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Effect of Physostigmine and Exercise on Choline Acetyltransferase and Acetylcholinesterase Activities in Fast and Slow Muscles of Rat

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BABU, S. R., S. M. SOMANI AND S. N. DUBE. *Effect of physostigmine and exercise on choline acetyltransferase and acetylcholinesterase activities in fast and slow muscles of rat.* PHARMACOL BIOCHEM BEHAV **45**(3) 713–717, 1993.—The interaction of physostigmine (Phy) and exercise on cholineacetyltransferase (ChAT) and acetylcholinesterase (AChE) have been studied in the fast extensor digitorum longus (EDL) and slow soleus muscles of rat. ChAT decreased significantly by trained exercise in EDL muscle and Phy prolonged this effect even up to 24 h. Soleus muscles showed a small increase of ChAT due to exercise but Phy + exercise did not change significantly. Both EDL and soleus showed a marked decrease in AChE activity due to subacute administration of Phy + trained exercise, exhibiting an additive effect. No recovery was observed in ChAT and AChE activities of EDL even after 24 h in Phy + trained exercise group. Our results suggest that Phy and exercise has significant effect on the synthetic (ChAT) and degradative (AChE) enzymes of acetylcholine in active EDL muscle. Exercise has prolonged the inhibitory effect of Phy on ChAT and AChE activities both in active EDL and passive soleus muscles. This study showed that Phy + exercise modified the functional activity of cholinergic system in EDL and soleus muscles.

Exercise Physostigmine Cholineacetyltransferase Acetylcholinesterase Extensor digitorum longus Soleus muscle

SOME muscle characteristics are influenced by the neurotransmitter ACh, a neurogenic substances conveyed by axoplasmic transport, and muscle electromechanical activity (8,19). These regulatory factors are affected by change in motor activity. The experimentally induced enhancement of neuromuscular use or disuse invariably leads to dramatic modifications in the muscle metabolism, contractile properties, and neuromuscular transmission-related molecules (3,13). ACh receptors, ChAT, and AChE have received much attention because of their usefulness as sensitive indicators of normal nerve-muscle interactions (8). AChE at the neuromuscular junction has an essential role of ending synaptic transmission (16); its regulation in skeletal muscles partly depends on nerve-evoked muscle activity (22). Fernandez and Donoso (8) showed that treadmill exercise significantly increases the G_4 form of AChE but not other AChE forms, and appears predominantly in fast twitch but not in slow twitch muscle of rat (8). Contrary to this, we have observed a decrease in total AChE in thigh muscle (7). Trained exercise induces several ultrastructural and protein metabolic changes in exercised skeletal muscle (6,15). Exercise also influences the effects of ChE inhibitors like Phy. We have previously reported that exercise enhances the inhibition of cholinesterase (ChE) activity in red blood cells (RBC) and brain elicited by Phy (7). We also reported the effect of Phy and exercise on ChAT and AChE activities in different regions of brain. We found that brain regions involved in the control of motor, autonomic, and congenitive functions were affected by subacute Phy and exercise (24). However, to date no information is available on simultaneous changes in synthetic ChAT and degradative AChE enzyme activities in fast twitch EDL and slow twitch soleus muscles due to treadmill exercise and/or ChE inhibitor (Phy). Phy, an anticholinesterase agent, was believed to be a potential drug for pretreatment of organophosphate intoxica-

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tions (25). Exercise alters the ChE activity due to Phy; this, in turn, would interfere with the work performance in the fields when Phy is used as a pretreatment drug. Intense fitness is required in the battlefield. The physical exercises would influence Phy-induced ChE activity in the muscle. Muscle constitutes the largest mass of the body. Therefore, exercises as a factor needs to be considered during development of a potential pretreatment agent and therapy regimen. The level of physical training may have a significant effect on how individuals will respond to treatment. Recently Turner et al. (1992) showed that exercise decreases the ChAT activity in adrenal gland of rats (28). Phy has also been a trial drug for improvement of memory function in patients with Alzheimer's disease (11,26). We have selected ChAT and AChE, the two most frequently used biochemical markers, to study the contractile properties of EDL and soleus muscle, which are the two most important muscles involved in exercise. This study was undertaken to determine whether subacute Phy and trained exercise or the combination of these two elicit alterations in ChAT and AChE activities in the EDL and soleus muscle.

METHOD

Phy free base, Acetyl-Co A and ACh chloride were obtained from Sigma Chemical Co. (St. Louis, MO). ^{3}H-Acetyl-Co A and ^{3}H ACh 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. The rats were 14–15 weeks old, and were young adults. The rats were divided into five groups (Gr) and trained as reported previously (24). These rats weigh 250–300 g at the time of sacrifice.

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

Gr II: was trained for 2 weeks as per protocol (Table 1). This group was subdivided into two subgroups. Group IIa were sacrificed 20 min after the last exercise and group IIb were sacrificed 24 h after the last exercise.

Gr III: received Phy (70 μg/kg, IM) twice daily for 2 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 2 weeks. Single 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.

TABLE 1

TRAINING PROTOCOL FOR EXERCISING RATS

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

Gr V: Phy was administered (70 μg/kg, IM) twice daily for 2 weeks and trained for 2 weeks 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 nine-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 (percent angle 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.

Rats from Gr IV were given a single bout of acute exercise (100% VO_2max) as reported earlier (25).

After completing the preceding protocols, rats were decapitated and EDL and soleus muscles were removed and frozen in liquid nitrogen. Tissues were stored at −70°C until analysis.

Enzyme Preparation

The EDL and soleus muscle were freed from tendon and were powdered under liquid nitrogen. The powdered muscle was weighed and 5% homogenates were prepared in 10 mM EDTA phosphate buffer (pH 7) using an ultrasonic processor. Fifty microliters 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 muscle until activities obtained were linear with tissue concentration.

Choline Acetyltransferase (ChAT) Assay

ChAT activity was determined using the radiochemical (1,10) 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 Phy 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 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 × 10^{-5} M, a selective inhibitor of BuChE activity. The incubation mixture contained 0.1 μCi^{3}H ACh (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 37°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 methods (20) 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 of ACh synthesized/mg protein/h and μmol of ACh hydrolyzed/mg protein/h, respectively, and are presented in Figs. 1 and 2 and Table 1. Data presented in Table 2 shows the percent of control of these two enzymes in both muscles at 20 min and 24 h after treatment. Endurance training (Gr II) decreased ChAT activity significantly ($p < 0.05$) to 68% of control in EDL whereas in soleus it increased to 124% of control (Table 2). AChE activity was decreased significantly ($p < 0.05$) in both muscles in this group. Subacute Phy (Gr III) decreased ChAT to 89% and 94% of control in EDL and soleus at 20 min but increased to 110% and 116% of control by 24 h (Fig. 1). AChE activity also decreased significantly ($p < 0.05$) to 56% and 57% of control in EDL and soleus at 20 min and remained depressed at 68% and 67% of control even after 24 h (Table 2, Fig. 2). Subacute Phy + single acute exercise (Gr IV) decreased ChAT activity in EDL and soleus to 96% and 90% of control which increased to 105% and 111% of control by 24 h, respectively. AChE activity in EDL and soleus decreased significantly to 63% and 32% of control at 20 min in Group IV; however, AChE recovered to 88% of control in EDL and 50% of control in soleus at 24 h (Fig 2). Subacute Phy + trained exercise (Gr V) decreased ChAT activity significantly ($p < 0.05$) in EDL and remained depressed up to 24 h, whereas, ChAT activity in soleus muscle remained unaffected (Fig. 1). AChE activity decreased significantly ($p < 0.05$) to 42% and 29% of control at 20 min in EDL and soleus, respectively. AChE activity remained depressed at 62% and 65% of control even after 24 h in EDL and soleus muscle, respectively. Our results showed a constant decrease in AChE activity in both muscles in all groups at 20 min and did not recover even after 24 h (Fig 2). On the other hand, ChAT showed a transient decrease at 20 min in both muscles and recovered to control level by 24 h. Only in sub-

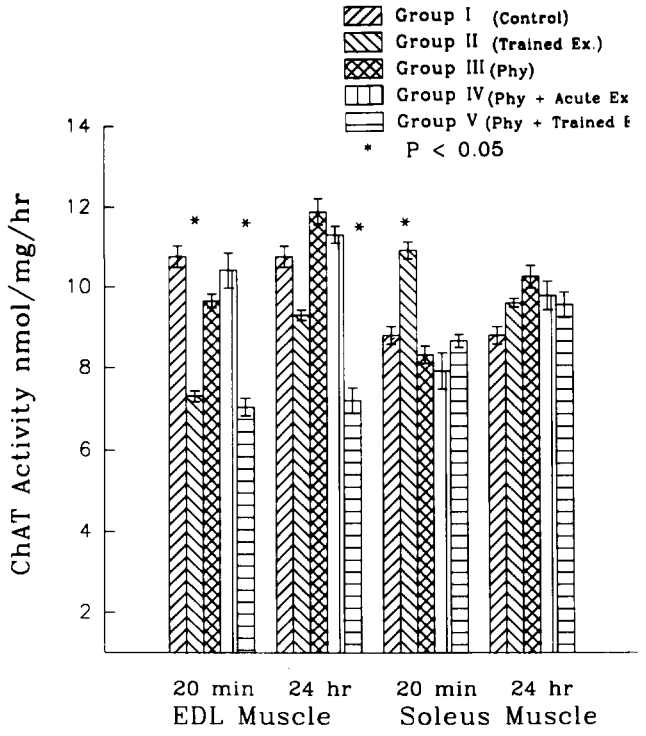

FIG. 1. Effect of trained exercise for 2 weeks (Gr II), subacute Phy administration (70 μg/kg, IM) for 2 weeks (Gr III), subacute Phy administration (70 μg/kg, IM) for 2 weeks + single acute bout of exercise (Gr IV), and subacute Phy administration (70 μg/kg, IM) for 2 weeks + trained exercise for 2 weeks (Gr V) on ChAT activity in EDL and soleus muscles of rat. Rats were sacrificed 20 min or 24 h after the last dose of Phy administration or exercise. Gr I are control animals. See the Method section for experimental protocols.

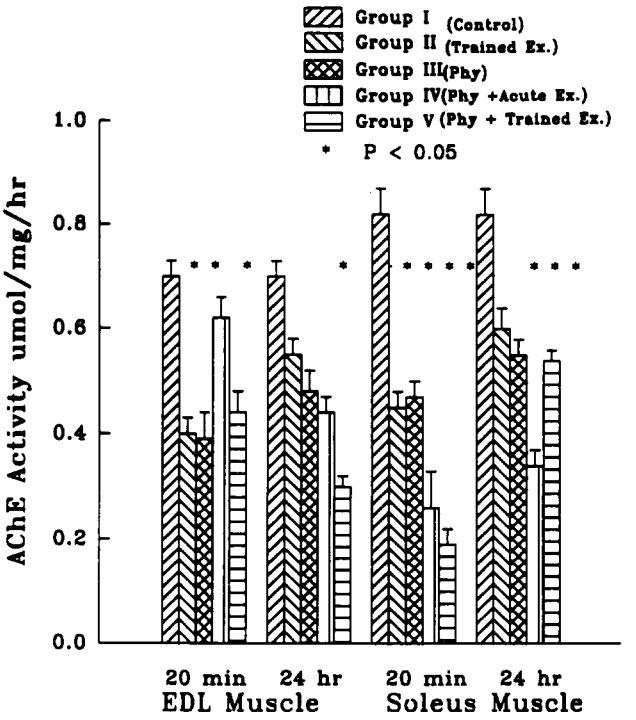

FIG. 2. Effect of trained exercise for 2 weeks (Gr II), subacute Phy administration (70 μg/kg, IM) for 2 weeks (Gr III), subacute Phy administration (70 μg/kg, IM) for 2 weeks + single acute bout of exercise (Gr IV), and subacute Phy administration (70 μg/kg, IM) for 2 weeks + trained exercise for 2 weeks (Gr V) on AChE activity in EDL and soleus muscle of rat. Rats were sacrificed 20 min or 24 h after the last dose of Phy administration or exercise. Gr I are control animals. See the Method section for experimental protocols.

TABLE 2

CHOLINEACETYLTRANSFERASE AND ACETYLCHOLINESTERASE ACTIVITIES (PERCENT OF CONTROL) IN FAST (EDL) AND SLOW (SOLEUS) MUSCLES IN SUBACUTE ADMINISTRATION OF PHY (70μg/kg, IM, TWICE DAILY FOR 2 WEEKS) AND/OR EXERCISED (ONCE DAILY FOR 2 WEEKS) RATS

			Cholineacetyltransferase		Acetylcholinesterase	
Group	Treatment	Time of Sacrifice After Treatment	EDL	Soleus	EDL	Soleus
I	Sedentary control	20 min	100%	100%	100%	100%
IIa	Trained exercise	20 min	67.8 ± 2.1*	123.9 ± 4.9*	57.1 ± 4.5	54.9 ± 3.6*
IIb	Trained exercise	24 h	86.5 ± 3.2	109.1 ± 2.9	79.2 ± 2.9	73.4 ± 2.8
IIIa	Subacute physostigmine	20 min	89.6 ± 3.3	94.4 ± 4.9	55.7 ± 7.1*	57.3 ± 7.3*
IIIb	Subacute physostigmine	24 h	110.5 ± 6.0	116.4 ± 4.8	68.6 ± 10.	67.1 ± 3.6*
IVa	Subacute physostigmine	20 min	96.7 ± 7.8	89.8 ± 11.1	62.8 ± 10.	31.7 ± 4.9*
	+					
IVb	Single acute exercise	24 h	105.1 ± 3.9	111.1 ± 8.9	88.5 ± 5.7	50.6 ± 7.3*
Va	Subacute physostigmine	20 min	65.3 ± 3.8*	98.4 ± 3.5	42.8 ± 2.8	29.2 ± 4.8*
	+					
Vb	Trained exercise	24 h	66.7 ± 5.6*	108.5 ± 7.2	62.8 ± 12.8	65.8 ± 4.8*

Values are mean ± SEM. Control value of ChAT in EDL 10.77 ± 0.23; soleus 8.81 ± 0.29 of ACh synthesized/mg protein/h. AChE control values in EDl 0.70 ± 0.13; soleus 0.82 ± 0.05 μmol/mg protein/h.
Statistical significance at $p < 0.05$.

acute Phy + trained exercise (Gr V) ChAT activity remained depressed even after 24 h in EDL muscle (Fig 1).

DISCUSSION

ChAT and AChE activities were found predominantly in cholinergic neurons (21) and specifically tend to be high at cholinergic synapses (12). Dettebarn has reported the distinctive difference in AChE activity in fast and slow muscles after reinnervation in rats (4). He also reported the effect of denervation and reinnervation on cholinergic enzymes in fast and slow muscles of rat (4). We observed a significant decrease in ChAT in fast twitch EDL muscle, but not in soleus muscle. This finding may be explained by considering the animal locomotion and the type of muscles involved in locomotion. Fast twitch muscles are active primarily during locomotion, whereas slow twitch muscles are active while the animals are at rest, regardless of their locomotive state (23,27). This active involvement of EDL during exercise may lead to a decrease in ChAT. But AChE was inhibited in both fast and slow muscles significantly due to endurance-trained exercise. This is contrary to Fernandez and Donoso (1988) who reported the increase in G_4 form AChE in fast twitch muscle due to exercise (8). We studied the total AChE instead of molecular forms and, moreover, the treadmill exercise was different from the protocol followed by the above authors. Their exercise protocol consisted of 1 to 2 h with a speed of 30–35 m/min once or twice a day, whereas our protocol was 20 min at progressive speeds only once a day (see the Method section, Table 1). The difference in exercise protocols may alter the enzyme activity. The total AChE activity decreased even the molecular forms (G_4 form) showed increase indicating the overall decrease of AChE. It was also reported that ChAT activity was reduced in the adrenal gland of young exercised rats, whereas there was no effect of exercise in the oldest group (28). Subacute Phy decreased ChAT activity slightly in both muscles but increased to above control level by 24 h. The initial decrease in ChAT indicates that Phy decreases the synthesis of ACh also, but the effect is temporary. AChE was significantly decreased in both muscles and remained depressed even after 24 h, possibly due to accumulation of Phy in these muscles after subacute Phy administration. Phy is a tertiary amine and is metabolized in peripheral tissue (29). Phy is a reversible ChE inhibitor; hence, one would expect AChE activity to return to control levels after a short time, but we observed a sustained inhibition of AChE even after 24 h. The administration of Phy to rats seems to alter the regulation of AChE as indicated by our results. In subacute Phy + single acute exercise group (Gr IV), only a transient decrease in ChAT was observed, whereas significant decrease was found in AChE in both muscles. In subacute Phy + endurance-trained exercise group (Gr V) a significant decrease in ChAT was observed in EDL but not in soleus, and remained depressed even after 24 h. Exercise decreased ChAT activity and Phy + exercise prolonged this effect up to 24-h period in EDL muscle. Soleus is not actively involved during exercise; hence, only a transient decrease in ChAT was observed in this muscle due to exercise. AChE was significantly decreased in both muscles in subacute Phy + trained exercise (Gr V) and remained depressed even after 24 h showing the cumulative effects of both stressors.

Exercise training is known to induce several ultrastructural and metabolic changes (15). We have reported these changes in metabolites, lactate, and pyruvate, in plasma due to exercise (2). Exercise decreased AChE activity in EDL muscle, and this muscle is very active during exercise. Our finding of reduction in ChAT activity in EDL muscle due to training is consistant with the recent finding that exercise decreases ChAT activity in adrenal gland of young rats (28). In trained exercise and Phy-administered rats, the AChE activities were significantly low even after 24 h in both the muscles. The inhibition was

more than 50% at 20-min period. However, subacute Phy + trained exercise affected ChAT only in EDL and not in soleus muscle. This is a significant observation because no change was expected in the passive soleus muscle. Our findings reveal that exercise not only affected the active EDL muscle in regard to ChAT activity but affected the slow soleus also by decreasing AChE activity. Several authors reported the altered AChE activity due to Phy administration (14,17,18). Exercise has prolonged the inhibition of AChE in both the muscles. Exercise and Phy not only has a cumulative effect immediately after exercise but also prolonged this effect up to 24 h. Recovery of AChE activity was observed in trained rats but not in trained + Phy-administered rats. Though further studies are needed to clearly elicit the mechanism of action of these two stressors, the present study indicates that a) physical exercise plays an important role in prolonging the action of the drug not only in active muscle like EDL but also in passive muscle like soleus; hence, it is important to consider other tissue along with the target tissue when evaluating for drug effects; and b) when evaluating an anticholinesterase agent for its effects, care should be taken to avoid a cumulative effect by a physical stressor like exercise. These results, when considered with our previous studies (24), provide evidence that the exercise and physostigmine additively decrease the AChE activity by modifying the functional activity of cholinergic system.

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