Effect of acetylcholinesterase inhibition with pyridostigmine on cardiac parasympathetic function in sedentary adults and trained athletes

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Submitted 8 December 2006; accepted in final form 20 February 2007

Dewland TA, Androne AS, Lee FA, Lampert RJ, Katz SD. Effect of acetylcholinesterase inhibition with pyridostigmine on cardiac parasympathetic function in sedentary adults and trained athletes. Am J Physiol Heart Circ Physiol 293: H86–H92, 2007. First published February 23, 2007; doi:10.1152/ajpheart.01339.2006.—Heart rate variability and postexercise heart rate recovery are used to assess cardiac parasympathetic tone in human studies, but in some cases these indexes appear to yield discordant information. We utilized pyridostigmine, an acetylcholinesterase inhibitor that selectively augments the parasympathetic efferent signal, to further characterize parasympathetic regulation of rest and postexercise heart rate. We measured time- and frequency-domain indexes of resting heart rate variability and postexercise heart rate recovery in 10 sedentary adults and 10 aerobically trained athletes after a single oral dose of pyridostigmine (30 mg) and matching placebo in randomized, double-blind, crossover trial. In sedentary adults, pyridostigmine decreased resting heart rate [from 66.7 (SD 12.6) to 58.1 beats/min (SD 7.6), P = 0.005 vs. placebo] and increased postexercise heart rate recovery at 1 min [from 40.7 (SD 10.9) to 45.1 beats/min (SD 8.8), P = 0.02 vs. placebo]. In trained athletes, pyridostigmine did not change resting heart rate or postexercise heart rate recovery when compared with placebo. Time- and frequency-domain indexes of resting heart rate variability did not differ after pyridostigmine versus placebo in either cohort and were not significantly associated with postexercise heart rate recovery in either cohort. The divergent effects of pyridostigmine on resting and postexercise measures of cardiac parasympathetic function in sedentary subjects confirm that these measures characterize distinct aspects of cardiac parasympathetic regulation. The lesser effect of pyridostigmine on either measure of cardiac parasympathetic tone in the trained athletes indicates that the enhanced parasympathetic tone associated with exercise training is at least partially attributable to adaptations in the efferent parasympathetic pathway.

Results. Resting and postexercise heart rate variability and heart rate recovery are used in human studies to assess cardiac parasympathetic tone (1, 30, 47). The high-frequency (HF) component of resting heart rate variability quantifies short-term changes in heart rate caused by the effects of central and peripheral respiratory reflexes on vagal efferent activity during a resting steady state with low sympathetic activation (1). Postexercise heart rate recovery assesses the complex interaction of baroreceptor and central command signals that mediate a rapid deceleration of heart rate in the setting of autonomic flux, profound exercise-induced sympathetic stimulation, and high minute ventilation (30).

Parasympathetic efferent control of heart rate is ultimately mediated by cholinergic signaling at the sinoatrial node. Acetylcholinesterase inhibitors increase parasympathetic neurotransmission by blocking the enzymatic breakdown of acetylcholine at cholinergic receptor sites in the autonomic nervous system (56). Pyridostigmine is a short-acting, reversible acetylcholinesterase inhibitor used in the treatment of myasthenia gravis. Since pyridostigmine does not readily cross the blood-brain barrier, its effects are limited to increasing the concentration of acetylcholine at the peripheral cholinergic receptor without influencing the central neural pathways that mediate acetylcholine synthesis and release (34).

The current investigation was undertaken to characterize the effects of acetylcholinesterase inhibition with pyridostigmine on parasympathetic regulation of resting heart rate variability and postexercise heart rate recovery. Given its mechanism of action, we hypothesized that the magnitude of effect of pyridostigmine would be proportional to pretreatment parasympathetic tone. To test this hypothesis, we recruited two cohorts with disparate baseline autonomic function: unfit sedentary subjects and fit trained athletes. In each cohort, the effects of pyridostigmine versus placebo on resting heart rate variability and postexercise heart rate recovery were assessed in a double-blind, randomized crossover study design.

METHODS

Study population. Subjects were recruited with posted advertisements on the campus of Yale University. Two cohorts of 10 sedentary subjects and 10 trained athletes were studied. Baseline characteristics of the two cohorts are provided in Table 1. Sedentary subjects had not participated in a regular exercise regimen for at least 3 mo before enrollment. Trained athletes participated in ≥30 min of aerobic exercise at least 5 times/wk for ≥3 mo and had a resting heart rate between 50–65 beats/min. For both cohorts, subjects with systolic blood pressure of <90 or >140 mmHg, diastolic blood pressure of <60 or >90 mmHg, resting heart rate of <50 beats/min, history of tobacco use, history of bronchospasm, or asthma, hypertension, diabetes mellitus, morbid obesity, or any other chronic medical condition were excluded. The study protocol was approved by the Yale University Human Investigation Committee. Subjects provided written, informed consent before participation.

Study design. A prospective, double-blind, randomized crossover study design was used. Two study visits with identical procedures were conducted 5–10 days apart. Drug administration and maximal exercise testing were performed in the morning or afternoon but always at the same time of day on both study days for each subject. Participants were instructed to abstain from caffeinated beverages and exercise for 24 h preceding each testing session and from all food and beverages for 8 h before testing.

The study drug consisted of a single dose of pyridostigmine (30 mg) or matching placebo administered as identical appearing oral

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First published February 23, 2007; doi:10.1152/ajpheart.01339.2006.
Table 1. Baseline characteristics of study cohorts

<table>
<thead>
<tr>
<th></th>
<th>Sedentary Subjects</th>
<th>Trained Athletes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age, yr</td>
<td>35 (SD 7)</td>
<td>27 (SD 8)</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>4/6</td>
<td>7/3</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.70 (SD 0.07)</td>
<td>1.76 (SD 0.10)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70.7 (SD 21.5)</td>
<td>71.0 (SD 10.2)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.3 (SD 6.0)</td>
<td>23.0 (SD 1.6)</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>84 (SD 7)</td>
<td>87 (SD 5)</td>
</tr>
<tr>
<td>HR beats/min</td>
<td>62 (SD 11)</td>
<td>53 (SD 7)</td>
</tr>
</tbody>
</table>

Values are means (SD); n, number of subjects. HR, heart rate.

tables supplied by the Investigational Drug Service at Yale-New Haven Hospital. A blocked randomization allocation scheme was implemented for each cohort through the same service. Pyridostigmine is an Food and Drug Administration-approved acetylcholinesterase inhibitor used for the treatment of myasthenia gravis. In healthy subjects, peak plasma levels and peak cholinesterase inhibition (15–20%) occur 90–150 min after oral dosing with minimal variation in drug levels and degree of cholinesterase inhibition during this time period (3, 39, 44). The study protocol is shown schematically in Fig. 1.

Resting heart rate and blood pressure were recorded in the seated period (3, 39, 44). The study protocol is shown schematically in Fig. 1. Exercise testing was performed on a motorized treadmill with graded increases in work rate at 3-min intervals according to a standard Bruce protocol. Electrocardiographic data (Cardiocontrol Cardioperfect MD) and breath-by-breath expired gas analysis (Medgraphics, St. Paul, MN) were recorded continuously during a 1-min preexercise rest period, throughout exercise, and during the first minute of recovery. The peak oxygen uptake was calculated as the median five of seven breaths with a moving average filter at the end of exercise. Peak exercise effort was confirmed in all subjects by a combination of at least two of the following criteria: achievement of age-predicted maximum heart rate (±10 beats), a plateau in oxygen uptake despite increasing work rate, and/or respiratory exchange ratio of >1.10. Since a plateau in oxygen uptake was not evident in all subjects, the term peak oxygen uptake rather than maximum oxygen uptake is used.

After completion of exercise, the treadmill was immediately stopped and subjects recovered at complete rest in a seated position. Heart rate recovery data were collected for 1 min. Heart rate at maximal exercise and at 1 min of recovery was calculated from the average of the R-R interval of four consecutive sinus beats taken from the ECG recording. Heart rate recovery was calculated by subtracting the heart rate obtained at 30 s and at 1 min after exercise cessation from the heart rate at peak exercise (2).

Heart rate variability analysis. Thirty minutes of Holter data were recorded on a magnetic tape beginning 90 min after study drug administration while the subject rested supine in a darkened, temperature-controlled room. To ensure all analyzed data were obtained during a steady state of supine rest, the first and last 5 min of each rest session were discarded. This yielded a 20-min window of data for resting heart rate variability analysis. Each tape was digitally sampled and analyzed using a GE-Marquette system (Milwaukee, WI) and then manually processed and edited. R-R interval data were generated, and each beat was labeled as normal, ectopic, or noise. The ectopic beats and noise were removed from the data set and replaced with interpolated linear splines.

A fast Fourier transform was used to calculate power spectrum data, which were then corrected for attenuation due to windowing and sampling. The power spectrum was integrated over the following frequency intervals (9): low frequency (0.04 to <0.15 Hz) and HF (0.15 to 0.40 Hz). Since the data were highly skewed, a natural log transformation was performed to normalize the distribution. Time domain measurements, including percentage of normal R-R intervals differing ≥50 ms from the preceding normal R-R interval (pNN50), root mean square difference among successive R-R normal intervals (rMSSD), and standard deviation of normal R-R intervals, were also calculated.

Data analysis. All continuous variables are expressed as means (SD), except where noted in RESULTS. The primary end points of the study were the time and frequency domain parasympathetic indexes of heart rate variability at rest [pNN50 (%), rMSSD (ms), and HF power (ln ms²)] and postexercise heart rate recovery at 1 min after maximal exercise testing (beats/min). Postexercise heart rate recovery at 30 s after maximal testing (beats/min) was analyzed as a secondary end point. Differences between pyridostigmine and placebo were analyzed in repeated-measures analysis of variance models appropriate for the crossover design. Estimates of the effect of pyridostigmine versus placebo did not differ in models with or without a carryover term; results from the simpler model are reported. Mixed effects models were used to determine whether clinical characteristics of the study samples (age, sex difference, body-mass index, and fitness level) were confounders or effect modifiers of the association between study drug treatment and postexercise heart recovery. Other clinical variables of interest were examined in relation to heart rate recovery and heart rate variability with linear regression analysis. Frequency domain indexes of parasympathetic activity normalized for total power and heart rate were also analyzed but did not differ from the main analysis and are not presented. For all analyses, a two-tailed P value <0.05 was used to infer statistical significance. Based on prior heart rate recovery data from our laboratory, a sample size of 10 subjects provided >80% power to detect a 5 beats/min change in heart rate recovery in response to pyridostigmine when compared with placebo with two-tailed α = 0.05. All analyses were performed with the Intercooled Stata Statistics Package (version 8.0, StataCorp, College Station, TX).

Fig. 1. Schematic of study design. Subjects were randomized in a double-blind, crossover trial to receive 30 mg pyridostigmine or matching placebo. Two study visits with identical procedures were conducted 5–10 days apart. A 30-min Holter recording was started 90 min after study drug administration. Maximal exercise testing (Ex) was performed 10 min after completion of the Holter recording, 130 min after study drug administration.
RESULTS

Effect of study drug on resting heart rate variability. Resting heart rate and blood pressure before administration of pyridostigmine or placebo did not differ in either group [sedentary subjects: heart rate; 69 (SD 7) vs. 70 beats/min (SD 7); and mean arterial pressure, 85 (SD 7) vs. 83 mmHg (SD 8); and trained athletes: heart rate, 59 (SD 5) vs. 58 beats/min (SD 5); and mean arterial pressure, 87 (SD 5) vs. 88 mmHg (SD 5); all comparisons, \( P > 0.5 \)]. Pyridostigmine decreased resting heart rate when compared with placebo in sedentary subjects but did not significantly change resting heart rate in trained athletes (Table 2). Time- and frequency-domain indexes of heart rate variability did not differ after administration of pyridostigmine versus placebo in sedentary subjects or trained athletes (Table 2).

Effects of study drug on postexercise heart rate recovery. Pyridostigmine significantly increased postexercise heart rate recovery compared with placebo in sedentary subjects [median 42.1 (interquartile range 12.8) to median 47.3 beats/min (interquartile range 4.3), \( P = 0.02 \)] but not in trained athletes [median 49.5 (interquartile range 17.0) to median 49.0 beats/min (interquartile range 12.0), \( P = 0.16 \); Fig. 2]. In multivariate analysis, there was no evidence of confounding or interaction effects between baseline characteristics (age, body-mass index, and peak oxygen uptake) and the effects of study drug on postexercise heart rate recovery within either cohort. A significant interaction between sex difference and response to study drug was noted in trained athletes (sex difference-by-study drug interaction term, \( P = 0.012 \)) but not in sedentary subjects. In male trained athletes, the increase in postexercise heart rate recovery after pyridostigmine versus placebo was greater than that observed in female trained athletes [males placebo 45.1 (SD 10.9) vs. males pyridostigmine 52.1 beats/min (SD 11.8); females placebo 49.7 (SD 6.7) vs. females pyridostigmine 46.3 beats/min (SD 3.2)]. In sedentary subjects, no difference in response to study drug between male and female subjects was observed [males placebo 33.8 (SD 13.2) vs. male pyridostigmine 39.3 beats/min (SD 11.4); females placebo 45.2 (SD 6.8) vs. female pyridostigmine 48.9 beats/min (SD 4.4)]. Postexercise heart rate recovery at 30 s after exercise demonstrated directional trends comparable with the observations at 1 min after exercise but did not achieve statistical significance in either cohort [sedentary subjects placebo 23.9 (SD 10.1) vs. pyridostigmine 26.9 beats/min (SD 8.7), \( P = 0.29 \); and trained athletes placebo 26.5 (SD 6.0) vs. pyridostigmine 26.6 beats/min (SD 5.1), \( P = 0.96 \).

Effects of study drug on exercise performance. There was a small but statistically significant increase in peak oxygen uptake after pyridostigmine when compared with that after placebo in trained athletes but not in sedentary subjects (Table 3). Other exercise performance variables did not differ after pyridostigmine versus placebo (Table 3).

Correlates of parasympathetic indexes. There was no significant correlation between postexercise heart rate recovery and pNN50, rMSSD, or HF power measured at rest. Regression analysis revealed no significant correlation between parasympathetic heart rate variability indexes; postexercise heart rate recovery; and any baseline, resting, or exercise variable in either sedentary controls or trained athletes.

DISCUSSION

Inhibition of acetylcholinesterase with 30 mg of pyridostigmine significantly decreased resting heart rate and increased postexercise heart rate recovery when compared with placebo in sedentary subjects. A smaller and nonsignificant effect of pyridostigmine on postexercise heart rate recovery was observed in trained athletes. Pyridostigmine did not alter parasympathetic indexes of resting heart rate variability when compared with placebo in either cohort. No significant correlation was found between parasympathetic indexes of resting heart rate variability and postexercise heart rate recovery in either cohort.

Previous studies with acetylcholinesterase inhibition. To our knowledge, this study is the first to concurrently investigate the effects of pyridostigmine on heart rate recovery and resting heart rate variability in sedentary adults and the first to study the effect of the drug on either of these variables in trained athletes. Previous studies of the effects of pyridostigmine on cardiac parasympathetic tone in healthy subjects have yielded inconsistent findings since both an increase in time-domain indexes (45) and decrease in the HF component of resting heart rate variability (19, 31) have been reported after drug administration. Patients with cardiovascular disease treated with pyridostigmine demonstrated an increase in both heart rate recovery at 1 min after exercise (2) and time-domain indexes of

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Pyridostigmine</th>
<th>( p ) value</th>
<th>Placebo</th>
<th>Pyridostigmine</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time domain</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Resting HR, beats/min</td>
<td>67 (SD 4)</td>
<td>58 (SD 2)</td>
<td>0.01</td>
<td>54 (SD 2)</td>
<td>51 (SD 2)</td>
</tr>
<tr>
<td>RR mean, ms</td>
<td>924 (SD 99)</td>
<td>1,001 (SD 85)</td>
<td>0.002</td>
<td>1,169 (SD 144)</td>
<td>1,232 (SD 168)</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>83 (SD 21)</td>
<td>95 (SD 38)</td>
<td>0.14</td>
<td>111 (SD 32)</td>
<td>110 (SD 40)</td>
</tr>
<tr>
<td>pNN50, %</td>
<td>35.3 (SD 17.5)</td>
<td>38.0 (SD 25.3)</td>
<td>0.64</td>
<td>42.8 (SD 16.0)</td>
<td>46.7 (SD 16.8)</td>
</tr>
<tr>
<td>rMSSD, ms</td>
<td>66.8 (SD 30.3)</td>
<td>78.5 (SD 47.3)</td>
<td>0.20</td>
<td>83.8 (SD 29.2)</td>
<td>81.1 (SD 31.2)</td>
</tr>
<tr>
<td>Frequency domain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF, ms(^2)</td>
<td>7.00 (SD 0.93)</td>
<td>6.95 (SD 1.42)</td>
<td>0.95</td>
<td>7.20 (SD 0.60)</td>
<td>7.16 (SD 0.56)</td>
</tr>
<tr>
<td>LF, ms(^2)</td>
<td>7.22 (SD 0.64)</td>
<td>7.16 (SD 0.99)</td>
<td>0.95</td>
<td>7.61 (SD 0.43)</td>
<td>7.62 (SD 0.34)</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.53 (SD 1.05)</td>
<td>2.02 (SD 1.92)</td>
<td>0.29</td>
<td>1.64 (SD 0.62)</td>
<td>1.89 (SD 1.33)</td>
</tr>
<tr>
<td>Total power, ms(^2)</td>
<td>8.46 (SD 0.48)</td>
<td>8.68 (SD 0.89)</td>
<td>0.20</td>
<td>9.03 (SD 0.60)</td>
<td>8.98 (SD 0.46)</td>
</tr>
</tbody>
</table>

Values are means (SD). SDNN, standard deviation of normal R-R intervals; pNN50, percentage of normal R-R intervals differing >50 ms from the preceding normal R-R interval; rMSSD, root mean square difference among successive R-R normal intervals; HF, high frequency; LF, low frequency.
Differences in pyridostigmine dosing, clinical status, and methodology may have contributed to these divergent findings.

Physiological determinants of heart rate variability and heart rate recovery. Although resting heart rate variability and postexercise heart rate recovery are both used to assess cardiac parasympathetic tone in human studies, these measures appear to provide discordant information in some settings (1, 6, 29, 49, 50, 52, 55a). In agreement with our findings, a lack of correlation between resting and postexercise indexes of cardiac parasympathetic tone has been reported in healthy, sedentary adults (32) and in patients with coronary artery disease (22). A recent observational study reported that parasympathetic indexes of resting heart rate variability were strongly linked with cardiorespiratory fitness (as assessed by maximal oxygen consumption), whereas postexercise heart rate recovery was more closely associated with training load (12). This observation led the authors to suggest that resting heart rate variability and postexercise heart rate recovery provide distinct and complementary information about parasympathetic function. The disparate effects of pyridostigmine on resting and postexercise indexes of parasympathetic tone may be attributable to underlying differences in the physiological determinants of these measures. Pyridostigmine inhibits the breakdown of acetylcholine at the sinoatrial nodal junction without modifying the pattern of phasic changes in neurotransmitter present during the respiratory cycle (56). Accordingly, our findings are consistent with the interpretation of resting heart rate variability as an index of phasic changes in vagal efferent activity (unaffected by pyridostigmine) and postexercise heart rate recovery as an index of mean cholinergic signaling in the sinoatrial nodal junction (augmented by pyridostigmine) (38).

Training and the parasympathetic nervous system. We examined the effects of pyridostigmine on indexes of parasympathetic function in sedentary adults and trained athletes. In previous cross-sectional studies, trained athletes exhibited increased heart rate recovery after exercise (30) and an increase in the parasympathetic indexes of resting heart rate variability (20, 54) compared with sedentary controls. Longitudinal studies confirm that participation in aerobic exercise training programs increases parasympathetic indexes of resting heart rate variability and heart rate recovery (23, 26, 36, 40, 57, 58). Accordingly, we anticipated that increased parasympathetic tone in our trained subjects (14) would be associated with increased synaptic levels of acetylcholine and therefore would provide more abundant substrate for pyridostigmine. The smaller effect of pyridostigmine on heart rate recovery and heart rate variability in the cohort of trained athletes in this study was an unanticipated finding.

Table 3. Peak exercise performance variables in study cohorts of sedentary subjects and trained after administration of placebo and pyridostigmine

<table>
<thead>
<tr>
<th></th>
<th>Sedentary Subjects</th>
<th>Trained Athletes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Pyridostigmine</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>173 (SD 12)</td>
<td>172 (SD 15)</td>
</tr>
<tr>
<td>%Maximum HR</td>
<td>93.4 (SD 5.9)</td>
<td>92.7 (SD 6.4)</td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>37.5 (SD 8.1)</td>
<td>34.9 (SD 5.6)</td>
</tr>
<tr>
<td>Tidal volume, l</td>
<td>1.8 (SD 0.5)</td>
<td>1.9 (SD 0.5)</td>
</tr>
<tr>
<td>Minute ventilation, l/min</td>
<td>63.4 (SD 15.9)</td>
<td>63.5 (SD 15.7)</td>
</tr>
<tr>
<td>V\textsubscript{O2}, ml·kg\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>29.4 (SD 6.2)</td>
<td>28.5 (SD 5.2)</td>
</tr>
<tr>
<td>RER</td>
<td>1.11 (SD 0.1)</td>
<td>1.13 (SD 0.1)</td>
</tr>
<tr>
<td>Exercise duration, s</td>
<td>670 (SD 212)</td>
<td>620 (SD 78)</td>
</tr>
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</table>

Values are means (SD). V\textsubscript{O2}, oxygen consumption; RER, respiratory exchange ratio.
The autonomic adaptations to physical training that contribute to increased parasympathetic activity have not been definitively characterized. Both altered baroreceptor stimulation secondary to increases in blood volume and changes in opioid and dopaminergic signaling have been suggested as mechanisms to account for training-induced augmentation of parasympathetic tone (11). Additionally, rat exercise training models demonstrate increased levels of acetylcholine in the atria (28) and a reduction in sinoatrial node sensitivity to acetylcholine (10) without an alteration of myocardial muscarinic receptor density (7). The lesser change in postexercise heart rate recovery after pyridostigmine in the trained athlete cohort may be attributed to a training-induced ceiling (saturation) effect. The existence of a physiological ceiling in parasympathetic control of heart rate is supported by previous human studies that demonstrate a plateau in the heart rate-lowering effects of vagal stimulation at depolarization rates of >25 Hz and by evidence of a nonlinear relationship between heart rate and time- and frequency-domain measures of resting parasympathetic tone at high R-R intervals (13, 24, 35). A ceiling effect may be related to the effects of competing homeostatic feedback mechanisms, including central autonomic reflexes regulating cerebral perfusion in the postexercise state. Alternatively, a parasympathetic ceiling effect may be attributable to saturation of parasympathetic neurotransmission related to training-induced changes in acetylcholine release, acetylcholine receptor characteristics, or post-receptor changes in the sinoatrial node. Although our study design cannot discern the precise mechanism(s) contributing to a ceiling effect, our observation that pyridostigmine significantly increased postexercise heart rate recovery in sedentary subjects with lesser, nonsignificant effects in trained athletes, coupled with its known peripheral mechanism of action, suggests that at least part of the training-induced autonomic changes in humans occur through alterations in cholinergic signaling at the sinoatrial node.

Sex difference and parasympathetic function. Our subgroup analysis based on sex difference indicates that female trained athletes demonstrated a reduction in postexercise heart rate recovery after administration of pyridostigmine, whereas male athletes demonstrated an increase in heart rate recovery after administration of pyridostigmine. The findings of this post hoc analysis must be interpreted with caution since the study sample was small and no comparable pattern was observed in the sedentary cohort. Previous studies suggest that premenopausal women have increased parasympathetic tone and reduced sympathetic tone when compared with age-matched men, possibly mediated by the central and peripheral effects of female gonadal hormones on autonomic neural transmission (18). A sex-based difference in pretreatment parasympathetic tone could make female athletes more susceptible to a ceiling effect that attenuates the pharmacological response to pyridostigmine. Alternatively, small sex-based differences in pyridostigmine pharmacokinetics could have contributed to our findings (39). The reduced response to pyridostigmine in female athletes cannot account for the overall differences between the two study cohorts, since our sedentary group tended to have more women than the trained athlete group.

Clinical implications. Patients with cardiovascular disease demonstrate pathological adaptations in the autonomic nervous system marked by parasympathetic withdrawal (21), reduced heart rate variability at rest (46), and impaired heart rate recovery after exercise (42) compared with healthy controls. Heart rate recovery is a validated clinical tool for assessing both autonomic imbalance and subsequent mortality risk (15, 16, 41–43, 53, 59). The results of this study suggest that heart rate recovery and heart rate variability may provide different and perhaps complementary information regarding parasympathetic changes that may be useful when applied in disease populations. Further investigation is warranted to more completely characterize how these indexes correlate in cardiovascular disease patients and to determine which variable is most closely associated with autonomic changes and mortality risk in this population. In a canine model of experimental myocardial infarction, greater heart rate recovery at 30-s postexercise was associated with decreased susceptibility to ventricular fibrillation (55). Pyridostigmine tended to increase heart rate recovery at 30-s postexercise in our sedentary cohort. In patients with heart failure, pyridostigmine treatment at a dose of 30 mg three times daily for 2 days was associated with reduction in frequency of ventricular ectopic beats during ambulatory electrocardiographic monitoring (8). Further studies in cardiac disease populations are warranted to determine the effects of pyridostigmine on susceptibility to ventricular arrhythmias. The unexpected finding of increased peak oxygen uptake in the trained athletes after administration of pyridostigmine could also have potential implications for enhancing performance in endurance athletes. Pyridostigmine at a dose of 45 mg has been noted to enhance left ventricular diastolic function during mental stress in normal subjects (51). Further work is needed to determine whether changes in left ventricular performance or other factors contribute to increased aerobic capacity during acetylcholinesterase inhibition.

Study limitations. Our study sample size was selected to assess within-group comparisons of postexercise heart rate recovery after placebo or pyridostigmine and was not sufficiently large to perform a formal analysis of interaction between training status and response to pyridostigmine. Accordingly, our qualitative comparison of findings in the two study cohorts must be interpreted with caution; additional studies with larger samples are needed to more fully characterize the effects of training on parasympathetic cardiac regulation. Small sample size also limited our power to detect changes in some of the measures of resting heart rate variability. We did not directly measure pyridostigmine levels or the degree of cholinesterase inhibition in our study subjects. Previous studies in healthy subjects and patients with myasthenia gravis indicate that pyridostigmine is primarily excreted unchanged in urine (5). Physical training is unlikely to alter renal excretion of pyridostigmine (48), but other effects of training, including reduced fat mass, increased basal metabolic rate, and longer duration of exercise testing in the study protocol, may have altered other aspects of pyridostigmine metabolism and thereby contributed to our findings. Moreover, individual differences in pyridostigmine pharmacokinetics related to age, sex, body habitus, or genetic factors are potential confounders that should be assessed in future studies (5, 27, 37, 39). Resting autonomic tone was not formally evaluated by pharmacological blockade with atropine in either cohort. However, we believe our inclusion criteria were sufficient to define two cohorts with distinctly different levels of resting parasympathetic function based on the large separation in level of cardiorespiratory...
fitness between groups. Our trained athlete cohort demonstrated a significantly lower resting heart rate and markedly increased minute ventilation and oxygen consumption at peak exercise compared with the sedentary controls after placebo administration, consistent with training effects. Additionally, the magnitude of heart rate recovery after peak exercise exhibited by both trained athletes and sedentary controls in our study is generally consistent with values attained in other populations of trained and untrained individuals, respectively (6, 17, 25, 33), although differences in recovery protocols confound the quantitative comparison of heart rate recovery values among published studies. Metronomic or volumetric methods were not employed to achieve respiratory synchronization during Holter data acquisition for heart rate variability analysis. However, respiratory rate and tidal volume measured both immediately before and at peak exercise did not change with drug administration in either cohort, suggesting that respiratory variables during supine rest were also likely unchanged with pyridostigmine dosing. Finally, we did not fully characterize or control the training mode, frequency, or intensity of our athlete cohort. Our crossover design with comparisons of within-subject differences minimizes the potential confounding effects of training load, but further work is needed to define the effects of these factors on cardiac parasympathetic indexes (12).

In conclusion, postexercise heart rate recovery increased in healthy, sedentary adults after the administration of 30 mg of pyridostigmine when compared with placebo. Smaller, nonsignificant changes were observed in aerobically trained athletes. Parasympathetic indexes of resting heart rate variability, including pNN50, rMSSD, and HF power, were unchanged with pyridostigmine compared with placebo in both cohorts. These findings suggest that postexercise heart rate recovery and resting heart rate variability characterize distinct aspects of cardiac parasympathetic regulation that can vary independently. Additionally, our data suggest that the enhancement of parasympathetic tone associated with exercise training is at least partially attributable to peripheral autonomic adaptations.

GRANTS

This study was supported in part by National Heart, Lung, and Blood Institute Grant HL-K24-04024 (to S. D. Katz).

REFERENCES


AJP-Heart Circ Physiol • VOL 293 • JULY 2007 • www.ajpheart.org


