Pharmacology Biochemistry and Behavior, Vol. 42, pp. 67–73, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Effect of Physostigmine on Plasma Lactate and Pyruvate in Untrained/Trained Rats

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Received 18 March 1991

BABU, S. R., P. BUCKENMEYER, R. G. KNOWLTON AND S. M. SOMANI. *Effect of physostigmine on plasma lactate and pyruvate in untrained/trained rats.* PHARMACOL BIOCHEM BEHAV **42**(1) 67–73, 1992.—Physostigmine (Phy) is metabolized to eseroline, a phenolic compound that appears to alter mitochondrial functions. The effect of Phy on recovery from exercise and on time course of plasma lactate and pyruvate levels following an acute bout of exercise (AE) was examined in untrained and trained (ET) rats. Phy alone elicited significantly higher plasma lactate and pyruvate levels than sedentary control. AE + Phy had a significantly higher plasma lactate and pyruvate levels compared to AE 2 min postadministration. From 5–30 min postexercise, lactate and pyruvate levels did not differ between these two acutely exercised groups. ET + Phy exhibited significantly lower levels of plasma lactate and pyruvate from 5–60 min postexercise compared to ET. The data show that the "additive" effect of Phy on postexercise plasma lactate and pyruvate levels can be attenuated by an enhanced fitness level in these rats.

Physostigmine Acute bout of exercise Untrained and trained Lactate Pyruvate

PHENOLIC drugs are known to alter mitochondrial function (20) in animals. A drug or metabolite that changes the mitochondrial redox state may alter the balance between aerobic and anaerobic energy substrates within the cell. Physostigmine (Phy), an anticholinesterase agent, was believed to be a potential pretreatment drug for organophosphate intoxication (14). Its treatment effects have significance relative to soldiers who may be exposed to chemical warfare. Phy has been a trial drug for the improvement of memory function in patients with Alzheimer's disease (5,19). Phy has not been sufficiently studied in an animal model, let alone a human model.

Phy is metabolized to eseroline, a phenolic compound, in plasma, muscle, brain, and liver (16,17). Recently Somani et al. (18) reported that eseroline causes neuronal cell death of mouse neuroblastoma (NiE 115), rat glioma (C_6), and neuroblastoma-glioma hybrid (ND 108-15) in in vitro studies. The mechanism of cell death seems to involve loss of cell adenosine triphosphate (ATP). King and Somani (9) studied the time course of Phy accumulation in brain subcellular fractions and reported that the mitochondrial radioactivity (RA) increased continuously up to 60 min. The increase of Phy and its degradation products, eseroline and rubreserine, in mitochondria may interfere with normal physiological function of this organelle, resulting in altered redox potentials and subsequent changes in lactate and pyruvate concentrations. Changes in cellular concentrations of lactate and pyruvate will usually be reflected in plasma levels of lactate since there is a concentration gradient and carrier-mediated "efflux" from the working muscle (11).

The level of blood lactate provides a fairly objective indication of the relative anaerobic demand of exercise (10). Upon its efflux from working skeletal muscle, lactate may be excreted in the urine or via sweat, it may be converted to glucose or protein, and it may be oxidized by various tissues in the body (4). A significant amount of lactate is oxidized and utilized as an energy source by working skeletal muscle. This process occurs not only during exercise but also during recovery from exercise. It is unknown if lactate would still be metabolized as readily during recovery if an individual was treated with Phy. For individuals exposed to chemical warfare, recovery from military exercises would be important in maintaining a state of "physical readiness."

The level of physical training may also have a significant effect on how individuals will be able to respond to treatment and pretreatment drugs used in chemical warfare. In endurance-trained individuals, the build-up of lactate concentra-

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tions in the blood is known to be attenuated compared to untrained individuals when both of these groups are subjected to the same absolute workload. Donovan and Brooks (1) attribute this occurrence to an increase in plasma lactate clearance in endurance-trained subjects. However, Favier et al. (2) suggest that endurance training induces a slower production of lactate in contracting skeletal muscle. In either instance, it would seem that physically trained individuals would have a greater "recovery" potential from acute bouts of exercise. When considering the potential effect of Phy on mitochondrial function, it is of interest to know what effect this may have on recovery from exercise performance, particularly in individuals in the Armed Forces exposed to anti-cholinesterase (ChE) agents. Therefore, this study was designed to investigate the effect of Phy on postexericise recovery relative to lactate accumulation in the blood. Dependent upon the effect of Phy on mitochondrial function, it is possible that the metabolism of pyruvate may also be affected. Therefore, pyruvate levels in the blood were also examined. The lactate/pyruvate (L/P) ratio was used as an indicator of intracellular or intravascular changes. Hence, L/P ratio was also calculated in the present study. The experimental protocol involves long and tedious exercise programs; hence, a rat model was designed and developed with a custom-built treadmill for these experiments. An animal model was used since untoward side effects of Phy administration have yet to be fully delineated.

METHOD

Chemicals

Phy free base was obtained from Sigma Chemical Co. (St. Louis, MO). Drierite (anhydrous $CaSO_4$), procured from W. A. Hammond Drierite Co. (Xenia, OH) was used. Diagnostic kits were purchased from Sigma Chemical Co. (St. Louis, MO) for the determination of lactate and pyruvate. All other chemicals were of analytical grade and were obtained from the usual commercial sources.

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_{2max}) (AE);
Group III – endurance trained + acute bout of exercise (80% VO_{2max}) (ET);
Group IV – Phy (70 μg/kg, IM) (Phy);
Group V – acute exercise (80% VO_{2max}) + Phy (70 μg/kg, IM) (AE + Phy);
Group VI – endurance trained + acute bout of exercise (80% VO_{2max}) + Phy (70 μg/kg, IM) (ET + Phy).

Endurance Training of Rats

Rats from Groups III and VI were acclimatized and then trained for 6 weeks, 5 days/week on a nine-channel motor-driven treadmill (custom-made in our Southern Illinois University School of Medicine workshop) using an incremental exercise program. During this program of exercising, the speed (m/min), angle of inclination (% grade), and duration (min) of exercise were varied to obtained progressive levels of exercise intensity as shown in Table 1.

Rats from Groups I, II, IV, and V were not trained but were maintained under similar environmental conditions to those of the endurance-trained rats.

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

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

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_{2max}. The speed of the belt and angle of inclination were increased at different stages as shown in Table 2.

The oxygen consumption and heat production in individual rats undergoing different stages of exercise were recorded once a week by 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, which served as sedentary control, received saline and were sacrificed immediately by decapitation. Rats from Group IV were administered [^{3}H]Phy (70 μg/kg, IM) via the gastrocnemius muscle and were sacrificed at 2, 5, 10, 15, 30, 45, and 60 min postinjection. Rats from Groups II and V were exercised until they reached 80% VO_{2max} and subsequently removed from the treadmill. Rats from Group V were then administered Phy (70 μg/kg, IM) and sacrificed at 2, 5, 10, 15, 30, 45, and 60 min postexercise in conjunction with rats from Group II. The 45- and 60-min time points were not done in Group II due to lack of animals at these time points. Rats from Group III, which were endurance trained, were subjected to an acute bout of

TABLE 2
PROTOCOL FOR EXERCISING RATS ON TREADMILL AT DIFFERENT GRADES AND SPEED 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

exercise (80% VO_{2max}) for 20 min using incremental exercise protocol and decapitated at 5, 15, 30, and 60 min. Rats from Group IV, which were endurance trained, were subjected to an acute bout of exercise at 80% VO_{2max}, administered Phy (70 μg/kg, IM), and sacrificed at 2, 5, 10, 15, 30, 45, and 60 min. Four to six rats were sacrificed at each time point.

Determination of Lactate and Pyruvate

Blood was collected into precooled centrifuge tubes after decapitation. Plasma was separated from blood immediately at 4°C by centrifugation for 10 min at 5000 RPM (Jouan Inc.), then deproteinized with 8% (w/v) perchloric acid. The supernatant was used for the estimation of lactate and pyruvate by the enzymatic method of Fleischer (3).

Statistical Analysis

The data was subjected to a parametric two-way analysis of variance (ANOVA) for unequal *n*'s using a general linear model approach. This approach tested the overall effect of experimental groups with time, both as independent factors. To compare experimental groups against the control group, a one-way ANOVA was performed at each time point. In addition, each time point was compared in each group to evaluate the effect of time. Follow-up tests were performed using Duncan's multiple-range test. Statistical significance was evaluated at the $p < 0.05$ level.

RESULTS

The Phy-dosed (Group IV) group showed a significantly elevated plasma lactate from 2–60 min postexericise (11.21 ± 0.76 to 4.86 ± 0.61 mM) (Fig. 1) compared to sedentary saline-dosed (Group I) rats (3.67 ± 0.52). The AE + Phy (Group V) had a significantly ($p < 0.05$) higher plasma lactate (7.40 ± 0.72) compared to the AE (Group II) (4.18 ± 0.3) without Phy administration at 2 min postexercise. Thereafter, plasma lactate values did not differ between the acutely exercised groups during recovery. ET rats treated with Phy (Group VI) had significantly ($p < 0.05$) lower plasma lactate values from 5–60 min postexercise (5.89 ± 1.0 to 4.36 ± 0.29) (Fig. 2) compared to ET rats without Phy (Group III) (6.50 ± 0.75 to 5.12 ± 0.61) (Fig. 3) (Table 3).

Plasma pyruvate levels of the Phy-dosed (Group IV) were significantly ($p < 0.05$) elevated above the saline-dosed sedentary control (Group I) for up to 30 min postinjection (0.26 ± 0.07 to 0.12 ± 0.06 vs. 0.13 ± 0.02, respectively) (Fig. 1). Group V elicited significantly higher plasma pyruvate levels at 2 min postexercise (0.28 ± 0.04) compared to Group II (0.20 ± 0.01). This trend appeared to continue up to 30 min postexercise for the acutely exercised groups. Among the endurance-trained animals, Group VI had significantly ($p < 0.05$) lower plasma pyruvate levels from 5–30 min postexercise (0.18 ± 0.06 to 0.10 ± 0.097) (Fig. 2) compared to Group III, which did not receive the drug (0.19 ± 0.05 to 0.13 ± 0.02) (Table 4; Fig. 3).

The L/P ratio, sometimes used as an indicator of intracellular or intravascular changes, was significantly ($p < 0.05$) high throughout 60 min postinjection for the Phy-dosed group (43.2–54.0, from 2–60 min postinjection, respectively) compared to saline-dosed sedentary control (Group I). In the acutely exercised groups, there was only a slight increase in L/P for both groups from 2–30 min postexercise (20.9–27.0 for the AE (Group II) vs. 22.9–25.2 for the AE + Phy (Figs. 4,5). The L/P ratio did not differ significantly through these time points for the acutely exercised groups. In the endurance-trained groups, the L/P was similar at 5 min postexercise for both Groups III and VI. But, by 60 min postexercise, there was a 17% increase in L/P ratio in the endurance-trained group receiving Phy vs. the endurance-trained group not receiving Phy (Figs. 2,3).

DISCUSSION

The results of this investigation showed that when acute exercise precedes Phy dosing the plasma levels of both lactate and pyruvate are diminished. Further, rats that are untrained and subjected to Phy following an acute bout of exercise are more likely to experience elevated lactate and pyruvate levels in the blood shortly after the cessation of exercise compared

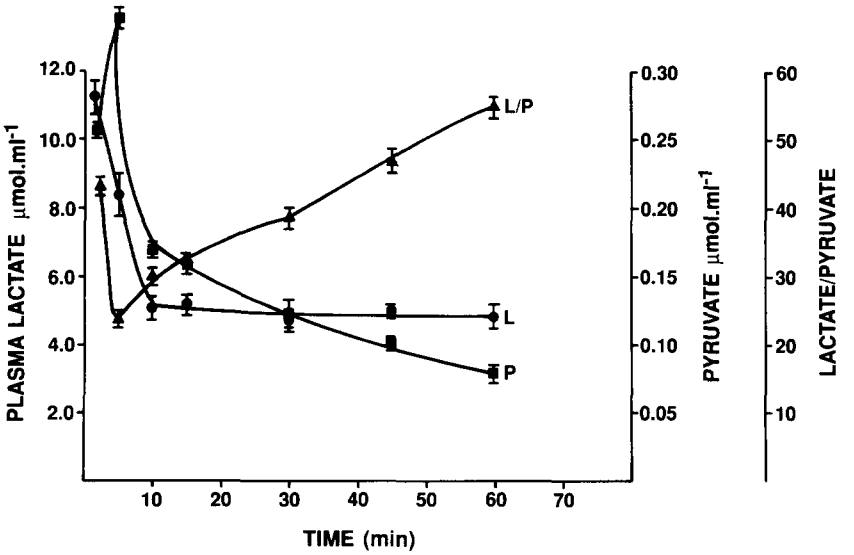

FIG. 1. Plasma lactate (L), pyruvate (P), and lactate to pyruvate ratio (L/P) after Phy administration (70 μg/kg, IM).

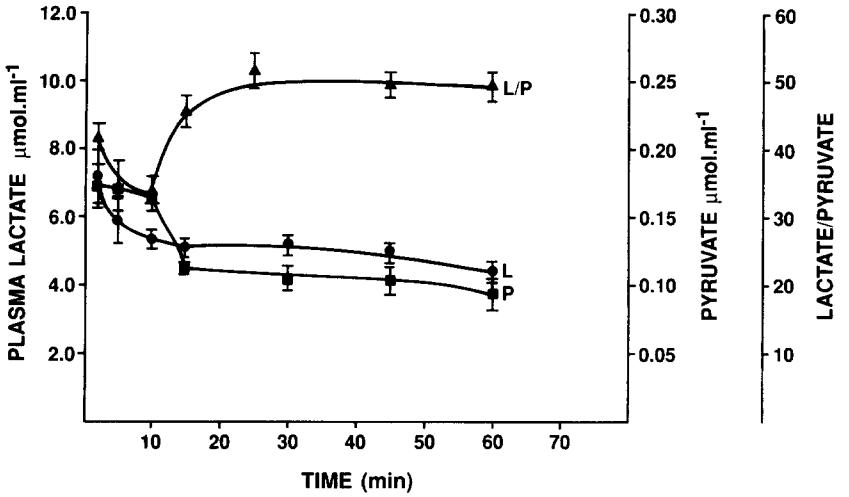

FIG. 2. Plasma lactate (L), pyruvate (P), and lactate to pyruvate ratio (L/P) during recovery period after endurance training and physostigmine administration (70 µg/kg, IM).

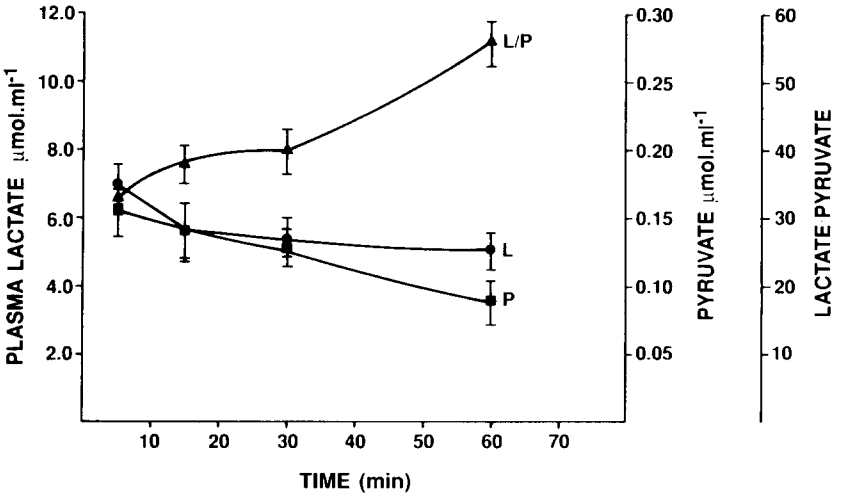

FIG. 3. Plasma lactate (L), pyruvate (P), and lactate to pyruvate ratio (L/P) during recovery period after endurance training (refer to Table 1 for training protocol).

TABLE 3

EFFECT OF EXERCISE (ACUTE AND ENDURANCE TRAINED), PHY, AND EXERCISE + PHY ON TIME COURSE OF PLASMA LACTATE LEVELS (mmol/L) IN RATS

Time (min)	AE (Group II)	ET (Group III)	Phy (Group IV)	AE + Phy (Group V)	ET + Phy (Group VI)
2	4.18 ± 0.30		11.22 ± 0.76*	7.40 ± 0.72†	7.40 ± 0.86‡
5	5.39 ± 0.88	6.50 ± 0.75	8.48 ± 1.68*	5.24 ± 0.34	5.89 ± 1.00‡
10	4.51 ± 0.37		5.12 ± 0.39*	4.92 ± 0.69	5.31 ± 0.27
15	3.68 ± 0.36	5.65 ± 0.99	5.32 ± 0.68*	2.59 ± 0.43	5.01 ± 0.41‡
30	3.78 ± 0.56	5.48 ± 0.61	4.88 ± 0.66*	4.29 ± 1.45	5.12 ± 1.45
45			5.09 ± 0.28*	4.49 ± 0.48	4.90 ± 0.35
60		5.12 ± 0.61	4.86 ± 0.61*	3.75 ± 0.57	4.36 ± 0.29‡

Values are mean of four observations ± SEM.
Sedentary control (Group I) = 3.67 ± 0.52 mmol/L.
*Significant difference was observed compared to Group I at $p < 0.05$.
†Significant difference was observed compared to Group II at $p < 0.05$.
‡Significant difference was observed compared to Group III at $p < 0.05$.

TABLE 4

EFFECT OF EXERCISE (ACUTE AND ENDURANCE TRAINED), PHY, AND EXERCISE + PHY ON TIME COURSE OF PLASMA PYRUVATE LEVELS (mmol/L) IN RATS

Time (min)	AE (Group II)	ET (Group III)	Phy (Group IV)	AE + Phy (Group V)	ET + Phy (Group VI)
2	0.20 ± 0.01		0.26 ± 0.07*	0.28 ± 0.04†	0.18 ± 0.04
5	0.20 ± 0.02	0.19 ± 0.05	0.35 ± .10*	0.18 ± 0.04	0.18 ± 0.06‡
10	0.16 ± 0.02		0.17 ± 0.03*	0.34 ± 0.03†	0.16 ± 0.01
15	0.16 ± 0.02	0.15 ± 0.01	0.16 ± 0.04*	0.18 ± 0.03†	0.11 ± 0.01‡
30	0.14 ± 0.02	0.13 ± 0.02	0.12 ± 0.06	0.17 ± 0.04	0.10 ± 0.09‡
45			0.11 ± 0.02	0.14 ± 0.04	0.10 ± 0.02
60		0.09 ± 0.01	0.09 ± 0.03	0.14 ± 0.03	0.09 ± 0.02

Values are mean of four observations ± SEM.
Sedentary control (Group I) = 0.1310.02 mmol/L.
*Significant difference was observed compared to Group I at $p < 0.05$.
†Significant difference was observed compared to Group II at $p < 0.05$.
‡Significant difference was observed compared to Group III at $p < 0.05$.

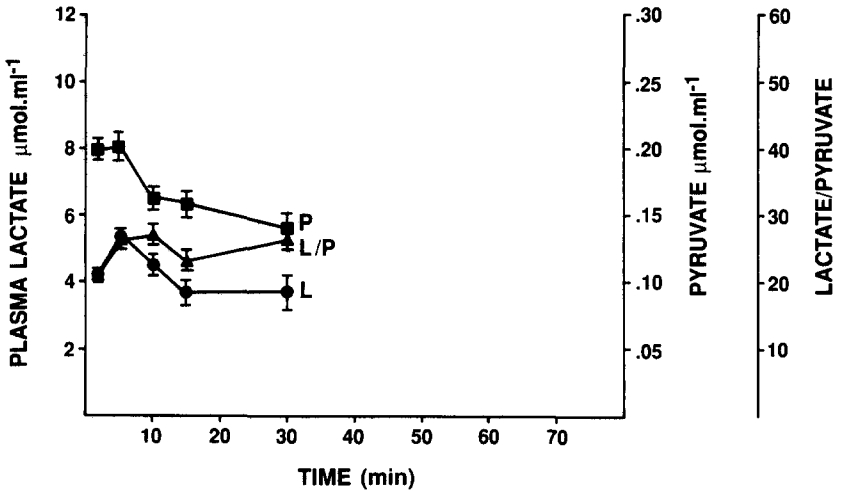

FIG. 4. Plasma lactate (L), pyruvate (P), and lactate to pyruvate ratio (L/P) during recovery period after acute exercise (refer to Table 2 for exercise protocol).

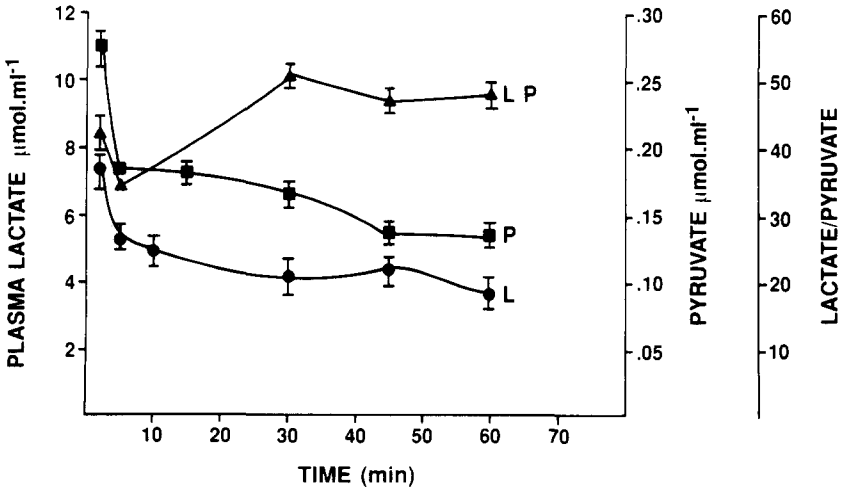

FIG. 5. Plasma lactate (L), pyruvate (P), and lactate to pyruvate ratio (L/P) during recovery period after acute exercise and physostigmine administration (70 µg/kg, IM).

to those not receiving the drug. However, this effect does not appear to linger beyond 2 min postexercise. When these rats are endurance trained, lower lactate and pyruvate levels appear in the blood after acute exercise when given Phy vs. when they do not receive the drug.

The Phy-dosed to sedentary controls rats raised lactate and pyruvate levels significantly compared to sedentary controls. This suggests that Phy is interrupting the "normal" oxidizing/reducing capacity of the mitochondria as suggested by King and Somani (9). If Phy and its metabolites (quinone compounds) accumulate in muscle cell mitochondria, they may interfere with the cell's redox state. Olgin et al. (10) reported that changes in the muscle reflect changes in muscle mitochondrial redox state and concluded that an increase in L/P ratio could reflect the redox state of mitochondria during exercise. Phy and its metabolites may create a type of stress similar to exercise. An accumulation of quinone-type compounds in muscle mitochondria may alter the NADH/NAD ratio, which is proportional to changes in cytosolic lactate/pyruvate ratio (12). As noted by Olgin et al. (10), these occurrences within the muscle would potentially be reflected in the blood due to the concentration gradient build-up between these two intervascular areas. We observed an L/P ratio of 43.2–54.0 from 2–60 min postinjection in plasma of Phy-dosed rats compared to 28.2 of saline-dosed rats. This significant "quantitative" difference lends support to the possibility that muscle mitochondrial function has been altered by Phy. Yet, other possibilities may exist that have not been experimentally explored, such as an enhancement of rate-limiting enzyme activities, particularly within the process of glycolysis.

One factor that altered the build-up of lactate and pyruvate in the plasma was the incorporation of exercise just prior to Phy dosing. In untrained animals subjected to acute exercise of 80% $\dot{V}O_{2max}$, the level of plasma lactate and pyruvate at 2 min postexercise was 63 and 24% lower, respectively, compared to the sedentary Phy-dosed group. When Phy was administered to animals acutely exercised, plasma lactate and pyruvate were attenuated less compared to the sedentary or Phy-dosed group. In this case, plasma lactate and pyruvate levels were 34% lower and 7% higher at 2 min postexercise compared to the sedentary Phy-dosed group. These results suggest that Phy only has a temporary effect on raising lactate levels in the blood in the early stage of recovery from a submaximal exercise effort at 80% $\dot{V}O_{2max}$. It is likely that since the blood flow is still elevated above "resting" in the early stages of recovery plasma lactate and pyruvate were more readily transported and metabolized at various target tissues. It may also be true that Phy was more readily metabolized during the early part of recovery since the metabolic rate is still elevated.

Another factor that has been shown to affect plasma lactate (and potentially plasma pyruvate) levels in response to a single exercise bout is endurance training. Favier et al. (2) reported a 28% less build-up of lactate in trained skeletal muscle compared to that in an untrained, sedentary animal in response to an acute exercise bout. However, contrary findings have been reported that show that submaximal exercise results in slightly greater lactate concentrations in skeletal muscle and blood of trained compared to untrained animals and humans (6,7,8,13). This latter finding would lend support to our study in that plasma lactate was slightly higher from 5–30 min postexercise in the trained groups compared to untrained, acutely exercised groups.

From this investigation, it appears that Phy dosing is associated with a metabolic stress as indicated by elevated plasma lactate and pyruvate levels observed in sedentary, untrained Phy-dosed rats. Exposure to Phy immediately postexercise in untrained animals only results in a temporary metabolic stress. In the case of endurance-trained rats, the metabolic stress of both the acute exercise and Phy dosing is reduced during recovery from a submaximal exercise effort.

The significance of these findings suggests that the metabolic stress induced by Phy is lessened in metabolically active animals (above resting levels). Therefore, we conclude that endurance-trained animals can readily adjust to the build-up of metabolic by-products caused by Phy administration. This finding is of particular importance when applied to soldiers who are likely to be exposed to chemical warfare like anticholinesterases.

ACKNOWLEDGEMENTS

The authors thank Donna Fectern, Roberta Melton, and Gina Huff for typing the manuscript. This study was supported by U.S. Army contract No. DAMD 17-88-C-8024.

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