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Experience affects exercise-induced changes in catecholamines, glucose, and FFA

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SCHEURINK, A. J. W., A. B. STEFFENS, G. H. DRETELER, L. BENTHEM, AND R. BRUNTINK. *Experience affects exercise-induced changes in catecholamine, glucose, and FFA.* Am. J. Physiol. 256 (Regulatory Integrative Comp. Physiol. 25): R169–R173, 1989.—The interference of the experimental conditions on the exercise-induced alterations in plasma catecholamines, plasma free fatty acids, and glucose and insulin concentrations was investigated in rats. Exercise consisted of strenuous swimming against a countercurrent (0.22 m/s) for 15 min in a pool with water of 33°C. Before, during, and after swimming, blood samples were taken through a permanent heart catheter. The blood component levels in rats that were confronted with exercise for the very first time were compared with the levels in rats that were well accustomed to the exercise conditions. The very first time rats swam caused an enhanced release of epinephrine from the adrenal medulla and a reduced output of norepinephrine from the sympathetic nerve endings. Furthermore, in the first time swim group, blood glucose levels were higher and plasma free fatty acid concentrations were lower compared with the well-accustomed animals. There were no differences in plasma insulin concentrations. It is concluded that the experimental conditions may interfere considerably with the hormonal and metabolic response to exercise. Furthermore the results reinforce the idea that the two parts of the sympathoadrenal system are functionally and metabolically dissociated.

emotional stress, epinephrine; norepinephrine; insulin; adrenal medulla; sympathetic nerve endings

EXERCISE requires hormonal and metabolic adjustments to increase the mobilization of energy substrates from liver and adipose tissue. In particular, the sympathoadrenal system is activated during exercise (for reviews see 3, 4). However, available data regarding the role of the adrenal medulla and the sympathetic nervous system on catecholamine alterations and actions during exercise are conflicting (2, 15, 19). These contradictory data may be explained by essential differences in the experimental conditions used in the different studies. According to Young et al. (22), a functional dissociation appears within the sympathoadrenal system between the activity of the sympathetic nervous system and the release of catecholamines by the adrenal medulla. This means that various physiological conditions may selectively activate one of the two parts of the sympathoadrenal system (11, 21, 22). As a consequence, changes in the stressfulness of the experimental environments may lead to marked differences in the catecholamine alterations and actions during exercise. For example, the hormonal and metabolic responses to exercise in rats that are unaccustomed to the exercise stimulus may essentially deviate from the responses that occur in rats that are thoroughly familiar with the experimental conditions (7, 19).

In the present study the exercise-induced changes in plasma catecholamines, free fatty acids, glucose, and insulin concentrations are investigated *1*) in rats that are exposed for the very first time to strenuous swimming and *2*) in rats that are well accustomed to the same experimental demands. The results demonstrate that the stressfulness of the experimental situation may interfere considerably with the hormonal and metabolic response to exercise.

MATERIALS AND METHODS

Animals and housing. Male Wister rats weighing 300–350 g at the beginning of the experiments were used. They were individually housed in clear 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 (17). This method allows frequent repeated blood sampling in unanesthetized undisturbed freely moving rats (16, 18). The experiments started 1 wk after insertion of the heart catheter when the rats were above preoperative weight.

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 level. This starting platform could be lowered into the water to the bottom of the swimming pool. A water pump (Loewe Silenta, FRG) provided a countercurrent (0.22 m/s) in the water that forced the animal to swim continuously. At the end of the exercise period, a removable resting platform (20 × 37 cm) at the upstream side of the swimming pool was offered to the swimming rat. In general, the rats climbed on this lighted and warmed platform within 2 min after it was presented.

Blood-sampling procedure and chemical determinations. Forty minutes before the start of an experiment, the animals were connected with a polyethylene blood-sampling tube (0.4 m length, 1.25 mm OD, and 0.75 mm ID) through which blood was sampled as described earlier (17). The rats were accustomed to the blood-sampling procedure for several times before the start of the experiments. During a whole experiment, 15 blood samples of 0.5 ml were withdrawn for determination of plasma catecholamines, blood glucose, plasma free fatty acids (FFA), and insulin concentrations. After each sample the same quantity of citrated donor blood was given to avoid diminution of the blood volume with related changes in hemodynamics. Donor blood was obtained from resting 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.

Blood samples were immediately transferred to chilled (0°C) centrifuge tubes containing 0.1% EDTA as an antioxidant and 10 μl 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 a 0.50-ml sample. The remaining 0.45 ml blood was centrifuged for 15 min at 5,000 rpm at 4°C. The supernatant was divided into three parts: 100 μl was immediately stored at −80°C for catecholamine measurements, 100 μl was used for FFA determination, and the remaining plasma was stored at −30°C for the insulin radioimmunoassay.

Determination of plasma catecholamine concentrations was performed by high-pressure liquid chromatography in combination with electrochemical detection as described previously (12) with minor modifications. The high-pressure liquid chromatography-electrochemical detection system included an LKB 2150 pump (LKB Instruments, Bromma, Sweden), a Rheodyne injection valve with a 50-μl loop, two reversed-phase Cp.sphere C_{18} cartridge columns in conjunction (Chrompack Ned) held at 30°C by a column stove (LKB), an ESA 5100 A electrochemical detector with a 5011 high-sensitive analytical cell and a 5020 guard cell (ESA), and a BD 41 two-channel flat recorder (Kipp). Guard cell potential in front of the injection valve was +450 mV, the potentials of the working electrodes were −50 mV and +350 mV, respectively. The mobile phase contained 0.05 M Na-acetate, 0.08% heptane sulfonic acid, 0.01% EDTA, 0.01% NaCl, and 5% methanol-95% H_2O (pH 4.75). The limit of the detection level for epinephrine was 0.010 ng/ml and 0.005 ng/ml for norepinephrine.

Plasma FFA were determined 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-μl samples. The bound and free ^{125}I-labeled insulin was separated by means of a polyethylene glycol solution (23.75% wt/wt) as described by Henquin et al. (5).

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 start of the experiment. To measure basal levels of the blood components, two blood samples in a 10-min interval ($t = -11$ and -1) 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 the home cage to the 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 during this time two blood samples were 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 during exercise. At the end of the exercise period ($t = 15$) the resting platform was lowered. Postexercise blood samples were taken at $t = 19$, 24, 29, and 39 min.

Experiments and statistics. The exercise-induced changes in plasma catecholamine, FFA, glucose, and insulin concentrations were investigated in *1*) rats that were exposed for the very first time to strenuous swimming and *2*) in rats that were well accustomed to the exercise method. The first group consisted of nine rats well accustomed to handling and the blood-sampling technique but unfamiliar with the swimming pool and the exercise conditions. This "first-swim" experiment was compared with a control experiment in which 12 rats, well accustomed to the whole experimental procedure, including swimming, were participating. These control rats can not be considered as physically well-trained animals, since they were made familiar with the swimming conditions only six times.

Data were expressed as average change ± SE from basal volume 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 of $t = -1$ min in the home cage. Differences between the first-swim and the control experiment for each sample point were determined by applying analysis of variance and the Mann-Whitney U test. The criterion of significance was set at $P < 0.05$.

RESULTS

Table 1 presents the absolute basal concentrations of plasma epinephrine (E) and norepinephrine (NE), blood glucose, plasma FFA, and insulin as measured at $t = -1$ min in the home cage in both experiments. There were no initial differences between the two groups. Figures 1 and 2 present the changes in the blood component levels in comparison with the basal values.

Basal levels of catecholamines as measured when the rats were sleeping or resting in the home cage were low (20–26 pg/ml for E and 138–177 pg/ml for NE), indicating that both groups of rats were completely unstressed and well accustomed to the blood-sampling method (18). A slight significant increase in both plasma E and NE

TABLE 1. *Basal values of plasma catecholamines, plasma FFA, blood glucose, and plasma insulin concentrations at t = −1 min in home cages*

	First Swim	n	Control Experiment	n
Plasma E, pg/ml	20±6	8	26±10	7
Plasma NE, pg/ml	138±31	8	177±37	7
Plasma FFA, μeq/ml	0.09±0.016	9	0.12±0.016	12
Blood glucose, mg/dl	112.9±3.5	9	105.5±2.4	12
Plasma insulin, μU/ml	39.0±3.0	9	43.7±4.3	12

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

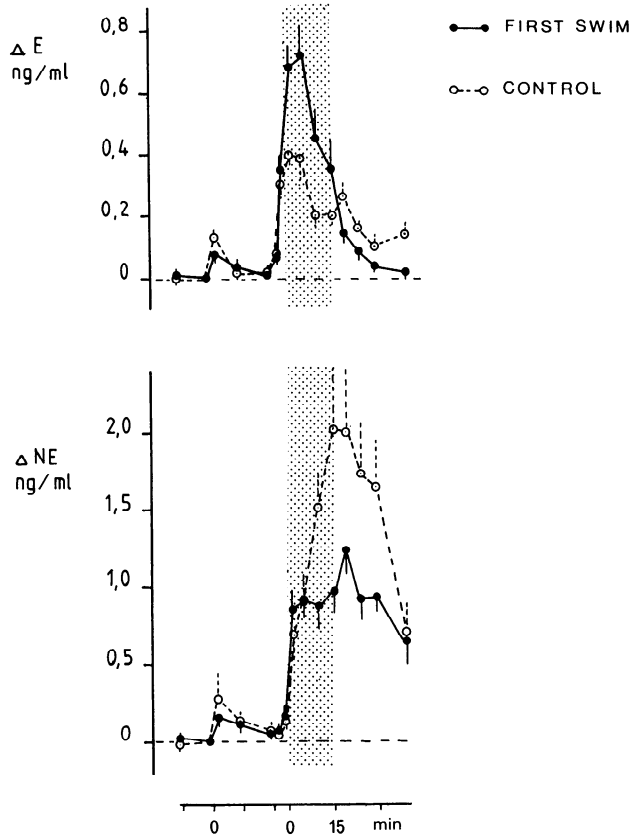

FIG. 1. Plasma epinephrine (E) and norepinephrine (NE) concentrations before, during, and after exercise in rats exposed for very first time to experimental exercise conditions (●——●) and rats well accustomed to whole experimental procedure (○- - -○). Data for E and NE are expressed as average changes ± SE from basal values. Swimming period is indicated by dotted area.

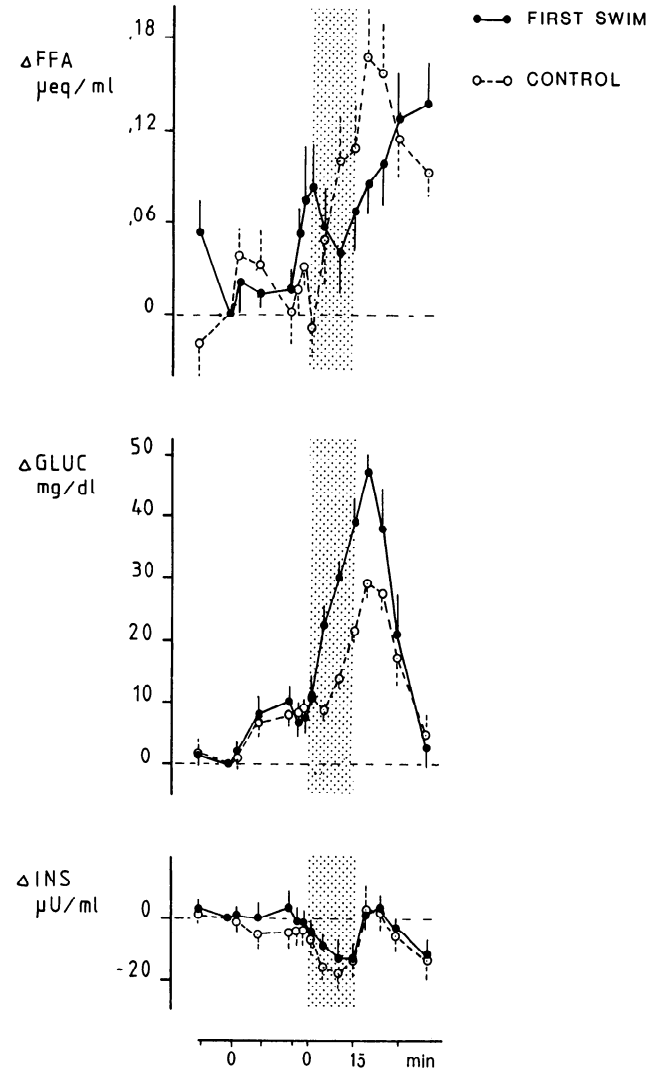

FIG. 2. Plasma free fatty acids (FFA), blood glucose (gluc), and plasma insulin (ins) concentrations before, during, and after exercise in rats exposed for very first time to experimental exercise conditions (●——●) and in rats well accustomed to whole experimental procedure (○- - -○). Data are expressed as in Fig. 1.

concentrations occurred at $t = 1.5$ min on the starting platform, caused by the transfer from the home cage to the new environment of the starting platform. Plasma catecholamine concentrations returned toward basal values at $t = 10$ and 20 min on the starting platform. During lowering of the starting platform, the experimental animals stayed almost immobilized. In this period a large significant increase in plasma E appeared, whereas plasma NE did not change. Concentrations in plasma reached a maximum at $t = 5$ min during exercise. Plasma NE concentrations were significantly increased during and after exercise in both groups. Maximal NE levels were reached at the end of the exercise period and in the early phase of the postexercise period when the animals were very active with grooming behavior. During exercise, plasma E levels of the first-swim group were nearly twice the values in the experiment with the accustomed animals (significant differences at $t = 1$, 5, 10, 19, 24, and 39 min). The exercise-induced increase in plasma NE concentrations in the first-swim group was significantly lower at $t = 24$ and 29 min in comparison with well-accustomed control rats.

Blood glucose concentrations increased during exercise. The increase was markedly enhanced in the first-swim group compared with the well-accustomed rats (significant at $t = 5$, 10, 15, 19, and 24 min). The exercise-induced increase in plasma FFA concentrations in the first swimmers was significantly different at $t = 1$ and 19 min from the values in the well-accustomed rats. In regard to insulin, there were no differences in plasma concentrations between the first-time swimmers and the well-accustomed rats before, during, or after exercise.

DISCUSSION

Some remarkable differences appeared between the response patterns of plasma E and NE in the different

phases of the control experiment. Plasma E increased during lowering of the starting platform, reached a maximum in the early phase of exercise, and remained moderately enhanced in both the last part of the swimming period and the resting period. NE was not affected by the lowering of the starting platform, but a marked increase occurred during exercise with the maximal level at the end of the swimming period. These results suggest that NE levels in plasma are well related to physical stress in the experimental procedure. Plasma E seems to be related to emotional stress. Immersion and swimming for the first time led to a markedly enhanced increase in plasma E concentrations (Fig. 1). This reinforces the notion that the concentration of E in plasma is related to emotional stress. In a previous study (13) we showed that in exercising rats, all E in plasma is of adrenal medullary origin and that NE in plasma originates entirely from the peripheral nerve endings of the sympathetic nervous system. The present findings therefore suggest that *1*) an emotional stress stimulus evokes the release of E by the adrenal medulla and *2*) physical activity leads to the release of NE from the nerve endings of the sympathetic nervous system.

A very intriguing result of the present study is the low exercise-induced increase in plasma NE concentrations in the first-time swimmers compared with the well-accustomed animals. This is in contrast to the idea that adrenal E stimulates the release of NE from the peripheral sympathetic nerve endings via activation of presynaptic β_2-adrenoceptors (8–10, 13). However, we showed recently (13) that in exercising rats high levels of E not only affected the stimulatory β_2-adrenoceptors but also stimulated inhibitory α_2-adrenoceptors on the peripheral sympathetic nerve endings. The net result was a relative reduction of the exercise-induced increase in plasma NE concentrations compared with controls or low levels of infused E (13). Therefore it is conceivable that the relatively low NE levels in the first swim experiment were due to an inhibitory presynaptic action of the high E levels on the peripheral nerve endings of the sympathetic nervous system.

With regard to the differences between naive and well-accustomed animals, an apparent discrepancy appears when the alterations in plasma catecholamine levels after the transfer of the rat from home cage to starting platform are compared with those after immersion and swimming. As expected, immersion and swimming led to much higher plasma E and lower plasma NE levels in naive animals compared with the accustomed animals. In contrast, after the transfer from home cage to starting platform, no difference between naive and accustomed rats appeared with respect to the increases in both plasma E and NE. This last effect is explainable, because both the accustomed animals as well as the naive animals were regularly handled and transferred from one cage into another before the actual onset of the exercise experiments. It is conceivable that it does not make any difference whether the rats are transferred to the starting platform instead of to another cage. In contrast, the combination of immersion and swimming may be considered far more stressful to the naive animals than to the accustomed ones, leading to an enhanced increase in plasma E in the early phase of exercise in the naive animals.

Both immersion and swimming can be considered as stressful events for the experimental animals. It therefore seems conceivable that during immersion a differential E response between the naive rats and the well-accustomed rats occurs. However, in the blood samples taken during immersion, a maximal response of the adrenal medulla has not yet been reached, so that one does not expect a difference in plasma E levels at that particular time point. Swimming per se might also have been an additional stress for the first-time swimmers, leading to an enhanced outflow of E from the adrenal medulla in comparison with the rats that are already conditioned. This is confirmed by the results of the present study, since after 10 min of exercise, plasma E levels in the naive animals were still significantly higher than control values. At this particular time point the effect of immersion on plasma E concentrations is neglectable because of the short half-life time of E in plasma (~1.5 min). Further experiments in which the experimental rats are subjected only to either slow immersion or exercise are needed to distinguish the effects of immersion and swimming as such on plasma E levels in naive rats compared with accustomed ones.

The high plasma E concentrations and the relatively reduced increase in plasma NE levels in the first-swim experiment led to enhanced blood glucose levels and a reduction in the exercise-induced increase in plasma FFA. These results can be explained by the findings of our previous study (14) that blood glucose concentrations are affected by adrenal medullary E and that plasma FFA levels are well related to the plasma concentrations of sympathetically released NE. However, it cannot be excluded that higher glucose levels and probably accompanying higher lactate levels in unaccustomed rats are responsible for inhibition of FFA release. Plasma insulin concentrations were seemingly unaffected by the enhanced emotional stress in the first-swim experiment. This is somewhat surprising because plasma insulin concentrations are influenced by activation of adrenoceptors on the pancreatic β-cell (6). However, plasma insulin concentrations are also affected by the ambient blood glucose level (20). It is possible that in the present study with the first swimmers the high plasma E concentrations caused an enhanced inhibition of the release of insulin by the pancreatic β-cell via activation of adrenoceptors but that this inhibition was counteracted by the high blood glucose levels. In addition, also the relatively reduced sympathetic activity in the first-swim experiment may have contributed to an attenuation of the effect of the high E levels on the pancreatic β-cell.

In summary, the results of the present study demonstrate that in rats the two branches of the sympathoadrenal system may be functionally and metabolically dissociated. Physical activity selectively leads to the release of NE by the peripheral nerve endings of the sympathetic nervous system. Activation of the adrenal medulla is evoked by an emotional stress factor and leads to an increase in E levels in the blood circulation. Blood glu-

cose levels were principally influenced by adrenal E, whereas plasma FFA levels are closely related to the concentration of NE in the blood circulation.

In addition, the findings of the present study demonstrate that the experimental conditions may considerably interfere with the hormonal and metabolic response to exercise. A high emotional stress component in the exercise conditions may lead to an exaggerated release of E by the adrenal medulla, affecting both the output of NE from the sympathetic nerve endings and the release of glucose and FFA into the blood circulation. Differences in experimental conditions may therefore at least partly explain the contradictory data in literature regarding sympathoadrenal function during exercise.

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