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Effects of Exercise on Behavioral Sensitivity to Carbamate Cholinesterase Inhibitors

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McMASTER, S. B. AND A. V. FINGER. *Effects of exercise on behavioral sensitivity to carbamate cholinesterase inhibitors.* PHARMACOL BIOCHEM BEHAV **33**(4) 811–813, 1989.—The interaction between exercise and drug response has not been studied extensively. The present study examined the relationship between both acute (15 minute) and chronic (10 week) treadmill exercise and behavioral response to the carbamates physostigmine and pyridostigmine. Rats trained on an operant task under a multi-component FR30 schedule were used to evaluate the interaction between exercise and performance following drug administration. The direct effects of both 10 weeks of exercise conditioning and a moderate exercise challenge, as well as the interaction between two were assessed. Results obtained with physostigmine show that acute exercise increased behavioral sensitivity. Chronic exercise resulted in behavioral tolerance. These results are consistent with previously reported studies of centrally acting compounds. In contrast, pyridostigmine, which has little or no central activity, produced no behavioral changes. This result was constant over exercise conditions.

Exercise Carbamate Rodent Operant Behavioral

ONE of the most interesting challenges faced by toxicologists today is the concept of medically pretreating individuals who have a high probability of exposure to toxic chemicals as a means of protecting them against the effects of poisoning. In addition to possessing the necessary antidotal properties, a pretreatment compound must be relatively nontoxic and allow the recipient to function in his normal work environment. Laboratory studies of potential pretreatment compounds often fail to consider job-related drug response variables, such as temperature and humidity conditions, sleep deprivation and physical exertion. The possibility that adaptation to these variables may occur over time, further influencing drug potency, is rarely considered in studies designed to evaluate pretreatment compounds.

The relationship between physical exercise and behavioral sensitivity to centrally-acting compounds has not been well characterized. Existing reports consistently confirm the influence of exercise on behavioral effects of centrally-acting compounds. Single exercise sessions have been reported to increase behavioral sensitivity to caffeine (1), as well as to cholinergic antagonists (7). Repeated exercise reportedly enhances the development of behavioral tolerance to cholinergic antagonists (9) and benzodiazepines (3).

The present study is a systematic evaluation of the interaction between exercise and the behavioral effects of two carbamate cholinesterase inhibitors that are under consideration for use as pretreatment compounds to protect against possible poisoning by organophosphorus (OP) agents. Carbamates and OPs exert their toxicity primarily by inhibiting the enzyme acetylcholinesterase. Because they occupy the same esteratic site, pretreatment with a carbamate prevents phosphonylation of the enzyme by an OP. Phosphonylation is considered irreversible; spontaneous decarbamylation occurs following treatment with physostigmine or pyridostigmine. Thus, although both classes can produce symptoms associated with excess levels of acetylcholine, recovery following carbamate treatment is relatively rapid. The tertiary carbamate physostigmine salicylate and pyridostigmine bromide, a quaternary carbamate, were studied in an unexercised control group and an exercise-conditioned group of rats. The behaviorally disruptive effects of these drugs were studied in both the presence and absence of a moderate predrug exercise challenge. Animals assigned to the control group were maintained under standard laboratory conditions, and are representative of the subject pool used for most behavioral studies conducted in rats. The exercise-conditioned group differed from the control group only in exercise history. Animals assigned to this group were required to run on a treadmill once a day, five days a week, for 10 weeks prior to drug testing.

The results of this study confirm both the increases in drug sensitivity in response to mild, acute exercise in normally sedentary animals and the drug tolerance produced in response to chronic exercise reported previously for other classes of compounds.

METHOD

Subjects

Sixteen experimentally-naive, male Sprague-Dawley-derived

rats were randomly assigned to an exercise conditioning (n = 8) or a control (n = 8) group. The animals were housed individually in suspended cages, under controlled temperature (74 ± 2°F) and humidity (50 ± 5%) conditions. A 12-hour light/dark cycle with lights on at 0600 was automatically maintained. Free-feeding weights, which ranged from 280–320 g, were determined at the end of a 2-week quarantine and acclimation period and adjusted upward to allow for normal growth-related increases. Unlimited access to water and a sufficient amount of lab chow to maintain each rat at 80% of his free-feeding body weight were provided daily.

Apparatus

Behavioral testing took place in standard rodent operant chambers (Lafayette Co., Lafayette, IN, No. 80001) enclosed in sound-attenuating, ventilated compartments. Operant training and test sessions were controlled by means of a microcomputer-based software system (OPN, Texas College of Osteopathic Medicine, Ft. Worth, TX) designed to initiate schedule contingencies, operate stimulus lights and record data (2). Exercise sessions were conducted on a rodent treadmill (Omnitech Inc., Columbus, OH, Omnipacer model).

Operant Training

All subjects initially were trained to lever press for food reward under a multiple time-out fixed ratio schedule of reinforcement (MULT TO FR). This schedule was composed of time-out (TO) components, during which no reinforcement was available, and fixed-ratio (FR) components during which each multiple of 30 lever presses (FR30) produced a 45-mg food pellet (BioServ, Inc., Frenchtown, NJ, No. F0021). The contingency in effect was signalled by the house light which was on during FR and off during TO components. Ten-minute TO and FR30 components alternated, for a total of four each, resulting in an 80-minute operant session. Training sessions were conducted 5 days per week between 1000 and 1300 hr. As training requirements progressed from continuous to FR30 reinforcement, intraperitoneal (IP) saline injections (0.5 ml injection volume) were introduced at the beginning of each TO component one day per week.

Exercise Training

When operant response rates for both groups had stabilized at approximately 2 responses per second and vehicle injections did not disrupt performance, one group of eight rats began an exercise-conditioning program. Treadmill running sessions took place once a day, five days per week, according to a schedule previously reported (8). Session duration and treadmill speed were gradually increased to encourage development of physical conditioning effects while minimizing stress. Conditioning was completed in ten weeks, with all animals reaching the training goal of a one-hour exercise session at a treadmill speed of 1.0 mph by week 6. Operant sessions were continued 2 or 3 times per week for both groups to maintain performance level; saline injections were made once a week. On days in which both exercise and operant sessions took place, the operant session preceded the exercise session by several hours. All animals were handled daily for weighing and assessment of general condition.

Drug Testing

The two groups of rats, differing only in exercise history, were tested under identical drug and exercise conditions. Half of the animals in each group were given physostigmine; the other half, pyridostigmine. The effect of the carbamate on MULT TO FR30 responding was evaluated twice in each group, once in rested and once in recently exercised animals. Rested animals were defined as having experienced no treadmill exercise within the previous 72 hours. Recently exercised animals had completed a 15-minute treadmill session at a speed of 0.5 mph 5 minutes prior to drug administration.

Each drug test consisted of a single 80-minute session under the MULT TO FR30 schedule. Physostigmine (Sigma Chemical, St. Louis, MO) and pyridostigmine (Hoffmann-La Roche, Nutley, NJ) were prepared daily in saline vehicle. Drugs were administered cumulatively, with one injection at the beginning of each TO component. This method produces a four-point dose-responsive curve within a single session (9). Injections were made IP at a volume calculated to deliver 5% of the LD_{50} dose, 50 μg/kg physostigmine or 180 μg/kg pyridostigmine, per injection (4). This procedure resulted in total cumulative doses of 50, 100, 150 and 200 μg/kg physostigmine. Comparable doses of pyridostigmine (180, 360, 540 and 720 νg/kg) were administered, as was a second series of doses ranging from 450 to 1800 μg/kg. The latter dose series is equivalent to 12.5–50% of the LD_{50} for this compound (4).

Behavioral effects were determined by comparing baseline nondrug response rates to the rate of responding at each dose level. To control for the inherent intersubject variability in baseline rate of responding, each animal's baseline rate was assigned a value of 100. Rates of responding under drug conditions were expressed as a percentage of this rate for individual animals and then combined for statistical analysis.

RESULTS

A three-factor mixed design ANOVA (6) revealed significant ($p<0.05$) main effects of chronic exercise, $F(1,6) = 20.39$, acute exercise, $F(1,6) = 7.21$, and dose of physostigmine, $F(3,18) = 3.52$, on performance. Interaction effects between chronic exercise and drug dose, $F(3,18) = 3.25$, and acute exercise and drug dose, $F(3,18) = 3.19$, also significantly affected performance.

Baseline rates of responding were not different between the two groups at the completion of operant training, or within the exercise-trained group when pre- and post-exercise conditioning data were compared. Two distinct exercise effects became apparent when physostigmine disruption of FR30 responding was evaluated. When physostigmine was administered under rested conditions, control animals displayed a typical dose-related decrement in response rate (10). The response of chronically-exercised animals, also tested under rested conditions, was significantly different from that of the control group.

Fifteen minutes of treadmill running at a speed of 0.5 MPH, completed five minutes before the start of an 80-minute MULT TO FR30 session, does not interfere with performance (8). In keeping with previous reports, response rates for both the control and chronically-exercised groups were within their normal range following this mild exercise challenge. When a 15-minute exercise session preceded dosing with physostigmine, the drug-induced disruption of behavior was significantly increased over that observed under rested conditions in the control group. The lowest dose tested, 50 μg/kg, decreased responding to approximately 85% of baseline under rested conditions and to 20% of baseline when coupled with acute exercise. Responding was completely suppressed by 150 and 200 μg/kg physostigmine following exercise in the control group. In contrast, performance by the chronically-exercised group was significantly less affected, with performance remaining above 60% of baseline across all doses despite the presession exercise challenge. These results are presented in Table 1.

TABLE 1

PERCENT OF BASELINE RESPONDING UNDER MULT TO FR30 SCHEDULE OF REINFORCEMENT FOLLOWING PHYSOSTIGMINE DOSING IN RATS TRAINED TO EXERCISE (n=4) AND A CONTROL GROUP WITH NO EXERCISE TRAINING (n=4)

Dose of Physostigmine (μg/kg)	Untrained Control Group		Exercise Trained Group	
	Rested	Exercised	Rested	Exercised
50	84.0± 3.9	17.0±11.23	92.8±5.5	70.2± 9.3
100	54.0±14.5	8.7± 6.3	90.2±3.1	70.8±10.7
150	27.3±22.3	0	84.0±3.9	76.4±13.6
200	31.0±25.5	0	81.4±5.7	66.8±17.8

Each group was tested under conditions of rest (a minimum of 72 hours since the most recent exercise session) and recent exercise (ending 15 min prior to initiation of the operant session).

No dose of pyridostigmine that was not completely incapacitating had any effect on operant performance maintained under a MULT TO FR30 schedule of reinforcement. This was observed regardless of exercise history (control or conditioned) or current exercise state (rested or following an acute exercise session). This finding supports the idea that the exercise effect is central in nature, since pyridostigmine is a quaternary compound that does not readily gain access to the CNS.

DISCUSSION

The results of this study are consistent with related reports of exercise-induced alterations in drug sensitivity (1, 3, 7, 9). Two distinct effects of treadmill exercise are consistently observed. Animals not specifically trained to exercise are significantly more sensitive to centrally-active drugs when a given dose is preceded by a very mild acute exercise challenge that is not itself disruptive (7). Long-term training not only eliminates the potentiated drug effect observed after mild exercise, but actually increases the amount of drug required to produce a performance decrement.

In exercise-trained subjects, the behavioral response to physostigmine in this study, as well as to scopolamine (9), benactyzine (9) and midazolam (3) described in previous reports can be described as a resistance to an expected drug effect. When compared to control animals, exercise-conditioned animals tolerate significantly higher levels of drug without disruption of behavior. This effect is measured in animals that have had no previous exposure to the drug under investigation or to any other drug. In this sense, the phenomenon violates the accepted definition of drug tolerance as a diminished responsiveness to a drug after previous administration of the drug or some related substance (5). The functional effect is identical, however.

The effect of acute exercise may be related to changes in blood flow and/or alterations in plasma levels of the drug. A transitory shunting away from the primary site of metabolism during and immediately following exercise may result in higher peak plasma levels. Chronic exercise, as suggested elsewhere, may produce a functional tolerance related to exercise-induced alterations in cortical levels of catecholamines and/or acetylcholine (9). Any explanation of the chronic exercise effect must account for its presence 72 hours post-exercise. Whatever the underlying mechanism, the results of this study suggest that exercise state and exercise history are both important variables affecting behavioral sensitivity to centrally-acting compounds, and, as such, should be considered in the development of pretreatment regimens.

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