Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue

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BAILEY, STEPHEN P., J. MARK DAVIS, AND ERNEST N. AHLBORN. *Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue.* J. Appl. Physiol. 74(6): 3006–3012, 1993.—Pharmacological manipulation of brain serotonergic [5-hydroxytryptamine (5-HT)] activity affects run time to exhaustion in the rat. These effects may be mediated by neurochemical, hormonal, or substrate mechanisms. Groups of rats were decapitated during rest, after 1 h of treadmill running (20 m/min, 5% grade), and at exhaustion. Immediately before exercise rats were injected intraperitoneally with 1 mg/kg of quipazine dimaleate (QD; a 5-HT agonist), 1.5 mg/kg of LY 53857 (LY; a 5-HT antagonist), or the vehicle (V; 0.9% saline). LY increased and QD decreased time to exhaustion (~28 and 32%, respectively; $P < 0.05$). At fatigue, QD animals had greater plasma glucose, liver glycogen, and muscle glycogen concentrations but lower plasma free fatty acid concentration than did V and LY animals ($P < 0.05$). In general, plasma corticosterone and catecholamine levels during exercise in QD and LY rats were similar to those in V rats. Brain 5-HT and 5-hydroxyindole-3-acetic acid concentrations were higher at 1 h of exercise than at rest ($P < 0.05$), and the latter increased even further at fatigue in the midbrain and striatum ($P < 0.05$). Brain dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were higher at 1 h of exercise ($P < 0.05$) but were similar to resting levels at fatigue. QD appeared to block the increase in DA and DOPAC at 1 h of exercise, and LY prevented the decrease in DA and DOPAC at fatigue ($P < 0.05$). These data indicate that the effects of pharmacologically altered brain 5-HT activity on fatigue are not likely due to substrate or hormonal mechanisms but may be related to changes in brain DA concentrations.

serotonin; dopamine; corticosterone; catecholamines; glycogen

FATIGUE DURING PROLONGED EXERCISE has traditionally been attributed to the occurrence of a "metabolic end point" where muscle glycogen concentrations are depleted, plasma glucose concentrations are reduced, and plasma free fatty acid (FFA) levels are elevated. Newsholme et al. (24) have recently hypothesized that fatigue during prolonged exercise may be influenced by activity of the brain serotonergic [5-hydroxytryptamine (5-HT)] system. This hypothesis is commonly referred to as the "central fatigue hypothesis."

The major premise of the central fatigue hypothesis is that increased brain 5-HT activity during prolonged exercise may cause fatigue by increasing lethargy and loss of central drive/motivation (24). The relationship between brain 5-HT and fatigue was initially based on the findings of several studies that indicate that sleepiness was increased after consumption of a meal rich in tryptophan (Trp), the precursor of 5-HT (18), and that motivation is reduced when 5-HT activity is increased (23).

To date, several investigations into the relationship between the availability of Trp (plasma free Trp) to the brain and the physical and mental performance in humans have been completed (5–7, 13). Although a number of confounding factors were present in these investigations, these authors have consistently reported an association between elevated levels of plasma free Trp and reduced physical and mental performance. In these investigations it was assumed that increased availability of Trp to the brain results in increased brain 5-HT activity, thus playing a role in fatigue. Although Chaouloff and co-workers (10, 11) have demonstrated that prolonged exercise results in increased availability of Trp to the brain and increased brain 5-HT and 5-hydroxyindole-3-acetic acid (5-HIAA) concentrations in the rat, a causal link between increased 5-HT activity and reduced physical and mental performance has not been established. Previous work in our laboratory has indicated that run time to exhaustion in the rat is positively and negatively affected by drugs that decrease and increase brain 5-HT activity (LY 53857 and quipazine dimaleate plus *m*-chlorophenyl piperazine, respectively; Ref. 4). It appears that these effects stem from the central nervous system, since administration of a peripherally restricted 5-HT antagonist (xylamidine tosylate) does not attenuate the reduction in run time to exhaustion (4); however, the specific mechanisms underlying the observed changes in run time to exhaustion because of altered brain 5-HT activity remain unclear.

Increased 5-HT activity has been shown to negatively affect brain dopamine (DA) synthesis and behavior (21, 28, 29). The importance of increased brain DA activity during exercise has been demonstrated in a classic investigation by Freed and Yamamoto (14). Altered 5-HT activity can also affect thermoregulation, pain tolerance, and the activity of the hypothalamic-pituitary-adrenocortical (HPA) and sympathoadrenal (SA) systems (1, 2, 17, 26). Manipulation of any of these systems could potentially alter performance; however, it is not clear which, if any, of these systems play a role in altered exercise performance due to administration of 5-HT agonists and antagonists.

The purpose of this investigation was to describe the effects of 5-HT agonist and antagonist drugs on *1*) substrate availability; *2*) brain concentrations of DA and

its primary metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC); and *3*) plasma concentrations of hormones produced by the HPA axis and SA system during prolonged exercise to fatigue in the rat.

METHODS

Animals

Seventy-two treadmill-accommodated male Wistar rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 300–400 g were placed into nine groups of eight rats each. Treadmill accommodation occurred in 3–4 wk, during which treadmill speed and running duration were slowly increased until the animals were able to easily maintain the desired speed and grade for 30 min. Animals were housed in a light- and temperature-controlled facility and were provided standard chow and water ad libitum. Animals were deprived of food and water for 3 h before the experiment. All experiments were performed between 1300 and 1500 h in a temperature-controlled room. Animals were killed in a room separate from but adjacent to the area where the experimental treatments were administered. Treatment of animals was in accordance with the guidelines of the University of South Carolina Animal Research Review Committee.

Experimental Design

Groups of animals were decapitated during rest, after 1 h of treadmill running (20 m/min, 5% grade), or after exhaustive treadmill running (20 m/min, 5% grade). Exhaustion or fatigue was defined as the point when animals were unable to keep pace with the treadmill despite constant physical prodding for 1 min. Immediately before the exercise treatment, animals were injected intraperitoneally with 1 mg/kg quipazine dimaleate (QD), 1.5 mg/kg LY 53857 (LY), or the vehicle (V; 0.9% saline). QD is a general 5-HT agonist, and LY is a 5-HT antagonist specific to 5-HT_{1C} and 5-HT_2 receptors. Both drugs were purchased from Research Biochemicals (Natick, MA). Dosages of drugs were those that were previously found to have the most profound effect on run time to exhaustion without reducing it to <1 h (4).

After decapitation the head was dropped in liquid nitrogen (~10 s), and the brain was then removed and dissected within 2 min. Midbrain (MB), striatum (ST), hypothalamus (HY), and hippocampus (HI) were dissected and deproteinized by sonication in 400 μl of 0.2 M perchloric acid. Trunk blood was collected in test tubes containing glutathione and ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid. Blood samples were then centrifuged, and the resultant plasma was saved at −80°C until analysis. Muscle (red gastrocnemius) and liver tissue were removed and wrapped in foil. All tissues were stored at −80°C until analysis.

Tissue Analyses

Metabolites. Plasma glucose and FFA concentrations were determined by specrophotometric techniques (20, 25). Muscle and liver glycogen concentrations were determined by the procedures described by Lo et al. (22).

Hormones. Plasma corticosterone concentrations were determined using a radioimmunoassay kit (minimal detectable level ~25 ng/l, CV = 6.5%; ICN Biomedical, Costa Mesa, CA). Plasma catecholamine (epinephrine and norepinephrine) concentrations were determined by high-performance liquid chromatography with electrochemical detection (HPLC-EC) techniques (minimal detectable levels for ~0.5 nmol for both norepinephrine and epinephrine, CV = 8.7 and 9.3%, respectively). Before HPLC analysis, plasma catecholamines were extracted from plasma as previously described (9). An ESA Coulochem II system (Bedford, MA) equipped with an ESA HR-80 column was used for catecholamine analysis. ESA CAT-A-PHASE was used as the mobile phase for these analyses.

Brain neurotransmitters. Deproteinized brain samples were analyzed for DA, DOPAC, 5-HT, and 5-HIAA concentrations with the use of an ESA Coulochem II HPLC-EC system (12). The system was equipped with a Keystone (Belle Fonte, PA) ODS Hypersil (3 μm) column, and 8% acetyl nitryl mobile phase (75 mM monobasic sodium phosphate, 1.4 mM 1-octane sulfonic acid, 10 μM EDTA; pH = 3.0) was used. 5-HT and 5-HIAA concentrations determined for V animals were used for statistical analysis. Brain 5-HT and 5-HIAA levels were not measured in animals treated with the 5-HT agonist and antagonist because these changes were not relevant to the purposes of this study.

Statistical Analyses

The effects of drug treatments on run time to exhaustion were assessed by a 1 × 3 analysis of variance (ANOVA). The effects of exercise on brain 5-HT and 5-HIAA concentrations in V animals were assessed by separate 1 × 3 ANOVAs. The effects of exercise and drug treatments on all remaining variables were assessed by 3 × 3 ANOVAs. Differences across exercise and drug treatments were determined using the Newman-Kuels post hoc procedure. When differences across treatments or treatment interactions were significant, multiple comparisons among means were made by using contrasts. Significance level for all analyses was set at $P \leq 0.05$. All data are presented as means ± SE.

RESULTS

Run Time to Exhaustion

Of the 72 animals included in the experiment, 24 ($n = 8$ under each drug condition) ran to exhaustion. Exhaustion occurred within ~110 min for V rats. This time was decreased ~32% and increased ~26% in QD and LY rats, respectively ($P < 0.05$; Fig. 1).

Metabolites

Plasma glucose. Plasma glucose was maintained after 1 h of exercise under all drug conditions (Fig. 2). Plasma glucose was significantly lower ($P < 0.05$) at exhaustion than at rest in the V and LY animals. Plasma glucose at exhaustion in the QD animals was similar to that at rest.

Plasma FFA. Plasma FFA levels were elevated ($P < 0.05$) after 1 h of exercise in all drug conditions (Fig. 2). FFA levels were increased to a greater extent ($P < 0.05$)

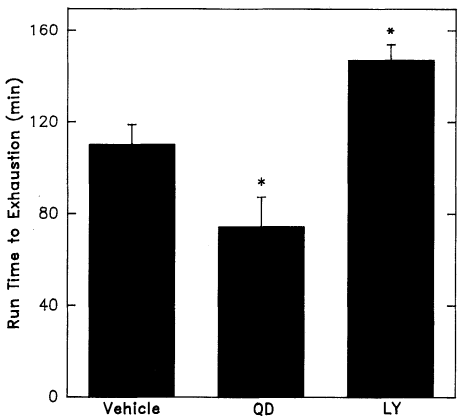

FIG. 1. Effect of quipazine dimaleate (QD; 1 mg/kg) and LY 53857 (LY; 1.5 mg/kg) on run time to exhaustion. * Significantly different from vehicle ($P < 0.05$).

at exhaustion in the V and LY animals than in the QD animals. The FFA level at exhaustion in the QD animals was similar to that at rest.

Muscle and liver glycogen. Muscle glycogen was lower ($P < 0.05$) after 1 h of exercise than at rest in the V and QD animals (Fig. 3). In LY animals, muscle glycogen at exhaustion was reduced to a level similar to that for V animals at exhaustion.

Liver glycogen was lower ($P < 0.05$) after 1 h of exercise in the V and LY animals (Fig. 3). Liver glycogen was further reduced ($P < 0.05$) at exhaustion in these two groups. In QD animals, liver glycogen was reduced ($P < 0.05$) at exhaustion; however, it was not as low as values observed in V and LY animals at exhaustion ($P < 0.05$).

Plasma Hormones

Corticosterone. Corticosterone increased ($P < 0.05$) as a result of exercise under all drug conditions (Fig. 4).

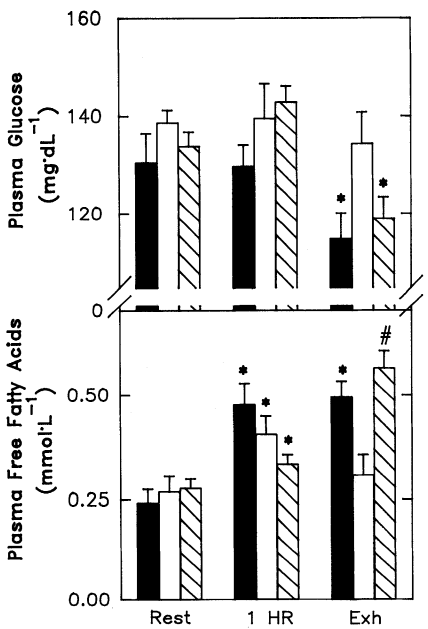

FIG. 2. Effect of exercise and drug treatments on plasma glucose and free fatty acids. Solid column, vehicle; open column, QD; hatched column, LY. Exh, exhaustion. Significantly different ($P < 0.05$) from same drug treatment: * at rest, # at rest and at 1 h of exercise.

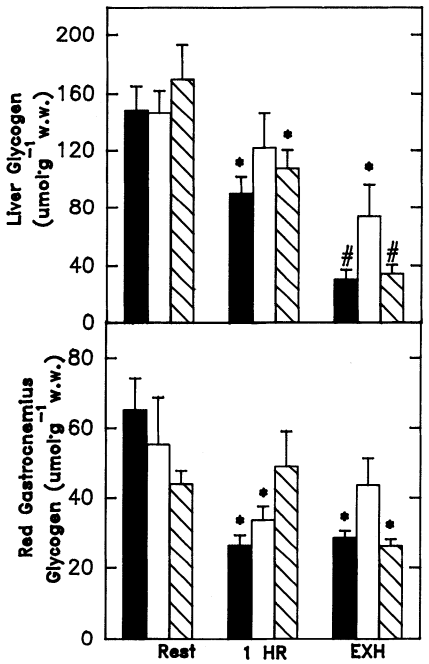

FIG. 3. Effect of exercise and drug treatments on liver and red gastrocnemius glycogen. Columns as in Fig. 2 legend. Significantly different ($P < 0.05$) from same drug treatment: * at rest, # at rest and at 1 h of exercise.

Corticosterone values were similar after 1 h of exercise and at exhaustion under all drug conditions. Corticosterone at rest tended to be greater for QD rats; however, this difference was not significant.

Catecholamines. Plasma epinephrine was elevated ($P < 0.05$) after 1 h of exercise in the V animals (Fig. 5). Epinephrine was greater ($P < 0.05$) at exhaustion in V and LY animals. In QD animals, epinephrine was greater ($P < 0.05$) than in the other two groups at rest and after 1 h of exercise. Epinephrine levels were similar under all drug conditions at exhaustion.

Plasma norepinephrine was elevated ($P < 0.05$) after 1 h of exercise in the V animals (Fig. 5). Norepinephrine was greater ($P < 0.05$) in the QD animals than the V animals both at rest and after 1 h of exercise. In LY animals, norepinephrine was elevated ($P < 0.05$) at rest;

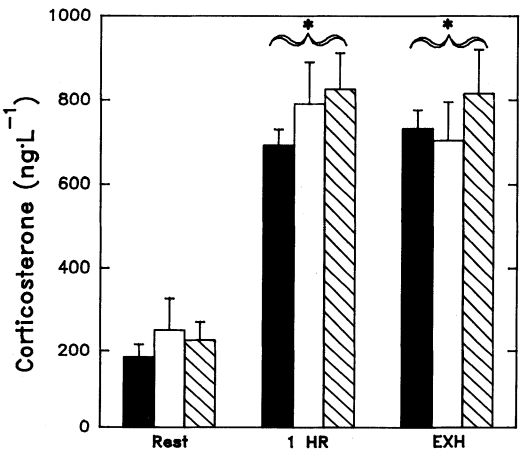

FIG. 4. Effect of exercise and drug treatments on plasma corticosterone. Columns as in Fig. 2 legend. * Significantly different ($P < 0.05$) from at rest.

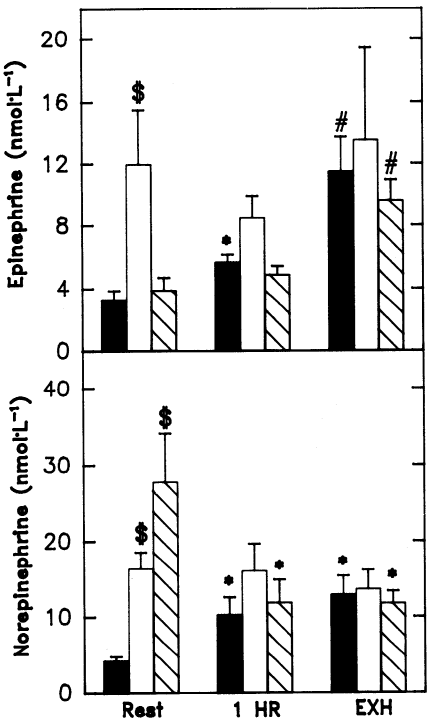

FIG. 5. Effect of exercise and drug treatments on plasma epinephrine and norepinephrine. Columns as in Fig. 2 legend. Significantly different ($P < 0.05$) from: * same drug treatment at rest, # same drug treatment at rest and at 1 h of exercise, $ vehicle at rest.

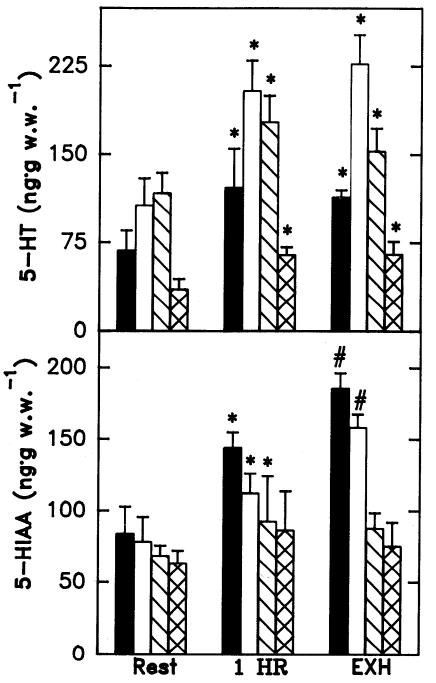

FIG. 6. Effect of exercise on serotonin [5-hydroxytryptamine (5-HT)] and 5-hydroxyindole-3-acetic acid (5-HIAA) in midbrain (solid column), striatum (open column), hypothalamus (hatched column), and hippocampus (cross-hatched column). Significantly different ($P < 0.05$) from same drug treatment: * at rest, # at rest and at 1 h of exercise.

however, after 1 h of exercise and at exhaustion it was similar to the values observed in V animals at these times.

Brain Neurotransmitters

5-HT. Of the 72 animals included in the experiment, 24 ($n = 8$ under each exercise condition) were administered V. In these animals brain areas were assayed for concentrations of 5-HT and 5-HIAA. 5-HT and 5-HIAA were greater ($P < 0.05$) after 1 h of exercise than at rest in the MB, ST, and HY (Fig. 6). In the HI only 5-HT was elevated ($P < 0.05$) by exercise. 5-HIAA was even greater ($P < 0.05$) in MB and ST after exhaustive exercise.

DA. DA and DOPAC concentrations were assessed in brain areas of all animals. As a result of 1 h of exercise alone in V rats, DA and DOPAC concentrations were elevated ($P < 0.05$) in MB, ST, and HY (Fig. 7). After exhaustive exercise, however, DA concentrations in V rats were not as great as after 1 h of exercise in MB, ST, and HY. In V animals DOPAC concentrations at exhaustion were also not as great as after 1 h of exercise in ST and HY. DA and DOPAC concentrations did not change as a result of exercise in HI.

DA concentrations were maintained near or below resting levels as a result of QD administration in all brain areas (Fig. 7). However, these values were not as great as those observed during V ($P < 0.05$). DA and DOPAC were elevated ($P < 0.05$) after 1 h of exercise and at exhaustion in MB, ST, and HY after LY administration.

DISCUSSION

The present study was completed to further examine the mechanisms underlying the effects of brain 5-HT activity on fatigue by examining the neurochemical, hormonal, and substrate effects of pharmacological manipulation of 5-HT activity during prolonged exercise to fatigue in rats.

The effects of increased (QD) and decreased (LY) 5-HT activity on run time to exhaustion are similar to those previously reported (4). QD animals ran ~32% less than did V animals, whereas LY animals exercised 26% longer than did V animals. QD and LY were chosen for this investigation because of their well-documented specificity for 5-HT receptors with little or no cross over to other neurotransmitter receptors (15). QD is a general 5-HT agonist, and LY is a 5-HT antagonist specific to 5-HT_{1C} and 5-HT_2 receptors (15). Consequently, it is reasonable to assume that the changes in run time to exhaustion in this study can be attributed to changes in 5-HT activity.

In V animals, the decrease in plasma glucose, liver glycogen, and muscle glycogen and the increase in plasma FFA during prolonged exercise are consistent with findings from previous reports (15). In contrast, in QD animals, plasma glucose and FFA at fatigue were similar to resting levels (Fig. 2). Moreover, the magnitudes of decline of muscle and liver glycogen were not as great at exhaustion in QD animals (Fig. 3). Consequently, it appears that QD animals did not fatigue because of a lack of substrate availability, suggesting that these animals stopped running as a result of some central 5-HT mechanism.

The changes in substrate availability in LY animals during exercise were similar to those observed in V animals. In all cases the magnitudes of change in these variables in LY animals were similar to those observed in V animals. An explanation for why it took longer for LY

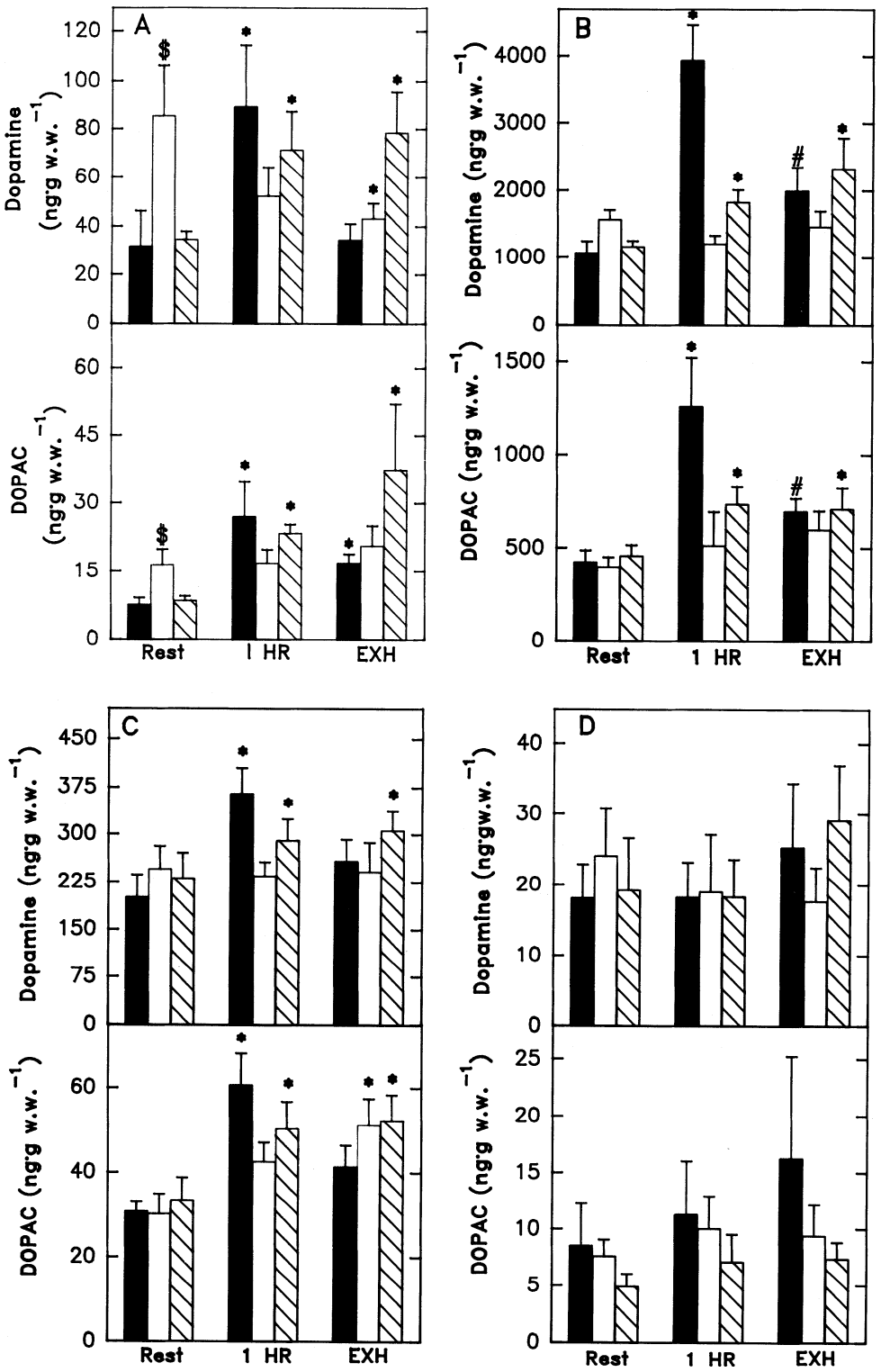

FIG. 7. Effect of exercise and drug treatments on dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) in midbrain (*A*), striatum (*B*), hypothalamus (*C*), and hippocampus (*D*). Columns as in Fig. 2 legend. Significantly different ($P < 0.05$) from: * same drug treatment at rest, # same drug treatment at rest and at 1 h of exercise, $ vehicle at rest.

animals to reach a metabolic end point similar to that observed in V animals is not evident.

Prolonged exercise has been shown to result in increased concentrations of 5-HT and 5-HIAA in various regions (HI, MB, and ST) of the brain (3, 10, 11). The data from this investigation are consistent with these findings; however, the magnitudes of increase in 5-HT and 5-HIAA concentrations were much greater here. These differences are probably due to differences in exercise intensity. For example, in a previous investigation 90 min of level treadmill running at 20 m/min resulted in increases of 9–20% for both 5-HT and 5-HIAA (10). In comparison, 60 min of treadmill running at 20 m/min and 5% grade in this investigation resulted in increases in 5-HT and 5-HIAA ranging from 50 to 90 and 34 to 71%, respectively. Another important observation of this investigation was that concentrations of 5-HIAA were even further elevated at exhaustion compared with at 1 h

of exercise in the MB and ST. These progressive increases in concentrations of 5-HIAA suggest that 5-HT synthesis and metabolism are progressively enhanced as a result of exercise to fatigue.

DA and DOPAC concentrations (indicators of DA synthesis and metabolism; Ref. 12) increased during the 1st h of exercise in the MB, ST, and HY (Fig. 7). At exhaustion, however, DA and DOPAC concentrations were consistently lower than after 1 h of exercise. The increases in brain concentrations of DA and DOPAC after acute exercise are consistant with findings of other investigators (8, 14, 19). To the best of our knowledge, however, this is the first investigation in which changes in brain concentrations of DA and DOPAC have been observed during prolonged exercise to fatigue. Therefore, the observation of the phenomenon of decreased concentrations of DA and DOPAC from 1 h of exercise to exhaustion is a novel one that may be important when investigating mechanisms of fatigue during prolonged exercise.

Evidence for this concept becomes even stronger when the effects of QD administration on brain concentrations of DA and DOPAC and run time to exhaustion are considered. After 1 h of exercise in QD animals, brain DA and DOPAC concentrations were similar to rest values of QD animals and lower than concentrations in V animals at 1 h of exercise in the MB, ST, and HY (Fig. 7). In contrast, brain DA and DOPAC concentrations were actually elevated at rest in QD animals compared with in V animals (MB and ST). The cause of these responses at rest are not clear; however, it is possible that elevated resting DA activity is a general response of altered brain function and behavior in QD animals. Regardless of these somewhat confusing resting data, it appears that fatigue was associated with attenuation of brain DA and DOPAC concentrations in QD animals.

Review of the effects of LY administration on changes in DA and DOPAC concentrations during exercise does not completely support the negative 5-HT–DA relationship described above. DA and DOPAC concentrations increased from rest to exercise in the MB, ST, and HY of LY animals (Fig. 7). At exhaustion, however, DA and DOPAC concentrations remained elevated compared with at rest. It is possible that the maintenance of DA and DOPAC concentrations in LY animals contributed to the animals' ability to exercise longer. This pattern of response, however, is not consistant with the premise that the onset of fatgiue during prolonged exercise is influenced by brain DA and DOPAC concentrations.

Increased 5-HT activity has been shown to significantly elevate plasma concentrations of hormones produced by the HPA axis (corticosterone) and the SA system (epinephrine and norepinephrine) in rats at rest (1, 30). Alteration of 5-HT activity did result in increases in plasma concentrations of both epinephrine (QD only) and norepinephrine (QD and LY) at rest; however, a similar effect was not observed for corticosterone. During exercise in this study, however, altered 5-HT activity (QD animals) resulted only in a significant elevation of epinephrine. Elevated levels of epinephrine in QD animals could have potentially hastened the onset of fatigue through its stimulatory effects on muscle glycogen breakdown (16). This does not appear to be the case, however, because muscle and liver glycogen concentrations at exhaustion were greater in QD than in V animals (Fig. 3).

As described above, norepinephrine was also elevated at rest in both QD and LY animals (Fig. 5). Elevated levels of norepinephrine at rest in QD and LY animals may raise some concern as to the effects of these levels on cardiovascular mechanisms of fatigue (27). However, it is unlikely that norepinephrine would have any effect on these mechanisms during exercise because plasma norepinephrine concentrations were similar between treatments during exercise. The data discussed above appear to indicate that the performance-altering effects due to manipulation of 5-HT activity are not mediated by changes in activity of HPA and SA systems. However, it must be kept in mind that the blood collection procedure used in this investigation (decapitation) may have caused an increase in sympathetic discharge that could have prevented the detection of small but important changes in the activity of these systems.

The purpose of this investigation was to examine potential neurochemical, hormonal, and substrate mechanisms underlying the effects of altered brain 5-HT activity on endurance performance. Increased brain 5-HT activity could also affect run time to exhaustion in the rat through its effects on thermoregulation and pain tolerance (17, 26). We do not believe that changes in thermoregulatory ability due to increased 5-HT activity influenced run time to exhaustion in this investigation because we did not observe any differences in colonic temperature during 30 min of exercise after QD administration (compared with after V administration) in a previous experiment (unpublished data). Furthermore, it is unlikely that increased brain 5-HT activity reduced run time to exhaustion via its effects on pain tolerance because increased brain 5-HT activity results in increased pain tolerance (15) and would be expected to have a positive rather than a negative effect on run time to exhaustion. Although it is our opinion that altered brain 5-HT activity probably does not result in changes in run time to exhaustion because of its effects on thermoregulation and pain tolerance, these possibilities must be further examined before a sound conclusion can be made.

In summary, run time to exhaustion in the rat is negatively and positively affected by drugs that increase and decrease brain 5-HT activity, respectively. These effects do not appear to be substrate dependent or due to alterations in HPA axis or SA system activity. Fatigue during prolonged exercise was associated with elevated brain levels of 5-HT and 5-HIAA and reduced brain levels of DA and DOPAC. Fatigue was also associated with an attenuation of brain DA and DOPAC concentrations in animals treated with a 5-HT agonist. Maintenance of brain DA and DOPAC concentrations during prolonged exercise may explain why animals treated with a 5-HT antagonist were able to exercise longer. However, an explanation as to why these animals eventually fatigued is still not evident. From the above it can be concluded that the interaction between brain 5-HT and DA systems may be important in determining the onset of fatigue during prolonged exercise.

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