Fundamental Biological Research of the Netherlands Organization for Scientific Research.

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Received 10 December 1987; accepted in final form 19 August 1988.

REFERENCES

- ANTONIS, A. Semiautomated method for the colorimetric determination of plasma free fatty acids. *J. Lipid Res.* 6: 307-312, 1965.
- ARNALL, D. A., J. C. MARKER, R. K. CONLEE, AND W. W. WINDER. Effect of infusing epinephrine on liver and muscle glycogenolysis during exercise in rats. *Am. J. Physiol.* 250 (*Endocrinol. Metab.* 13): E641-E650, 1986.
- CARLSON, K. I., J. C. MARKER, D. A. ARNALL, M. L. TERRY, H. T. YANG, L. G. LINDSAY, M. E. BRACKEN, AND W. W. WINDER. Epinephrine is unessential for stimulation of liver glycogenolysis during exercise. *J. Appl. Physiol.* 58: 544-548, 1985.
- CHRISTENSEN, N. J., AND H. GALBO. Sympathetic nervous activity during exercise. *Annu. Rev. Physiol.* 45: 139-153, 1983.
- EXTON, J. H. Mechanisms involved in alpha-adrenergic effects of catecholamines on liver metabolism. *J. Cyclic Nucleotide Res.* 5: 277-287, 1979.
- GALBO, H. *Hormonal and Metabolic Adaptation to Exercise*. Stuttgart, FRG: Thieme, 1983.
- GAUTHIER, C., K. EL TAYEB, M. VRANIC, AND H. L. LICKLEY. Glucoregulatory role of cortisol and epinephrine interactions studied in adrenalectomized dogs. *Am. J. Physiol.* 250 (*Endocrinol. Metab.* 13): E393-E401, 1986.
- HENQUIN, J. C., P. MALVAUX, AND A. E. LAMBERT. Glucagon immunoassay using polyethylene glycol to precipitate antibody-bound hormone. *Diabetologia* 10: 61-68, 1974.
- HOLM, E. Adrenergic regulation of insulin release. *Acta Med. Scand. Suppl.* 672: 21-25, 1983.
- LAFONTAN, M., M. BERLAN, AND C. CARPENE. Fat cell receptors: inter- and intraspecific differences and hormone regulation. *Int. J. Obesity* 9: 117-128, 1985.
- RICHTER, E. A. Influence of the sympatho-adrenal system on some metabolic and hormonal responses to exercise in the rat. With special reference to the effect on glycogenolysis in skeletal muscle. *Acta Physiol. Scand. Suppl.* 528: 1-42, 1984.
- RICHTER, E. A., H. GALBO, B. SONNE, J. J. HOLST, AND N. J. CHRISTENSEN. Adrenal medullary control of muscular and hepatic glycogenolysis and of pancreatic hormonal secretion in exercising rats. *Acta Physiol. Scand.* 108: 235-242, 1980.
- SCHEURINK, A. J. W., A. B. STEFFENS, H. BOURITIUS, G. DRETELER, R. BRUNTINK, R. REMIE, AND J. ZAAGSMA. Adrenal and sympathetic catecholamines in exercising rats. *Am. J. Physiol.* 256 (*Regulatory Integrative Comp.* 26): R155-R160, 1989.
- SHIMAZU, T. Neuronal control of intermediate metabolism. In: *Neuroendocrinology*, edited by S. Lightman and B. Everitt. Oxford UK: Blackwell, 1986, p. 304-339.
- SMITH, U. Adrenergic control of lipid metabolism. *Acta Med. Scand. Suppl.* 672: 41-47, 1983.
- SONNE, B., K. J. MIKINES, E. A. RICHTER, N. J. CHRISTENSEN, AND H. GALBO. Role of liver nerves and adrenal medulla in glucose turnover in running rats. *J. Appl. Physiol.* 59: 1640-1646, 1985.
- STEFFENS, A. B. Blood glucose and FFA levels in relation to the meal pattern in the normal rat and the ventromedial hypothalamic lesioned rat. *Physiol. Behav.* 4: 215-225, 1969.
- STEFFENS, A. B. A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol. Behav.* 4: 833-836, 1969.
- STEFFENS, A. B., G. DAMSMA, J. VAN DER GUGTEN, AND P. G. M. LUITEN. Circulating free fatty acids, insulin, and glucose during chemical stimulation of the lateral and ventromedial areas of the hypothalamus in the rat. *Am. J. Physiol.* 247 (*Endocrinol. Metab.* 10): E765-E771, 1984.
- STUDER, R. K., AND A. B. BORKE. Differences between male and female rats in the regulation of hepatic glycogenolysis. *J. Biol. Chem.* 257: 7987-7993, 1982.
- WIERSMA, J., AND J. KASTELIJN. A chronic technique for high frequency blood sampling/transfusion in the freely behaving rat which does not affect prolactin and corticosterone secretion. *J. Endocrinol.* 107: 285-292, 1985.
- WINDER, W. W. Control of hepatic glucose production during exercise. *Med. Sci. Sports Exercise* 17: 2-5, 1985.
- WINDER, W. W., M. A. BEATTIE, C. PIQUETTE, AND R. T. HOLMAN. Decrease in liver norepinephrine in response to exercise and hypoglycemia. *Am. J. Physiol.* 244 (*Regulatory Integrative Comp. Physiol.* 13): R845-R849, 1983.
- WINDER, W. W., S. F. LOY, D. S. BURKE, AND S. J. HAWKES. Liver glycogenolysis during exercise in adrenalectomized male and female rats. *Am. J. Physiol.* 251 (*Regulatory Integrative Comp. Physiol.* 13): R1151-R1155, 1986.
- WOODS, S. C., G. J. TABORSKY, JR., AND D. PORTE, JR. CNS control of nutrient homeostasis. In: *Handbook of Physiology: The Nervous System. Intrinsic Regulatory Systems of the Brain*. Bethesda, MD: Am. Physiol. Soc., 1986, sect. 1, vol. IV, chapt. 7, p. 365-412.
- YAMAGUCHI, N. Evidence supporting the existence of presynaptic alpha-adrenoceptors in the regulation of endogenous noradrenaline release upon hepatic sympathetic nerve stimulation in the dog liver in vivo. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 321: 177-184, 1982.