Objective – Parasympathetic dysfunction is an independent risk factor in individuals with coronary artery disease, and cholinergic stimulation is a potential therapeutic option. We determined the effects of pyridostigmine bromide, a reversible anticholinesterase agent, on electrocardiographic variables of healthy individuals.

Methods – We carried out a cross-sectional, double blind, randomized, placebo-controlled study. We obtained electrocardiographic tracings in 12 simultaneous leads of 10 healthy young individuals at rest before and after oral administration of 45 mg of pyridostigmine or placebo.

Results – Pyridostigmine increased RR intervals (before: 886±27 ms vs after: 1054±37 ms) and decreased QTc dispersion (before: 72±9ms vs after: 45±3ms), without changing other electrocardiographic variables (PR segment, QT interval, QTc, and QT dispersion).

Conclusion – Bradycardia and the reduction in QTc dispersion induced by pyridostigmine may effectively represent a protective mechanism if these results can be reproduced in individuals with cardiovascular diseases.

Keywords - autonomic nervous system, QTc dispersion, pyridostigmine
Methods

Ten healthy volunteers (3 males and 7 females) with a mean age (mean ± standard deviation) of 28±6 years, weighing 67.4±16.5 kg, and measuring 170±6 cm of height underwent a cross-sectional, double-blind, randomized protocol on two different mornings. After resting in the dorsal decubitus position, a surface electrocardiogram with 12 simultaneous leads (software ErgoPC®, Micromed, Brazil) was performed on each volunteer, before and 2 hours after oral administration of 45 mg of pyridostigmine bromide (Mestinon®, Roche, Brazil) or placebo. All individuals were nonsmokers in a fasting period and were not on any medication. They were instructed not to ingest any substance containing alcohol or caffeine and to avoid strenuous physical exercise during the 2 days preceding the examination. These individuals were considered healthy based on normal results of clinical and electrocardiographic examinations, two-dimensional echocardiography with Doppler, and cardiopulmonary exercise testing.

All volunteers gave written consent to take part in the study after being informed about the procedures they would undergo and risks they would be exposed to. The study was approved by the Institutional Committee on Ethics.

The same observer measured the following variables in the 12 electrocardiographic leads: PR segment, RR and QT intervals. In addition, QTc interval, and QT and QTc dispersions were calculated. The software used recorded the 12 electrocardiographic leads simultaneously in a digital form, storing the signals for later analysis. During the analysis, tracings and time recording could be widened, allowing measurements with greater resolution. To compare the values calculated, we used the arithmetic mean of the results obtained in the 12 leads. The simultaneous 12-lead recording is an indispensable tool for calculating spatial dispersion of QT and QTc intervals at the same time.

The QT interval was measured from the first deflection of the QRS complex until the return point of the T wave to the base line or the lowest point between the T and U waves7. Correction of the QT interval for the heart rate (QTc) was obtained through Bazett's formula (QT/√RR)6-10. Dispersions of the QT and QTc intervals were calculated, respectively, as the subtraction between the greatest and the smallest QT and QTc intervals in the 12 electrocardiographic leads11-13.

Statistical assessment of data was based on a repeated measurements analysis of variance (ANOVA). When the F value was significant, the ANOVA was followed by the Student-Newman–Keuls test for post-hoc paired comparisons. For the statistical analysis of the symptoms reported by the patients while using pyridostigmine and placebo, we used the chi-square test. The results were considered statistically significant when p<0.05.

Results

Even though undesired effects occurred with a greater frequency with the use of pyridostigmine (p=0.041), the symptoms were mild, self-limited, and were reported by only 4 individuals as follows: sialorrhea (n=3), abdominal colic and diarrhea (n=1), and epigastric discomfort (n=1).

Two hours after administration of pyridostigmine, we observed significant bradycardia (p=0.01) (table I) shown as an increase in the duration of the RR intervals and reduction in QTc dispersion. No significant differences were observed in the other electrocardiographic variables studied (QT and QTc intervals, QT dispersion, and PR segment).

Discussion

Autonomic nervous system dysfunction due to sympathetic hyperactivity or parasympathetic hypoactivity is associated with an increase in the risk of arrhythmias and other cardiac events in patients with heart disease 4,14. Therefore, dysautonomia increases cardiovascular morbidity and mortality. Adrenergic hyperactivity after acute myocardial infarction has been known for decades, and its clinical relevance has been soundly characterized by a reduction in mortality in patients treated with beta-blockers15, a management currently considered standard in the postacute myocardial infarction treatment16. More recently, analysis of neuroendocrine features of congestive heart failure has culminated with the indication for use of beta-blockers also in this disease17. Even though a reduction in mortality in congestive heart failure has been observed specifically with the use of carvedilol18, the general concept that a reduction in the noxious effects of the sympathetic hyperactivity on the heart has been characterized as an efficacious way of reducing cardiac events.

On the other hand, parasympathetic dysfunction gained importance from studies showing its role as an independent risk factor in patients after acute myocardial infarction19, and, more recently, also in patients with congestive heart failure19. Paradoxically, studies aiming to investigate possible specific therapeutical measures for vagal hypofunction are scarce. Aerobic training may promote an increase in vagal cardiac activity as shown by the greater variability heart rate of patients undergoing this type of training10. In regard to possible pharmacological alternatives, in the beginning of the '90s, four independent groups21-24 published studies about the cardiovascular effects of scopolamine after acute

<table>
<thead>
<tr>
<th>Variable (ms)</th>
<th>Placebo</th>
<th>Pyridostigmine</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR Segment</td>
<td>87±5</td>
<td>88±5</td>
</tr>
<tr>
<td>RR Interval</td>
<td>913±35</td>
<td>936±51</td>
</tr>
<tr>
<td>QT Interval</td>
<td>366±8</td>
<td>368±9</td>
</tr>
<tr>
<td>QT Dispersion</td>
<td>55±5</td>
<td>54±7</td>
</tr>
<tr>
<td>QTc Interval</td>
<td>387±7</td>
<td>383±7</td>
</tr>
<tr>
<td>QTc Dispersion</td>
<td>60±6</td>
<td>60±7</td>
</tr>
</tbody>
</table>

*P <0.05 vs pre pyridostigmine; QTc- QT interval corrected by the preceding RR interval according to the Bazett's formula (QT/√RR).
myocardial infarction. Scopolamine is a muscarinic cholinergic antagonist that may have a paradoxical effect when administered at low doses (0.4 to 0.6mg) 25. All those studies 21-24 showed a reduction in heart rate and an increase heart rate variability, therefore, suggesting that scopolamine might have a protective effect against cardiac events after acute myocardial infarction. However, the only study that specifically investigated this hypothesis has not confirmed the speculation of the above cited authors 26. Hull Jr et al 26 found that, even though the drug increases heart rate variability in chronically instrumented, dogs it was not able to prevent ventricular fibrillation during physical exertion and artificially induced coronary artery occlusion. Therefore, no experimental or clinical studies exist indicating a pharmacological therapeutical alternative for parasympathetic hypoaetivity that may protect against arrhythmogenic sudden death and cardiac mortality in general. Thus, the search for drugs that may cause cholinergic cardiac stimulation and reactivate the possibility of treating vagal hypoaetivity present in diverse cardiovascular diseases still continues.

Pyridostigmine bromide is a reversible anticholinesterase agent that does not cross the blood-brain barrier at usual doses and causes a cholinomimetic action by decelerating hydrolysis of endogenous acetylcholine and, consequently, increasing its concentration in the synaptic cleft. Its mostly known clinical indication is for the treatment of the skeletal muscular paralysis of myasthenia gravis 27, in which the daily dosage may reach 720mg. Pyridostigmine may also be used in multiple sclerosis, amyotrophic lateral sclerosis, spinal myotrophies, and paresis consecutive to poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indications of pyridostigmine include prevention of disorders after lumbar puncture, poliomyelitis. Other less common indica...
inducing bradycardia, pyridostigmine bromide at the dose used showed a potential cardioprotective effect in regard to the occurrence of ventricular arrhythmias and sudden death. The study here described was carried out in healthy young individuals and, therefore, may not be necessarily reproducible in individuals with cardiovascular diseases. In addition, all studies performed so far by our team have involved the use of a single dose 29,30,32,33, or a maximum 3 doses 31 of pyridostigmine bromide. Studies with patients may analyze the effect of pyridostigmine bromide administration in fractionated doses at long-term.

If the effects demonstrated so far in healthy individuals may be reproduced in these patients, the performance of controlled studies aiming to evaluate a potential protective effect of pyridostigmine may be justifiable.

Acknowledgements

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