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Effect of Concurrent Exercise and Physostigmine on Lactate and Pyruvate in Plasma, Muscle, and Brain Tissue of Rats

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BUCKENMEYER, P. J., S. R. BABU, R. G. KNOWLTON AND S. M. SOMANI. *Effect of concurrent exercise and physostigmine on lactate and pyruvate in plasma, muscle, and brain tissue of rats.* PHARMACOL BIOCHEM BEHAV 47(4) 779–788, 1994. – The purpose of this investigation was to determine the effect of physostigmine (Phy) and/or concurrent exercise on lactate, pyruvate, and L/P ratio in plasma, skeletal muscle, and brain tissue in male Sprague–Dawley rats. The Phy-dosed (Phy-D) and Phy-dosed + concurrent acute exercise (Phy-D + CAE) groups elicited significantly higher L/P ratios in plasma compared to the acutely exercised (AE) group at 30 min postexercise. Physostigmine dosing, with or without exercise, resulted in significantly lower muscle pyruvate levels, from 30 to 50 min postdrug administration, in Phy-D and Phy-D + CAE groups compared to the AE group. In the brain, lactate values were significantly elevated in the acutely exercised groups at 5 min postexercise with or without Phy dosing. However, at 15 to 30 min postexercise, lactate values were significantly elevated in the Phy-D + CAE compared to the AE group. These data suggest that when Phy is administered prior to a 20-min moderately intensive exercise bout, there is an accumulation of lactate for a prolonged period of time in recovery.

Physostigmine Concurrent acute exercise Lactate Pyruvate Plasma Muscle and brain

PHYSOSTIGMINE (Phy), an anticholinesterase agent, is a trial drug for Alzheimer's disease (5,18,19). It is also considered to be a potential pretreatment drug for organophosphate intoxication (14). It is extensively used as an antidote for tricyclic antidepressant drug overdose. Physostigmine is metabolized to eseroline and is converted to rubroserine, a "quinone-type" compound (16). Quinones are known to disturb mitochondrial function (21). Because the mitochondria serves as an important "energy processor" during exercise, it is perceivable that Phy could affect exercise performance. The effect of exercise on the disposition of other drugs has been reviewed (17). We have recently shown that Phy, like acute exercise, can significantly increase plasma lactate and pyruvate shortly after injection (2). Because Phy and its metabolites are known to accumulate in rat brain cells up to 60 min postinjection (9), we felt it was important to measure lactate and pyruvate over an extended time frame following drug dosing. If Phy accumulates in muscle cell mitochondria as it does in brain cell mitochondria, one might observe an alteration in its redox state as noted by Weinbach and Garbus (21). This occurrence could suggest an increase in the lactate/pyruvate (L/P) ratio; hence, an increase in lactate accumulation or decrease in pyruvate (as it is directed to acetyl-CoA or alanine) within the muscle cell. The changes in cellular concentrations of lactate and pyruvate will usually be reflected in plasma levels of lactate because there is a concentration gradient and carrier-mediated "efflux" from the working muscle (12).

The role of lactate during exercise has been the subject of much interest in recent years due to its diverse metabolic role. Conflicting results have been reported regarding the accumulation of lactate in blood and muscle during exercise (3). Lac-

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tate can be formed by mass conversion of pyruvate, resulting in a change in L/P ratio. The mitochondrial membrane proton shuttle may be too slow to reoxidize the reduced cytosolic NAD, resulting in the conversion of pyruvate to lactate. This results in an increase of L/P ratio (20). Lactate will also be formed as a net result of a higher rate of pyruvate production via glycolysis compared to the rate at which the citric acid cycle can turn over pyruvate to acetyl-CoA (10). Subsequently, an accumulation of pyruvate in conjunction with any increase in the content of protons or NADH can cause a shift in the lactate dehydrogenase reaction towards lactate formation (11). It is possible that this could readily occur if Phy does indeed disrupt the "aerobic" functioning of the mitochondria of the skeletal muscle cell. Individuals who are exercising while being exposed to anticholinesterase agents, such as Phy, could have their work performance hampered. A scenario that reflects this circumstance might be soldiers becoming exposed to chemical warfare. Because lactate and pyruvate serve as markers of the aerobic/anaerobic status of the cell, it is important to determine the lactate and pyruvate concentration in plasma, muscle, and brain, to evaluate the effects of a drug like Phy under different exercising conditions. Therefore, this study was carried out to investigate the effect of Phy, concurrent exercise, and the combination of these two factors on the lactate, pyruvate, and L/P ratio in plasma, muscle, and brain.

METHOD

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) weighing 175-200 g were used in this study. The rats were divided into four groups:

- saline-dosed (S-D)
- acute exercise (80% VO_2max for 20 min) (AE)
- Phy-dosed (70 μg/kg, IM) (Phy-D)
- Phy-dosed (70 μg/kg, IM) + concurrent acute exercise (80% VO_2max) (Phy-D + CAE).

Physostigmine Administration and Exercising of Rats on Treadmill

Physostigmine free base was obtained from Sigma Chemical Co. (St. Louis, MO). Physostigmine was not synthesized in our lab due to its instability. [^{3}H]Physostigmine (13 Ci/mmol) was custom synthesized by Amersham Corporation (Chicago, IL). ^{3}H was utilized because it was just as effective of a radioligand as ^{14}C, and more easily attained. Also, because this study is a continuation of a project investigating the effect of exercise on pharmacokinetics and pharmacodynamics of Phy in rats, we needed to be consistent with our "tracer" as well as the site of injection (IM, thigh). We did not trace the ^{3}H-labelled Phy in this present study.

Physostigmine was labeled with tritium on both ortho positions to the carbamate chain on the aromatic ring of physostigmine. [^{3}H]Phy was diluted with unlabelled 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 pH range. The purity of Phy was assessed using high performance liquid chromatography (HPLC) and an ultraviolet detector, and also by monitoring the [^{3}H]Phy in the eluant. The solution used in all experiments was greater than 95% pure. Diagnostic kits were purchased from Sigma Chemical Co. for the determination of lactate and pyruvate. All other chemicals were of analytical grade and were obtained from the usual commercial sources.

The oxyscan system and omnipacer treadmill (Omnitech, Inc., Columbus, OH) were used to determine maximal oxygen consumption (VO_2max) of the animals (15). Rats from AE and Phy-D + CAE were subjected to a progressive treadmill protocol as described in Table 1 to obtain the VO_2max of each rat.

Determination of VO_2max (maximal oxygen consumption) was carried out to determine the workload necessary for exercising the rats at 80% VO_2max. Measurement of VO_2max was considered valid only if the animal ran until it could no longer maintain pace with the treadmill. Three days following the determination of VO_2max, AE rats were exercised at different speeds and inclinations for 20 min, corresponding to approximately 80% VO_2max. Rats from Phy-D + CAE were subjected to the same level of intensity as AE but were administered Phy prior to exercising for 20 min (see Fig. 1). The rats of AE and Phy-D + AE were sacrificed at 20 (immediate postexercise), 22, 25, 30, 35, and 50 min after the start of exercise. Rats from Phy-D were administered Phy (70 μg/kg, IM) and were sacrificed at 20, 22, 25, 30, 35, and 50 min postdosing. The S-D rats were administered saline (IM in the thigh), and were sacrificed after 20 min. In previous studies from this lab, metabolites have been shown to be stable after saline injection. A minimum of four animals were sacrificed by decapitation in each group (at each time point examined) between 0800 to 1100 h to minimize circadian cycle effects. The brain and gastrocnemius muscle of the leg were quickly dissected and plunged into liquid nitrogen after decapitation. The frozen brain and muscle were wrapped in aluminum foil and stored at −70°C until analysis.

Determination of Lactate and Pyruvate in Plasma

Blood was collected into precooled, heparinized centrifuge tubes after decapitation in a different room away from sedentary and exercising animals. Plasma was separated from blood immediately at 4°C by centrifugation for 10 min at 5000 rpm (Jouan, Inc.) and deproteinized with 8% (w/v) perchloric acid immediately. The supernatant was used for the estimation of lactate and pyruvate. Lactate and pyruvate were determined by the enzymatic method of Fleischer (4) and expressed as mmol/l.

Determination of Lactate and Pyruvate in Brain and Muscle

The frozen tissues were weighed, powdered under liquid nitrogen, and then 10% (w/v) cold perchloric acid was added to the powdered tissue before it thawed. Frozen tissue powder was sonicated for 30 s with an ultrasonic processor probe in two intervals of 15 s each and a 10% homogenate was prepared by adding the required amount of precooled perchloric acid. The

TABLE 1

TREADMILL PROTOCOL FOR DETERMINATION OF VO_2max IN RATS

Stage	Grade/Degrees	Speed (m/min)	Duration (min)
0	0	2	5
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

Stage 0 represents minimal activity for acclimation to treadmill.

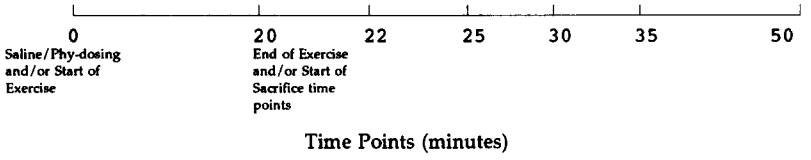

FIG. 1. Drug dosing and exercise protocol.

homogenization was done below 0°C to minimize enzymatic reactions in the tissues. The protein precipitate was removed by centrifugation (Sorvall, Dupont) and the supernatant was neutralized with potassium carbonate. The supernatant was then separated by further centrifugation and used for assays. Muscle and brain lactate and pyruvate were determined by the modification of enzymatic methods of Gutmann and Wahlefeld (6) and were expressed as mg/g wet weight of the tissue.

Statistical Analysis

The data were 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 S-D group, a one-way ANOVA was performed at each time point. In addition, each time point was compared in each group using the effect of time. Follow-up tests were performed using Duncan's multiple range test. Statistical significance was evaluated at the 5% level.

RESULTS

The effect of Phy, acute exercise, and Phy + concurrent exercise on post-Phy administration or postexercise time courses of lactate, pyruvate, and L/P ratio in plasma is shown in Fig. 2A, Fig. 2B, and Fig. 2C, respectively.

Plasma

At 20 min post-Phy administration, lactate concentration was 24% above S-D level (3.23 ± 0.27 mM). In the Phy-D group, lactate values then declined within the next 10 min (90% of S-D) before returning to a similar concentration reached at the 20-min post-Phy administration time point. The effect of exercise alone resulted in a lactate concentration that was 19% above S-D at the 20-min time point, and increased to 66% above S-D (5.4 ± 0.9 mM) at 25 min before returning to S-D levels (3.2 ± 0.27 mM) at the 50-min time point. The combined effect of Phy and exercise also showed a significant increase in lactate concentration 44% above S-D (4.7 ± 0.5 mM) at the 22-min time point, which declined steadily and returned to S-D levels by 50 min. There was no

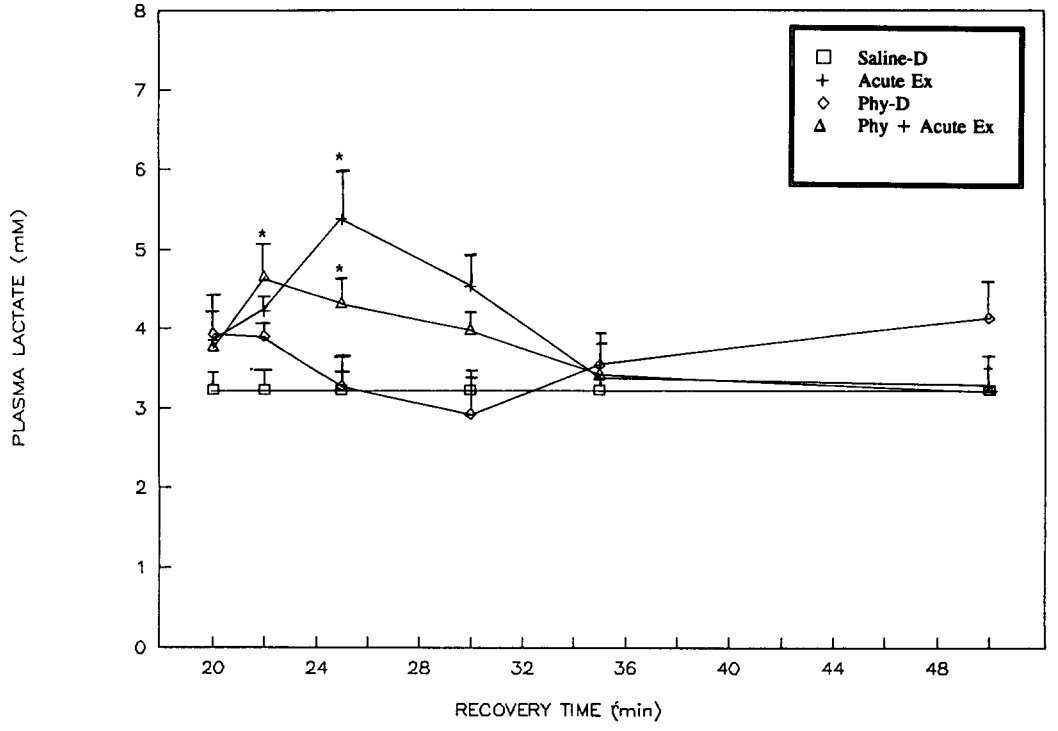

FIG. 2A. Plasma lactate at post-Phy administration (70 μg/kg, IM) or post-initiation of exercise time points in saline-dosed, acutely exercised, Phy-dosed, and Phy + acutely exercised groups. Mean ± SEM of four to six animals. *Significantly different than Saline-D at $p < 0.05$.

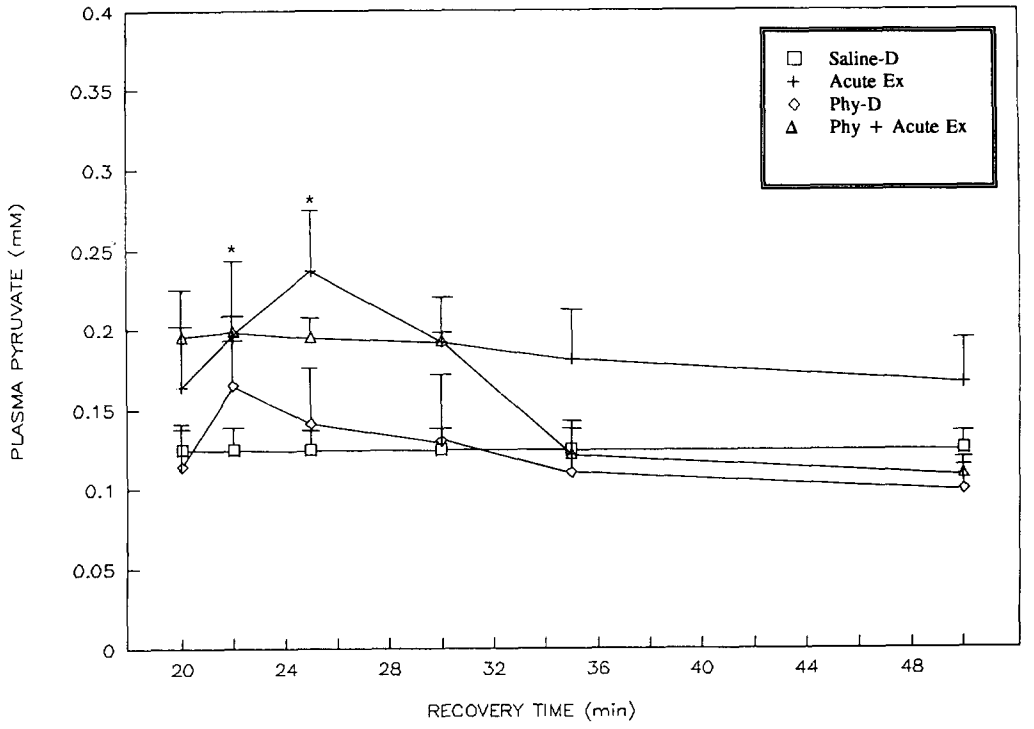

FIG. 2B. Plasma pyruvate at post-Phy administration (70 μg/kg, IM) or post-initiation of exercise time points in saline-dosed, acutely exercised, Phy-dosed, and Phy + acutely exercised groups. Mean ± SEM of four to six animals. *Significantly different than Saline-D at $p < 0.05$.

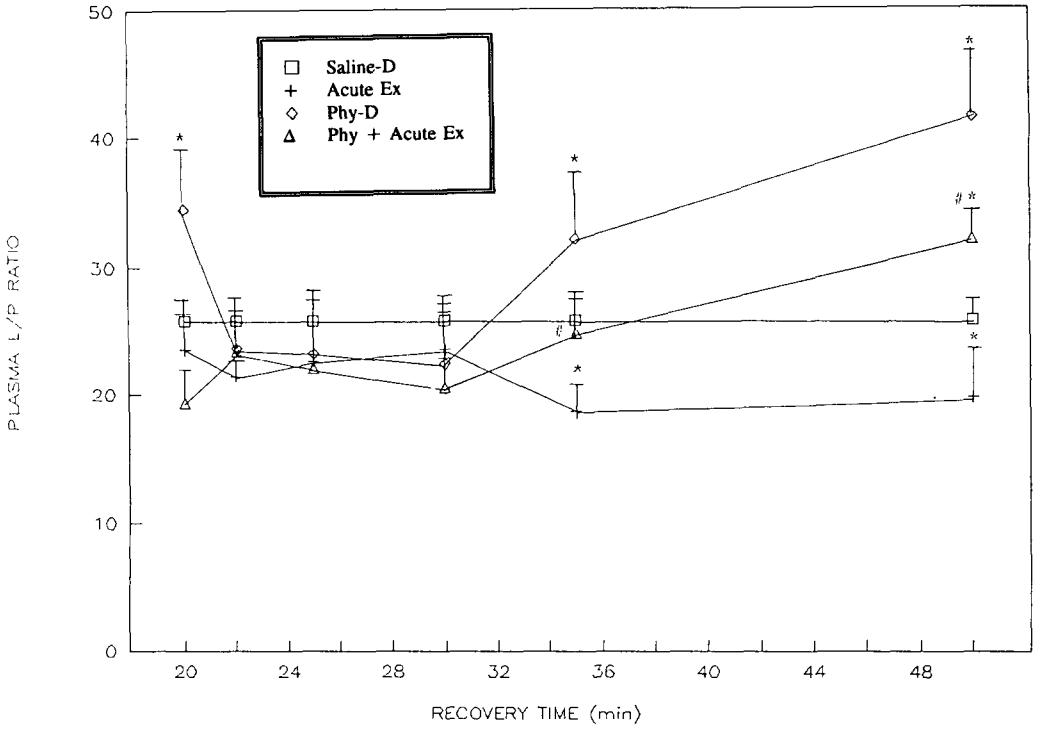

FIG. 2C. Plasma L/P ratio at post-Phy administration (70 μg/kg, IM) or post-initiation of exercise time points in saline-dosed, acutely exercised, Phy-dosed, and Phy + acutely exercised groups. Mean ± SEM of four to six animals. *Significantly different than Saline-D at $p < 0.05$. #Significantly different than Acute Ex at $p < 0.05$.

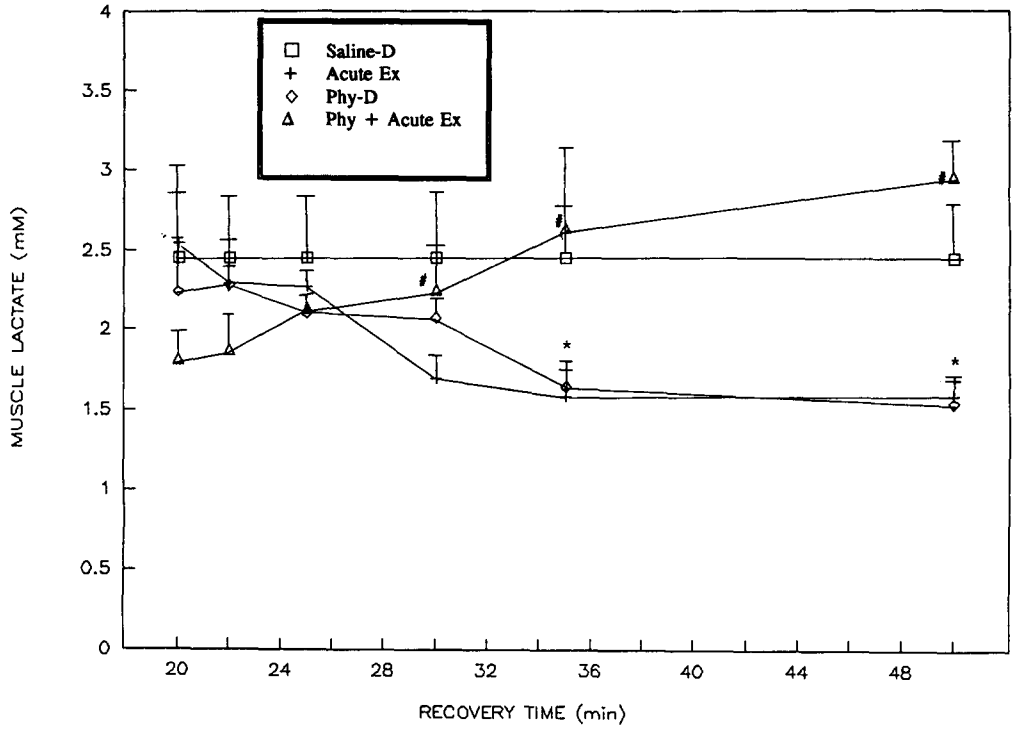

FIG. 3A. Muscle lactate at post-Phy administration (70 μg/kg, IM) or post-initiation of exercise time points in saline-dosed, acutely exercised, Phy-dosed, and Phy + acutely exercised groups. Mean ± SEM of four to six animals. *Significantly different than Saline-D at $p < 0.05$. #Significantly different than Acute Ex at $p < 0.05$.

significant difference between AE and Phy-D + CAE relative to plasma lactate levels at any of the time points.

Plasma pyruvate was 31% above S-D (0.125 ± 0.012 mM) with Phy administration at the 22-min time point and then decreased to 80% of S-D by 50 min post-Phy administration. Exercise alone elicited a 57% and 89% increase in pyruvate at 22 and 25 min, respectively. Pyruvate concentration was still elevated 33% above S-D at the 50-min time point. Phy-D + CAE resulted in a pyruvate concentration 56% above S-D at the 20-min time point and decreased to 87% of S-D by the 50-min time point. The level of plasma pyruvate did not significantly differ between AE and Phy-D + CAE at any of the time points.

The L/P ratio, an indicator of cell redox state, will increase when the cell redox state is reduced, suggesting the conversion of pyruvate to lactate. At 20 min post-Phy administration, L/P ratio was 34.5 ± 7.0, which was significantly above the S-D value of 25.8 ± 2.3. It declined to 22.4 ± 5.4 at the 30-min time point, then steadily increased to 41.5 ± 6.3 at the 50-min time point. Exercise alone did not elicit any significant change in L/P ratio at any of the postexercise time points and was actually 24% below S-D level at the 50-min time point. In Phy-D + CAE rats, L/P ratio was initially below S-D level but increased gradually thereafter until reaching 32.2 ± 2.5 at the 50-min time point, slightly above S-D level. At the 35- and 50-min time points, the Phy-D + CAE group elicited significantly greater L/P ratios compared to the AE group.

Muscle

The effects of Phy, exercise, and Phy + concurrent exercise on post-Phy administration or postexercise time courses of muscle lactate, pyruvate, and L/P ratios are shown in Fig. 3A, Fig. 3B, and Fig. 3C, respectively. From 20 min post-Phy administration in the Phy-D group, lactate concentration decreased from 91% to 71% of S-D (2.24 ± 0.3 to 1.54 ± 0.1 mM) at the 50-min time point. In response to the acute exercise bout, muscle lactate in AE decreased from 18% above S-D values at the 20-min time point to 26% below S-D values by the 50-min time point. Phy-D + CAE increased muscle lactate levels 84% to 137% of S-D values (1.81 ± 0.2 to 2.96 ± 0.2 mM) from the 20- to 50-min time points, respectively. In comparing Phy-D + CAE to AE, the Ph-D + CAE rats elicited significantly greater lactate values than AE from 30- to 50-min time points.

Pyruvate was 71% of S-D (0.015 ± 0.003) at 20 min post-Phy administration and further decreased to 33% of S-D by 50 min in Phy-D. Though the pyruvate level of the AE group was 74% of S-D at 20 min, pyruvate increased to S-D level by 50 min, which is an expected phenomenon. But in the Phy-D + CAE group, pyruvate was lowest (50% of S-D) at the 20-min time point compared to the Phy-D or AE group and remained significantly lower than S-D throughout the recovery period. These trends were reflected in L/P ratios.

In the Phy-D group L/P ratio increased to 238.7 ± 9.4 by 25 min post-Phy administration but declined to 158 ± 14.6 by the 35-min time point. The increase in L/P ratio early in recovery was due to the decrease in pyruvate at the 25-min time point. The L/P ratio increased again to 216 ± 28.1 by the 50-min time point, possibly indicating the conversion of pyruvate to acetyl-CoA since lactate values were declining. In the AE group, a decreasing trend (160.8 ± 15.1 to 72.0 ± 7.7) in L/P ratio was observed from the 20- to 50-min time points. An interesting observation was that Phy-D + CAE

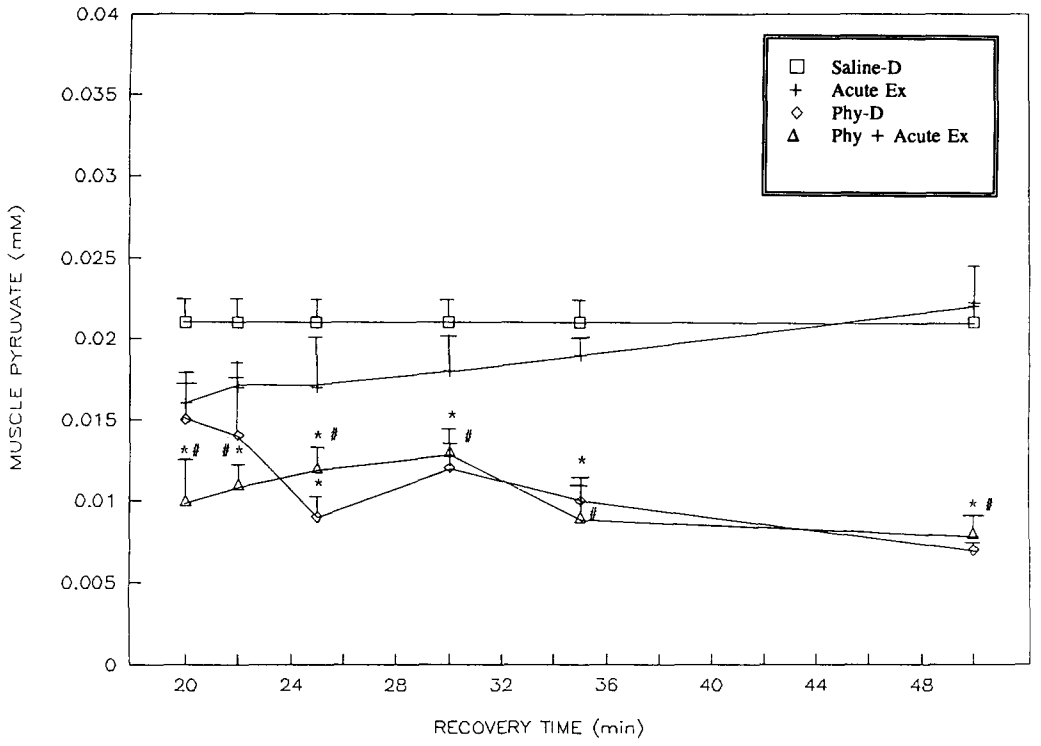

FIG. 3B. Muscle pyruvate at post-Phy administration (70 μg/kg, IM) or post-initiation of exercise time points in saline-dosed, acutely exercised, Phy-dosed, and Phy + acutely exercised groups. Mean ± SEM of four to six animals. *Significantly different than Saline-D at $p < 0.05$. #Significantly different than Acute Ex at $p < 0.05$.

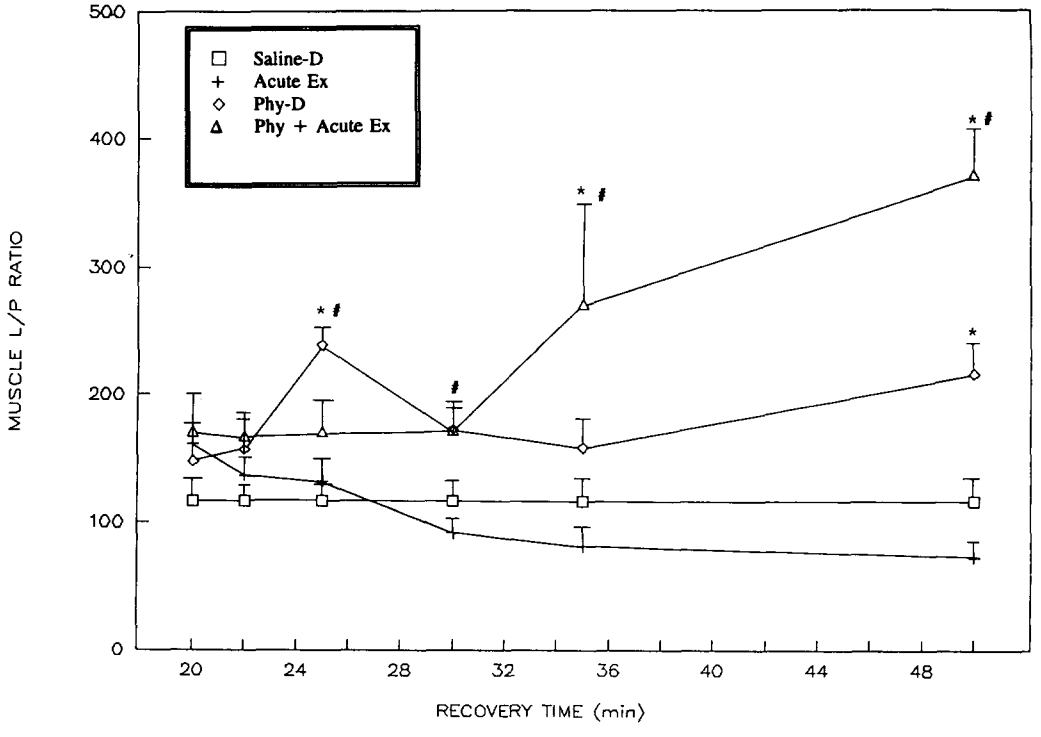

FIG. 3C. Muscle L/P ratio at post-Phy administration (70 μg/kg, IM) or post-initiation of exercise time points in saline-dosed, acutely exercised, Phy-dosed, and Phy + acutely exercised groups. Mean ± SEM of four to six animals. *Significantly different than Saline-D at $p < 0.05$. #Significantly different than Acute Ex at $p < 0.05$.

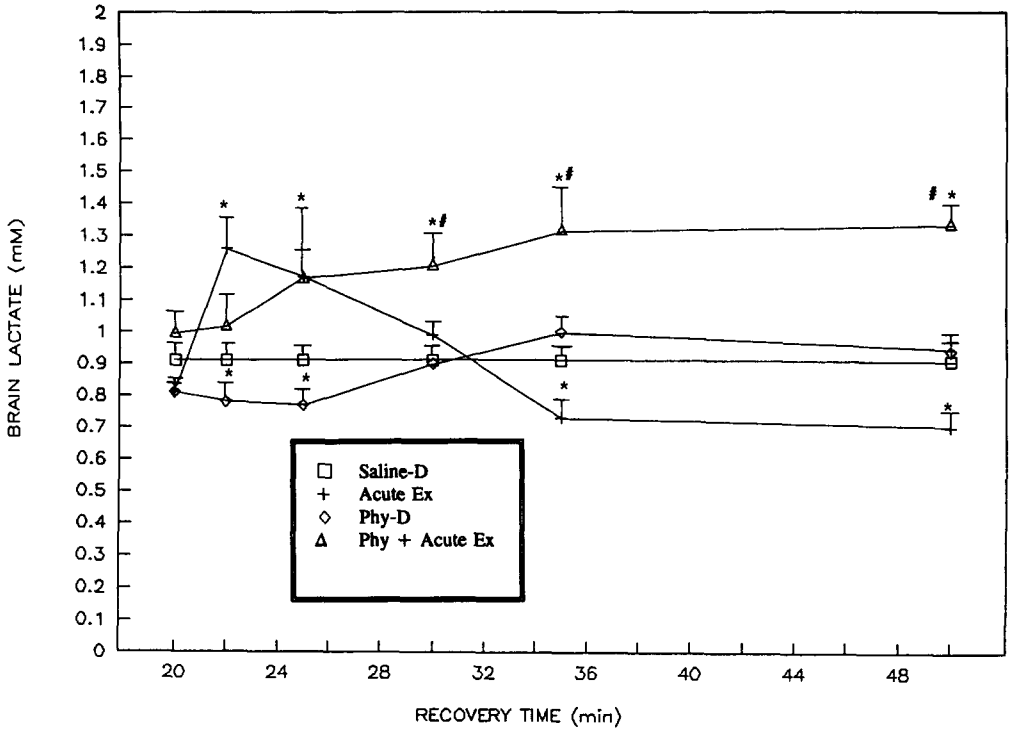

FIG. 4A. Brain lactate at post-Phy administration (70 μg/kg, IM) or post-initiation of exercise time points in saline-dosed, acutely exercised, Phy-dosed, and Phy + acutely exercised groups. Mean ± SEM of four to six animals. *Significantly different than Saline-D at $p < 0.05$. #Significantly different than Acute Ex at $p < 0.05$.

increased the L/P ratio to several fold above S-D values, indicating the conversion of pyruvate to lactate even up to the 50-min time point. In contrast to the AE group, Phy-D + CAE exhibited the opposite trend in L/P ratio from the cessation of exercise (20-min time point) to the 50-min time point. This suggests that the presence of Phy is contributing to an increase in lactate accumulation beyond the amount caused by exercise alone.

Brain

The effects of Phy, exercise, and Phy + concurrent exercise on post-Phy administration or postexercise time courses of lactate, pyruvate, and L/P ratio in brain are shown in Fig. 4A, Fig. 4B, and Fig. 4C, respectively. In the Phy-D group, lactate decreased to 85% of S-D up until 25 min post-Phy administration and then returned to S-D level by the 30-min time point. However, exercise alone elicited a 37% higher lactate value (1.26 ± 0.1 mM) at the 22-min time point, and decreased 23% below S-D (0.7 ± 0.01 mM) by the 50-min time point. The combined effect of Phy-D + exercise showed a steady increase in lactate from the 20- to 50-min time point. This resulted in a significant difference between Phy-D + CAE and AE from the 30- to 50-min time points.

Pyruvate was below S-D levels at all time points (45–57% of S-D) following Phy administration in the Phy-D group. The AE group showed a similar level of pyruvate as the S-D group at 22 min, but it decreased to 67% of S-D by the 50-min time point. Compared to the AE group, Phy-D + CAE rats had a significantly higher level of pyruvate at 20 min but did not differ thereafter.

A small increase in pyruvate and decrease in lactate of the Phy-D group decreased the L/P ratio from 12.5 ± 2.5 to 8.2 ± 1.8 at the 25-min time point. The L/P ratio returned to 11.5 ± 1.3 by the 50-min time point. In the AE group, L/P ratio gradually decreased from 9.5 ± 2.0 at 20 min to 7.4 ± 2.5 at the 50-min time point. In contrast to the AE group, L/P ratio of the Phy-D + CAE gradually increased from 20 to 50 min such that L/P ratios were significantly different between these two groups at and beyond the 25-min time point. Therefore, it appears that the Ph-D + CAE group was continuing to convert pyruvate to lactate from 20 to 50 min, and the AE group was more readily converting pyruvate to another metabolite besides lactate within the same time frame.

DISCUSSION

The findings of this investigation suggest that Phy administration to sedentary rats results in an enhanced L/P ratio in muscle and brain tissue. The effect of Phy was mimicked in the AE group relative to lactate levels in muscle. This suggests that physostigmine has some effect on the aerobic/anaerobic energy system. Phy-D + CAE further enhanced this effect, particularly in the latter stages of exercise recovery. In plasma, the Phy-D group did not elicit lactate values significantly different than S-D group levels between 20 and 50 min. In our previous study (2), Phy was administered soon after AE and was found to increase plasma lactate significantly above sedentary S-D levels. Plasma lactate was significantly elevated at 25 min in the AE and Phy-D + CAE groups before returning to S-D levels. This shows that Phy did not alter the normal rise in plasma lactate following an acute exercise. Plasma pyruvate also significantly increased shortly after exercise in the two exercise groups with no significant effect from Phy. These

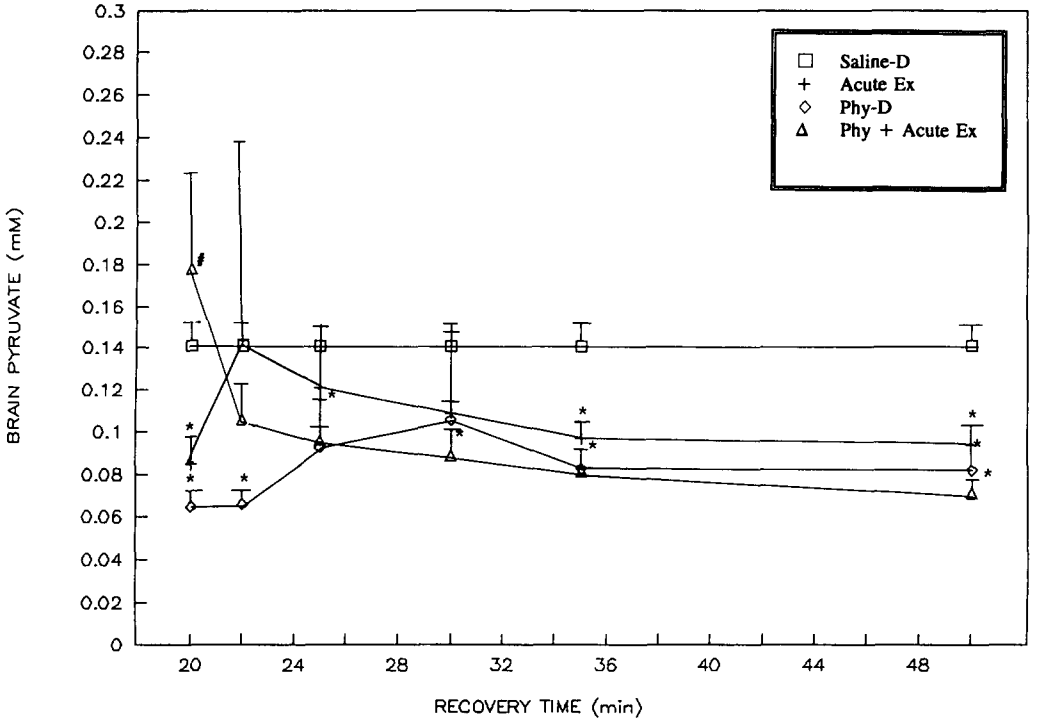

FIG. 4B. Brain pyruvate at post-Phy administration (70 μg/kg, IM) or post-initiation of exercise time points in saline-dosed, acutely exercised, Phy-dosed, and Phy + acutely exercised groups. Mean ± SEM of four to six animals. *Significantly different than Saline-D at $p < 0.05$. #Significantly different than Acute Ex at $p < 0.05$.

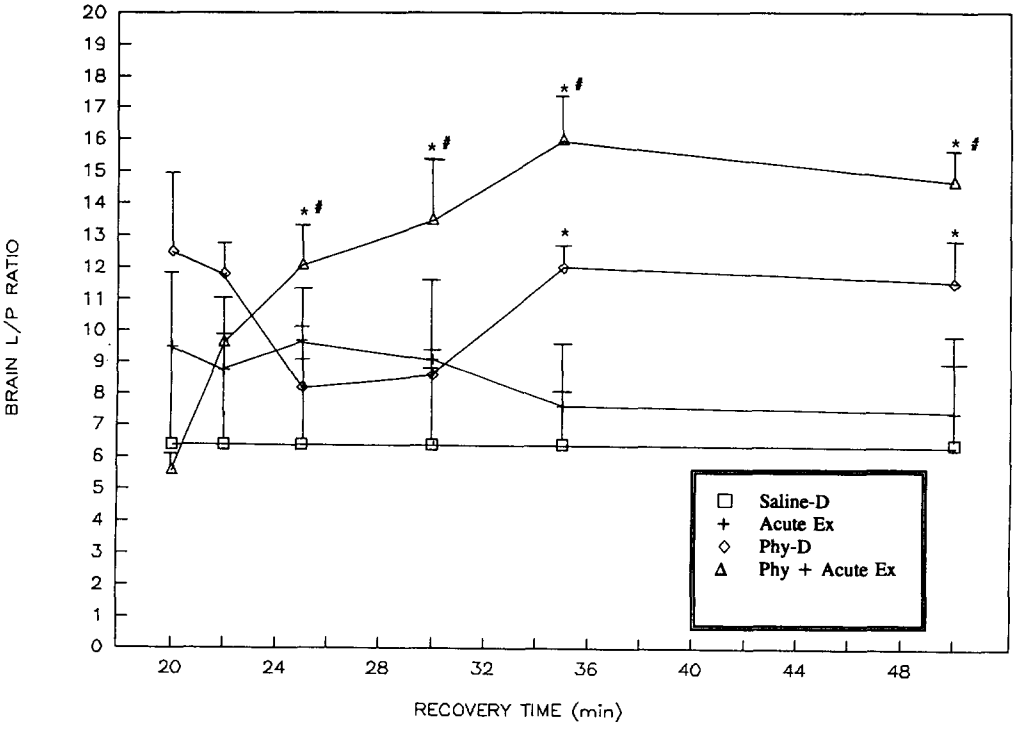

FIG. 4C. Brain L/P ratio at post-Phy administration (70 μg/kg, IM) or post-initiation of exercise time points in saline-dosed, acutely exercised, Phy-dosed, and Phy + acutely exercised groups. Mean ± SEM of four to six animals. *Significantly different than Saline-D at $p < 0.05$. #Significantly different than Acute Ex at $p < 0.05$.

changes seem to be a reflection of changes taking place in muscle as reported by Karlsson et al. (7) and Roth and Brooks (12). It is well known that during exercise, lactate formation in muscle increases (8) and continues to increase up to 5 min after exercise. Wasserman et al. (20) reported that the increased blood lactate may be due to the diffusion of lactate from muscle to blood. Following an acute exercise bout, blood pyruvate has been reported to rise (13), in conjunction with blood lactate. This finding is consistent with our data. However, after 5 min postexercise (25-min time point), blood lactate decreased more rapidly than pyruvate, resulting in a decrease in L/P ratio. But in Phy-D and Phy-D + CAE groups, the plasma L/P ratio showed an increasing trend from the 30- to 50-min time points, indicating that Phy may be interfering with energy metabolism of the cell. Of comparable interest, L/P ratio was increasing, beyond 10 min postexercise, in the Phy-D + CAE group and decreasing in AE rats. This was due to a more rapid drop in plasma pyruvate levels in Phy-D + CAE, suggesting a more apparent uptake of pyruvate by other tissues. It is unclear why this would more readily occur in plasma of exercised rats receiving Phy vs. exercised rats not exposed to this drug.

In the Phy-D group, both muscle lactate and pyruvate decreased up to 50 min postinjection, whereas the Phy-D + CAE group increased their muscle lactate level. In the presence of Phy, the pyruvate level was significantly lower than S-D values with or without exercise. This resulted in significantly higher L/P ratios in muscle at all time points for the two groups receiving Phy. Sahlin et al. (13) have reported a decrease in muscle lactate and an increase in muscle pyruvate after exercise. This is consistent with our data in the AE group. This results in a linear decrease in L/P ratio. Similar findings have also been reported by Wasserman et al. (20). These findings suggest that pyruvate in the muscle becomes more readily available for aerobic energy resynthesis while lactate diffuses slowly into the blood or is oxidized by the muscle for energy needs. In contrast to the AE group, the continuous decrease in muscle pyruvate, increase in lactate, and increase in L/P ratio of the Phy-D + CAE group suggests that pyruvate is continuously converted into lactate even after the cessation of exercise, particularly at 10 to 30 min postexercise. Although we are unsure of the mechanism involved, it appears that Phy has an effect on the recovery process of the muscle cell. This is an interesting observation and has not been previously reported. It suggests that when Phy is administered to exercising rats, the lactate-producing effect of moderately intensive exercise is prolonged by this drug. It may be that Phy could be interfering with the mitochondrial membrane proton shuttle. This is supported by King and Somani (9), who found an increased accumulation of Phy and its metabolites in the mitochondria during Phy administration. Interference of the mitochondria's redox state can lead to a decrease in aerobic metabolism resulting in an increase in muscle lactate. We have shown that Phy + CAE prolonged the inhibition of cholinesterase (ChE) in the muscle compared to Phy alone. The rate of decarbamylation (K_d) significantly decreased in muscle following Phy + CAE ($0.0135\ min^{-1}$) compared to Phy alone ($0.0308\ min^{-1}$) (4). This study showed that lactate concentration increased in muscle due to Phy + CAE. Phy is more stable in acidic environment, and enhanced lactate formation and accumulation possibly provides the acidic environment in the muscle, which allows Phy to act on ChE enzyme, thereby prolonging its effect in this tissue.

In the brain, pyruvate levels seemed to be affected more than lactate. When comparing the Phy-D group to the S-D group, pyruvate levels were significantly lower in the Phy-D group at all time points. Because lactate values did not differ when comparing Phy-D to S-D, it would appear that pyruvate in the brain was more readily being converted to another metabolite, such as acetyl-CoA, instead of lactate. Acute exercise elicited slightly higher brain lactate values at 2–5 min postexercise compared to the S-D condition, much like that observed in plasma, although lactate levels were well below S-D levels by 30 min postexercise. A similar pattern was observed with pyruvate values in the acutely exercised rats. Hence, L/P ratio for the AE group did not change appreciably following exercise. However, like muscle tissue, an increase in L/P ratio of the Phy-D + CAE group, from immediate to 30 min postexercise, did occur. This again suggests that Phy prolongs the lactate-producing effect of moderately intensive exercise even in the brain. Only in the Phy-D + CAE group did it seem apparent that the decreasing pyruvate level was related to an increase in lactate accumulation. It is interesting to note that in both muscle and brain tissue, the Phy-D + CAE group showed an increase in L/P ratio from immediate to 30 min postexercise compared to the AE group, which had the opposite decreasing trend in L/P ratio. The primary cause of this contrast was the tendency of lactate levels to decrease shortly after exercise in the AE group, and the increase in lactate values, following exercise, in the Phy-D + CAE rats. It therefore appears that there may be a prolonged effect of physostigmine following an acute bout of exercise at 80% $\dot{V}O_2max$. If physostigmine does accumulate in the mitochondria of muscle and brain cells, it may have an effect on the recovery phase of an acute exercise bout. If cellular oxygen content is unable to be utilized for mitochondrial oxidative phosphorylation, due to physostigmine interference, then lactate might accumulate more readily. At an exercise intensity of 80% $\dot{V}O_2max$, the animals need a significant amount of oxygen to keep up with increased metabolic processes, subsequently resulting in increased oxygen tension in the tissues. If the tissues are also subjected to physostigmine during exercise and recovery it would seem, from our results, that available oxygen in the cell is not able to be oxidized as readily; hence, increases in lactate accumulation.

The significance of these results suggests that Phy does induce a temporary metabolic stress similar to an acute exercise bout. This is supported from a previous investigation (2). In addition, at later time points in exercise recovery (10–30 min postexercise), Phy appears to be associated with an attenuation of pyruvate levels in plasma, muscle, and brain. This suggests that Phy, with or without exercise, affects the metabolic use of pyruvate within muscle and brain tissue in later stages of exercise recovery.

Based upon these results, and the available literature, we conclude:

1. the potential for conversion of pyruvate to lactate is enhanced up to 30 min after exercise at 80% VO_2max when Phy is administered;
2. the effect of Phy is more pronounced in the muscle than the brain after exercise; and
3. Phy appears to decrease O_2 tension in muscle and brain, thereby increasing the normal formation of lactate after exercise.

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