Pharmacology Biochemistry & Behavior, Vol. 39, pp. 337–343. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Effect of Cholinesterase Inhibitor and Exercise on Choline Acetyltransferase and Acetylcholinesterase Activities in Rat Brain Regions

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Received 16 October 1990

SOMANI, S. M., S. R. BABU, S. P. ARNERIC AND S. N. DUBE. *Effect of cholinesterase inhibitor and exercise on choline acetyltransferase and acetylcholinesterase activities in rat brain regions.* PHARMACOL BIOCHEM BEHAV **39**(2) 337–343, 1991.—This study sought to determine whether the choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) enzymes in the brain were affected in a regionally selective manner by chemical and physical stressors: 1) subacute administration of physostigmine (Phy); 2) exercise; and 3) the combination of these two stressors. ChAT and AChE activities in corpus striatum were significantly decreased due to Phy as well as Phy + exercise. This suggests that corpus striatum is affected by chemical stressors but more so by the combination of chemical and physical stressors. The brainstem is the only region which showed inhibition of ChAT activity due to exercise. Subacute Phy also inhibited brainstem ChAT activity. The hippocampus showed significant decrease in ChAT activity due to Phy + exercise but not due to Phy alone. These results suggest that the brain regions involved with control of motor, autonomic and cognitive functions were affected by subacute Phy and exercise. These data are consistent with the hypothesis that the responsiveness of these brain regions to different stressors is a function of the level of ongoing cholinergic activity and that elevations in ACh levels due to AChE inhibition may have long-term effects on the regulation of ChAT and AChE activities through a negative feedback mechanism.

Exercise Physostigmine Choline acetyltransferase Acetylcholinesterase Brain regions

ALTERATIONS in central neurotransmitter systems due to physical exercise and/or chemical stressors have not received much attention. Of the few studies that have specifically examined biochemical markers of the brain cholinergic system, the two most frequently measured indices have been the biosynthetic enzyme of acetylcholine (ACh), choline acetyltransferase (ChAT), and the degradative enzyme, acetylcholinesterase (AChE).

Apparent discrepancies in the literature regarding the effects of stressors on cholinergic function have been common. For example, ChAT activity diminished in rat brains exposed to immobilization stress (15). ChAT activity increased in the rat cerebral cortex after acute and repeated electroshock (16,20). Unchanged ChAT activity after exposure to immobilization for 2 h was also reported in hypothalamic regions: brainstem, striatum and hippocampus (12, 13, 15, 27). None of these previous studies directly compared both cholinergic enzymes, and none directly compared the effects of physical exercise and chemical stressors such as Phy, a cholinesterase (ChE) inhibitor. Phy was considered to be a potential pretreatment agent against organophosphate poisoning (26). There is even greater discrepancy concerning the measurement of AChE in the brain exposed to different drugs and stressors (1, 7, 11, 25).

We have reviewed Phy—an overview as pretreatment drug for organophosphate intoxication (26). We have recently reported the effect of Phy administration and different levels of exercise on ChE activity in red blood cells (RBC) and various tissues of the rat (5). We observed that exercise enhances the inhibition of ChE activity in RBC and whole brain elicited by Phy. In order to more clearly delineate these effects on central cholinergic function, this study sought to determine: will trained exercise, subacute Phy administration, or the combination of these two treatments, elicit adaptive changes in the biosynthetic or degradative enzymes for ACh? If so, are these changes differentially expressed within subregions of the brain?

METHOD

Physostigmine (Phy) free base, acetyl-Co A and acetyl choline chloride were obtained from Sigma Chemical Co. (St. Louis,

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TABLE 1
TRAINING PROTOCOL FOR EXERCISING RATS

Week	Belt Speed (m/min)	Inclination (% grade)	Duration at Each Speed (min)
1	8.2, 15.2, 19.3	6	10
2	8.2, 15.2, 19.3	6	10

MO). ^{3}H-Acetyl-Co A and ^{3}H acetyl choline iodide were obtained from Amersham Corp. (Chicago, IL). Ready-Solv was procured from Beckman Instruments, Inc. (Fullerton, CA). All other chemicals were analytical grade and were obtained from the usual commercial sources.

Animals

Male Sprague-Dawley rats (weight 150–175 g) were obtained from Harlan Industries, Indianapolis, IN. These rats weigh 250–300 g at the time of sacrifice. The rats are 14–15 weeks old, and they are young adults. The rats were divided into five groups (Gr).

Gr I: received saline and served as sedentary controls. These rats were sacrificed on the day of the experiment.

Gr II: was trained for two weeks as per the protocol (Table 1) and exercise was stopped 24 h prior to sacrifice.

Gr III: received Phy (70 μg/kg, IM) twice daily for two weeks. This group was divided into two subgroups. Group IIIa was given Phy on the day of the experiment and sacrificed after 20 min; Group IIIb was given Phy 24 h prior to sacrifice.

Gr IV: Phy was administered (70 μg/kg IM) twice daily for two weeks. Acute exercise (100% $VO_{2\ max}$) was given 24 h before sacrifice. These rats were divided into two subgroups. Group IVa was given Phy on the day of the experiment and sacrificed after 20 min; Gr. IVb was given Phy 24 h prior to sacrifice.

Gr V: Phy was administered (70 μg/kg, IM) twice daily for two weeks and trained for 30 min as per protocol (Table 1). This group was also divided into two subgroups. Group Va received Phy on the day of experiment and were sacrificed after 20 min; Group Vb received Phy 24 h prior to sacrifice.

Training of Rats

Rats from Gr II and Gr V were acclimatized to a treadmill in the beginning and were trained on a 9-channel motor driven treadmill (custom built at SIU) using an incremental exercise program. During this program of exercising, the speed (meters/min), angle of inclination (% grade), and the duration (min) of exercise were varied to obtain different levels of exercise intensity as shown in Table 1.

Rats from Gr III and IV were not trained but were maintained under similar conditions to those of the trained rats. Each rat's weight was recorded daily before exercising the rats on the treadmill in order to determine the body weight changes during the entire period of training.

After completing the preceding protocols, rats were decapitated and different regions of brain (corpus striatum, cerebral cortex, brainstem and hippocampus) were removed and frozen in liquid nitrogen. Tissues were stored at −70°C until analysis.

Enzyme Preparation

Frozen tissues were thawed and 10% homogenates were prepared in 10 mM EDTA phosphate buffer (pH 7) using an ultrasonic processor. Fifty μl of this aliquot was transferred to Eppendorf tubes for protein assay. To the remaining homogenate, equal volumes of Triton X-100 (0.4%) and bovine serum albumin (0.2%) were added to release full enzyme activity. The concentration of the homogenate was diluted for each brain region until activities obtained were linear with tissue concentration.

Choline Acetyltransferase (ChAT) Assay

ChAT activity was determined using the radiochemical (2,10) method. The incubation mixture contained 0.1 μCi ^{3}H acetyl-Co A, 300 mM NaCl, 50 mM Na phosphate buffer (pH 7.4), 8 mM choline chloride, 5 mM EDTA, and 0.1 mM physostigmine sulfate. The mixture was incubated for 40 min at 37°C and the reaction stopped with 0.5 ml of 2-heptanone containing sodium tetraphenylboron (10 mg/ml). The contents were vortexed, centrifuged and the organic phase was removed into scintillation vials. This step was repeated, and to 1 ml of organic phase 15 ml of Ready-Solv (Beckman) was added. The contents were vortexed and counted in a Beckman liquid scintillation counter (LS 5800) ChAT activity was calculated as nmol ACh synthesized per h per mg protein.

Acetylcholinesterase (AChE) Assay

The AChE assay was performed using a radiochemical method (9) with slight modifications. The homogenate was preincubated for 30 min at 37°C with iso-OMPA $Cl \times 10^{-5}$ M, a selective inhibitor of BuChE activity. The incubation mixture contained 0.1 μCi^{3}H acetylcholine (0.5 mM), 20 mM sodium phosphate buffer (pH 7.2) and bovine serum albumin (0.8 mg/ml). The final incubation volume was 100 μl and incubation was carried out at 30°C for 30 min. After incubation, 0.4 ml of ice cold 10 mM sodium phosphate buffer, pH 7.4, was added and tubes were placed on ice. After centrifugation the ketone layer was aspirated and the aqueous phase was washed once more with ketonic sodium tetraphenylboron. The final aqueous layer was transferred into scintillation vials and 16 ml of scintillation cocktail was added. The vials were vortexed, counted, and AChE activity was calculated as μmol per h per mg protein.

Protein concentrations were determined with the Coomassie blue protein-binding method (21) using bovine serum albumin as standard.

Calculations and Statistics

The data was expressed as the mean ± SEM of four rats. The statistical analysis was performed on the absolute values of the data obtained from each experimental group. Data was analyzed and the differences detected by Student's paired *t*-test. The criterion of statistical significance was $p<0.05$.

RESULTS

The results of ChAT and AChE activities are expressed as nmol/mg of protein/h and μmol/mg of protein/h, respectively, for the different brain regions studied and are shown in Figs. 1–4.

Corpus Striatum

ChAT activity did not change in corpus striatum (98% of control) due to endurance training (Gr II). ChAT activity significantly decreased to 76%, 95% and 91% of control in Phy-administered (IIIa), Phy + acute exercise (IVa) and Phy + trained

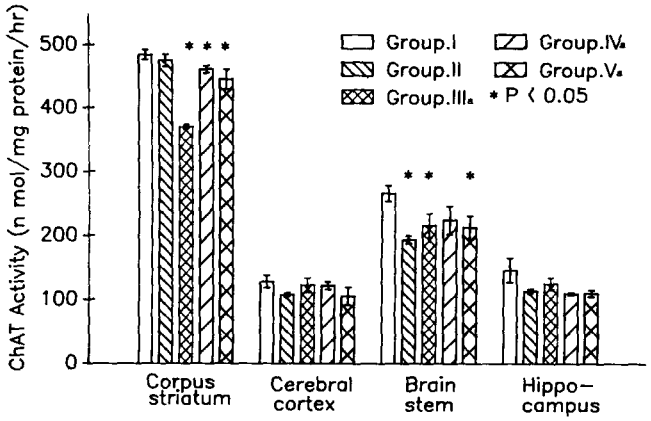

FIG. 1. Effect of trained exercise for two weeks (Gr II), subacute Phy administration (70 μg/kg IM) for two weeks (Gr IIIa), subacute Phy administration (70 μg/kg IM) for two weeks + single acute bout of exercise (Gr IV) and subacute Phy administration (70 μg/kg IM) for two weeks + trained exercise for two weeks (Gr Va) on ChAT activity in different regions of the rat brain. Rats were sacrificed 20 min after the last dose of Phy administration.

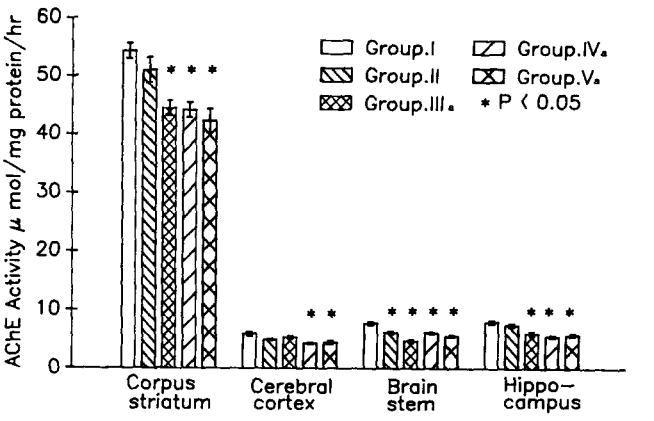

FIG. 3. Effect of trained exercise for two weeks (Gr II), subacute Phy administration (70 μg/kg IM) for two weeks (Gr IIIa), subacute Phy administration (70 μg/kg IM) for two weeks + single bout of exercise (Gr IVa) and subacute Phy administration (70 μg/kg IM) for two weeks + trained exercise for two weeks (Gr Va) on AChE activity in different regions of the rat brain. Rats were sacrificed 20 min after the last dose of Phy administration.

exercise (Va) rats, respectively, which were sacrificed after 20 min of Phy administration (Fig. 1 and Table 1). ChAT activity also decreased to 88%, 68% and 88% of control in Phy-administered (IIIb), Phy + acute (VIb) and Phy + trained exercise (Vb) rats, respectively, which were sacrificed after 24 h of Phy administration (Fig. 2 and Table 2).

AChE activity significantly decreased in subacute Phy-administered (Gr IIIa) and subacute Phy + acute exercise or trained exercise (Gr IV and Gr Va) rats after 20 min of sacrifice (Fig. 3 and Table 2). AChE activity remained at 89%, 87% and 90% of control in Phy-administered (IIIb), Phy + acute exercise (VIb) and Phy + trained exercise (Vb), respectively, even after 24 h (Fig. 4 and Table 3).

Cerebral Cortex

ChAT activity in cerebral cortex of trained rat (Gr II) was low (84% of control). There was no significant change in ChAT activity after subacute Phy + acute exercise (Gr IVa and IVb) in cerebral cortex of rats sacrificed at 20 min or 24 h after Phy administration (Figs. 1 and 2, and Table 1). However, ChAT activity decreased to 89% of control in cerebral cortex of rats sacrificed after 24 h of Phy administration (Gr IIIb). This enzyme activity decreased significantly ($p<0.05$) in rats sacrificed 24 h after subacute Phy + training (Gr Vb).

AChE activity was low in endurance trained rats (Gr II) and in subacute Phy (Gr IIIa), as shown in Fig. 3 and Table 2.

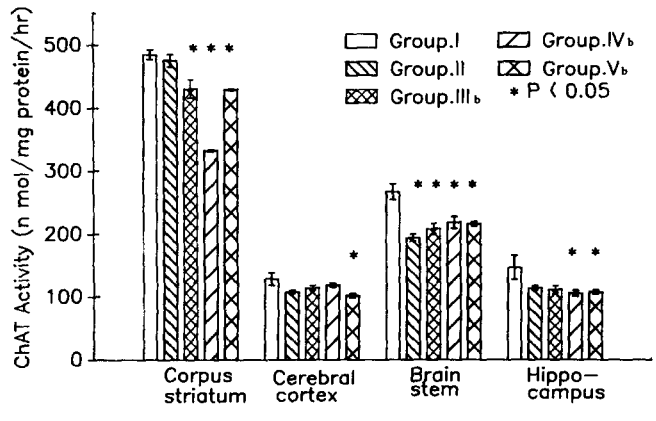

FIG. 2. Effect of trained exercise for two weeks (Gr II), subacute Phy administration (70 μg/kg IM) for two weeks (Gr IIIb), subacute Phy administration (70 μg/kg IM) for two weeks + single bout of exercise (Gr IVb) and subacute Phy administration (70 μg/kg IM) for two weeks + trained exercise for two weeks (Gr Vb) on ChAT activity in different regions of the rat brain. Rats were sacrificed 24 h after the last dose of Phy administration.

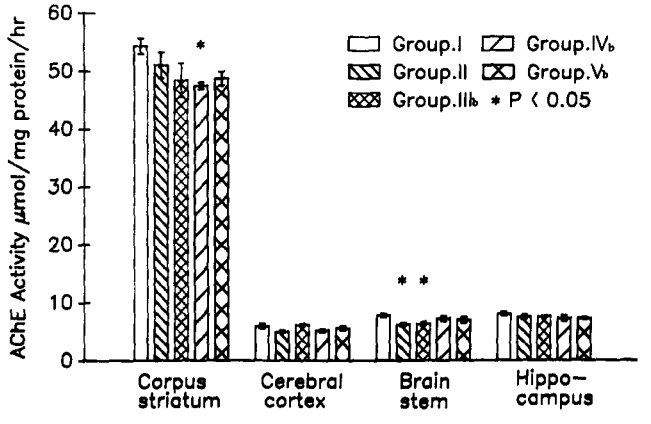

FIG. 4. Effect of trained exercise for two weeks (Gr II), subacute Phy administration (70 μg/kg IM) for two weeks (Gr IIIb), subacute Phy administration (70 μg/kg IM) for two weeks + single bout of exercise (Gr IVb) and subacute Phy administration (70 μg/kg IM) for two weeks + trained exercise for two weeks (Gr Vb) on AChE activity in different regions of the rat brain. Rats were sacrificed 24 h after the last dose of Phy administration.

TABLE 2

EFFECT OF REPEATED DOSE OF PHYSOSTIGMINE (70 μg/kg, IM) DAILY FOR 2 WEEKS AND TRAINED EXERCISE FOR 2 WEEKS ON CHOLINE ACETYL TRANSFERASE (ChAT) ACTIVITY (% OF CONTROL) IN DIFFERENT BRAIN REGIONS OF RATS

Group	Treatment	Corpus Striatum	Cerebral Cortex	Brain Stem	Hippocampus
II	Trained Exercise	98.1 ± 4.0	84.04 ± 2.2	72.8* ± 2.2	77.8 ± 2.2
III	Subacute Phy				
IIIa	Sacrificed 20 min after Phy	76.3* ± 0.7	95.6 ± 8.5	81.1* ± 7.0	85.5 ± 6.2
IIIb	Sacrificed 24 hr after Phy	88.7* ± 2.9	89.0 ± 3.4	78.0* ± 3.2	75.9 ± 4.2
IV	Subacute Phy + Acute Exercise				
IVa	Sacrificed 20 min after Phy	95.1* ± 1.2	95.3 ± 4.6	84.3 ± 8.2	74.9 ± 1.3
IVb	Sacrificed 24 hr after Phy	68.5* ± 0.3	92.5 ± 4.6	81.8* ± 3.5	72.1* ± 3.2
V	Subacute Phy + Trained Exercise				
Va	Sacrificed 20 min after Phy	91.9* ± 3.3	82.4 ± 10.7	80.1* ± 6.4	75.3 ± 3.5
Vb	Sacrificed 24 hr after Phy	88.5* ± 0.2	79.4* ± 2.3	81.1* ± 1.2	73.3* ± 2.1

*Significant at $p<0.05$.

AChE activity remained at 89%, 87% and 90% of control in Phy-administered (IIIb), Phy + acute exercise (IVb) and Phy + trained exercise (Vb) rats, respectively, even after 24 h (Fig. 4 and Table 2).

However, AChE activity significantly decreased to 72% and 73% of control in subacute Phy + acute exercise (Gr IVa) and in subacute Phy + trained groups (Gr Va) (Fig. 3). AChE activity recovered in all groups by 24 h after Phy administration (Fig. 4). These results indicate that AChE activity in cerebral cortex was inhibited by Phy + exercise (acute or trained) at 20 min of Phy administration and recovered to control level by 24 h.

Brainstem

ChAT activity decreased significantly in brainstem in all groups, indicating that this region is more sensitive to ChAT activity by Phy as well as exercise. ChAT activity decreased to 73% of control ($p<0.05$) in trained exercise rats (Gr II). ChAT activity also decreased significantly in all groups of rats sacrificed after 20 min or 24 h of Phy administration with or without exercise (Fig. 2 and Table 1).

AChE activity significantly decreased ($p<0.05$) in brainstem in all groups except in Phy + acute exercise (IVb) and Phy + trained exercise (Vb) rats sacrificed after 24 h (Fig. 4 and Table 2). AChE activity decreased to 81%, 61% and 82% of control in trained exercise (Gr II), subacute Phy sacrificed after 20 min (Gr IIIa) and subacute Phy sacrificed after 24 h (Gr IIIb), respectively (Fig. 3 and 4). AChE activity also decreased significantly to 79% of control in subacute Phy + acute exercise (Gr IVa) and 75% of control in subacute Phy + trained exercise (Gr Va) rats sacrificed after 20 min of Phy administration (Fig. 3 and Table 2). AChE activity was significantly low in subacute Phy (Gr IIIb) rats sacrificed after 24 h of Phy administration (Fig. 4). However, AChE activity recovered to 93% and 90% of control in Phy + acute exercise (IVb) and Phy + trained exercise (Vb) rats sacrificed after 24 h, respectively (Fig. 4 and Table 2).

Hippocampus

ChAT activity decreased to 77% of control in hippocampus in trained rats (Gr II). ChAT activity significantly decreased ($p<0.05$) to 72% control in subacute Phy + acute exercise (Gr IVb) and in subacute Phy + trained exercise (Gr Vb) rats sacrificed after 24 h of Phy administration (Fig. 2 and Table 1). ChAT activity was low in all other groups (Figs. 1 and 2 and Table 1).

AChE activity decreased in all groups but significantly decreased to 76%, 69% and 72% of control ($p<0.05$) in Phy-administered (IIIa), Phy + acute exercise (IVa) and Phy + trained exercise (Va) rats sacrificed after 20 min, respectively (Fig. 3 and Table 2).

DISCUSSION

This study suggests that the biosynthetic and degradative enzymes for ACh in brain regions involved with control of motor,

TABLE 3

EFFECT OF REPEATED DOSE OF PHYSOSTIGMINE (70 μg/kg, IM) DAILY FOR 2 WEEKS AND TRAINED EXERCISE FOR 2 WEEKS ON AChE ACTIVITY (% OF CONTROL) IN DIFFERENT BRAIN REGIONS OF RATS

Group	Treatment	Corpus Striatum	Cerebral Cortex	Brain Stem	Hippocampus
II	Trained Exercise	94.0 ± 4.0	84.3 ± 2.5	80.5* ± 2.6	94.1 ± 3.6
III	Subacute Phy				
IIIa	Sacrificed 20 min after Phy	81.8 ± 2.4	90.8 ± 4.1	61.2* ± 3.1	75.6* ± 5.0
IIIb	Sacrificed 24 hr after Phy	89.3 ± 6.0	104.1 ± 3.2	82.1* ± 4.7	94.7 ± 2.2
IV	Subacute Phy + Acute Exercise				
IVa	Sacrificed 20 min after Phy	81.4* ± 2.4	71.6* ± 2.0	78.7* ± 1.8	68.7* ± 2.7
IVb	Sacrificed 24 hr after Phy	103.8 ± 1.0	87.0 ± 4.4	93.2 ± 4.9	90.8* ± 5.5
V	Subacute Phy + Trained Exercise				
Va	Sacrificed 20 min after Phy	77.9* ± 3.8	74.7* ± 4.9	71.6* ± 2.1	72.1* ± 2.7
Vb	Sacrificed 24 hr after Phy	104.5 ± 3.0	93.6 ± 5.7	90.5 ± 5.8	90.8* ± 1.5

*Significant at $p<0.05$.

autonomic and cognitive functions are affected by trained exercise and subacute Phy in a regionally selective pattern that appears to depend on the type and interaction of these two stressors. Rats were sacrificed at 20 min and 24 h after subacute administration of Phy in order to observe the short- and long-term effects of the drug on ChAT and AChE activities, as well as the effects of single acute exercise or trained exercise. The data are consistent with the hypothesis that the responsiveness of these brain regions to these different stressors is a function of the level of ongoing cholinergic transmission and that elevations in ACh levels due to AChE inhibition may have long-term effects on ChAT and AChE activities through a negative feedback mechanism.

Previous work from this laboratory (5) suggested that inhibition of whole brain ChE activity by Phy was enhanced with exercise. However, it was unclear whether all brain regions responded similarly, since both the spontaneous activity and the regulation of that activity are differentially controlled in brain (23,24). Moreover, it was uncertain whether other enzymes involved in cholinergic transmission may also be affected.

This study observed marked (up to 5–20-fold) differences in the regional distribution of ChAT and AChE, consistent with the known cholinergic innervation to the areas examined (6). Remarkably, the short- and long-term changes in ChAT and AChE activity elicited by the different stressors also showed regional selectivity. For example, the only brain region where ChAT activity responded to trained exercise was the brainstem, a region which is involved in maintaining critical autonomic functions related to the cardiopulmonary system and where ACh has potent actions (3). However, an alternative interpretation is that the changes in ChAT and AChE activities may have occurred with the different motor neurons within the brainstem, since they provide a major contribution to the detected enzyme activities. ChAT activity in cerebral cortex is not affected by any individual stressor, which may suggest the relative sparing of cholinergic systems in higher association centers involved with cognitive function. However, combination of these two stressors (Phy + trained exercise) did show an interaction to reduce ChAT activity in cortex and in hippocampus, an area of brain involved in learning and memory processes. In contrast, ChAT activity in corpus striatum was depressed significantly in all groups receiving Phy, and remain depressed even 24 h following withdrawal of Phy. Thus, the cholinergic system in corpus striatum, which is normally involved in motor control, is essentially unaffected by exercise, but susceptible to a chemical stressor such as Phy.

Results by others examining the effects of Phy have been mixed. In contrast to this study, ChAT in cortical regions decreased, but no significant changes were shown in hippocampus and striatum after continuous minipump infusion of Phy for two weeks (19). Similarly, ChAT and AChE decreased in cerebral cortex due to Phy administration (18). It is unclear whether the differences in these studies may be explained by the doses used or the continuous nature of the Phy administered.

However, cholinergic parameters in various regions of brain react differently to altered stress conditions, such as cold and swimming (8, 14, 17). This study supports this concept, since regional differences in cholinergic activities were also observed for Phy and exercise.

The molecular/physiological mechanisms that govern the lasting changes in ChAT and AChE activity remain equivocal at this time. However, three comments can be made. First, a single dose of ^{3}H-Phy followed by trained exercise decreased the clearance of Phy significantly. This decrease in clearance will cause an increase in Phy (unpublished data from our lab) which cannot fully account for the inhibition of AChE activity seen at the 24-h period, since trained exercise alone without Phy reduces AChE. Second, in brainstem the mechanism to decrease ChAT may be the same for exercise and Phy, since the combination of the two stressors did not show additive effects. Third, elevating tissue concentrations of ACh may ultimately govern these long-term effects independent of the mechanisms that initiate these effects. This premise is best illustrated in the brainstem where respiratory and cardiovascular functions are regulated. Trained exercise alone increases blood pressure and heart rate (22) and down regulates ChAT activity. Increases in medullary tissue concentrations of ACh by ChE inhibitors also elevate blood pressure and respiration (4). It is plausible that when tissue levels of ACh rise, ChAT activity is down regulated as a compensatory mechanism to normalize cholinergic transmission and, hence, blood pressure. Mechanistically, these changes may be initiated as follows. Under control conditions tissue levels of ACh are regulated by the net synthesis and degradation of ACh by ChAT and AChE, respectively. Immediately following AChE inhibition, tissue levels of ACh rise and initiate processes that decrease both ChAT and AChE activity. Chronically elevated ACh concentrations eventually down regulate ChAT and AChE activities to normalize cholinergic transmission, whereas acute exercise initially increases tissue ACh levels by enhancing biosynthesis without affecting degradation. This phenomenon does not appear to be expressed in corpus striatum, hippocampus or cerebral cortex. However, chronically elevated levels of ACh still down regulate ChAT and AChE activities, although these activities are initiated via different mechanisms. In order to verify this hypothesis, both tissue concentrations and the turnover of ACh need to be measured. Experiments are in progress to further support this hypothesis.

To summarize, Phy, exercise or the combination of both decreases brain ChAT and/or AChE activities in a regionally selective pattern. These data are consistent with the hypothesis that elevations in ACh concentrations down regulate the level of ongoing cholinergic neurotransmission via a negative feedback mechanism.

ACKNOWLEDGEMENTS

The authors gratefully thank the helpful comments of Ezio Giacobini, M.D., Ph.D., and the skillful assistance of Mrs. Roberta J. Melton for manuscript preparation. This work was supported, in part, by the U.S. Army Medical Research and Development Command under contract DAMD 17-88-C-8024.

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