Cholinergic stimulation with pyridostigmine increases heart rate variability and baroreflex sensitivity in rats

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Abstract

Objective: Impaired parasympathetic modulation increases the risk for sudden death in patients with heart diseases. Therefore, cholinergic stimulation may have a potential protective role. The aim of this study was to verify the effects of pyridostigmine bromide, a reversible cholinesterase inhibitor, on heart rate (HR), blood pressure (BP), HR and BP variability, and baroreflex sensitivity (BS).

Methods: Male Wistar rats were divided in two groups: (1) treated with pyridostigmine in drinking water (7 days, \( n = 10 \); PYR) and (2) a control group (\( n = 12 \); CTR). BP was recorded in freely moving rats, and HR and BP variability were quantified by the standard deviation (S.D.) of the mean values during a 30-min period and by spectral analysis. BS was assessed by the ratio between pulse interval and BP power spectra (spontaneous BS) and also by the changes on HR produced by phenylephrine and sodium nitroprusside-induced BP changes.

Results: Treated rats had a PYR intake of \( 7.91 \pm 1.90 \) mg/day (\( \approx 31 \) mg/kg/day). There were no differences between groups concerning resting HR (\( P = 0.158 \)), systolic BP (\( P = 0.481 \)), and BP variability (\( P = 0.201 \)). On the other hand, treatment with PYR increased HR variability on the time domain (S.D.—PYR: 13.5 \( \pm 5.3 \) ms vs. CTR: 9.9 \( \pm 3.6 \) ms; \( P = 0.034 \)) and frequency domain (Total power—PYR: 208.3 \( \pm 157.7 \) ms\(^2\) vs. CTR: 109.2 \( \pm 65.6 \) ms\(^2\); \( P = 0.030 \)). BS was also augmented with PYR for both the spontaneous method (High frequency band—PYR: 2.55 \( \pm 0.60 \) ms/mm Hg; \( P = 0.033 \)) and the drug-induced reflex bradycardia (PYR: 2.48 \( \pm 1.02 \) bpm/mm Hg vs. CTR: 1.54 \( \pm 0.58 \) bpm/mm Hg; \( P = 0.024 \)) and reflex tachycardia (PYR: 4.08 \( \pm 1.04 \) bpm/mm Hg vs. CTR: 2.95 \( \pm 1.30 \) bpm/mm Hg; \( P = 0.037 \)).

Conclusions: In conclusion, treatment with pyridostigmine increased HR variability and BS in normal rats with no modifications on basal hemodynamic parameters. Considering that reduced HR variability and baroreflex sensitivity are independent risk factors in heart disease, the present results support the concept that cholinergic stimulation with pyridostigmine may become a therapeutic option for vagal dysfunction.

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1. Introduction

The autonomic nervous system plays a pivotal role in the integration and regulation of physiological function. Concerning the cardiovascular system, beat-to-beat blood pressure (BP) adjustments, which are necessary for optimal peripheral blood flow supply, operate mainly via reflex autonomic modulation of cardiac output and peripheral vascular resistance. Therefore, both the spontaneous fluctuations in heart rate (HR) and its reflex changes in response to BP increases or decreases have been used to investigate the function of the autonomic nervous system (Smyth et al., 1969; Sleight et al., 1995; Mancia et al., 1998; Parati et al., 2000). Although there is a general pattern of reciprocal changes in sympathetic and parasympathetic activities, rapid oscillations in HR seem to depend mainly on the fluctuations of vagal drive to the sinus node (Japundzic et al., 1990).

In the clinical setting, autonomic dysfunction, characterized by increased sympathetic activity and reduced vagal modulation, is involved in the pathogenesis of life-threatening ventricular arrhythmias and cardiac sudden death of
patients with heart disease (La Rovere et al., 2001). Actually, HR variability (HRV) and baroreflex sensitivity (BS) have been shown to carry prognostic value after myocardial infarction (La Rovere et al., 1998), as well as in patients with heart failure (Mortara et al., 1997; Nolan et al., 1998). Inhibiting the cardiac effects of increased sympathetic activity with beta-blockers has been demonstrated to be an effective means to reduce mortality (Yusuf et al., 1996), but few studies have focused on the concept that increasing parasympathetic modulation may be a therapeutic alternative (Vybiral et al., 1990; De Ferrari et al., 1993; Pedretti et al., 2003). Nevertheless, cholinergic stimulation has been shown (Stramba-Badiale et al., 1991) and to interrupt ventricular arrhythmia in experimental models (Stramba-Badiale et al., 1991) and to interrupt ventricular tachycardia in humans (Waxman and Wald, 1977).

It has been previously shown by our group that cholinergic stimulation with pyridostigmine bromide (PYR), a reversible cholinesterase inhibitor, increases HRV (Nobrega et al., 2001) and blunts the hemodynamic (Nobrega et al., 1999) and cardiac (Sant’anna et al., 2003) response to mental stress in healthy subjects. In addition, administration of PYR increased HRV and decreased ventricular arrhythmias in heart failure patients (Behling et al., 2003). However, it would be important to determine whether this agent would modify the dynamic mechanisms of autonomic cardiovascular integration. A single previous publication has reported the effect of cholinergic stimulation with PYR and stress on baroreflex function in mice (Joaquim et al., in press). Therefore, the aim of this study was to determine the effects of PYR on HRV and BP variability, and the BS in conscious and unrestrained rats.

2. Materials and methods

Groups of adult male Wistar Kyoto rats (200–300 g) were housed singly in plastic cages with controlled temperature (23 °C) and with 12-h light/dark cycle and rat chow ad libitum. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. National Institute of Health, NIH Publication No. 85-23, revised 1996). Control rats (CTR, n = 12) had unlimited access to drinking water (tap), while treated rats (PYR, n = 10) had similar access to water containing pyridostigmine bromide (0.14 mg ml⁻¹, Sigma). Water consumption was monitored during the 7-day treatment period for both PYR and CTR groups. Experiments were performed on the seventh day of treatment with PYR or placebo. The day before, i.e., on the sixth day of treatment, catheters were inserted in the left femoral vein and artery under anesthesia with ketamine (80 mg kg⁻¹, Parke-Davis) and xilazine (12 mg kg⁻¹, Bayer) to allow drug infusions and BP recordings, respective. Catheters were flushed with heparinized saline solution (100 U/ml) immediately after placement and the next day, before BP signal was recorded.

2.1. Data acquisition and analysis

BP was recorded from the left femoral artery during 90 min in conscious rats, using pressure transducers (Statham, USA) connected to an amplifier (Stemtech, USA). Previous to the analog to digital conversion, blood pressure is low pass-filtered (fc = 50 Hz, Butterworth, fourth order; Bioengineering Division, InCor) for high-frequency noise removal, and recorded in a microcomputer (Gateway 2000, 4DX2-66 V) using a commercially available software (Codas, Dataq, USA) with a 2-kHz sampling frequency. Pulse interval (PI) was estimated considering the intervals between consecutive diastolic BP events.

The first 45-min period of recording was assigned for animal adaptation to laboratory environment and equipment, and for signal quality adjustments. The following 20-min period was used for HR and BP time and frequency analysis that provided with more than 6500 events for each hemodynamic parameter. BP waveforms were analyzed by specific semiautomatic monitor (MATLAB 6.0, Mathworks, USA). Systolic and diastolic values were detected after parabolic interpolation, and signals artifacts were visually identified and removed.

2.2. Heart rate and blood pressure variability

Time-domain analysis consisted in calculating mean PI and systolic BP (SBP), PI variability and SBP variability as the standard deviation from its respective time series.

For frequency domain analysis, the whole 20-min time series of PI and SBP were cubic-spline-interpolated (250 Hz) and -decimated (18) to be equally spaced in time. Following linear trend removal, power spectral density was obtained by the Fast Fourier Transformation using the Welch’s method over 16,384 points with a Hanning window (512) and 50% overlapping. Spectral power for very low- (VLF 0–0.20 Hz), low- (LF 0.20–0.75 Hz), and high- (HF 0.75–4.0 Hz) frequency bands was calculated by means of power spectrum density integration within each frequency bandwidth, using a customized routine (MATLAB 6.0, Mathworks). Considering that in freely moving animals, the proportional contribution of the VLF component may increase in 20-min-long recordings, data were submitted also to another processing routine, where the 20-min recordings of the decimated PI signal (2048 points) were segmented in 2.5-min periods, and only steady segments were processed as described above. Spectral power within the LF- and HF-frequency bands in each animal along the 20-min recordings was averaged across the 2.5-min segments and used for analysis. This approach should allow for a more specific evaluation of the effects of PYR on the LF and HF components.

2.3. Baroreflex sensitivity

Spontaneous BS was assessed by calculating the square root of the ratio between PI and BP spectral powers,
known as the α-index (Parati et al., 2000), separately for the LF (α-LF) and HF (α-HF) bands, obtained as described above. BS was also determined by the method using pharmacological stimulation with vasoactive drugs both in CTR \( (n = 6) \) and PYR \( (n = 10) \). After the 90-min baseline recordings, BS was assessed by reflex changes in HR induced by phenylephrine (PHE)- and sodium nitroprusside (SNP)-provoked BP increases and decreases, respective. Increasing doses of PHE (0.5, 1, 2, 4, 8, 16 \( \mu \)g ml\(^{-1} \)) and SNP (2.5, 5, 10, 20, 40 and 80 \( \mu \)g ml\(^{-1} \)) were infused in bolus injections of 100 \( \mu \)l volume. For the BS analysis, only changes in SBP up to 40 mm Hg (decreases or increases) were considered, because extreme changes in SBP may not represent a physiological response. Reflex responses to each dose of PHE or SNP \( \Delta \text{HR/} \Delta \text{BP} \) (bpm/mm Hg) were calculated from peak BP and HR values after drug infusion subtracted from baseline, and the average response across the doses was taken as the individual rat BS index for reflex bradycardia and tachycardia, respective. The identification of the appropriate BP and HR values, as well as all calculations, was performed using a customized algorithm (MATLAB 6.0, Mathworks). BS was also compared between CTR and PYR by means of linear regression analysis. Data sets for reflex bradycardia and tachycardia for each rat were plotted \( (\Delta \text{BP} \times \Delta \text{HR}) \). Individual linear regression lines were generated for each animal, and one mean regression line for reflex bradycardia and another one for tachycardia were used to determine the effects of PYR against CTR.

2.4. Acetylcholinesterase activity

To determine the magnitude of acetylcholinesterase activity inhibition caused by the treatment with PYR in the drinking water, an independent group of 20 rats had 200 \( \mu \)l of blood drawn from the femoral artery before and after the 7-day period of treatment. Blood samples were centrifuged at 5000 rpm for 15 min and 100 \( \mu \)l of plasma samples were frozen at \(-20^\circ\text{C}\). Acetylcholinesterase activity was determined by radiometric procedure (Johnson and Russell, 1975) as described by Rotundo and Fambrough (1979). The reaction started after 20 \( \mu \)l of plasma samples was incubated with 5 \( \mu \)l of \([^3\text{H}]\text{-ACh}\) (0.1 \( \mu \)Ci; 24 mM; specific activity: 55.20 \( \mu \)Ci/mmoll) as substrate. The enzyme activity was assayed in the presence of the butyrylcholinesterase inhibitor, tetraisopropyl pyrophosphoramide (Iso-OMPA, 10 \( \mu \)M). After 15 min, the reaction was interrupted with the addition of 1.5 ml of glicine/HCl pH 2.5 buffer, and the AChE activity (dpm h\(^{-1} \)) was measured and expressed as percentage of inhibition when comparing PYR against CTR.

2.5. Statistical analysis

All data are reported as mean and standard deviation. The unpaired Student \( t \)-test was used to compare the hemodynamic parameters in time and frequency domains and the BS between control and PYR-treated groups. The regression lines generated in the pharmacological BS experiments were compared by a modified \( t \)-test according to Zar (1984). A \( P \) value \( < 0.05 \) was considered statistically significant. All procedures were performed with the GraphPad Prism (GraphPad Prism Software, San Diego, CA, USA).

3. Results

Water consumption was similar between CTR and PYR rats (CTR: 49.93 ± 12.51 ml/day; PYR: 44.69 ± 4.75 ml/day; \( P = 0.321 \)). Treated rats had a PYR intake of 7.91 ± 1.90 mg/day, corresponding to approximately 31 mg/kg/day. In the group of rats where the effect of the 7-day treatment with pyridostigmine on plasma acetylcholinesterase activity was tested, enzymatic activity decreased from 10900 ± 1778 to 6443 ± 3523 units, an average inhibition of 40% from pretreatment values \( (P < 0.001) \). No changes in motor behavior were observed in the rats treated with PYR.

3.1. Baseline hemodynamics

Treatment with pyridostigmine did not change baseline HR (CTR: 346 ± 16 bpm vs. PYR: 337 ± 24 bpm; \( P = 0.158 \)), systolic BP (CTR: 122 ± 13 mm Hg vs. PYR: 122 ± 12 mm Hg; \( P = 0.481 \)), or diastolic BP (CTR: 93 ± 12 mm Hg vs. PYR: 94 ± 10 mm Hg; \( P = 0.387 \)).

3.2. Heart rate and blood pressure variability

Time domain analysis revealed that treatment with pyridostigmine increased PI variability (CTR: 9.9 ± 3.6 ms vs. PYR: 13.5 ± 5.3 ms; \( P = 0.034 \)), but had no effect on SBP variability (CTR: 6.2 ± 1.7 mm Hg vs. PYR: 5.7 ± 1.7 mm Hg; \( P = 0.278 \)). Frequency domain analysis on the 20-min tracings also showed increased PI variability but no differences for SBP between PYR and CTR groups (Fig. 1; Table 1). The results of the alternative approach where spectral analysis was applied to short tracings revealed that PYR increased total power (CTR: 30.2 ± 24.8 ms\(^2\); PYR: 83.8 ± 75.1 ms\(^2\); \( P = 0.040 \)) and HF (CTR: 6.0 ± 3.9 ms\(^2\); PYR: 15.4 ± 14.2 ms\(^2\); \( P = 0.049 \)), but had no effect on LF component (CTR: 2.9 ± 2.9 ms\(^2\); PYR: 5.6 ± 5.2 ms\(^2\); \( P = 0.122 \)) or the LF/HF ratio (CTR: 0.42 ± 0.24; PYR: 0.33 ± 0.17; \( P = 0.219 \)).

Although respiration was not directly monitored, the frequency of HF peak power, known to be mainly determined by respiratory rhythm, was similar in both groups of rats (CTR: 1.49 ± 0.19 Hz; PYR: 1.55 ± 0.19 Hz; \( P = 0.288 \)), suggesting that PYR had no major effect on the respiratory pattern.
3.3. Baroreflex sensitivity

Spontaneous BS in the LF band was similar between PYR (1.02 ± 0.5 ms/mm Hg) and CTR (0.79 ± 0.35 ms/mm Hg; \(P=0.107\)), but in the HF band, it was higher for PYR (2.55 ± 1.06 ms/mm Hg) compared to CTR (1.85 ± 0.60 ms/mm Hg; \(P=0.033\); Fig. 2, right panel).

BS, assessed by the magnitude of HR response to drug-induced BP changes (Fig. 2, left panel) was augmented in PYR compared to CTR for both reflex bradycardia (PYR: 2.48 ± 1.02 bpm/mm Hg vs. CTR: 1.54 ± 0.58 bpm/mm Hg; \(P=0.024\)) and reflex tachycardia (PYR: 4.08 ± 1.04 bpm/mm Hg vs. CTR: 2.95 ± 1.30 bpm/mm Hg; \(P=0.037\)). Rats treated with PYR also presented different linear regressions when compared to CTR both for reflex bradycardia (\(P=0.045\); Fig. 3, upper panel) and tachycardia (\(P=0.015\); Fig. 3, lower panel).

4. Discussion

The present results show that treatment with oral administration of PYR, an indirect cholinomimetic drug, increases HRV and BS in conscious rats, but had no significant effect on resting hemodynamic parameters or the BP variability. These results suggest that PYR increases parasympathetic modulation of cardiovascular function, an effect that may increase the physiological capacity for controlling BP.

The autonomic control of the cardiovascular system has been studied by the analysis of spontaneous fluctuation of HR and BP, as well as by the reflex responses to different stimuli, such as physical and mental stress or vasoactive drugs. The spontaneous and provoked responses seem to provide complementary information, because it is possible to probe not only the cardiovascular autonomic function at basal conditions but also during a physiological challenge. Thus, the spontaneous approach may represent the tonic component of the control mechanisms, whereas the reflex responses assess the phasic component (Malik and Camm, 1993; Hohnloser et al., 1994). This aspects may have particular interest because marked changes in cardiovascular modulation may be observed only when the system is stressed but not during resting conditions (Levy, 1971; Stramba-Badiale et al., 1991; Hohnloser et al., 1994). This study employed a model of conscious, unrestrained rats, where the confounding effect of anesthesia on autonomic function is avoided and the mechanisms of cardiovascular

<table>
<thead>
<tr>
<th>Pulse Interval</th>
<th>Control</th>
<th>Pyridostigmine</th>
<th>(P) value</th>
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<tbody>
<tr>
<td>Total power (ms²)</td>
<td>109.2 ± 65.6</td>
<td>208.3 ± 157.7</td>
<td>0.030</td>
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<tr>
<td>VLF power (ms²)</td>
<td>26.0 ± 18.5</td>
<td>44.3 ± 29.1</td>
<td>0.044</td>
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<tr>
<td>LF power (ms²)</td>
<td>3.1 ± 2.9</td>
<td>4.9 ± 4.3</td>
<td>0.138</td>
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<tr>
<td>HF power (ms²)</td>
<td>4.9 ± 3.6</td>
<td>9.3 ± 10.4</td>
<td>0.093</td>
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<tr>
<td>LF/HF</td>
<td>0.6 ± 0.4</td>
<td>0.6 ± 0.3</td>
<td>0.420</td>
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<tr>
<th>Systolic blood pressure</th>
<th>Control</th>
<th>Pyridostigmine</th>
<th>(P) value</th>
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<tbody>
<tr>
<td>Total power (ms²)</td>
<td>44.2 ± 25.9</td>
<td>35.5 ± 21.1</td>
<td>0.201</td>
</tr>
<tr>
<td>VLF power (ms²)</td>
<td>11.3 ± 6.0</td>
<td>9.2 ± 5.7</td>
<td>0.250</td>
</tr>
<tr>
<td>LF power (ms²)</td>
<td>4.5 ± 3.2</td>
<td>4.2 ± 2.2</td>
<td>0.434</td>
</tr>
<tr>
<td>HF power (ms²)</td>
<td>1.3 ± 0.4</td>
<td>1.1 ± 0.5</td>
<td>0.258</td>
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Values are mean ± S.D.
control can be studied in a condition closer to the physiological situation.

PYR is a reversible cholinesterase inhibitor that has a quaternary carbamine group, and because of its structure and charge it does not cross the blood–brain barrier (Taylor, 1996), hence its main action occurs in the peripheral synaptic cleft, inhibiting the acetylcholine hydrolysis released by cholinergic neurons. In the mammalian heart, the main action of PYR seems to be mainly on the M2-type muscarinic receptors (Dhein et al., 2001). In the clinical setting, this drug is used in the treatment of myasthenia gravis but has been extensively used as prophylactic measure against organophosphorous compounds, such as sarin, soman, or butan gases during the Gulf War (Keeler et al., 1991).

In healthy humans, PYR produced bradycardia (Nobrega et al., 1996), without modifying cardiac function (De Pontes et al., 1999), increased HRV (Nobrega et al., 2001) and blunted the rate–pressure product increase during mental stress (Nobrega et al., 1999). In heart failure patients, PYR reduced the ventricular arrhythmia density and increased HRV (Behling et al., 2003). Using animal models, PYR administration showed that in anesthetized rats, the drug blunted the increase in myocardial oxygen consumption elicited by intracerebroventricular infusion of L-glutamate (Grabe-Guimaraes et al., 1999). The present results have shown that chronic oral administration of PYR increases HRV and BS in rats, suggesting a parasympathetic modulatory effect of the drug of cardiovascular function. Considering that VLF component was also increased in this study, two other potential mechanisms could also be involved, namely, PYR-induced increases in motor activity and/or respiratory irregularities. Concerning motor behavior, no changes were observed, which is in agreement with a previous study where a similar dose of PYR (6.6 mg/day) was used in drinking water in a 7-day treatment (Francesconi et al., 1986). In relation to potential fluctuations of respiratory rhythm, although it was not specifically monitored, the fact that the frequency of HF peak power of HRV and its standard deviation were not different (see results), suggests that this possibility is very unlikely.

Two recent publications employed a similar approach in mice to determine the effects of PYR on cardiovascular function (Bernatova et al., 2003; Joaquim et al., in press). These authors found that, although resting BP and HR did not change, a 3-day treatment with PYR combined with intermittent shaker stress induced significant increases in HRV and BS, an effect not seen with either intervention separately. In this study, we observed that treatment with PYR increased HRV and BS in rats that were not specifically submitted to stressor stimuli. This apparent contradiction may represent species differences, but it is also possible that, in our experiment, there was still some underlying stress
induced by the insertion of catheters the day before. Although these procedures are minimal as compared to open-chest and abdominal surgeries and thus induce less pronounced organic responses, this mechanism could partially explain the modulatory effects of PYR on cardiovascular control found in this study. Nevertheless, this study and that of Joaquim et al. (in press) have important differences. While in the former, PYR increased HRV via augmented VLF and HF components suggesting a vagal-mediated effect, in the later, PYR plus stress caused marked increases only in the LF component without changes in HF. Considering that increases in LF/HF ratio may indicate an adrenergic predominance in autonomic balance (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), PYR may have exposed the underlying sympathetic activation caused by stress through the well-known “accentuated antagonism”, in which vagal modulation of HR is pronounced in face of increased adrenergic background (Levy, 1971; Stramba-Badiale et al., 1991). On the other hand, the LF/HF ratio has been questioned as a valuable tool in the murine model (Just et al., 2000).

Previous studies have employed pharmacological alternatives to increase vagal modulation to the heart in the rat experimental model. The so-called “bradycardic agents”, such as zatebradine (Kruger et al., 2000) improved autonomic markers like HRV and BS in control and myocardial-infarcted rats. Intravenous amiodarone increased the spontaneous BS and shifted the autonomic balance towards vagal predominance in normotensive and spontaneous hypertensive BS and shifted the autonomic balance towards vagal predominance (Dias et al., 2002). Although promising, the clinical significance of this approach remains to be determined (Nobrega and Teixeira de Castro, 2000).

4.1. Resting hemodynamic parameters

In this study, mean values for BP and HR of treated animals were similar to the control group. Although bradycardia is commonly observed after acute pyridostigmine administration, no difference was observed after 7 days of treatment in PYR animals. Previous studies have shown also that subcutaneous (Bernatova et al., 2003) or intravenous (Bataillard et al., 1990) infusions of PYR had no effects on hemodynamic parameters in mice and rats, respective.

4.2. Heart rate variability

Time domain analysis showed increased HRV in treated rats compared to controls. Spectral analysis showed that PYR treatment increased total VLF and HF power. The mechanisms underlying the origin of the VLF oscillations are not completely understood. However, it was shown that atropine almost abolish the VLF power and all other spectral components, suggesting that HRV also in the VLF band is driven by parasympathetic modulation (Taylor et al., 1998). In addition, this HRV component may carry clinical implications because reduced VLF, in combination with other variables, may identify heart failure patients at increased risk of cardiovascular events (Yamada et al., 2003).

The HF power is determined mainly by vagal modulation of HR and when augmented, it strongly suggests a higher contribution of parasympathetic modulation. In this study, no changes were observed in the LF power, which represent in rats both sympathetic and parasympathetic outflows to the heart (Japundzic et al., 1990).

4.3. Blood pressure variability

Spectral analysis of BP oscillations showed that the slow component (LF) is determined mainly by the sympathetic and vasomotor activities, and the fast component (HF) is modulated by mechanical factors, such as stroke volume and cardiac output (Japundzic et al., 1990). In treated rats, BP variability in time and frequency domains showed no difference from untreated animals. These data suggest that at this chronic dose, PYR treatment did not modify BP dynamics.

4.4. Baroreflex sensitivity

BS was markedly enhanced by PYR treatment. Both reflex bradycardia and reflex tachycardia were increased by treatment as evaluated by the vasoactive drug method. As the main action of pyridostigmine is peripheral, the increased reflex bradycardia provoked by PHE infusions observed in treated rats could be explained by a long lasting action of the acetylcholine released from vagal nerves in the atria. Acetylcholine release to the ventricles is markedly increased after PHE-induced BP elevation (Kawada et al., 2001). It was also shown that neostigmine, another anticholinesterase agent modifying the degradation rate of the released acetylcholine in the neuroeffector junction, plays a key role in determining the increase in the gain of the transfer function that represents the dynamic properties of the vagus nerve activity to the heart (Nakahara et al., 1998). In this study, not only reflex bradycardia but also reflex tachycardia was increased after treatment. Reflex bradycardia is mainly dependent on vagal activation, while tachycardia is modulated by parasympathetic withdrawal and sympathetic activation (Head and McCarty, 1987). In both situations, vagal control of HR plays a key role. In CTR and PYR rats, SNP induced similar decreases in BP, while the magnitude of reflex tachycardia was always higher in treated animals than controls for the same BP levels. Because it is well-known that parasympathetic activity plays an important role in controlling sympathetic drive (Levy, 1971), it is possible that inhibition of the augmented vagal tone during decreases in BP caused not only a direct effect on the sinus node but also increased HR by exerting a lower presynaptic inhibition of norepinephrine release by adrenergic nerve endings.
BS measured by the $\alpha$-index was increased in the HF band in the pyridostigmine-treated rats. Mancia et al. (1999) have shown that sinoaortic denervation in the cat decreased $\alpha$-LF, and the relative number of segments where PI and SBP were coherent, while $\alpha$-HF was also significantly decreased without reducing the relative number of coherent segments. These findings based the conclusion that $\alpha$-LF may reflect more specifically a baroreflex modulation of the sinus node, although the $\alpha$-HF also involves baroreflex-mediated responses (Mancia et al., 1999). HRV increased in the HF band that is determined by vagal modulation, hence this result may represent an improved capacity to HR adjustments in relation to BP fluctuations, which origin seems to be mainly the parasympathetic improvement due to PYR treatment. Taken together, our results strongly suggest that PYR treatment enhances vagal modulation of HR and the BS.

### 4.5. Potential clinical implications

HRV and BS are autonomic markers that have independent prognostic value for mortality after myocardial infarction (La Rovere et al., 2001). In addition, the capacity to increase BS as an adaptation to physical training identifies myocardial infarction patients with lower mortality rates when compared to those who did not increase the BS or did not train (La Rovere et al., 2002). In this study, both HRV and BS were increased after PYR treatment in rats. The clinical and physiological importance of these findings still need to be tested in pathophysiological conditions in animals and humans. Vagal electrical stimulation over 6 weeks prevented cardiac remodeling and improved survival rates in chronic heart failure rats (Li et al., 2004). In humans, results obtained by our group are promising. In heart failure patients, 48 h of PYR treatment (45 mg/8 h) reduced the number of ventricular arrhythmias and increased HRV (Behling et al., 2003). Prospective trials should evaluate whether the long-term administration of PYR can be of value in reducing hard endpoints, such as mortality in individuals with cardiovascular diseases.

In conclusion, treatment with pyridostigmine during 7 days induced an improvement on heart rate variability and baroreflex sensitivity in conscious unrestrained rats with no modifications on basal hemodynamic parameters. These results support the hypothesis that pharmacological cholinergic stimulation may contribute to potentiate vagal control of heart rate during spontaneous oscillations and drug-induced changes of blood pressure. Further studies are needed to confirm the effectiveness of pyridostigmine treatment on autonomic markers, cardiovascular risk factors, and morbidity mortality.

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