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Chronic Exercise Produces Tolerance to Muscarinic Antagonists in Rats[1]

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McMASTER, S B AND J M. CARNEY *Chronic exercise produces tolerance to muscarinic antagonists in rats* PHARMACOL BIOCHEM BEHAV 24(4) 865–868, 1986 —Previous work has shown that exercise can modify behavioral sensitivity to antimuscarinic compounds The present study examined the effect of 10 weeks of endurance exercise on atropine and scopolamine potency The behaviorally disruptive effects of these compounds were evaluated in rats trained to respond under a MULT TO FR30 schedule of reinforcement for food reward Following 10 weeks of endurance exercise, atropine and scopolamine dose response curves were significantly altered The ED50 values were increased 10 and 40-fold, respectively Tolerance to atropine or scopolamine has been reported previously only in response to chronic drug administration The present data demonstrate that non-drug factors can significantly influence behavioral response to muscarinic antagonists

Exercise Rats Scopolamine Atropine Anticholinergic sensitivity Drug tolerance

THE number of people who regularly engage in physical activity for recreational and/or health related purposes has increased dramatically over the past decade [7] Many of these individuals run moderate distances three or more times per week [6]. In addition, the number of prescriptions for centrally active compounds written annually also increased steadily during the last decade [18]. We have previously demonstrated that acute exercise increases behavioral sensitivity to centrally active drugs [16]. Simple population statistics predict some overlap between the group exercising and the group for whom prescriptions are ordered. Moreover, a regular exercise program is often recommended as part of a comprehensive treatment plan and could result in a change in drug effects [3]. The commonly held belief that physical fitness level and mental health are positively correlated [8] implies an exercise-induced alteration in brain function If some change in brain function does occur due to exercise, then such an alteration should produce measurable behavioral changes in human and non-human subjects.

One method available to probe the central nervous system (CNS) for functional changes involves the effects of centrally active drugs on behavior. The relationships between exercise state, fitness level and CNS drug sensitivity have not been studied. If daily exercise produces an adaptation involving a specific system within the CNS, post-exercise responses to drugs affecting the same system also would be expected to be altered We have reported an increase in behavioral sensitivity to muscarinic blocking agents following as little as 15 minutes of acute exercise in normally sedentary subjects [16] Exercise-induced changes in drug sensitivity were not observed in animals that had completed an 8 week exercise training program. These findings suggest that exercise may have both short and long term effects on CNS functioning

The exercise program studied previously is comparable to one typically followed by individuals desiring to increase cardiovascular and overall fitness level. While many people consider the end-point of such an introductory exercise program a final goal, others strive for increased endurance. The present experiment evaluated the impact of an additional two weeks of intense exercise training on sensitivity to the behavioral effects of atropine and scopolamine. The resulting data indicate that this more strenuous exercise program not only protects against the exercise-induced increase in drug sensitivity observed in non-trained subjects, but also changes drug sensitivity under rested conditions. Tolerance of comparable magnitude is observed following chronic drug administration [19] and attributed to changes in receptor density This is the first report of the development of drug tolerance in response to chronic exercise.

METHOD

Subjects

Twelve male Sprague-Dawley derived rats (Sasco, Omaha, NB) weighing 250–300 g at the start of the experiment were assigned to a control (n=6) or exercise conditioning (n=6) group. The animals were housed individually in

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suspended cages, under a 12 hour light/dark cycle, with unlimited access to water. They were fed once a day with a sufficient amount of lab chow to maintain each rat at 80% of his free-feeding body weight

Apparatus

Behavioral testing took place in standard operant chambers (Lafayette Co #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 [17] Exercise sessions were conducted on a rodent treadmill (Quinton, Inc #4215)

Drugs

Atropine sulfate and scopolamine hydrobromide 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.

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

Exercise Training

When response rates had stabilized at or above one response per second, six of the rats began an exercise training program Treadmill running sessions took place once a day, five days per week. The first eight weeks of training followed a schedule previously reported in detail [16] Beginning with a minimal requirement of 15 minutes at 0 5 M.P H. during week one, treadmill sessions were gradually increased until the training goal of 60 minutes at 1 0 M P.H was reached. This occurred during week six; weeks seven and eight served to allow the animals to adjust to this level of exercise Thereafter, rats were required to run daily for progressively increasing durations On day one of week nine, an additional 15 minute session was completed. The duration of the treadmill session was increased by 15 minutes each successive day for eight days and by 30 minutes on each of the following two days Thus, the final training requirement was a single treadmill session of four hours duration at a speed of 1 0 M P.H The rats assigned to the control group (n=6) received no exercise training

Drug Testing

The two groups of rats, differing only in exercise history, were tested under identical conditions. The behavioral effects of atropine and scopolamine were evaluated in rested animals (i e , a minimum of 72 hours had elapsed since their most recent treadmill session and stable baseline rates of responding had been recovered). Each drug test session consisted of a single 80 minute session under the MULT TO FR30 schedule Drugs were administered cumulatively, with one injection at the beginning of each TO component This method produces up to four data points within a single operant session [13]. When more than four doses were required for characterization of the dose-response curve, a second drug test was conducted a minimum of three days after the first Both groups were tested with atropine first Two drug sessions were required to fully evaluate the effects of atropine on FR30 responding for each group However, because the third dose administered on the second day caused behavior to drop to zero in the control group, a fourth dose was not administered to those animals. Following a full week with no drug exposure, both groups were tested with scopolamine The control group was tested on two days, with four doses administered during the first session and an additional two doses three days later Scopolamine was tested in the exercised group only once

Behavioral sensitivity to atropine and scopolamine was determined by calculating the dose required to produce 50% disruption (ED50) of baseline response rates Due to the inherent intersubject variability in response rate, each animal's stable non-drug rate of responding was assigned the 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 A straight line linear regression model was employed to determine the statistical significance of differences in drug sensitivity between the control and exercise conditioned groups [12]

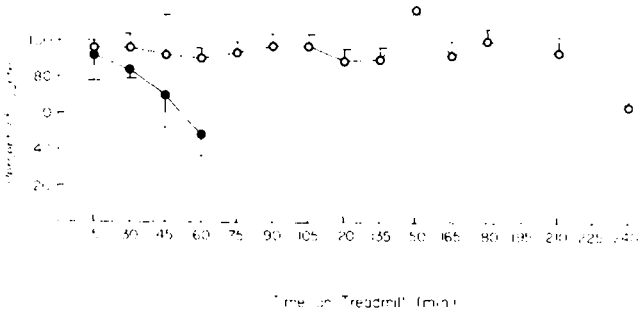

FIG 1 Operant responding under a MULT TO FR30 schedule of food reinforcement, expressed as percent of baseline level, in groups of rats with no exercise training (closed symbols) and following 10 weeks of exercise training (open symbols) Each point represents the mean (±S E) for 6 rats Following treadmill sessions of 15, 30, 45 and 60 min duration at 1 0 M P H , animals not trained to exercise displayed a duration dependent disruption of operant behavior Within-session recovery occurred after all but the 60 min condition Completion of an exercise training program protected against the disruptive behavioral effects of treadmill exercise A four hr treadmill session was required to produce disruption of operant responding in the trained group and recovery occurred by the second FR component, less than 25 min post-exercise

RESULTS

The ratio and pattern of FR responding in the present study were similar to those previously reported for FR30 responding The rates of lever pressing (S E) in each of the four FR components were 1 2 (±0 12), 1 11 (±0 15), 1 11 (±0 13) and 0 96 (±0 20) responses/second Relatively few

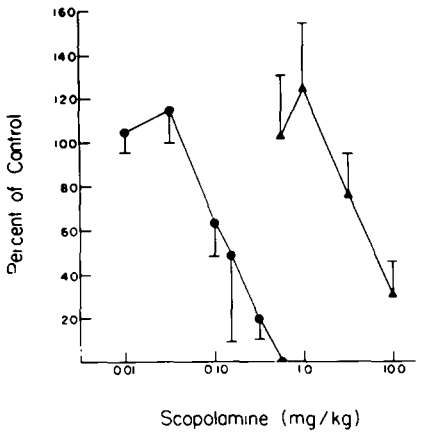

FIG 2. Scopolamine potency in subjects with no history of exercise training (●) and following completion of a 10 week exercise training program (▲) The behaviorally disruptive effects of scopolamine, expressed as percent of non-drug operant rates, were measured in animals trained to perform an operant task under a MULT TO FR30 schedule of food reinforcement The dose producing 50% disruption (ED50) in the non-exercise trained group was 0 15 mg/kg (0 07–0.32 mg/kg) Following 10 weeks of exercise training, the ED50 was 6 5 mg/kg (1 68–12 52 mg/kg) This reflects a significant ($p<0.001$) decrease in sensitivity to the behavioral effects of scopolamine as a result of chronic exercise

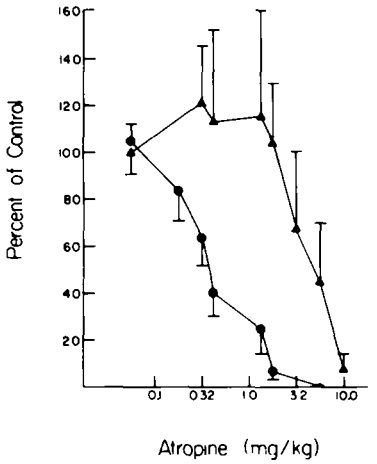

FIG 3 Atropine potency in subjects with no history of exercise training (●) and following completion of a 10 week exercise training program (▲) The behaviorally disruptive effects of atropine, expressed a percent of non-drug operant rates, were measured in animals trained to perform an operant task under a MULT TO FR30 schedule of food reinforcement The dose producing 50% disruption (ED50) in the non-exercise trained group was 0 42 mg/kg (0 21–0.80 mg/kg) Following 10 weeks of exercise training, the ED50 was 4 53 mg/kg (1 94–10 5 mg/kg) This reflects a significant ($p<0$ 01) decrease in sensitivity to the behavioral effects of atropine as a result of chronic exercise

responses occurred during the TO components that alternated with the food reinforced FR components

Prior to any exercise conditioning, acute exercise produced a graded decrease in FR responding as the duration of time on the treadmill at a speed of 1.0 M P.H. was increased (Fig. 1) For example, a 60 minute treadmill exercise session reduced responding to approximately 50% of control performance After completion of the exercise training program, it was necessary to extend the pre-session exercise period to 240 minutes before any effects on operant performance were observed Rates of responding were not significantly altered by the 10 week exercise training program; the post-training baseline performance of exercise trained subjects did not deviate from pre-training levels

Both atropine and scopolamine produced dose-related decreases in FR responding (Figs 2 and 3). The effect of exercise training on behavioral sensitivity to these drugs was a significant decrease in potency. In the absence of exercise training, the ED50 for scopolamine-induced disruption of responding was 0.15 mg/kg. Following exercise training, the scopolamine dose-effect curve was shifted significantly to the right The post-training ED50 of 6 5 mg/kg reflects a 40-fold difference in the amount of drug required to produce an equivalent effect in the two groups. A similar decrease in potency was observed when atropine was administered to the exercise trained subjects The atropine ED50 for the non-conditioned group was 0.42 mg/kg in contrast to 4.53 mg/kg in the exercise trained group, reflecting a ten-fold shift to the right. These changes in potency of both drugs are statistically significant ($p<0.001$).

DISCUSSION

Muscarinic antagonists such as atropine and scopolamine have been shown to reliably disrupt operant behavior [20] However, the schedule of reinforcement is a significant factor in the determination of their qualitative effects For example, these compounds produce response rate increases under fixed-interval [1] and differential reinforcement of low rate [5,14] schedules of reinforcement, and response rate decreases under fixed-ratio schedules [1,20]. In the present study, atropine and scopolamine had the anticipated, dose related, rate decreasing effects in the subjects not exposed to exercise training. However, following completion of a ten week exercise training program, normally disruptive doses had no effect on FR responding. Disruption of responding in these animals did not occur at doses less than 10 times as large as the ED50 in control animals

It is important to note that completion of the ten week exercise program did not alter operant response rate *per se* Exercise trained animals continued to perform at pre-exercise training baseline levels throughout the training and evaluation period. In the absence of an exercise or drug challenge, operant performance by exercise trained and non-trained groups was indistinguishable. Furthermore, no obvious changes in other behaviors were observed. All animals were weighed daily No significant differences in body weight, or the amount of lab chow required to maintain individual animals at the desired weight, were noted between the groups.

The term tolerance is used to describe a decrease in sensitivity to a drug following some treatment Traditionally, the treatment producing drug tolerance consists of administration of either the same drug or one which is metabolized via the same pathway. Nevertheless, the term tolerance applies equally well to the effect produced by exercise in the current experiment An organism is said to have developed tolerance to a compound when the same dose of a drug produces a lesser effect, or when a larger dose of the drug is required to produce a given effect [11]. Both conditions are met by the present results

The mechanisms which underlie the development of drug tolerance are often classified as either dispositional or functional [11] Dispositional tolerance is related to changes in factors such as absorption, distribution, excretion and metabolism that influence the bioavailability of a compound Functional tolerance relates more directly to the properties of the target tissue

The mechanism through which exercise alters behavioral sensitivity to muscarinic blocking agents was not addressed by the present experiment Additional experiments measuring plasma and/or brain levels of atropine and scopolamine in exercise trained and control subjects as well as receptor binding studies are required to clearly differentiate between dispositional and functional tolerance However, data from other sources allows some speculation and provides guidance for future investigations

There is no evidence that exercise alters the absorption or excretion of atropine or scopolamine However, their distribution could be influenced by exercise Although cerebral blood flow remains unchanged during and following exercise [21], exercise clearly induces changes in peripheral blood flow that include shunting away from the liver, kidney and intestine [9]. Changes in regional blood flow to areas involved in the metabolism of a drug could result in higher circulating levels It is unlikely, however, that this is the mechanism underlying the present findings Even if one assumes that an extensive period of exercise training does not result in any attenuation of, or adaptation to, this effect, exercise-induced changes in blood flow cannot reasonably be expected to persist for 72 hours post exercise Further, dispositional tolerance is usually restricted to no more than a 100% increase in the ED50 [10] We believe the exercise-induced tolerance observed is more likely to represent an alteration in the target tissue, or functional tolerance Both the magnitude of the tolerance produced by exercise and the fact that it was observed in rested, rather than recently exercised, animals support this hypothesis Changes in the target tissue of atropine and scopolamine relate directly to the drug-receptor complex A change in receptor density or kinetics, involving either the generation of new receptors, or conformational changes in existing receptors may occur in response to exercise

Drug-induced regulation of muscarinic receptor density has been demonstrated both *in vivo* and *in vitro* [11] Chronic administration of drugs such as atropine and scopolamine has been shown to result in an increase in the number of muscarinic receptors in the cortex [15,19] This is assumed to represent an adaptive response, directed toward the maintenance of a steady level of cholinergic activity Catecholamines have also been implicated in the regulation of muscarinic receptor kinetics [4] Increased levels of dopamine have been reported to inhibit cortical release of acetylcholine [2] Physical exercise is known to increase CNS concentrations of catecholamines

Any theory advanced to explain the effect of exercise on drug sensitivity must account for the observation that acute and chronic exercise produce opposite effects In rats not previously exposed to exercise, a single 30 min treadmill session increases sensitivity to the behaviorally disruptive effects of atropine and scopolamine [16] In contrast, rats that have completed a 10 week exercise training program display no increase in drug sensitivity following exercise Moreover, exercise trained animals are far less sensitive to atropine and scopolamine under rested conditions

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