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Endurance Training Changes Central and Peripheral Responses to Physostigmine

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SOMANI, S. M. AND S. N. DUBE. *Endurance training changes central and peripheral responses to physostigmine.* PHARMACOL BIOCHEM BEHAV **41**(4) 773-781, 1992.—Whether the pharmacodynamics of physostigmine (Phy) [rate of decarbamylation of cholinesterase (ChE) enzyme] (K_d) is altered due to acute and/or trained exercise in brain and various tissues of rat has been addressed. Acute exercise (AE) + Phy increased, whereas endurance training (ET) + Phy decreased ChE activity in brain, red blood cells (RBC), and various tissues as compared to Phy alone. The K_d of brain ChE was significantly increased (181% of control) by AE + Phy and decreased (66% of control) by ET + Phy as compared to Phy alone. There was a slight increase (114% of control) in K_d of RBC-ChE in AE + Phy as compared to Phy alone. The K_d of heart ChE was significantly decreased (44% of control) by ET + Phy as compared to Phy alone. The K_d of diaphragm ChE was significantly increased (384% of control) in AE + Phy and decreased (80% of control) in ET + Phy as compared to Phy alone. The K_d of muscle ChE significantly decreased (67% of control) by AE + Phy as compared to Phy alone, but ET + Phy did not affect the K_d in muscle. These results suggested that AE and ET have opposite effects on K_d after Phy administration.

Cholinesterase in RBC and tissues Endurance-trained exercise Acute exercise Physostigmine
Rate of decarbamylation

PHYSICAL exercise evokes a number of enzymatic changes in the body, especially in muscles and liver (8,12,33). Exercise is one of the important factors that alter ChE activity (21,24). The intensity of these changes depends upon the type and severity of exercise (14,20).

Physostigmine (Phy), a reversible cholinesterase (ChE) inhibitor, is a centrally acting carbamate. Phy is a flow-limited, poorly plasma bound and highly extracted drug (29). Phy and its analogues have been shown to improve memory function (32) and are considered a potential pretreatment agent against organophosphate intoxication (7,9,10,27). Reversible ChE inhibitors are likely to be used on the battlefield or crop fields when a person is engaged in strenuous work. McMaster and Carney (19) demonstrated that acute exercise increases the behavioral sensitivity to Phy. We recently reported the in vivo kinetics of dose response of Phy and ChE activity in red blood cells (RBC) and tissues of rats (28). Earlier, Somani and Khalique (25,30,31) reported the pharmacokinetics and pharmacodynamics of Phy in rat after IV, IM, and oral administration.

The interaction of various drugs and exercise in man and animals has been reported widely (15,23). However, few reports are available regarding the interaction of exercise on the disposition and pharmacodynamics of drugs. The different forms of physical exercise do not necessarily alter the processes of drug absorption to the same extent, resulting in the varied pharmacodynamic action of drugs. If 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; how the different types of physical exercises would influence Phy-induced ChE activity needs to be considered during development of a potential pretreatment agent and therapy regimen. The combined effect of physical exercise and chemical stressor such as Phy on the cholinergic system has not received much attention.

Recently, we showed that Phy or physical exercise or combination of two stressors depressed choline acetyltransferase (ChAT) and/or acetylcholinesterase (AChE) activities in different brain regions differently and inconsistently, depending on the level of stress. The magnitude of the changes in ChE activity depends upon the type and severity of exercise. We have reported the K_d of butyryl cholinesterase (BuChE) in plasma and ChE in brain to be 0.11 min^{-1} and 0.027 min^{-1}, respectively, after administration of [^{3}H]-Phy (100 μg/kg, IV) (33). These rates were considerably higher than the elimination rate of Phy in plasma (0.046 min^{-1}) and in brain (0.063

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min^{-1}), indicating a longer-lasting effect of Phy than reflected by the drug level. It is likely that acute or trained exercise modifies the effects of reversible ChE inhibitors by altering the time course of ChE activity in RBC and tissues of rat. Therefore, this investigation addresses the question of whether the pharmacodynamics of Phy (K_d of ChE enzyme) alter due to acute and/or trained treadmill exercise in RBC and various tissues of rat.

METHOD

Chemicals

Physostigmine free base was obtained from Sigma Chemical Co. (St. Louis, MO). Ready-Solv scintillation cocktail was procured from Beckman Instruments, Inc. (Fullerton, CA). Drierite (anhydrous $CaSO_4$), procured from W.A. Hammond Drierite Co. (Xenia, OH), was used. The diagnostic kit was purchased from Sigma Chemical Co. for the determination of blood hemoglobin (Hb). [^{3}H]-acetylcholine (ACh) was obtained from DuPont Company, New England Nuclear Research Products (Boston, MA). All other chemicals were of analytical grade and were obtained from the usual commercial sources.

Animals

Male Sprague-Dawley rats (initial weight 160–200 g) were used.

Rats were divided into six groups:

- Group I—sedentary control (SC), saline administration
- Group II—acute exercise (80% $VO_{2\,max}$) (AE)
- Group III—endurance trained + acute exercise (80% $VO_{2\,max}$) (ET)
- Group IV—Phy (70 μg/kg, IM) (Phy)
- Group V—acute exercise (80% $VO_{2\,max}$) + Phy (70 μg/kg, IM) (AE + Phy)
- Group VI—endurance trained + acute exercise (80% $VO_{2\,max}$) + Phy (70 μg/kg, IM) (ET + Phy).

Endurance Training of Rats

Rats from Groups III and VI were acclimatized to treadmill in the beginning and were trained on a nine-channel motor-driven treadmill (built in our Southern Illinois University, School of Medicine workshop), utilizing an incremental exercise program 5 days a week for 6 weeks. During this program of exercise, the speed (meters/min), angle of inclination (degrees), and duration (min) of exercise were varied to obtain different levels of exercise intensity as shown in Table 1. In the first 2 weeks, conveyor belt speeds were 8.2, 15.2, and 19.3 m/min and the angle of inclination was 6°. Exercise duration at each speed was 5 min the first week and 10 min the second week. In the third and fourth weeks, speeds were maintained at 19.3, 26.8, and 30.3 m/min. The duration of exercise at each speed was 10 min. The angle of inclination was 6° during the third week and 9° in the fourth week. The final 2 weeks of exercise involved sustaining speeds of 19.3, 26.8, and 30.3 m/min at a 9° angle of inclination for 10 min at each speed.

TABLE 1

ENDURANCE TRAINING PROTOCOL FOR EXERCISING RATS

Week	Belt Speed (m/min)	Angle of Inclination (°)	Duration at Each Speed (min)
1	8.2, 15.2, 19.3	6	5
2	8.2, 15.2, 19.3	6	10
3	19.3, 26.8, 30.3	6	10
4	19.3, 26.8, 30.3	9	10
5	19.3, 26.8, 30.3	9	10
6	19.3, 26.8, 30.3	9	10

TABLE 2

PROTOCOL FOR EXERCISING RATS ON TREADMILL AT DIFFERENT GRADES AND SPEEDS FOR CONSTANT DURATION

Stage	Grade (°)	Speed (m/min)	Duration (min)
1	0	8.2	5
2	5	15.2	5
3	10	19.3	5
4	10	26.8	5
5	12.5	26.8	5
6	12.5	30.3	5
Recovery	0	2	5

Determination of maximum oxygen consumption ($VO_{2\,max}$) was carried out in the beginning of the training protocol to determine the $VO_{2\,max}$ for each rat. Measurement of maximal oxygen consumption (100% $VO_{2\,max}$) was considered valid only if the animal ran until it could no longer maintain pace with the treadmill. During the training, $VO_{2\,max}$ was determined for each rat the fifth day of every week.

Acute Bout of Exercise to Rats

Rats from Groups II, III, V, and VI were given an acute bout of exercise on the treadmill (Omnitech Electronics, Inc., Columbus, OH) at 80% $VO_{2\,max}$. The speed of the belt and angle of inclination were increased at different stages as shown in Table 2.

Oxygen consumption and heat production in individual rats undergoing different stages of exercise have been recorded once a week by an Omnitech Oxyscan Analyzer. Body weights were recorded every day for all groups.

Dosing and Sacrificing of Rats

On the day of the experiment, rats from Group I (SC) (eight rats) were administered saline and sacrificed. Rats from Group II (AE) were subjected to an acute bout of exercise (80% of $VO_{2\,max}$) and sacrificed at 2, 5, 10, 15, and 30 min. Rats from Group III (ET) were endurance trained, subjected to an acute bout of exercise (80% $VO_{2\,max}$) for 30 min using incremental exercise protocol, and decapitated at 5, 15, 30, and 60 min. Rats from Group IV (Phy) were administered Phy (70 μg/kg, IM) and sacrificed at 2, 5, 10, 15, 30, 45, and 60 min. Rats from Group V (AE + Phy) were subjected to acute exercise at 80% $VO_{2\,max}$ and soon after exercise Phy was administered (70 μg/kg, IM) and rats sacrificed at 2, 5, 10, 15, 30, 45, and 60 min. Rats from Group VI (ET + Phy) were endurance trained and subjected to an acute bout of exercise for 30 min at 80% $VO_{2\,max}$ and then administered Phy (70 μg/kg, IM) and sacrificed at 2, 5, 10, 15, 30, 45, and 60

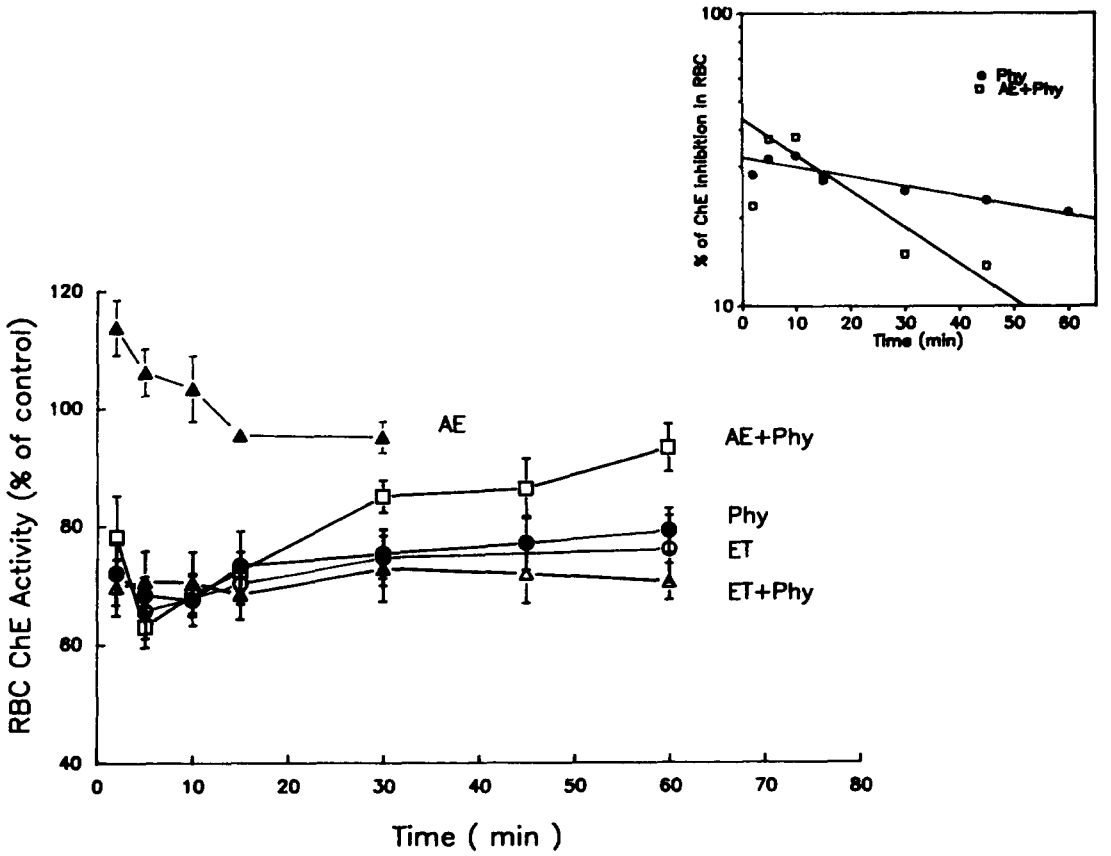

FIG. 1. Interaction of acute exercise (AE), endurance-trained exercise (ET) and physostigmine (Phy) (70 μg/kg, IM) on time course of % control ChE activity in RBC of rats. Values are mean ± SEM. Inset figure shows the rate of decarbamylation of ChE in RBC.

min. Four to six rats were sacrificed at each time point. Blood, brain, heart, diaphragm, and thigh muscle were collected for analysis of ChE activity.

Determination of ChE Activity

The ChE enzyme activity was determined by the radiometric method in RBC, brain, heart, diaphragm, and thigh muscle (16). The ChE values of RBC are expressed as μmol ACh hydrolyzed/min/g Hb content, whereas the tissue ChE values are expressed as μmol ACh hydrolyzed/min/g of wet weight of tissue.

Determination of Hb

Hb content of blood was determined by Sigma diagnostic kit using a Beckman Spectrophotometer at 540 nm.

Determination of K_d

The percent ChE inhibition was plotted on a semilog graph to obtain a declining slope representing the K_d of the enzyme. The best-fit lines were obtained by linear regression analysis and the correlation coefficient (r) was determined. However, the time points prior to maximum enzyme inhibition were excluded in estimating the best-fit line since they do not represent the recovery phase of the enzyme. The half-time of enzyme recovery ($T_{½}$) is the time taken for the percent inhibition to be reduced to 50% of its maximum value.

Statistical Analysis

Statistical analysis of six treatment groups were examined over eight time periods (0, 2, 5, 10, 15, 30, 45, and 60 min) using independent t-test. The criterion of statistical significance was $p < 0.05$.

RESULTS

Effect of Exercise on ChE Activity and K_d

The interaction of AE, ET, and Phy (70 μg/μCi/kg, IM) on time course of % control ChE activity in RBC and tissues are shown in Figs. 1-5. The K_d of ChE activity and $T_{½}$ for recovery of enzyme in RBC and tissues are presented in Table 3.

RBC

Acute exercise showed a transient increase in ChE activity of RBC (114% of control) at 2 min that returned back to almost control level after 10 min. Phy produced decrease in ChE activity with a peak effect at 10 min (67% of control) and recovered back to 79% of control at 60 min. ET decreased ChE activity to 66% of control at 5 min and was almost maintained up to 60 min (76% of control). ET + Phy further decreased ChE activity in RBC as compared to Phy alone (Fig. 1). Phy, AE + Phy, and ET + Phy produced maximum ChE

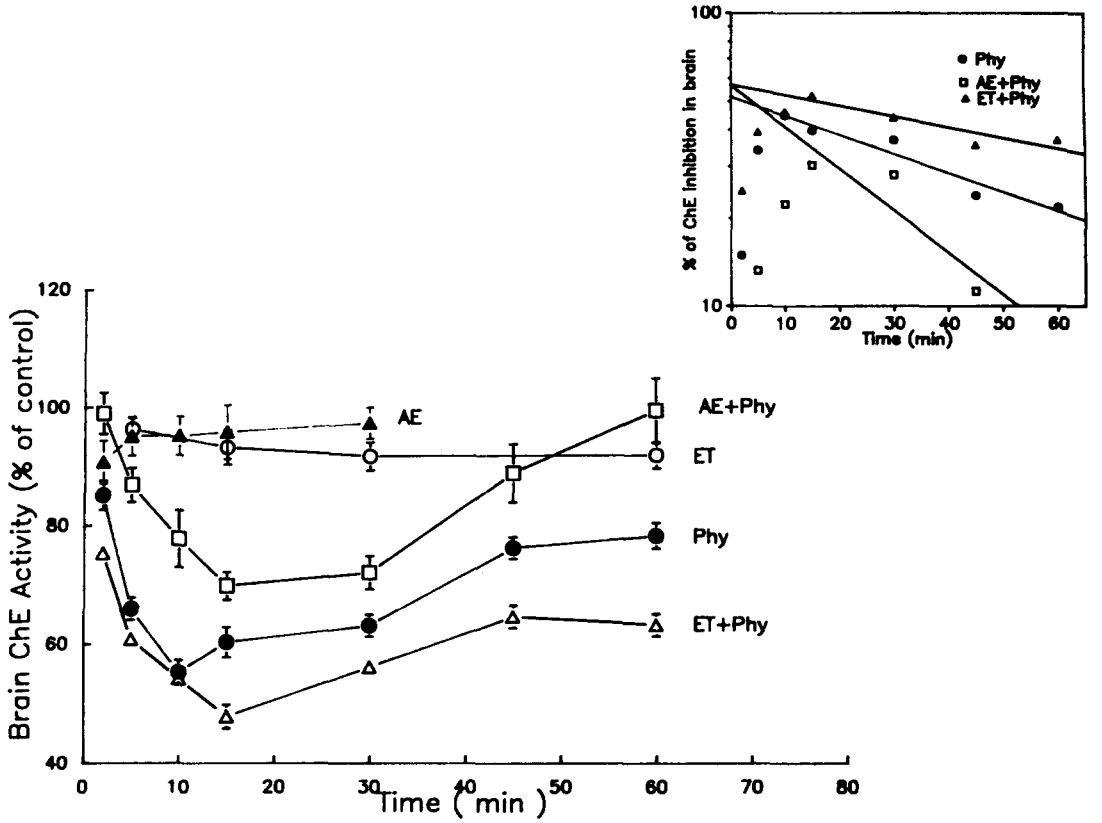

FIG. 2. Interaction of acute exercise (AE), endurance-trained exercise (ET) and physostigmine (Phy) (70 μg/kg, IM) on time course of % control ChE activity in brain of rats. Values are mean ± SEM. Inset figure shows the rate of decarbamylation of ChE in brain.

inhibition in RBC at 5–10 min. It seems that AE enhanced recovery, whereas training delayed the recovery, of Phy-inhibited ChE activity.

There was a slight increase (114% of control) in K_d of RBC-ChE in AE + Phy (0.024/min) as compared to Phy alone (0.021/min), with $T_{1/2}$ of enzyme recovery 29 and 33.5 min, respectively (Table 3).

Brain

AE, as well as ET, produced a slight decrease in ChE activity of brain (91–97% of control) at various time points, which was not statistically significant. AE + Phy showed an increase in ChE activity (70% of control) as compared to Phy alone (60% of control) at 15 min, which recovered to 99% of control at 60 min. Et + Phy showed further decrease in ChE activity (48% of control) ($p < 0.02$) at 15 min, which recovered to 64% of control ($p < 0.05$) at 60 min (Fig. 2). It seems that exercise delayed ChE recovery; however, there was almost complete recovery in AE + Phy (99% of control) and slower recovery in ET + Phy (63% of control) ($p < 0.05$) as compared to Phy alone (78% of control) at 60 min.

The K_d was significantly ($p < 0.02$) increased (181% of control) by AE (0.025/min) and decreased (66% of control) ($p < 0.05$) by ET (0.009/min) as compared to Phy alone (0.014/min) in brain (Table 3). The $T_{1/2}$ of recovery of enzyme was 50, 27.5, and 75 min in Phy, AE + Phy and ET + Phy groups, respectively (Table 3) (Fig. 2 inset).

Heart

AE slightly decreased the ChE activity of heart (89–95% of control) at various time points. AE + Phy showed a faster recovery of ChE activity (100% of control) as compared to Phy alone (89% of control) at 60 min. ET decreased ChE activity (85–75% of control) from 5–60 min. ET + Phy produced further decrease in ChE activity (58% of control) ($p < 0.05$) as compared to Phy alone (69% of control) or training alone (80% of control) at 30 min (Fig. 3). AE completely recovered ChE activity (101% of control); however, training further depressed ChE activity to 73% of control as compared to Phy alone (89% of control) at 60 min.

The K_d was significantly ($p < 0.02$) decreased (44% of control) by ET (0.008/min) as compared to Phy alone (0.019/min). ET significantly ($p < 0.05$) increased the $T_{1/2}$ of recovery of enzyme (85 min) as compared to Phy alone (37.5 min) (Fig. 3 inset).

Diaphragm

AE did not produce any significant effect on ChE activity of diaphragm (95–98% of control) at various time points. AE + Phy showed an increased ChE activity (73% of control) as compared to Phy alone (66% of control) at 15 min. ET decreased ChE activity 85–78% of control from 5–60 min. ET + Phy showed further decrease in ChE activity (50% of control) as compared to Phy alone at 15 min (Fig. 4). There was

faster ChE inhibition, as well as recovery by AE (102% of control), as compared to Phy alone (82% of control) at 60 min. ET delayed ChE inhibition, as well as recovery of ChE activity.

The K_d of ChE activity was very significantly ($p < 0.01$) increased (384% of control) in AE + Phy (0.039/min) and decreased (80% of control) in ET + Phy rats (0.008/min) as compared to Phy alone (0.01/min) (Fig. 4 inset). The $T_{½}$ of recovery of enzyme was 67.5, 17.5, and 84 min in Phy, AE + Phy, and ET + Phy groups, respectively (Table 3).

Muscle

AE did not produce any significant effect on ChE activity in muscle (99–105% of control) at various time points. ET produced decrease in ChE activity (89–79% of control) from 5–60 min. AE + Phy, as well as ET + Phy, further depressed ChE activity up to 10 min, which was maintained up to 60 min in ET + Phy. However, AE + Phy showed increased ChE activity from 15–45 min as compared to Phy alone (Fig. 5). AE enhanced recovery, whereas training delayed recovery of ChE activity.

The K_d of ChE activity significantly ($p < 0.05$) decreased (67% of control) by acute exercise (0.008/min) as compared to Phy alone (0.012/min). However, Et did not affect the K_d (Fig. 5 inset). The $T_{½}$ of enzyme recovery was 55, 83.5, and 60 min in Phy, AE + Phy, and ET + Phy groups, respectively.

DISCUSSION

Effect on Metabolic Variables and Weight Gain

Prolonged exercise for 6 weeks did not produce any significant effect on the metabolic variables, especially during the initial stages of an incremental exercise protocol. However, the oxygen (O_2) consumption was slightly decreased due to ET of rats over a period of 6 weeks as compared to age-matched sedentary control rats.

Effect of Exercise on ChE Activity and K_d

The K_d of Phy-inhibited ChE varies in different tissues. The K_d is highest in RBC (0.021/min) and least in diaphragm (0.01/min) with intermediate rate in heart, brain, and muscle (0.019–0.012/min). The K_d in descending order is RBC > heart > brain > muscle > diaphragm. The $T_{½}$ of recovery of enzyme also follows the same pattern. AE (about 80% $VO_{2\,max}$) showed a transient increase in ChE activity of RBC (114% of control) at 2 min that returned back to control level after 10 min. We recently reported (4) that AE (80% $VO_{2\,max}$) produced a slight increase in ChE activity of RBC (112% of control). This increase in ChE activity of RBC may be due to secondary effect of hypoxia, increased hemoconcentration, and sequestration of RBC from spleen during initial time points to cope with the increased demand of the body during exercise (20). Later, there may be a cholinergic acclimatization

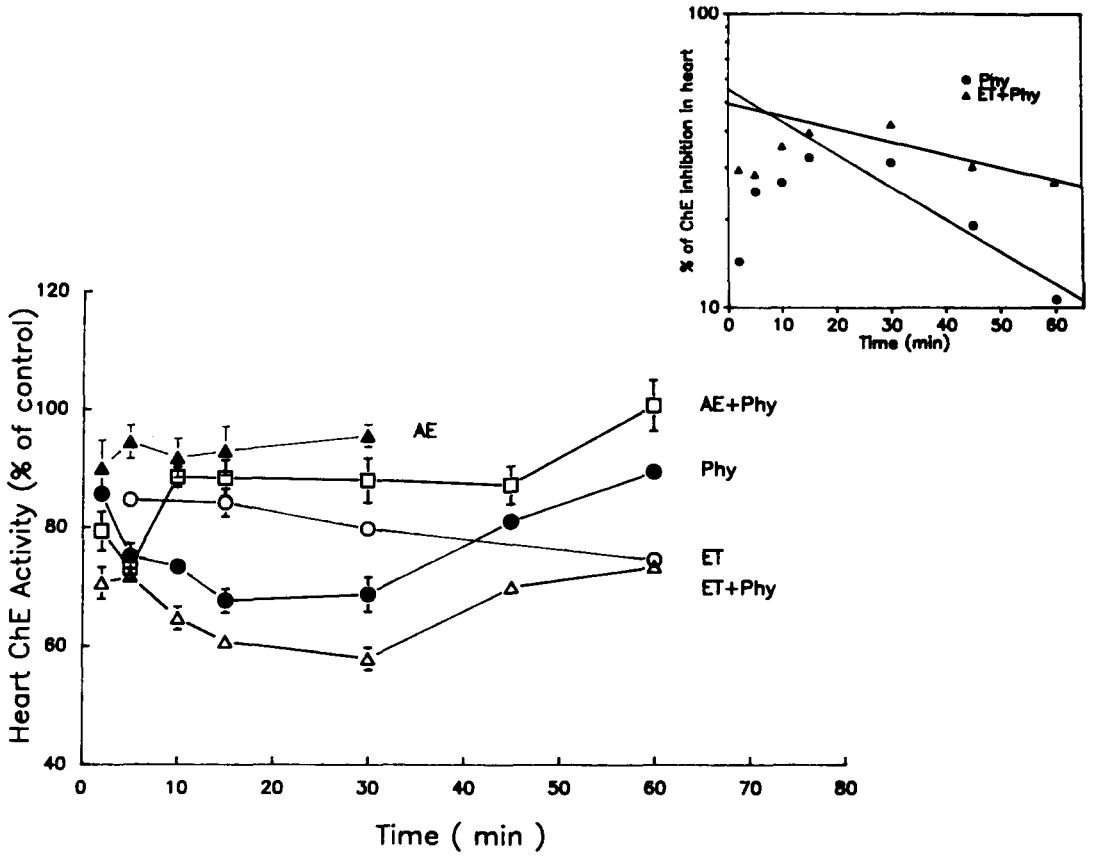

FIG. 3. Interaction of acute exercise (AE), endurance-trained exercise (ET) and physostigmine (Phy) (70 μg/kg, IM) on time course of % control ChE activity in heart of rats. Values are mean ± SEM. Inset figure shows the rate of decarbamylation of ChE in heart.

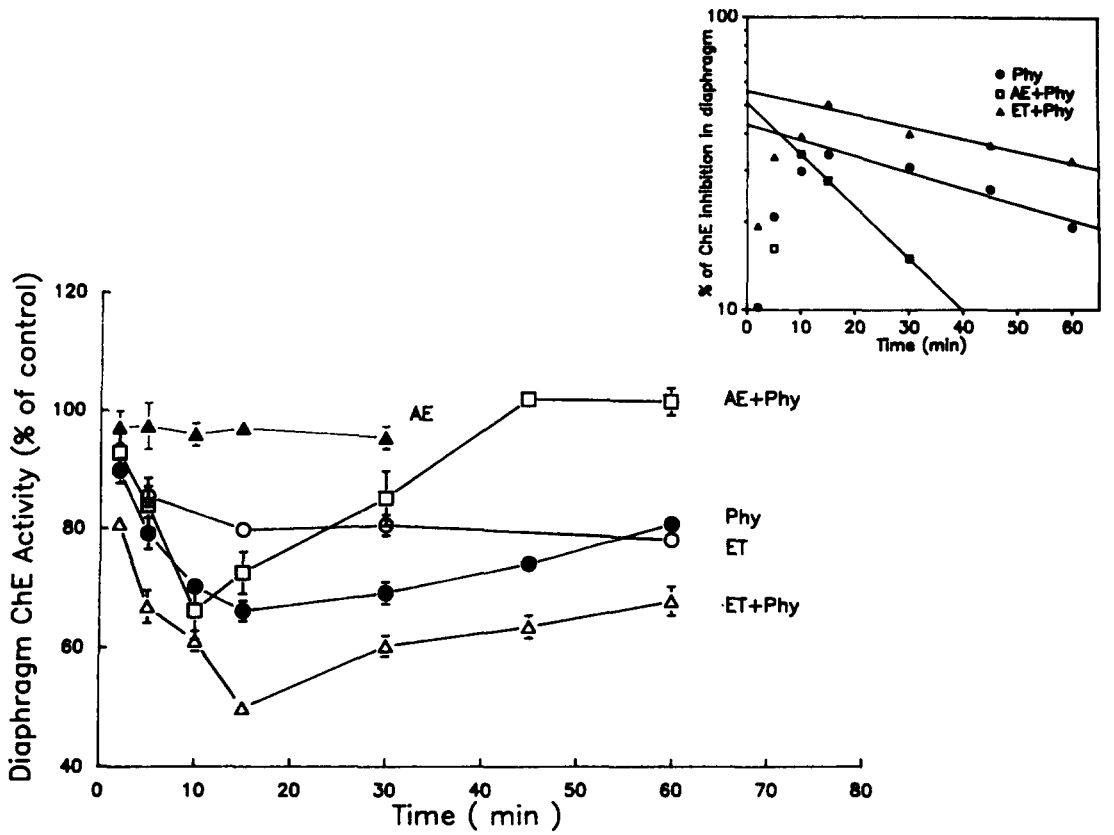

FIG. 4. Interaction of acute exercise (AE), endurance-trained exercise (ET) and physostigmine (Phy) (70 μg/kg, IM) on time course of % control ChE activity in diaphragm of rats. Values are mean ± SEM. Inset figure shows the rate of decarbamylation of ChE in diaphragm.

after 10 min. Endurance training followed by an acute bout of exercise significantly decreased the ChE activity of RBC during 5–60 min.

The results show that Phy alone (70 μg/kg, IM) produced a significant decrease in ChE activity in RBC and various tissues, which corresponded with our earlier results (26). The maximum ChE inhibition varied 23–40% between 5 and 15 min. Several workers (9,10,13) have also shown the maximum effect of Phy to be within 15–30 min after its IM administration.

There appears to be controversy regarding the extent of ChE inhibition by Phy in various tissues of several animal species following different routes of administration (13,18). Harris et al. (10) reported 58% ChE inhibition in whole blood at 15 min with 70-μg/kg IM dose of Phy to rat. Heyl et al. (13) have also shown ChE inhibition (70%) in whole blood at 15 min after 250 μg/kg Phy, IM, to rabbits. These authors determined the total ChE present in whole blood, which comprises true cholinesterase (AChE) and pseudocholinesterase (BuChE). It is understandable that our value of ChE inhibition in RBC (27%) is lower because we determined ChE enzyme in RBC and not in whole blood. The present findings on the extent of ChE inhibition are well corroborated with our earlier findings (28).

AE enhanced the recovery of Phy-induced ChE inhibition in RBC and various tissues. However, ET potentiated the effect of Phy on ChE inhibition, thereby resulting in slower recovery of the enzyme. The modulatory effect of acute and endurance-trained exercise on the pharmacodynamic effect of Phy may be due to several factors such as blood flow, rate of metabolism, clearance, plasma protein binding, and pH changes.

Phy is a flow-limited drug; therefore, its disposition and pharmacokinetics are influenced by exercise (29). We have shown that trained exercise increased the area under the curve (AUC) from 579 to 834 ng/ml min and half-life from 8.8 to 15.7 min as compared to Phy alone (26). We also reported the decrease in clearance from 121 to 84 ml/min/kg, as well as rate of elimination from 0.08 to 0.04/min as compared to Phy alone. Several authors have reported a decrease in clearance of many drugs by exercise [see the review by Somani et al. (29)].

AE enhanced the K_d in RBC, brain, and diaphragm; however, there was a decrease in K_d in muscle by AE. ET decreased the K_d in brain, heart, diaphragm, and muscle. Different types of exercises have been reported to affect the blood flow in different ways (15). Change in blood flow will affect the amount of drug reaching the receptor sites in tissues, thus affecting pharmacodynamic activity. The main factor affecting drug absorption following IM administration is a change in blood flow (11).

During exercise, there is considerable increase in cardiac output with a redistribution of blood flow to different organs (15,29). The blood flow to other organs changes with exercise. Several studies demonstrated the relationship between blood flow and exercise (15,23). Studies on the effect of different

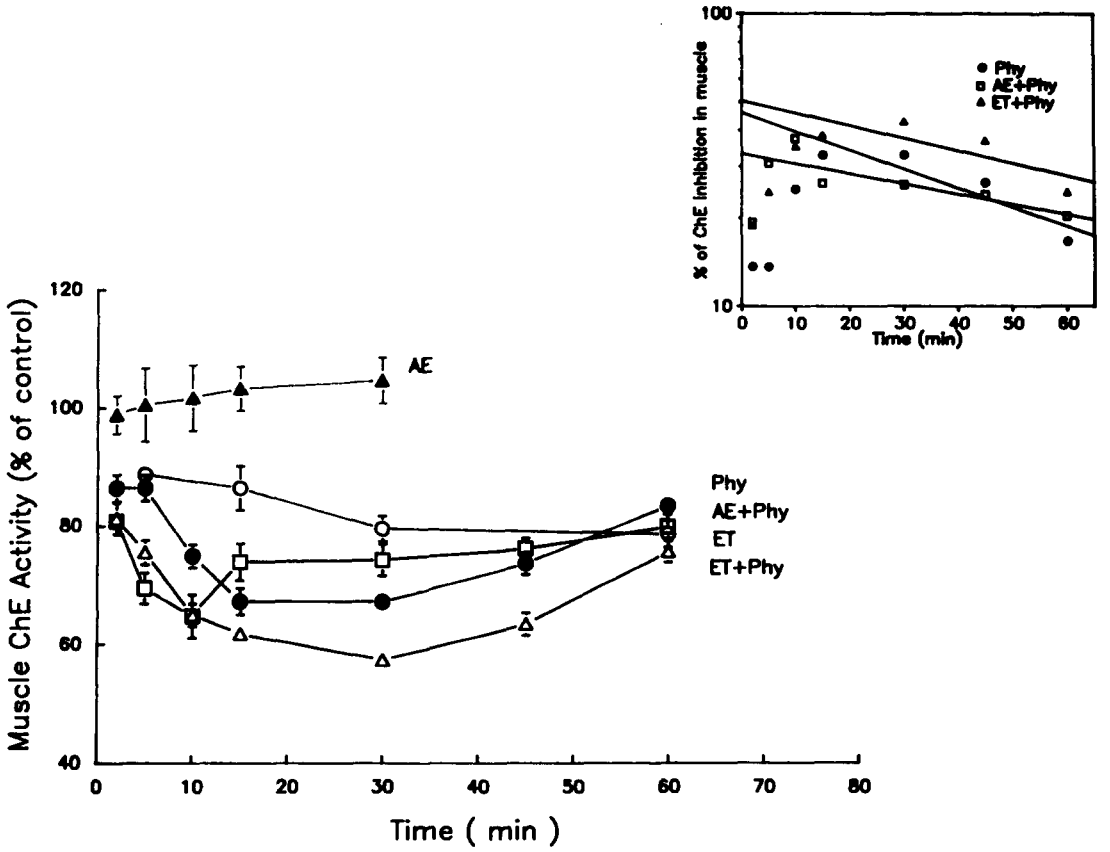

FIG. 5. Interaction of acute exercise (AE), endurance-trained exercise (ET) and physostigmine (Phy) (70 μg/kg, IM) on time course of % control ChE activity in muscle of rats. Values are mean ± SEM. Inset figure shows the rate of decarbamylation of ChE in muscle.

TABLE 3

EFFECT OF ACUTE OR TRAINED EXERCISE ON RATE OF DECARBAMYLATION (K_d) OF ChE

		Group IV (Phy)	Group V (AE + Phy)	Group VI (ET + Phy)
RBC	K_d min^{-1}	0.021	0.024	–
	r	0.93	0.95	–
	$T_{½}$ min	33.5	29	–
Brain	K_d min^{-1}	0.014	0.025	0.009
	r	0.97	0.90	0.91
	$T_{½}$ min	50.0	27.5	75.0
Heart	K_d min^{-1}	0.019	–	0.008
	r	0.95	–	0.89
	$T_{½}$ min	37.5	–	85.0
Diaphragm	K_d min^{-1}	0.01	0.039	.008
	r	0.97	0.99	0.98
	$T_{½}$ min	67.5	17.5	84.0
Muscle	K_d min^{-1}	0.012	0.008	0.012
	r	0.91	0.89	0.79
	$T_{½}$ min	55.0	83.5	60.0

In min^{-1} of ChE in RBC and tissues of rat. r is the correlation coefficient for % ChE inhibition vs. time for the declining curve. $T_{½}$ is the half-time in min for recovery of ChE enzyme.

intensities of exercise in human beings have shown that moderate exercise increases the blood flow to threefold in heart and tenfold in muscle without affecting brain. The total blood flow increases to threefold by moderate exercise (15). In the present studies, AE (80% $VO_{2\,max}$) corresponds to moderate exercise. Thus, AE helped in quicker absorption, and possibly an increased disposition of Phy, resulting in faster recovery of ChE activity as compared to Phy alone. However, the prolonged exercise (ET for 6 weeks) delayed disposition and metabolism of Phy (26), which might be the cause for prolonged effect of Phy, producing further decrease in ChE activity as compared to Phy alone. Ramos et al. (22) and Day et al. (3) have also shown that chronic exercise decreased the metabolism of hexobarbitone, resulting in increased sleeping time. It is possible that prolonged moderate exercise (ET) might be decreasing the hepatic blood flow, resulting in decreased clearance and rate of elimination of Phy (26), thereby potentiating the effect of Phy on ChE inhibition.

In the present studies, we determined the ChE activity of thigh muscle in general and not in slow and fast muscle separately. Hence, we could not observe any significant change in ChE activity of muscle by acute exercise. It has been reported that muscle blood flow can vary as much as 19% (5) in different muscles, and there are large individual variations in drug absorption. Pedzikiewicz et al. (21) reported an increase in muscle ChE activity (20%) after a short physical exercise. This increase may be related to an increase in blood flow in skeletal muscles (1). However, muscarinic cholinergic receptors do not

play a significant role in elevating muscle blood flow in conscious rats either during the anticipatory phase or during slow locomotor exercise (2). Selective increase in G_4 AChE activity of adult male Fischer rats subjected to treadmill exercise has been reported by Fernandez and Donoso (6).

The present study also indicates that acute or endurance-trained exercise for 6 weeks did not significantly alter ChE activity in brain. This is in agreement with the commonly held belief that physical exercise does not alter blood flow to the brain. However, AE, as well as training, both modify the pharmacodynamic effect of Phy on ChE activity in brain. It is possible that free drug concentration might be decreased during AE and may be increased during Et in the brain compared to Phy administered to the sedentary control rat.

In conclusion, AE transiently increased ChE activity in RBC, which returned to normal within 10–15 min. ET decreased ChE activity of RBC without affecting other tissues. AE enhances the K_d and thus decreases $T_{1/2}$ of recovery of enzyme as compared to Phy alone. However, ET potentiates ChE inhibition in RBC and various tissues due to decreased clearance of Phy. ET for 6 weeks may be beneficial to prolong and potentiate the ChE inhibition by Phy. Phy has a low margin of safety—a slight increase in dose causes toxic symptoms. ET may help reduce the required dose of Phy, thereby reducing its toxic effects. This may be advantageous if Phy is used as a pretreatment drug against organophosphate intoxication on the battlefield.

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