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Concurrent Acute Exercise Alters Central and Peripheral Responses to Physostigmine

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DUBE, S. N., S. M. SOMANI AND S. R. BABU. *Concurrent acute exercise alters central and peripheral responses to physostigmine.* PHARMACOL BIOCHEM BEHAV 46(4) 827–834, 1993.—This study reports the modulatory effects of physostigmine (Phy) and concurrent acute exercise on the time course of cholinesterase (ChE) activity, the rate of decarbamylation (K_d), and half-time of recovery of ChE in red blood cells (RBC) and various tissues of rats. Acute exercise equivalent to 80% VO_2max (maximal oxygen consumption) transiently increased the RBC ChE activity, whereas Phy decreased ChE activity in RBC and various tissues. Physostigmine along with concurrent acute exercise increased the K_d in RBC, brain, and heart by 56.4%, 66.7%, and 139%, respectively, compared to Phy alone. The K_d in diaphragm and muscle decreased to 14.1% and 56.2%, respectively, compared to Phy alone. The variation in K_d might be due to the effect of concurrent acute exercise on the redistribution of Phy in various tissues of rat as a result of changes in blood flow.

Cholinesterase in RBC and tissues Concurrent acute exercise Physostigmine Rate of decarbamylation

PHYSOSTIGMINE (Phy) is a centrally acting reversible anticholinesterase agent and is considered to be a potential prophylactic agent against organophosphate intoxication (7,9, 13,20). Physostigmine and its analogues have been shown to improve memory function (6). The pharmacodynamics of Phy are likely to be altered by concurrent exercise due to changes in blood flow to liver and changes in pH values of muscle. During exercise, cardiac output increases with the increase in intensity of workload, and concomitant changes in regional blood flow distribution occurs. Thus, the blood flow to skeletal muscle and skin is greatly increased, while the hepatic blood flow decreases during exercise (1,4,10). Exercise increases the functional capacity of the cardiovascular system and decreases myocardial oxygen demand (14). The rates of absorption, distribution, metabolism, and excretion are most important in determining the duration of action of Phy, which are likely to be altered during exercise (24). In turn, these processes will also affect the ChE activity because the enzyme is inhibited by Phy. The changes that occur in the cholinesterase system due to physical exercise and centrally acting anticholinesterases have not received much attention.

We have previously reported that the postexercise administration of Phy influenced the ChE inhibition in RBC and brain, irrespective of the intensity of exercise (3). Physostigmine, exercise, and the combination of both decrease brain choline acetyltransferase (ChAT) and/or acetylcholinesterase (AChE) activities in a regionally selective pattern (19). Physostigmine and trained exercise decreased the ChAT and AChE levels in extensor digitorum longus (EDL) and soleus muscles of rat (2). We have also reported that post acute exercise and post endurance training followed by Phy administration have opposite effects on rate of decarbamylation of this drug (22). One potential use of reversible anticholinesterases (such as Phy) is as a pretreatment drug against nerve agents. In this case, the drug(s) will be administered to military personnel who undergo intense physical trainng. Since the time course of a flow-limited drug such as Phy is influenced by exercise dynamics (24) and since Phy has a low margin of safety (a slight increase in dose causes toxic symptoms), any alterations in the half-life of Phy due to physical exercise would alter the pharmacodynamic effect. Therefore, the purpose of this investigation is to study the effect of concurrent exercise on the pharmacodynamics of Phy (or any other carbarmates), which would potentially be administered under combat field

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TABLE 1

PROTOCOL FOR EXERCISING RATS ON TREADMILL, AT DIFFERENT GRADES (ANGLES) AND SPEED FOR CONSTANT DURATION

Stage	Angle of Inclination	Speed (m/min)	Duration at Each Speed (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

conditions. It is important to determine the change in ChE activity in RBC and various tissues of rat following concurrent exercise to develop a rational therapy regimen.

METHOD

Chemicals

Physostigmine (Phy) free base was obtained from Sigma Chemical Co. (St. Louis, MO); [^{3}H]Phy (13 Ci/mmol) was custom synthesized by Amersham Corporation (Chicago, IL). Ready-Solv EP was procured from Beckman Instruments Inc. (Fullerton, CA). Drierite (anhydrous $CaSO_4$), procured from W. A. Hammond Drierite Co. (Xenia, OH). Diagnostic kit for the determination of blood hemoglobin was purchased from Sigma Chemical Co. All other chemicals were analytical grade and were obtained from the usual commercial sources.

[^{3}H]Physostigmine Solution

Physostigmine was labeled with tritium on both ortho positions to the carbamate chain on the aromatic ring of Phy. [^{3}H]Physostigmine was diluted with unlabeled Phy (162.07 μCi/140 μg/ml). The solution was prepared using physiological saline (0.9% w/v) in which 10 μl of hydrochloric acid was added to assure that the solution was in an acidic range. The purity of Phy was assessed using high performance liquid chromatography (HPLC) by ultraviolet detector and also by monitoring the [^{3}H]Phy in the eluant using liquid scintillation counter. The solution used in all experiments was greater than 95% pure.

Animals

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) weighing 175-200g were divided into four groups:

- Group I—sedentary control (SC) saline administration
- Group II—acute exercise (80% VO_2max) (AE)
- Group III—Phy (70 μg/kg, IM) (Phy)
- Group IV—Phy (70 μg/kg, IM) + concurrent acute exercise (80% VO_2max) (CAE + Phy).

Physostigmine Administration and Exercising of Rats on a Treadmill

The Oxyscan System and Omnipacer Treadmill (Omnitech. Inc., Columbus, OH) were used to monitor the maximal oxygen consumption (VO_2max) as reported earlier (22). The rats from groups II and IV were exercised on the treadmill as described in Table 1 to obtain 100% VO_2max for each rat. After 3 days, the rats from group II were exercised at different speeds and inclinations for 20 min, corresponding to approximately 80% VO_2max. Rats from group IV were administered Phy and then exercised for 20 min. The rats from groups II and IV were sacrificed at 20, 22, 25, 30, 35, and 50 min after Phy administration or start of exercise. Rats from group III were administered Phy (70 μg/kg, IM) and were sacrificed at 20, 22, 25, 30, 35, and 50 min. Sedentary control rats (group I) were administered saline and were sacrificed after 15 min.

A minimum of animals were sacrificed in each group between 0800 and 1100 h to minimize circadian cycle effects. Each group consisted of four rats. Soon after the decapitation, blood, brain, heart, diaphragm, and thigh muscle were collected. The blood was processed for the determination of RBC ChE. The tissues were stored at −80°C until analysis for ChE determination.

Determination of ChE Activity

The ChE enzyme activity was determined by the modified radiometric method of Johnson and Russell (11). The ChE activity in RBC, brain, heart, diaphragm, and thigh muscle

TABLE 2

RATE OF DECARBAMYLATION (K_d) OF ChE IN RBC AND TISSUES OF RAT

	Physostigmine			Physostigmine + Exercise		
	r	K_d (min^{-1})	$T_{1/2}$ (min)	r	K_d (min^{-1})	$T_{1/2}$ (min)
RBC	0.96	0.0165	42.0	0.91	0.0258*	26.8
Brain	0.98	0.0231	30.0	0.90	0.0385*	18.0
Heart	0.92	0.0252	27.5	0.98	0.0602*	11.5
Diaphragm	0.85	0.0078	88.8	0.88	0.0067	103.4
Muscle	0.95	0.0308	22.5	0.94	0.0135*	51.3

These rats were administered Phy (70 μg/kg,IM) and Phy + concurrent acute exercise (80% VO_2 max)(n = 4).

r = the correlation coefficient for % ChE inhibition vs. time for the declining curve

$T_{1/2}$ = half time in min for recovery of ChE enzyme.

*Statistical significance at $p < 0.05$ compared to Phy alone.

was determined as reported in our previous paper (21). In this procedure, [^{3}H]ACh is used as the substrate. This method measured the radioactivity due to [^{3}H]acetate formed by the enzymatic hydrolysis of [^{3}H]ACh. The substrate is prepared daily by mixing 0.5 M Tris buffer (0.25 M Trizma base, 0.25 M Tris-HCl, 1.2 M NaCl, pH 7.4), ACh chloride (Cl) (0.1 mmol for RBC, diaphragm, heart, and thigh muscle; 1 mmol for brain) and [^{3}H]acetylcholine iodide (AChI) (1 mCi/0.01 mmol).

The hemoglobin content of blood was determined by Sigma Diagnostic Kit using a Beckman Spectrophotometer at 540 nm. The ChE values of RBC were expressed as μmol of ACh hydrolyzed/min/g of hemoglobin, whereas the tissue ChE values were expressed as μmol of ACh hydrolyzed/min/g wet weight of tissue.

Determination of Rate of Decarbamylation

The percent ChE inhibition vs. time in RBC and various tissues was plotted on semilog graph to obtain a declining slope representing the rate of decarbamylation (K_d) of the enzyme (23).

Statistical Analysis

The best fit lines were obtained by linear regression analysis for various time periods and the correlation coefficient (r) was determined. The ChE values were subjected to Student's t-test. Significant differences were accepted at $p < 0.05$.

RESULTS

Effect on ChE Activity and Rate of Decarbamylation

The effect of Phy + concurrent acute exercise on time course of ChE activity in RBC and various tissues of rats has been presented in Figs. 1-5. The rate of decarbamylation (K_d) of ChE enzyme is shown as the inset in Figs. 1-5 and in Table 2.

RBC

The ChE activity in RBC was 112%, 103%, and 95% of control at 20, 30, and 50 min, respectively, after acute exercise (Fig. 1). Phy depressed ChE activity 74%, 79%, and 84% ($p < 0.01$) of control at 20, 30, and 50 min, respectively. Phy + concurrent acute exercise further depressed the ChE activity significantly to 53% and 73% of control at 20 and 30 min, respectively, and recovered to 83% of control by 50 min (Fig. 1). There was no significant change in ChE activity at 50 min in Phy alone or Phy + concurrent acute exercise.

Figure 1 inset and Table 2 show that there was a significant increase (156% of control) in rate of decarbamylation of ChE enzyme in Phy + concurrent acute exercise (0.0258 min^{-1}) compared to Phy alone (0.0165 min^{-1}) in RBC.

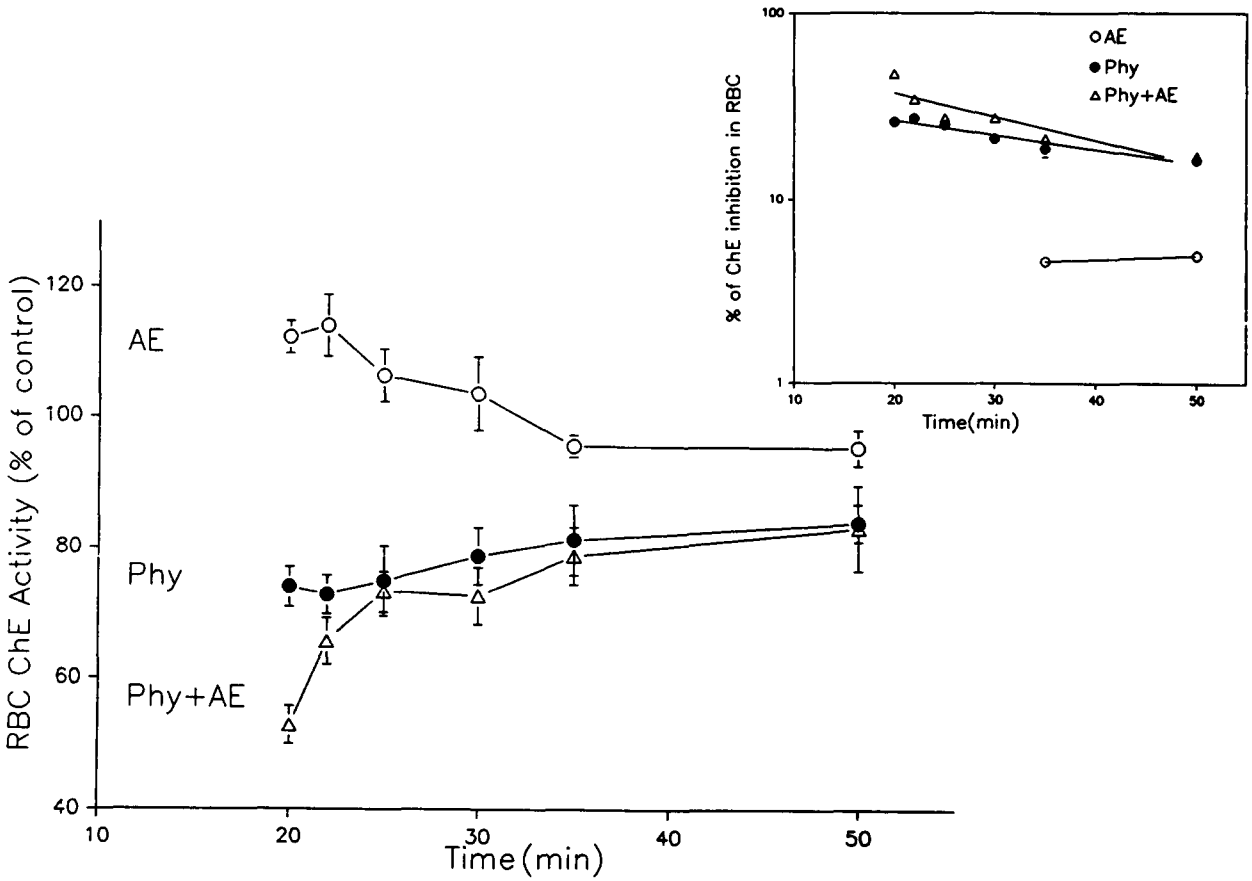

FIG. 1. Effect of acute exercise (AE) (80% VO_2max), Phy (70 μg/kg), and Phy then concurrent AE on time course of percent control ChE activity in RBC of the rat. Values are mean ± SEM. Inset shows the rate of decarbamylation (K_d) of ChE enzyme in RBC.

Brain

Acute exercise showed a small decrease in ChE activity in the brain (88–97% of control from 20–50 min). Phy decreased ChE activity 67%, 77%, and 85% ($p < 0.01$) of control at 20, 30, and 50 min, respectively. Phy + concurrent acute exercise significantly depressed the ChE activity to 54% ($p < 0.05$) and 70% of control at 20 and 25 min, respectively (Fig. 2), but recovered to 85–86% of control within 50 min.

There was a significant increase (167% of control) in the rate of decarbamylation of ChE enzyme in Phy + concurrent acute exercise (0.0385 min^{-1}) compared to Phy alone (0.0231 min^{-1}) in the brain (Fig. 2 inset and Table 2).

Heart

Acute exercise decreased ChE activity in the heart to 87% ($p < 0.05$), 92%, and 95% of control at 20, 30, and 50 min, respectively (Fig. 3). Phy also depressed ChE activity 68%, 75%, and 85% ($p < 0.01$) of control at 20, 30, and 50 min, respectively. Phy + concurrent acute exercise showed 78%, 85%, and 95% of control ChE activity at 20, 30, and 50 min, respectively, indicating that concurrent acute exercise has decreased the effect of Phy by increasing the ChE activity at 20, 22, 30, and 50 min ($p < 0.05$) compared to Phy alone.

The rate of decarbamylation was significantly increased to approximately 2.5-fold (239% of control) in Phy + concurrent acute exercise (0.0602 min^{-1}) compared to Phy alone (0.0252 min^{-1}) in the heart (Fig. 3 inset and Table 2).

Diaphragm

Acute exercise did not alter ChE activity in the diaphragm from 20–50 min (Fig. 4). Phy depressed ChE activity significantly 67%, 70%, and 75% of control at 20, 30, and 50 min, respectively ($p < 0.01$). Phy + concurrent acute exercise produced ChE activity 70%, 76%, and 77% of control at 20, 30, and 50 min, respectively (Fig. 4).

The rate of decarbamylation of ChE activity was slightly decreased (86% of control) in Phy + concurrent acute exercise (0.0067 min^{-1}) compared to Phy alone (0.0078 min^{-1}) in the diaphragm (Fig. 4 inset and Table 2).

Muscle

Acute exercise did not alter the muscle ChE activity at 20, 30, and 50 min (Fig. 5). Phy depressed ChE activity 59%,

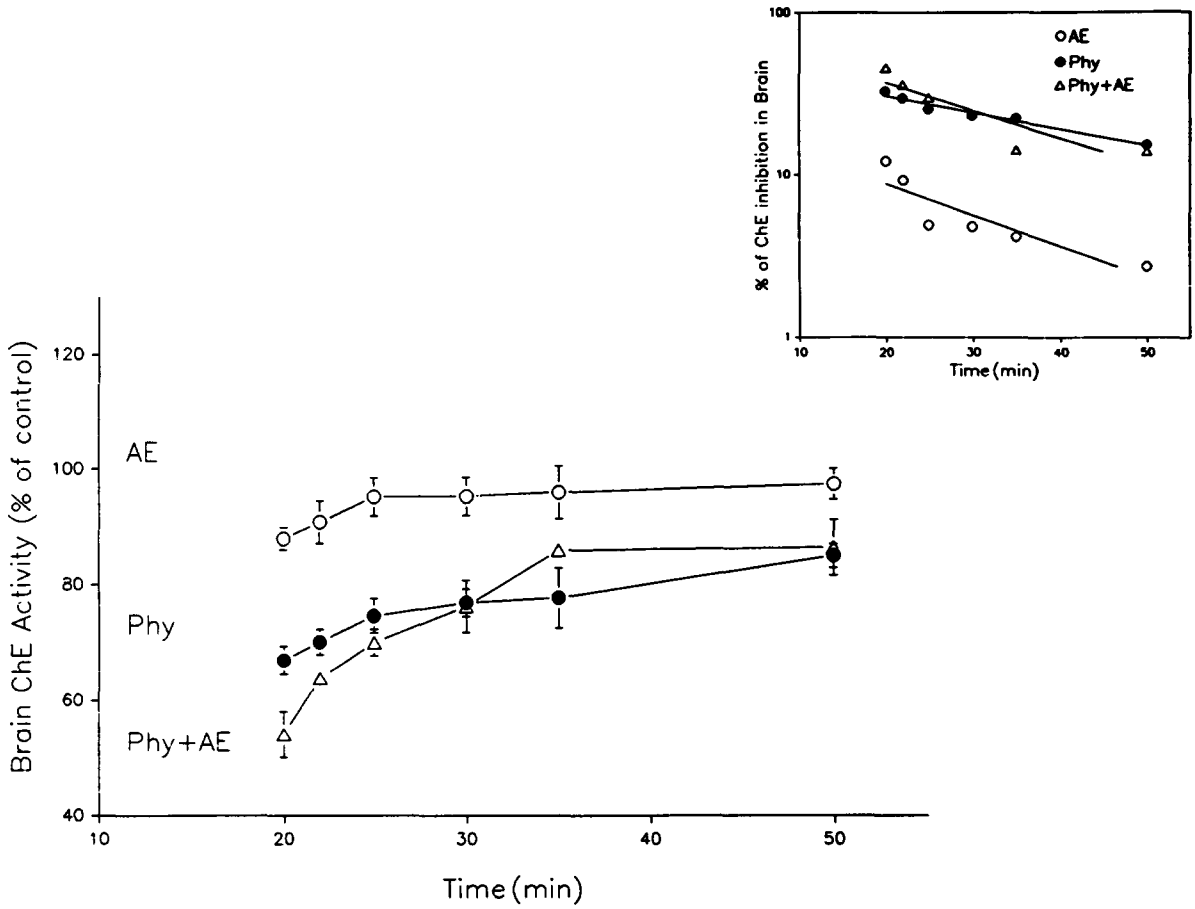

FIG. 2. Effect of acute exercise (AE) (80% VO_2max), Phy (70 μg/kg), and Phy then concurrent AE on time course of percent control ChE activity in brain of rat. Values are mean ± SEM. Inset shows the rate of decarbamylation (K_d) of ChE enzyme in the brain.

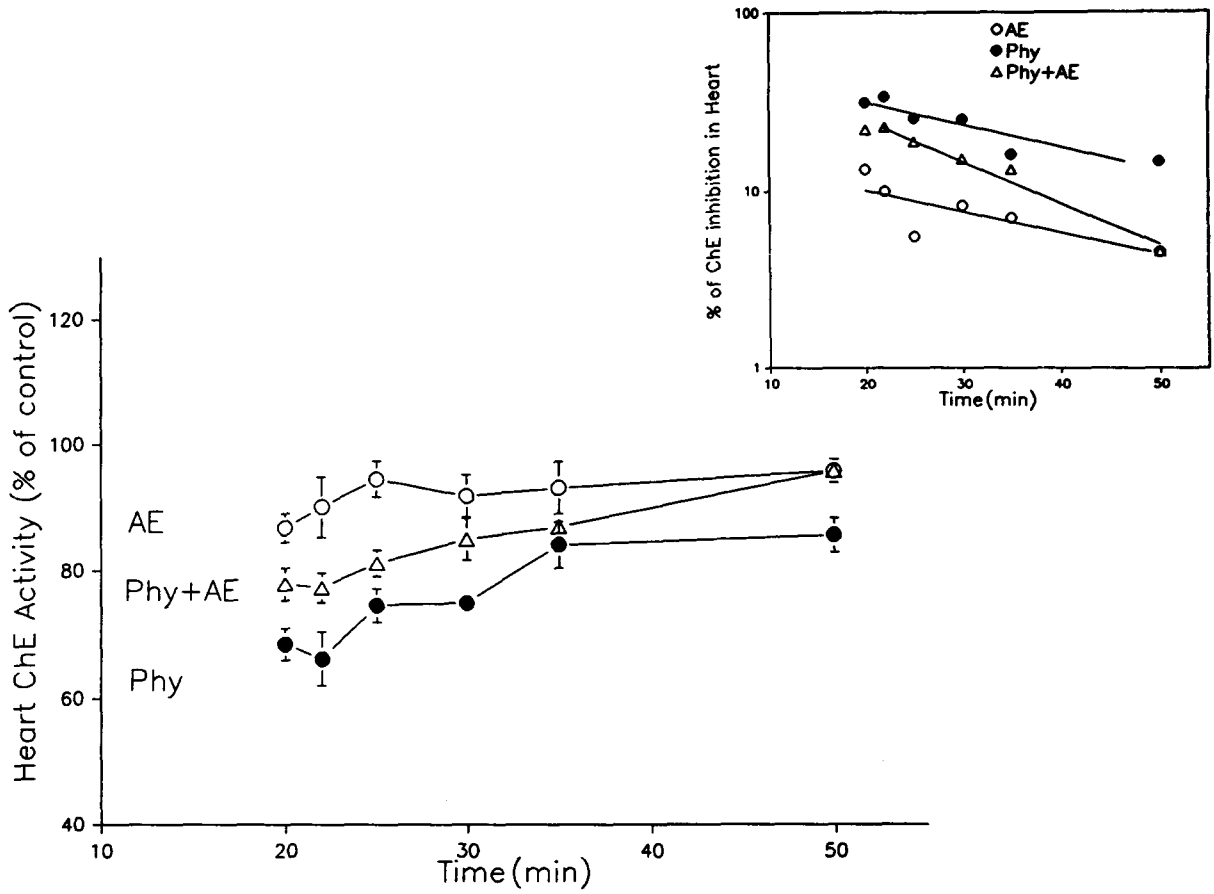

FIG. 3. Effect of acute exercise (AE) (80% VO_2max), Phy (70 μg/kg), and Phy then concurrent AE on time course of percent control ChE activity in the heart of the rat. Values are mean ± SEM. Inset shows the rate of decarbamylation (K_d) of ChE enzyme in the heart.

69%, and 83% of control at 20, 30, and 50 min, respectively. Phy + concurrent acute exercise showed 58%, 60%, and 69% of control ChE activity at 20, 30, and 50 min, respectively (Fig. 5).

The rate of decarbamylation of ChE enzyme was significantly decreased (44% of control) in Phy + concurrent acute exercise (0.0135 min^{-1}) compared to Phy alone (0.0308 min^{-1}) in muscle (Fig. 5 inset and Table 2).

DISCUSSION

The decarbamylation rate is an indirect indication of the recovery of the tissue from ChE inhibition. The rate of decarbamylation of Phy-inhibited ChE varied in different tissues; the rate was highest in muscle (0.0308 min^{-1}) and lowest in the diaphragm (0.0078 min^{-1}), with intermediate rate (0.0252–0.0165 min^{-1}) in the heart, brain, and RBC. The results showed the rate of decarbamylation in descending order is muscle > heart > brain > RBC > diaphragm. Physostigmine + concurrent acute exercise showed significant increase in the rate of decarbamylation in RBC, brain, and heart, and a marked decrease in muscle and diaphragm. This variation in rate of decarbamylation may be attributed to a difference in selective metabolism of Phy in various tissues.

Acute exercise-induced transient increase in ChE activity of RBC is in agreement with our earlier work (3). This increase may be due to secondary effect of hypoxia, increased hemoconcentration and sequestration of RBC from spleen during initial stress time points to cope up with the increased demand of body during exercise (15). Later on there may be a cholinergic acclimatization after 20–25 min.

Physostigmine + concurrent acute exercise significantly decreased ChE activity in RBC and the brain from 20–25 min, and in muscle throughout the 50-min period compared to Phy alone. However, ChE activity increased in the heart and diaphragm following Phy + concurrent acute exercise compared to Phy alone. The modulatory effect of concurrent acute exercise on pharmacodynamics of Phy may be due to several factors, such as rate of decarbamylations, blood flow, bile flow, rate of metabolism, and half-life of Phy. Different types of exercise have been reported to affect the blood flow in different ways (10). Change in blood flow will affect the amount of drug reaching the receptor site, thus affecting the pharmacodynamic activity. The main factor affecting drug absorption following IM administration is a change in blood flow (8,10). This may be one of the causes for the increase in the recovery of ChE inhibited by Phy and exercise compared to Phy alone.

The increase in ChE activity of the heart may be attributed to increase in cardiac output with a redistribution of blood flow to different organs (10,24). Exercise increases cardiovas-

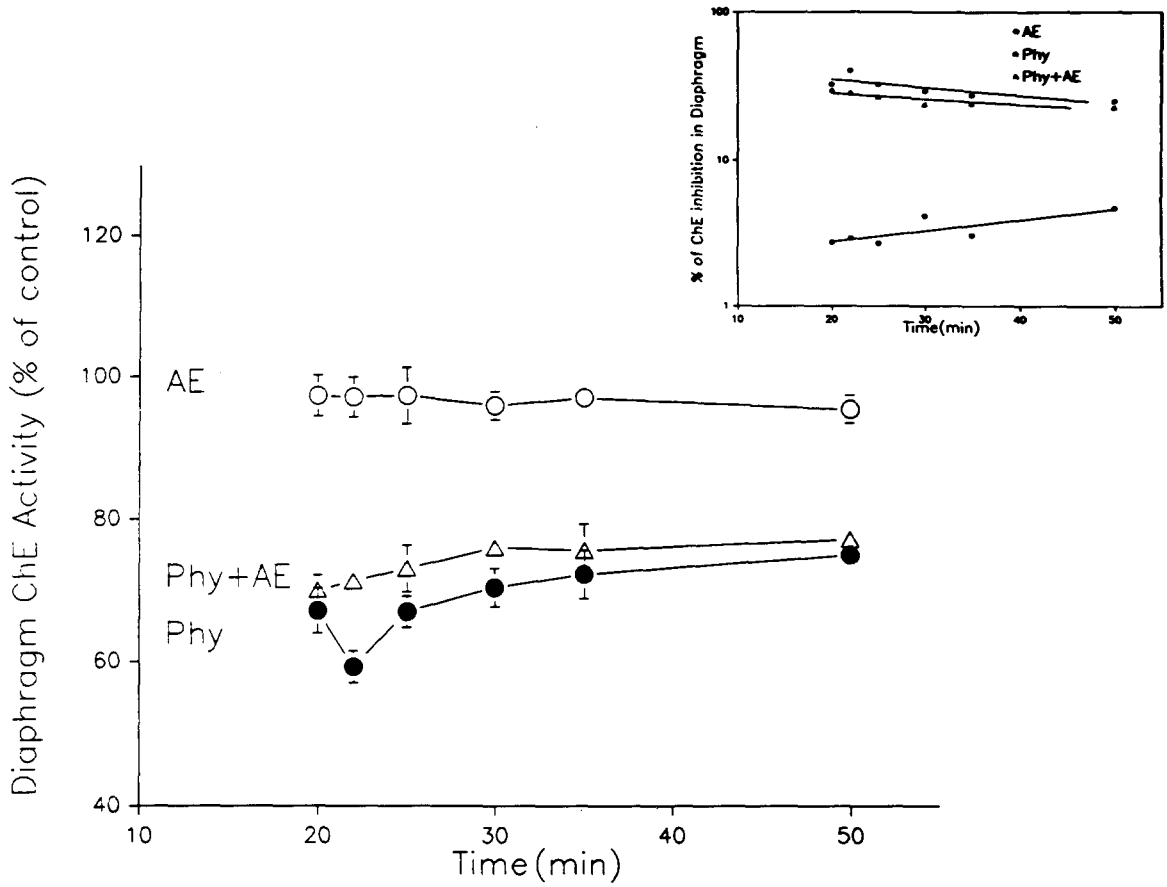

FIG. 4. Effect of acute exercise (AE) (80% VO_2max), Phy (70 μg/kg), and Phy then concurrent AE on time course of percent control ChE activity in diaphragm of the rat. Values are mean ± SEM. Inset shows the rate of decarbamylation (K_d) of ChE enzyme in the diaphragm.

cular functional capacity and decreases myocardial oxygen demand (14). Several studies have demonstrated the relationship between blood flow and exercise. Moderate exercise in human beings has been reported to increase blood flow threefolds in the heart (17). In the present study, the concurrent acute exercise (80% VO_2max) corresponds to moderate exercise. It is also possible that free drug concentration might be decreased during Phy + concurrent acute exercise because of increased blood flow in the heart and diaphragm leading to increased ChE activity or decreased Phy concentration in these tissues.

Since the bile flow is very slow compared to hepatic blood flow, it is possible that any effect on altered bile flow will be counteracted by the effect of altered hepatic blood flow (12,18). Exercise decreases the hepatic blood flow, thereby resulting in decreased clearance and rate of elimination of Phy.

We could observe any significant change in ChE activity of muscle by concurrent acute exercise. We have recently shown the effect of Phy and exercise on cholineacetyltransferase (ChAT) and AChE activities in fast and slow muscles of the rat (2). We have shown that these activities are affected differentially due to exercise, depending on the function of muscle. It has been reported that muscle blood flow can vary as much as tenfold in muscles after moderate exercise, and there is large individual variations in drug uptake in this tissue. Pedzikiewicz et al. (16) have reported an increase in muscle ChE activity (20%) after a short physical exercise. These authors reported an increase in ChE activity due to an increase in blood flow in skeletal muscles (1). Selective increase in G4 acetylcholinesterase activity of adult male Fischer rats subjected to treadmill exercise has been reported by Fernandez and Donoso (5).

We have shown that Phy + concurrent acute exercise increased the rate of decarbamylation in RBC, brain, and heart; however, the extent of increase in K_d varied from tissues to tissues. In contrast to the previous observation (22), we have observed that K_d decreased in diaphragm and muscle following Phy + concurrent exercise. This might be due to change in the exercise protocol in the present investigation where acute exercise has been given 20 min after Phy administration. It is also possible that preexercise and postexercise might be modulating the pharmacodynamic response of Phy differently. It is known that muscle and diaphragm actively take part in the physical exercise and the blood flow increased more in the muscle compared to other tissues, affecting the decrease in K_d in Phy + concurrent acute exercise.

In conclusion, Phy + concurrent acute exercise enhances the rate of decarbamylation of Phy-inhibited ChE in RBC, brain, and heart, indicating that concurrent exercise decreases the effect of Phy in these tissues. On the other hand, muscle and diaphragm showed an opposite effect, prolonging the

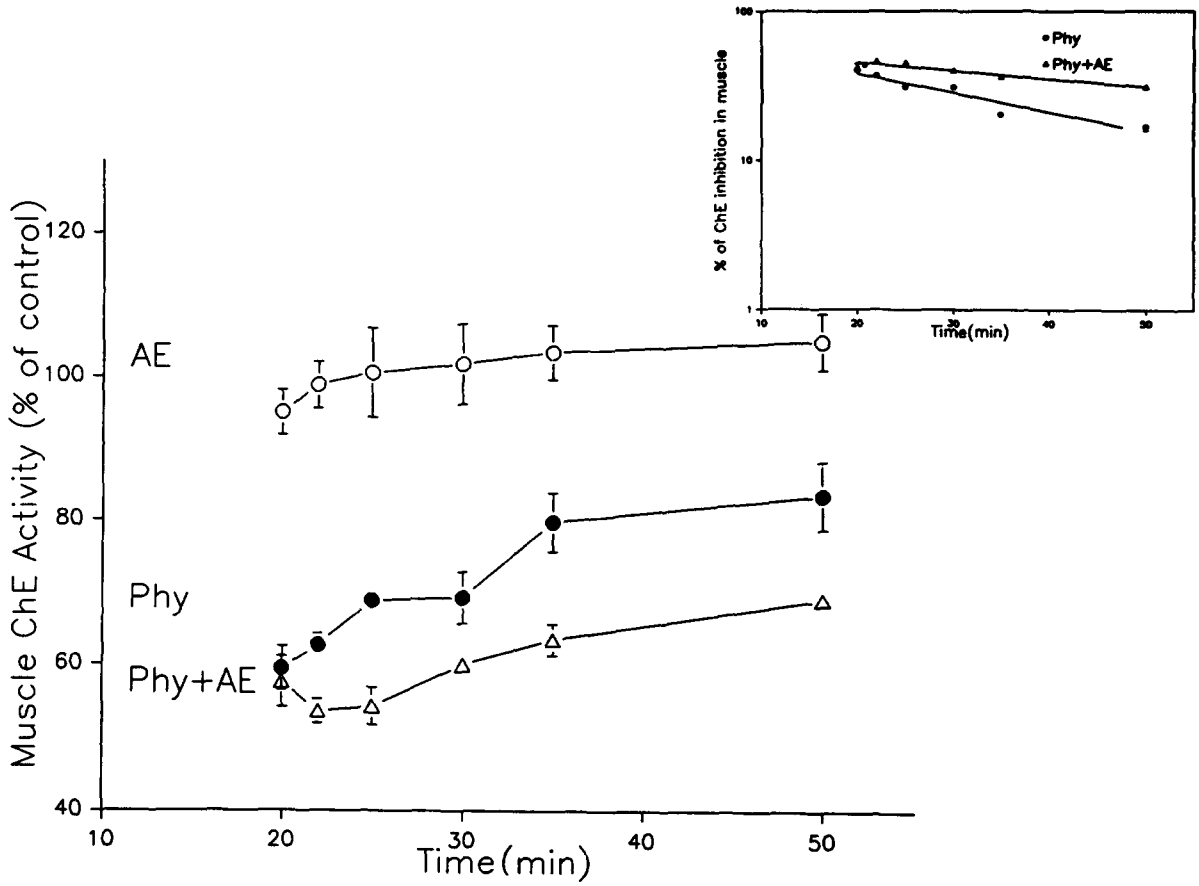

FIG. 5. Effect of acute exercise (AE) (80% VO_2max), Phy (70 μg/kg), and Phy then concurrent AE on time course of percent control ChE activity in muscle of rat. Values are mean ± SEM. Inset shows the rate of decarbamylation (K_d) of ChE enzyme in muscle.

cholinesterase inhibition by Phy. This study would be useful in understanding the effect of exercise in the prophylaxis of Phy during organophosphorus intoxication in the development of a rational therapy regimen.

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