Pharmacology Biochemistry & Behavior, Vol. 23, pp. 191–194, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Changes in Drug Sensitivity Following Acute and Chronic Exercise

S. B. McMASTER*[1] AND J. M. CARNEY†

**Department of Psychiatry and Behavioral Science and †Department of Pharmacology*
University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

Received 11 June 1984

McMASTER, S. B. AND J. M. CARNEY. *Changes in drug sensitivity following acute and chronic exercise.* PHARMACOL BIOCHEM BEHAV **23**(2) 191–194, 1985.—A number of factors are known to influence drug sensitivity. These include biological variables such as genetics, age, endocrine status and gender, as well as environmental variables such as operant schedules, ambient temperature and sleep deprivation. Additional factors function as either biological or environmental variables in different situations. For example, chronic drug administration can produce tolerance and cross tolerance and function as a biological variable. Acute administration of the same compound can function as an environmental variable. The present study examined exercise as both a biological and an environmental variable influencing drug sensitivity. Chronic exercise leads to relatively long term changes in physical fitness level, and functions as a biological variable. Fitness level did not influence drug sensitivity when physically conditioned animals and non-exercised control subjects were compared under rested conditions. Mild acute exercise, an environmental variable, increased sensitivity to muscarinic antagonists in the control subjects but not in the exercise trained animals. These results indicate that exercise state should be considered as an environmental variable capable of influencing drug response and that biological fitness level modifies this effect.

Exercise Rodent Scopolamine Benactyzine Citrate synthase Anticholinergic sensitivity

THE response of an individual organism to a particular compound is determined by a multitude of factors. Many of these are inherent properties of the individual. Among the biological factors known to influence drug sensitivity are genetics [14], age, endocrine status and gender [17]. Environmental variables also influence drug sensitivity. Experimentally studied examples include operant schedule [3], ambient temperature [12] and sleep deprivation time [13]. A third category of variables influencing drug sensitivity is composed of factors that can act as either biological or environmental variables, depending upon the situation. For example, chronic administration of compound A can result in the development of tolerance and cross tolerance. A subject tolerant to compound A may display an atypical response to compound B. In this case, the first drug is acting as a biological variable. On the other hand, acute administration of compound A may also interact with compound B if the two are administered so that their effects are temporally concurrent. This short term effect is the result of drug A acting as an environmental variable.

Physical exercise can also operate as either a biological or an environmental variable. Chronic exercise results in relatively long term biological changes in a number of systems. Acute exercise affects the same systems but in a more transient manner. The changes that occur in the cardiovascular and respiratory systems as a result of acute or chronic exercise have been the subject of countless investigations. The possibility that similar changes occur in the central nervous system (CNS) has not received an equal amount of attention. One approach that can be used to study potential CNS changes that result from exercise involves the use of centrally active drugs. Changes in drug response following acute and/or chronic exercise can be attributed to exercise-induced changes in the system(s) affected by the drug.

The present study examined the effect of acute and chronic exercise on response to three muscarinic antagonists; scopolamine hydrobromide, scopolamine methylbromide and benactyzine. These compounds represent two prototypic muscarinic antagonists, differing only in their ease of access to the CNS, and one synthetic analog. The synthetic compound, benactyzine, has been reported to have negligible peripheral effects [8]. The two naturally occurring compounds, the tertiary and quaternary forms of scopolamine, act both peripherally and centrally. Scopolamine methylbromide enters the CNS less readily than scopolamine hydrobromide, but is the more potent of the two [1].

METHOD

Subjects

Twenty-four male Sprague-Dawley derived rats (Sasco, Omaha, NB) weighing 250–300 g at the start of the experiment were assigned to a control (n=12) or exercise condi-

[1]Requests for reprints should be addressed to Commander, US Army Medical Research Institute of Chemical Defense, ATTN: SGRD-UV-YN/McMaster, Aberdeen Proving Ground, MD 21010-5425.

tioning (n=12) group. They were maintained at 80% of their ad lib body weight. Rats had unlimited access to water and were housed individually in suspended cages under a 12 hr light/dark cycle throughout the experiment.

Apparatus

Behavioral testing took place in standard operant chambers (Lafayette Co. No. 80001) enclosed in sound attenuating, ventilated compartments. An AIM-65 microcomputer based system was used to determine schedule contingencies, control stimulus lights and record data [10]. Exercise sessions were conducted on a rodent treadmill (Quinton Inc. No. 4215).

Operant Training

Eighteen of the twenty-four rats 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 produced a 45 mg food pellet (BioServ). Ten minute TO and FR components alternated, for a total of four each, resulting in an 80 min operant session.

Exercise Training

When response rates had stabilized at approximately one response per second, six of the rats began an eight week exercise training program. Treadmill exercise sessions took place once a day, five days per week, according to the following schedule:

week 1	15 min at 0.5 mph
week 2	30 min at 0.5 mph
week 3	45 min at 0.5 mph
week 4	30 min at 1.0 mph
week 5	45 min at 1.0 mph
week 6	60 min at 1.0 mph
week 7	60 min at 1.0 mph
week 8	60 min at 1.0 mph

The animals undergoing exercise training also completed the operant task two or three days per week in order to maintain the response rate established during training. Exercise and operant sessions were conducted several hours apart on days when both occurred, with the operant session always preceding the exercise session.

Biochemical Verification of Training Effects

The remaining group of six animals completed the eight week exercise training program but received no operant training. Following the eighth week of exercise training, these animals were sacrificed by decapitation. Muscle samples were removed from the hind limbs of each animal. Additional samples were obtained from six age, weight and sex matched control animals. The tissue was immediately frozen by immersion in liquid nitrogen and placed in storage at −65°C. Citrate synthase activity was measured according to the method of Sere [15].

Procedure

The 18 rats trained on the operant task were tested following six drug/exercise treatments. Three muscarinic antagonists were administered, under both rested and exercised conditions, to each animal. Rested animals had a minimum of 72 hr between their most recent treadmill session and drug testing. Exercised animals completed a 15 min exercise session at a treadmill speed of 1.0 mph a maximum of five minutes prior to their first drug dose.

Tests conducted in exercised animals began by placing the animals on the moving treadmill. After 15 minutes of steady running at 1.0 mph they were removed and placed in the behavioral chambers. Transfer of a group of six animals was accomplished within three to five minutes. Each animal then completed an 80 min operant session under a MULT TO FR30 schedule. Drugs were administered in a cumulative sequence, with one injection at the beginning of each TO component. This allowed determination of a four point dose response curve within a single session. Testing rested animals required direct transfer from the home cages to the behavioral chambers. Drug administration and evaluation procedures were identical under rested and exercised conditions.

Drugs

Scopolamine hydrobromide, scopolamine methylbromide and benzylic acid β-diethylaminoethyl ester (benactyzine) were purchased from Sigma Chemical in crystalline form. They were prepared for intraperitoneal injection by dissolving the salt in saline at concentrations calculated to deliver the correct dose, calculated as the free base, at an injection volume of 1.0 ml per kg of body weight.

Data Analysis

Operant response rates under test conditions were converted to percent of baseline response rates for individual animals. The resulting scores were then combined and group means and standard errors calculated. The effect of exercise *per se* was evaluated using a 2 (group) × 3 (exercise duration) × 4 (component) mixed factorial design with both the treatment and within session components as repeated measure factors [19]. A probit transformation was performed on drug data. Statistical comparisons between the various treatment conditions were computed based on a straight line linear regression model [6]. Citrate synthase levels were statistically analyzed by means of a *t*-test [11].

RESULTS

Exercise Training

Following eight weeks of exercise training, FR30 responding remained at the level observed under rested conditions when an exercise challenge of 15 or 60 min duration preceded the operant test session. Responding by subjects not specifically trained to exercise was disrupted by pre-operant session treadmill running of 60 min duration, $F(2,32)=4.21$, $p<0.05$, but not by a 15 min exercise session.

Exercise Training and Drug Sensitivity

Completion of eight weeks of exercise training did not alter sensitivity to scopolamine hydrobromide, scopolamine methyl bromide or benactyzine when the drugs were administered under rested conditions. The ED50 values for disruption of operant performance under the MULT TO FR schedule of reinforcement appear in Table 1.

TABLE 1

EXERCISE-INDUCED CHANGES IN BEHAVIORAL SENSITIVITY TO MUSCARINIC BLOCKING AGENTS

Exercise State		ED50 (mg/kg) Control n=12	ED50 (mg/kg) Exercise Conditioned n=6
Rested	Scop HBr	0.15	0.14
	Scop MeBr	0.26	0.20
	Benactyzine	11.22	10.32
Exercised	Scop HBr	0.06	0.20
	Scop MeBr	0.04	0.18
	Benactyzine	5.31	6.70

The behaviorally disruptive doses defined as the dose producing a 50% decrease in operant response rate of scopolamine hydrobromide (Scop HBr), scopolamine methylbromide (Scop MeBr) and benactyzine were shifted to the left following a 15 min exercise session in animals not specifically trained to exercise (control subjects). This shift was not observed in 8 week exercise conditioned subjects when scopolamine is administered.

Exercise State and Drug Sensitivity

When drugs were administered following a 15 min exercise session, significant increases in sensitivity to scopolamine hydrobromide ($p<0.01$) and scopolamine methylbromide ($p<0.001$) were observed in animals not specifically trained to exercise. Dose-response curves remained parallel to those observed in rested animals, but were shifted significantly to the left. No change in sensitivity to these compounds was observed in the exercise trained animals following an identical exercise challenge. Sensitivity to benactyzine was increased following 15 min of running in both the exercise trained group ($p<0.05$) and the group not trained to exercise ($p<0.05$). These results are summarized in Table 1.

Citrate Synthase Assay

Spectrophotometric analysis of muscle tissue homogenates revealed significant differences, $t(5)=3.01$, $p<0.05$, in citrate synthase activity in samples from rats that had completed the eight week exercise training program and control subjects. The mean value for the control group was 33.08($\pm$0.33) micromoles/microliter/min/g in contrast to 41.12 ($\pm$1.5) micromoles/microliter/min/g for the exercised group.

DISCUSSION

The behavioral effects of anticholinergic drugs have been studied in experimental animal and human subjects. One consistent finding resulting from animal studies is the ability of these compounds to disrupt schedule controlled behavior [2, 4, 8, 18]. Anticholinergics reliably produce dose-related decreases in operant response rates under fixed-ratio schedules of reinforcement [2,18]. Human studies demonstrate reliable dose-related changes in cognitive behaviors such as alertness [9], reaction time [5], memory, judgement and perception of time [7].

Although there is general agreement regarding effective doses and relative potencies across studies, the magnitude of a particular individual's response to a particular drug is determined by a number of factors. Many of these are biological variables, such as genetics and gender, that remain constant over the lifespan of the organism. Other biological variables, such as age and endocrine status, vary, but in a steady, predictable manner. Biological variables appear to exert comparable influence over drug sensitivity in many mammalian species, and are therefore routinely considered both in the design of experiments and when drugs are prescribed. Environmental variables can also influence drug sensitivity, but generally receive less attention.

The present study examined the influence of physical exercise on sensitivity to three anticholinergic drugs. Exercise was employed as both an environmental and a biological variable. This was accomplished by exposing rats to acute exercise sessions as well as a chronic exercise program. The effectiveness of the exercise training program was verified via analysis of skeletal muscle samples from chronically exercised subjects. Exercise training produces measurable increases in the concentration of many of the enzymes involved in aerobic metabolism and oxidative phosphorylation [16]. The present study included an analysis of one such enzyme. Citrate synthase activity was significantly increased in the exercise trained animals as compared to the non-trained group, indicating that conditioning had occurred.

Acute exercise resulted in increased behavioral sensitivity to scopolamine hydrobromide, scopolamine methylbromide and benactyzine. Chronic exercise for a period of eight weeks did not directly alter sensitivity to any of these compounds. When rats that had completed the eight week exercise program were evaluated while in a rested state, dose response curves were not different from those obtained prior to exercise training (Table 1). Exercise training did, however, alter the response to the drug/acute exercise combination when scopolamine hydrobromide and scopolamine methylbromide were evaluated. When animals that had completed the eight week exercise program were required to run immediately prior to drug evaluation, the resulting dose response curves were not different from those measured in rested animals (exercise trained and non-trained). Thus, the biological variable did not have a measurable influence on drug sensitivity until the challenge of acute exercise, an environmental variable, was in effect.

Benactyzine potency was shifted by exercise in both the exercise trained and non-trained rats. The rested ED50 doses for these groups were 11.22 and 10.32 mg/kg, respectively. The demands of acute exercise resulted in an ED50 of 5.31 mg/kg in the non-trained group. This was not significantly greater in the exercise trained group, which had a calculated ED50 dose of 6.70 mg/kg. The reason for this observation is not clear. Benactyzine and scopolamine differ in a number of ways. Perhaps the most relevant in this case is the extent of their peripheral nervous system (PNS) effects. If the exercise/drug interaction is produced by a combination of central and peripheral effects, the portion mediated by the PNS may not be observed in the benactyzine treated animals.

Given the widespread acceptance of animal models in the study of exercise effects, potential applications of these findings to a human population are important. An individual considering the initiation of a physical fitness program should be aware of potential changes in sensitivity to currently prescribed medication early in the program. In addition to an increased primary effect, side effects of a drug may also be experienced as more severe. Based on the present

findings, it is predicted that as fitness level increases, drug response will be less influenced by exercise state. The effect of loss of physical conditioning on drug sensitivity has not, as yet, been addressed.

ACKNOWLEDGEMENTS

This research was supported in part by: DAMD 17-81-C-1246. The views, opinions and findings in this report are those of the authors and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other official documentation.

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