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Adrenal and sympathetic catecholamines in exercising rats

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SCHEURINK, A. J. W., A. B. STEFFENS, H. BOURITIUS, G. H. DRETELER, R. BRUNTINK, R. REMIE, AND J. ZAAGSMA. *Adrenal and sympathetic catecholamines in exercising rats.* Am. J. Physiol. 256 (Regulatory Integrative Comp. Physiol. 25): R155–R160, 1989.—The effects of adrenodemedullation and/or adrenoceptor agonists and antagonists on plasma epinephrine (E) and norepinephrine (NE) concentrations during exercise were investigated in rats. Exercise consisted of strenuous swimming against a countercurrent 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. E was not detectable in plasma of adrenodemedullated (Adm) rats. A marked reduction in the normal exercise-induced increase in plasma NE concentrations occurred in both Adm rats as well as in intact rats injected with the β_2-selective adrenoceptor antagonist ICI 118551. Intravenous infusion of either E or the β_2-selective agonist fenoterol restored the increase in plasma NE in Adm rats. Injection of the α_2-selective antagonist yohimbine in combination with infusion of the β_2-selective agonist fenoterol into Adm rats caused an enormous increase in plasma NE. It is concluded that all NE in plasma as released during exercise originates from the peripheral nerve endings of the sympathetic nervous system. Adrenal E influences the release of NE via activation of presynaptic β_2-adrenoceptors.

exercise; epinephrine; norepinephrine; adrenodemedullation; presynaptic α_2- and β_2-adrenoceptors

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IT IS WELL ESTABLISHED that during exercise the sympathoadrenal system is activated, which results in increased plasma concentrations of epinephrine (E) and norepinephrine (NE) (5, 8). However, available data do not give information about the relative role of the adrenal medulla and the sympathetic nervous system in the catecholamine alterations during exercise. For example, physiological mechanisms such as presynaptic actions of adrenal catecholamines on the release of NE by the peripheral sympathetic nerve endings (7, 11, 13, 14) may strongly interfere with the catecholamine concentrations in plasma. Moreover, the origin of NE in plasma during exercise is not clearly defined. In several studies in exercising rats, adrenodemedullation was reported to cause a marked reduction in plasma NE concentrations (1, 18, 21), suggesting that the major part of NE in plasma originates from the adrenal medulla. Others (27, 28), however, failed to detect any differences in plasma NE concentrations between intact and adrenodemedul-

lated rats, suggesting that either the adrenal medulla does not produce NE or that a compensatory increase in sympathetic nervous activity may occur after adrenodemedullation.

The aim of the present study was to distinguish the relative contribution of the adrenal medulla and the sympathetic nervous system to plasma catecholamine concentrations in exercising rats. Therefore plasma E and NE concentrations were measured under different experimental conditions such as adrenodemedullation and/or pharmacological interference. The results demonstrate that the release of E by the adrenal medulla is independent of the release of NE by the nerve endings of the sympathetic nervous system. However, NE release by the sympathetic nerve endings is strongly influenced by adrenal E via a presynaptic adrenergic regulatory mechanism, suggesting that a complete dissociation between adrenal medulla and sympathetic nervous system may not occur under physiological conditions.

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 regime (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 (0.95 mm OD, 0.50 mm ID) through the right jugular vein externalized on the top of the skull according to the techniques described earlier (23). This method allows frequent repeated blood sampling in unanesthetized undisturbed freely moving rats (22, 26). Whenever intravenous infusions were to be performed, the rats were provided with a second smaller silicon heart catheter (0.64 mm OD, 0.28 mm ID) implanted in the contralateral (left) jugular vein (24). Because of its small size, the second catheter did not prevent venous return from the head of the animals. The rats did not lose weight after implantation and they did not show signs of edema in the head. The experiments started 1 wk after insertion of the heart catheter(s).

In certain experiments the rats were surgically adrenodemedullated 2 wk before the insertion of the heart

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catheter(s) to enable adrenocortical regeneration. During the recovery period, the rats hardly required saline as drinking water. Adrenodemedullation was verified histologically after termination of the experiments. Medullary tissue was completely absent in the recovered adrenal after correct adrenodemedullation.

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 down to the bottom of the swimming pool. A water pump (Loewe Silenta, FRG) 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) at the upstream side of the swimming pool was offered to the swimming rat. 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 (22). During the whole experiment, 14 blood samples of 0.25 ml were withdrawn for determination of plasma catecholamines. 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 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 anticoagulant.

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 plasma catecholamines, two blood samples in a 10-min interval ($t = -11$ 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 on 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 after ~2 min, one blood sample was taken. The moment the rat started to swim was defined as $t = 0$ min. 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. During the resting period 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 to investigate the physiological relevance of the presynaptic regulatory mechanisms.

Administration of the adrenergic agonists E and fenoterol was achieved by infusion of the drug through the small 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 lowering of the 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 agonist fenoterol (Boehringer-Ingelheim) was infused at a rate of 7 µg in 0.1 ml saline/min (17). The adrenergic antagonists ICI 118551 (β_2-selective, ICI-Pharma) and yohimbine (α_2-selective, Sigma) were given in a single dose (respectively, 0.1 and 0.13 mg/rat) before exercise. They were dissolved in 0.1 ml saline and injected through the heart catheter 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 as antioxidant and 10 µl heparin solution (500 IU/ml) as anticoagulant. Blood was centrifuged at 4°C for 15 min at 5,000 rpm, and 100 µl of the supernatant were stored at −80°C for the catecholamine measurements.

Determination of plasma catecholamine concentrations was performed by high-pressure liquid chromatography in combination with electrochemical detection as described previously (17), with some small modifications. The high-pressure liquid chromatography-electrochemical detection system included a 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, and the potentials of the working electrodes were −50 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). Absolute detection levels for E and NE in plasma were 0.010 and 0.005 ng/ml, respectively.

Statistics. Data are expressed as means ± SE. Wilcoxon matched-pairs signed-rank test was used when the levels of E and NE at a certain time during the experiment were compared with the basal value at $t = -1$ min in the home cage. Analysis of variance and the Mann-Whitney U test were applied to determine for each sample point the significant differences between the results of an experiment and the controls. 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 measure the effect of exercise on plasma levels of E and NE under the given experimental conditions. Seven rats, well accustomed to the experimental procedure, participated in this study.

The results are presented in Fig. 1. Basal levels of

catecholamines were 0.03 ± 0.01 and 0.18 ± 0.04 ng/ml for E and NE, respectively. A slight, significant increase in both plasma E and NE concentrations occurred at t = 1.5 min on the starting platform, caused by handling and transferring the rat from the home cage to the starting platform. Regarding plasma E, a large significant increase occurred before exercise during the lowering of the starting platform. E levels reached a maximum level (0.42 ± 0.06 ng/ml) in the early phase of swimming whereupon a decrease followed. A significant increase in plasma NE occurred during and after exercise. Maximal NE level was 2.20 ± 0.43 ng/ml at t = 15 min at the end of the exercise period.

Experiment II. Adrenodemedullation. Nine bilaterally adrenodemedullated (Adm) rats were submitted to swimming to investigate the relative contribution of the adrenal medulla to plasma E and NE levels during exercise.

The results are presented in Fig. 2. Epinephrine was not detectable (<10 pg/ml) in plasma of Adm rats. The exercise-induced increase in plasma NE was reduced in Adm rats in comparison with the control experiment (significant at t = 10, 15, 19, 24, and 29 min, i.e., during and after swimming).

Experiment III. Presynaptic regulatory mechanisms. Data from pharmacological literature indicate that adrenal catecholamines may influence the release of NE by the peripheral nerve endings of the sympathetic nervous
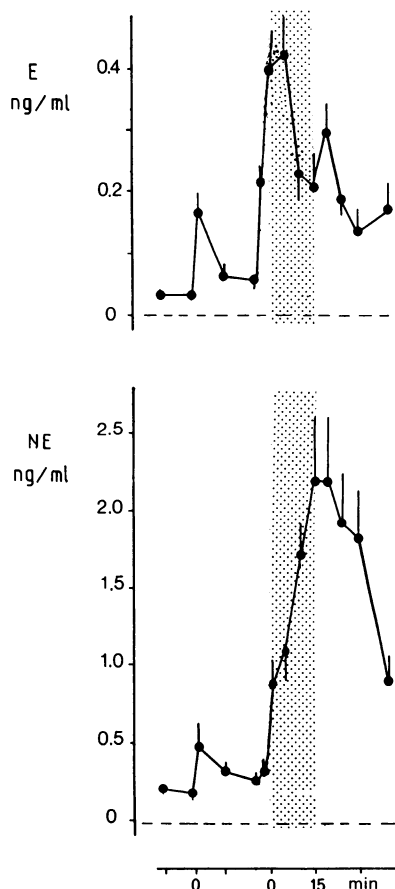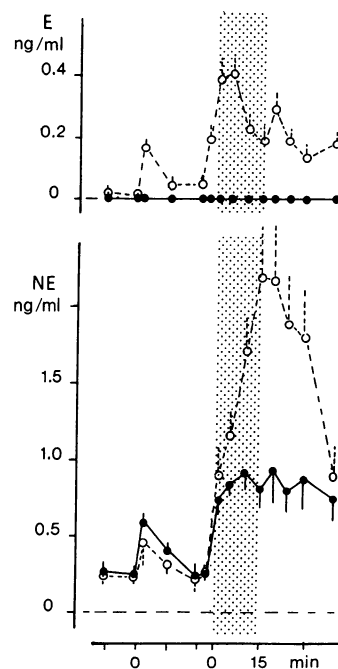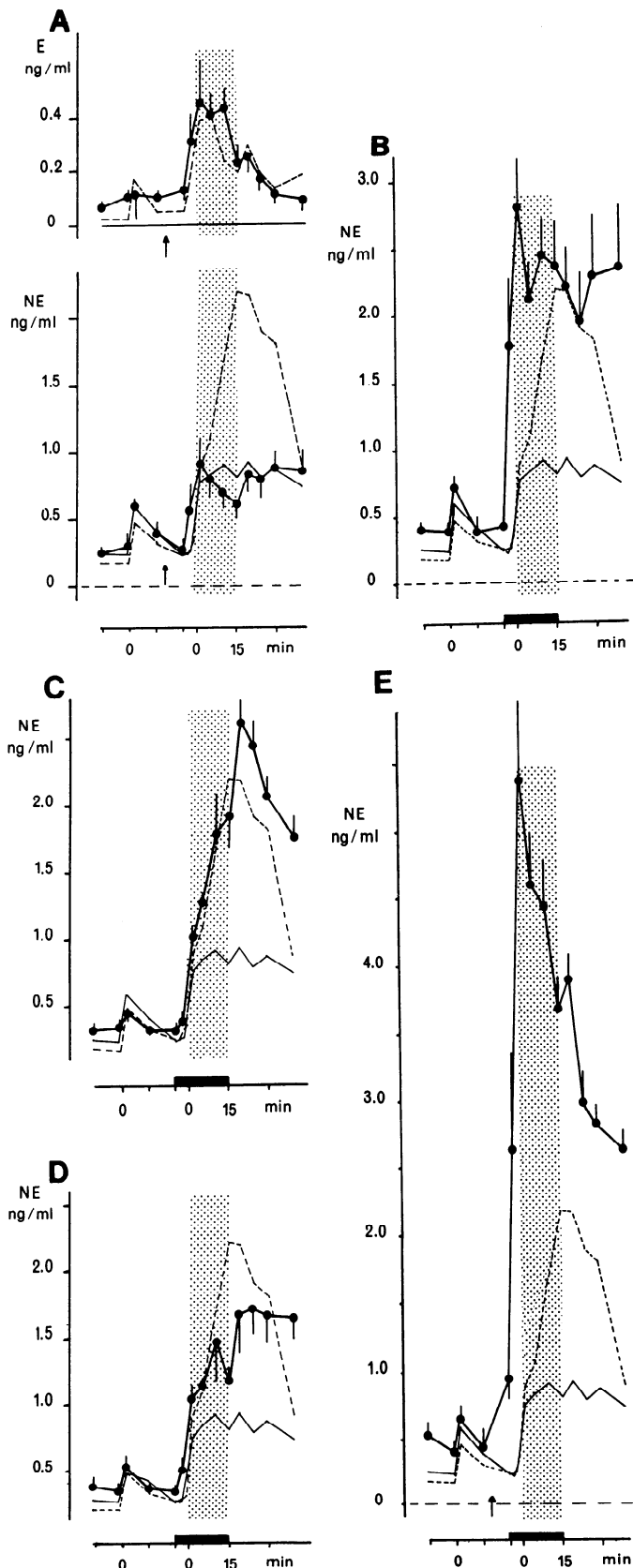

FIG. 2. Effect of adrenodemedullation on plasma epinephrine (E) and norepinephrine (NE) concentrations before, during, and after exercise (●——●). Control experiment with intact rats is depicted (○- - -○). Data for E and NE are expressed as means ± SE. Swimming period is indicated by dotted area.

system via presynaptic regulatory mechanisms. The aim of *experiment III* was to investigate, in particular, the physiological relevance of presynaptic β_2-adrenoceptors on the sympathetic nerve endings in exercising rats.

The role of presynaptic β_2-adrenoceptors was investigated in *experiment IIIa* and *IIIb*. *Experiment IIIa* consisted of a single intravenous injection of the β_2-selective adrenoreceptor antagonist ICI 118551 in seven intact rats. The results are presented in Fig. 3A. Plasma E concentrations were not affected by the β_2-antagonist and were similar to the control values. The exercise-induced increase in plasma NE levels was reduced after β_2-blockade (significantly lower than control values at t = 10, 15, 19, 24, and 29 min). Plasma NE concentrations after β_2-blockade were similar to the values in the Adm rats. In *experiment IIIb*, the β_2-selective adrenoceptor agonist fenoterol was intravenously infused during exercise into five Adm rats. The results are presented in Fig. 3B. Plasma E was not measurable in these animals. Plasma NE concentrations were markedly increased during fenoterol infusion compared with the Adm rats (significant during and after infusion).

The aim of the *experiments IIIc* and *IIId* was to study the role of E on the release of NE by the nerve endings of the sympathetic nervous system. Therefore E was intravenously infused into exercising Adm rats. In *experiment IIIc*, a dose of 20 ng E/min was infused into seven Adm rats. This dose restored glucose and free fatty acid patterns in Adm rats toward control values [data in a companion paper (19)], and we therefore considered this dose of E as a physiological one. Measurements of plasma E levels in the right jugular vein would not give any information about the content of E in the general circulation. Therefore only plasma NE concentrations are

FIG. 1. Plasma epinephrine (E) and norepinephrine (NE) concentrations before, during, and after exercise (●——●). Data for E and NE are expressed as means ± SE. Swimming period is indicated by dotted area.

measured in the experiments dealing with intravenous E infusion. Plasma NE concentrations in *experiment IIIc* are presented in Fig. 3C. When compared with results in Adm rats, the dose of 20 ng E/min caused an enhanced increase in plasma NE levels during exercise (significant at all sample points during and after swimming). Plasma NE levels were remarkably similar to the values in the control experiment. In *experiment IIId*, a higher dose of E (50 ng/min) was intravenously infused into Adm rats. Plasma NE concentrations from this experiment with five Adm rats are shown in Fig. 3D. The exercise-induced increase in plasma NE was enhanced compared with the data from the Adm rats (significant at $t = 19$, 24, 29, and 39 min) but was lower than the control experiment (significant at $t = 15$) and the 20-ng E/min infusion in Adm rats (significant at $t = 15$, 19, and 24 min).

The physiological importance of the adrenergic presynaptic regulatory mechanism was investigated in *experiment IIIe* in which a single intravenous injection of the α_2-selective adrenoceptor antagonist yohimbine before exercise was combined with an intravenous infusion of the β_2-selective adrenoceptor agonist fenoterol during swimming. Four rats were participating in this study. The results are presented in Fig. 3E. Plasma E was not detectable in these animals. The injection of yohimbine caused an significant increase in plasma NE concentration at $t = 20$ min of the starting platform. Infusion of fenoterol led to a further increase in plasma NE concentrations. The combined α_2-inhibition and β_2-stimulation ultimately led to extremely increased plasma levels of NE (significant for all time points during and after exercise when compared with both control and Adm rats).

DISCUSSION

In rats, catecholamine-producing cells are identified in the adrenal medulla, the peripheral nerve endings of the sympathetic nervous system, and the extra-adrenal chromaffin tissue (25). In the present study, E was not detectable in plasma of Adm rats before, during, and after exercise. This suggests that in rats all E measurable in the blood circulation originates from the adrenal medulla and that extra-adrenal production of E does not contribute to plasma E levels. The effect of adrenodemedullation on plasma NE concentrations is less pro-

FIG. 3. *A*: effect of intravenous administration of β_2-selective adrenoceptor antagonist ICI 118551 on plasma epinephrine (E) and norepinephrine (NE) concentrations before, during, and after exercise (●——●). Control experiments with intact (– – –) and adrenodemedullated (——) (Adm) rats are depicted. Data are expressed as means ± SE. Swimming period is indicated by dotted area. Injection time is indicated by an arrow. *B*: effect of intravenous infusion of β_2-selective adrenoceptor agonist fenoterol (7 μg/min) on plasma NE concentrations in exercising Adm rats (●——●). Control experiments with intact (– – –) and Adm (——) rats are depicted. Period in which infusion was given is indicated by a horizontal bar at bottom of graph. Data are expressed as in Fig. 3A. *C*: effect of intravenous infusion of 20 ng E/min on plasma NE concentrations in exercising Adm rats (●——●). Control experiments with (– – –) and Adm (——) rats are depicted. Data are expressed as in Fig. 3B. *D*: effect of intravenous infusion of 50 ng E/min on plasma NE concentrations in exercising Adm rats (●——●). Control experiments with intact (– – –) and Adm (——) rats are depicted. Data are expressed as in Fig. 3B. *E*: effect of intravenous administration of α_2-selective adrenoceptor antagonist yohimbine in combination with intravenous infusion of the β_2-selective adrenoceptor agonist fenoterol on plasma NE concentrations in exercising Adm rats (●——●). Control experiments with intact (– – –) and Adm (——) rats are depicted. Injection time is indicated by an arrow. Data are expressed as in Fig. 1 and 3B.

nounced. Basal and starting platform levels of NE in Adm rats were almost similar to control values, but the normal exercise-induced increase in plasma NE was markedly reduced. These findings seemingly suggest that ~60% of the plasma NE content during exercise originates from the adrenal medulla. Extra-adrenal sources, in particular the peripheral nerve endings of the sympathetic nervous system, may then produce the remaining part of the circulating NE during exercise. However, this generally accepted view (9, 12, 15, 30) ignores possible actions of adrenal medullary hormones on the release of NE by the peripheral sympathetic nerve endings. In vitro studies in particular have provided evidence for the existence of adrenergic presynaptic receptor mechanisms in the peripheral sympathetic system (for reviews see 10, 13). In the first place, these studies suggest that NE released by the sympathetic nerve endings inhibits a further release of NE through a negative-feedback mechanism via presynaptic α_2-adrenoceptors (29). Recently Remie and Zaagsma (17) demonstrated in unanaesthetized freely moving animals the in vivo relevance of this presynaptic α_2-autoreceptor mechanism. Beside this inhibition via α_2-adrenoceptors, a facilitation of the (stimulus-evoked) release of NE was found after activation of presynaptic β_2-adrenoceptors. According to Majewski (11) and others (3, 4, 10, 13), these presynaptic β_2-adrenoceptors are principally activated by blood-borne E or E taken up by the sympathetic nerve endings and released as a cotransmitter with NE. These in vitro data suggest that in *experiment II* adrenodemedullation might have affected the release of NE from the peripheral sympathetic nerve endings in particular via a reduced activation of the presynaptic β_2-adrenoceptors. This means that the reduction in the exercise-induced increase in plasma NE in Adm rats cannot be explained solely by a diminished adrenal NE production but also by a decreased presynaptic stimulation of sympathetic NE release. In addition, the suggestion that activation of β_2-adrenoceptors may play a substantial physiological role in the release of NE during exercise is confirmed by the reduced increase in plasma NE after intravenous administration of the β_2-selective adrenoceptor antagonist ICI 118551 to normal rats. Moreover, this reduction in NE levels after β_2-blockade was nearly identical to the effect of adrenodemedullation on plasma levels of NE. This suggests that the effect of Adm on plasma NE levels during exercise may entirely be explained by a diminished activation of presynaptic β_2-adrenoceptors on the peripheral nerve endings of the sympathetic nervous system. Accordingly, it may be concluded that during exercise practically all NE in plasma originates from the peripheral nerve endings and that the noradrenergic cells in the adrenal medulla do not essentially contribute to the NE content in plasma (2).

Infusion of a physiological dose of E (20 ng/min) into adrenodemedullated rats completely restored the exercise-induced increase in NE. The effect of infusion of E into Adm rats on plasma NE concentrations was exactly opposite to the effect of β_2-adrenoceptor blockade in intact animals. This reinforces the idea that the presynaptic β_2-adrenoceptors on the peripheral nerve endings

are activated by circulating E. In addition, these results also suggest that production of NE by the adrenal medulla is not necessary to explain the exercise-induced increase in NE concentrations.

The infusion of a dose of 50 ng E/min into exercising Adm rats caused an exaggerated increase in blood glucose levels [data in a companion paper (19)], indicating that 50 ng E/min was an unphysiological high dose. The high dose caused only a moderate increase in NE release. NE levels were significantly enhanced compared with Adm rats, but the exercise-induced increase in plasma NE was reduced compared with the Adm rats infused with the dose of 20 ng E/min. The most plausible explanation is a combined action of the high dose of E on both the stimulatory β_2- as well as the inhibitory α_2-adrenoceptors on the peripheral nerve endings of the sympathetic nervous system. This implies that only high levels of circulating E may affect the inhibiting α-adrenoceptors. This is in accordance with the view that a certain threshold concentration of NE (and also E) in the synaptic cleft is needed for the activation of the α_2-presynaptic autoreceptors (10).

The infusion of the selective β_2-agonist fenoterol into exercising Adm rats immediately caused a steep increase in plasma NE. Plasma NE levels were even higher than in the intact controls. These results confirm the prominent role of the presynaptic β_2-mechanism as we recently found in the nervous plexus of the electrically stimulated portal vein of the freely moving rat where both basal and evoked release were strongly facilitated by β_2-adrenoceptor stimulation (16). The physiological relevance of the whole presynaptic regulatory mechanism is further emphasized by plasma NE levels after the combination of an injection of an α_2-antagonist and an infusion of a β_2-agonist into exercising Adm rats. This α_2-blockade and β_2-stimulation led to maximal presynaptic stimulation with extremely high plasma NE levels, indicating that presynaptic mechanisms are an important regulating factor for the release of NE from the nerve endings of the sympathetic nervous system during exercise.

The results of the present study suggest that during exercise adrenal E may regulate ~60% of the total NE release via a presynaptic β_2-adrenoceptor mechanism. However, it should be noticed that plasma levels of NE reflect the overflow of NE from the synaptic cleft into the blood circulation. This implies that plasma NE levels not only reflect the release of NE by the sympathetic nerve endings but also that factors like neuronal reuptake of NE, breakdown of NE, or hemodynamic changes may be included as well (6). Therefore, although pharmacological (11, 13) and in vivo (7, 14) studies provided enough evidence for a stimulating β_2-adrenoceptor mechanism localized on the presynaptic nerve endings, a decreased transcapillary flux or an increase in the neuronal reuptake of NE after β_2-blockade cannot be completely ruled out at present (6).

Finally, it should be noted that an increase in E occurred even before the start of exercise during lowering of the starting platform (Fig. 1). This increase is probably caused by emotional stress accompanying immersion that may mask the effects of exercise as such on E levels

(20). To unravel this masking effect of immersion on exercise-induced alterations on plasma E levels, the effect of immediate swimming without slow immersion and slow immersion without swimming will be investigated in future experiments.

In summary, the results of the present study show that in rats all E in plasma is produced by the adrenal medulla. NE in plasma originates from the peripheral nerve endings of the sympathetic nervous system. Circulating E stimulates NE release from the peripheral nerve endings of the sympathetic nervous system via activation of presynaptic β_2-adrenoceptors. This implies that the release of E by the adrenal medulla is crucial for a high NE release during exercise. Very high levels of E may also affect inhibitory presynaptic α_2-adrenoceptors on the peripheral sympathetic nerve endings.

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