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Assessment of cardiac autonomic tone in conscious rats

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ABSTRACT

Cardiac autonomic tone can be assessed either by estimating separately vagal and sympathetic tones or by evaluating the net effect of their interaction, the so-called sympathovagal balance (SVB). To compare the most commonly used methods in rats, telemetric recordings of the electrocardiogram were performed in normotensive WKY rats, and in groups of spontaneously hypertensive (SHR) rats that were either untreated or chronically treated with the cholinesterase inhibitor, pyridostigmine, to enhance vagal tone. Cardiac autonomic blockers were administered alone and in combination, so that heart rate (HR) could be measured (1) under resting conditions, (2) with either autonomic branch blocked, and (3) with both branches blocked (which provided intrinsic HR, iHR). SVB was assessed as the ratio of resting HR to iHR. This calculation pointed to a sympathetic predominance in untreated SHRs and even more so in WKY rats, and to a marked vagal predominance in pyridostigmine-treated SHRs. By contrast, the ratio between low and high frequency components (LF/HF) of RR interval spectra did not significantly differ between the groups. Each autonomic tone was quantified as the HR change induced by its selective blocker or as the difference between iHR and HR after blockade of its counterpart. Both pharmacological methods indicated vagal enhancement in treated SHRs, but provided opposite results in terms of vagal vs. sympathetic predominance. These data seriously question the use of the LF/HF ratio as an index of SVB, and the possibility to reliably estimate vagal and sympathetic tones separately through current pharmacological approaches in conscious rats.

1. Introduction

Assessment of cardiac autonomic function is of major diagnostic and prognostic importance in numerous cardiovascular diseases (Deyell et al., 2015; Floras and Ponikowski, 2015; Pellman and Sheikh, 2015). Cardiac autonomic tone can be assessed either by estimating vagal and sympathetic tones separately or by estimating the net effect of their interaction, the so-called sympathovagal balance (SVB). The most straightforward and undisputable way of assessing SVB is to compare heart rate (HR) or RR interval (RRI) with intrinsic HR (iHR) or intrinsic RRI (Goldberger, 1999). iHR is measured after the combined administration of a muscarinic antagonist and a β-adrenoceptor antagonist. It is the frequency of spontaneous discharge of pacemaker cells of the sinus node in the absence of autonomic modulation. To quote Goldberger: "When vagal effects predominate, the heart rate is less than the intrinsic heart rate; when sympathetic effects predominate, the heart rate is greater than the intrinsic heart rate". This approach, however, involves the infusion of autonomic blockers, and is, therefore, impracticable in the routine clinical setting. For this reason, noninvasive indices of SVB have been proposed in humans, chiefly indices extracted from the frequency domain analysis of short-term HR variability (Pagani et al., 1986).

In conscious rats, iHR can easily be measured, either acutely (de La Fuente et al., 2013; Lataro et al., 2013) or continuously over long periods of time (Oosting et al., 1997). When autonomic blockers are administered sequentially, it is theoretically possible to estimate separately vagal and sympathetic tones. Two methods of calculation have been proposed which can both be supported by valid arguments. One method consists in equating the HR change induced by a muscarinic antagonist with vagal tone, and the HR change induced by a β-adrenoceptor antagonist with sympathetic tone (Lataro et al., 2013). The other method equates the difference between iHR and HR after β-adrenoceptor blockade alone with vagal tone, and the difference between iHR and HR after muscarinic blockade alone with sympathetic tone (de La Fuente et al., 2013; Guasch et al., 2013).

Considering the lack of consensus for assessing cardiac autonomic tone in rats, we compared the aforementioned methods in groups of rats with distinctly different SVB, namely aging spontaneously hypertensive rats (SHR) that were either untreated or under chronic pyridostigmine treatment (Sayin et al., 2015). Pyridostigmine is a peripherally acting acetylcholinesterase inhibitor which inhibits degradation of acetylcholine in the synapse, and thus enhances endogenous vagal effects (Santos-Almeida et al., 2015). We also included data from age-matched normotensive Wistar–Kyoto (WKY) rats, which were

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expected to differ from SHRs regarding SVB (Murphy et al., 1991; Scridon et al., 2012).

2. Material and methods

2.1. Animals

Twelve 46 week-old male rats of the SHR strain and 8 sex- and age-matched rats of the WKY strain were used (Janvier Labs, Le Genest Saint Isle, France). Five of 12 SHRs were chronically treated with pyridostigmine: rats were implanted subcutaneously with osmotic minipumps (Alzet model 2ML4; DURECT Corporation, Cupertino, CA, USA) containing the solution of pyridostigmine bromide, which provided a continuous infusion of 15 mg/kg/day of the drug at a fixed flow rate of 2.5 μl/h. Pyridostigmine was infused during 3 weeks (Sayin et al., 2015). During the third treatment week, cardiac autonomic tone was assessed as described below.

Four days before surgery for telemetric probe implantation, rats were housed individually for acclimatization to the recording conditions (temperature: 21–22 °C; 12 h light/dark cycle with lights on at 7:00 A.M.; standard rat chow and tap water ad libitum). At the end of the experiment, all rats were euthanized with a lethal dose of pentobarbital sodium (>100 mg/kg, i.p.).

The study protocol was previously approved by the Ethics Committee for Animal Experimentation of the University Claude Bernard Lyon 1.

2.2. Recording of the electrocardiogram in conscious rats

The electrocardiogram (ECG) was recorded by means of telemetry transmitters (Model TA11CA-F40; Data Sciences International, DSI, St. Paul, MN, USA). Under isoflurane anesthesia (2.5% in oxygen), the transmitter was placed in the abdominal cavity, parallel to the body axis, and attached to the muscle wall. The two ECG leads were tunneled subcutaneously and secured in a lead II configuration (Scridon et al., 2012). To alleviate pain, rats were given carprofen (Rimadyl®; 5 mg/kg, s.c.) 20 min before surgery, then 2 times per day during 3 days following surgery. All rats were allowed at least 2 weeks to recover before recordings were initiated. In pyridostigmine-treated rats, minipumps were implanted after a one-week recording without pharmacological interventions (Sayin et al., 2015).

The ECG signal was captured by a receiver plate (RPC-1; DSI) placed under the rat's cage and was sent to the DSI data exchange matrix. The ECG was sampled at 2000 Hz and stored on a computer with Dataquest A.R.T software (Version 4.31 Gold; DSI). Offline processing operations were performed using custom software written in the LabVIEW language (National Instruments, Austin, TX, USA). Artifacts and arrhythmic cardiac beats were automatically detected and eliminated, as previously described (Gallet et al., 2013), so that only sinus rhythm beats were included in the analyses.

2.3. Experimental protocol

In the 3 groups of rats, HR was measured before and after sequential blockade of the two branches of the autonomic nervous system. The peripherally acting muscarinic receptor antagonist, methylatropine, and the β-adrenoceptor antagonist atenolol were used. Methylatropine (2 mg/kg, s.c.) was first administered. Fifteen to 20 min later, atenolol (2 mg/kg, s.c.) was injected, and HR could thus be measured after total cardiac autonomic blockade, which provided iHR. The next day, the sequence of injections was inverted (first atenolol, then methylatropine). Under each condition, 5-min periods of stable HR were selected and used for calculations. The SVB was estimated as the ratio of resting HR (rHR) to iHR. rHR was calculated over a 5-min period preceding drug administrations when the rat was in a quiet awake state. Great care was taken to ensure that rats were in the same environmental conditions and behavioral state on both trial days, and that measurements of rHR and iHR were not separated by more than 40 min to avoid the potential confounding effect of slow changes in iHR.

2.4. Analysis of short-term HR variability

For the purpose of comparison with previously published studies (Scridon et al., 2012), short-term HR variability was analyzed using RRI data. Oscillations of RRI were studied by using spectral methods based on a fast Fourier transform algorithm (Bertram et al., 2005). Briefly, RRI time series were resampled at 10 Hz and the 5-min baseline periods were split into consecutive 25.6-s periods. Slow oscillations related to blood pressure Mayer waves (Julien, 2006) were quantified as the spectral power in the low-frequency (LF, 0.3–0.6 Hz, expressed in ms^2) band, and faster oscillations synchronous with respiration (Rubini et al., 1993) were quantified as the spectral power in the high frequency (HF, 0.6–2.5 Hz, expressed in ms^2) band. The spectral index of SVB was calculated as the ratio between low and high frequency powers (LF/HF) of RRI spectra (Dias da Silva et al., 2002; Kawaguchi et al., 2005; Pagani et al., 1986).

2.5. Statistics

Statistical analysis was performed using the Statistica software version 6.1 (StatSoft, Tulsa, OK, USA). Within-group differences were tested for significance with the Wilcoxon signed-rank test. Between-group differences (WKY rats vs. untreated SHRs and pyridostigmine-treated SHRS vs. untreated SHRs) were tested by the Mann–Whitney test. Differences were considered statistically significant at $P < 0.05$. Day-to-day variations of variables were assessed according to Jones and Payne (1997). Data are expressed as mean ± SEM.

3. Results

3.1. Assessment of SVB

In the 3 groups of rats, rHR significantly differed from iHR, pointing to autonomic modulation (Table 1). The SVB index (ratio of rHR to iHR) measured over 2 consecutive days was highly reproducible (day-to-day variations were 5.4, 2.3 and 5.1% in WKY rats, control and treated SHRs, respectively). This was due to the reproducibility of both resting HR and iHR (Table 1). The SVB index was >1 in both WKY rats and control SHRs, thus indicating sympathetic predominance in both strains of rats. Moreover, it was significantly higher in WKY rats than in SHRs,

Table 1
Effects of cardiac autonomic blockers on HR in conscious rats.

	WKY (n = 8)	SHR (n = 7)	Treated SHR (n = 5)
rHR (bpm)			
Day 1	308 ± 4*	285 ± 6	242 ± 6*
Day 2	323 ± 6*	278 ± 9	237 ± 6*
Mean	316 ± 4*	281 ± 7	239 ± 4*
HR atropine (bpm)	407 ± 6†	381 ± 17†	413 ± 10†
HR atenolol (bpm)	264 ± 4†	246 ± 7†	225 ± 3*
iHR (bpm)			
Day 1	267 ± 5	269 ± 4	297 ± 7*
Day 2	274 ± 5	262 ± 6	293 ± 6*
Mean	270 ± 4	266 ± 4	295 ± 6*
rHR/iHR			
Day 1	1.16 ± 0.02*	1.06 ± 0.01	0.82 ± 0.03*
Day 2	1.18 ± 0.03*	1.06 ± 0.02	0.81 ± 0.02*
Mean	1.17 ± 0.02*	1.06 ± 0.01	0.81 ± 0.02*

rHR, resting heart rate; iHR, intrinsic heart rate.
* $P < 0.05$ vs. SHR.
† $P < 0.05$ for HR after atropine vs. rHR on Day 1 or HR after atenolol vs. rHR on Day 2.