Modulation of sympathetic activity and heart rate variability by ivabradine

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1. Introduction

Reduction of sinus rate by ‘pure’ bradycardic agent (i.e. drugs directly inhibiting pacemaker function) is an emerging therapeutic concept, corroborated by the positive outcome of clinical studies on ivabradine (IVA) in the treatment of ischaemic heart disease\textsuperscript{1} and congestive heart failure.\textsuperscript{2} Nevertheless, in both such conditions, autonomic activity plays a pivotal role as a pathogenetic factor and a prognostic index; hence, the influence of therapeutic agents on the autonomic balance is of considerable interest.

Heart rate regulation is a central element in the baroceptive control of blood pressure. I\textsubscript{f} inhibition may blunt the response of sino-atrial node to autonomic activation, thus interrupting the negative feedback loop normally existing between arterial pressure and adrenergic activity. Thus, unless other mechanisms prevail, selective I\textsubscript{f} inhibition in the sinus node is expected to shift the autonomic balance towards sympathetic activation. Previous reports concerning the effect of I\textsubscript{f} blockade on catecholamine levels\textsuperscript{3,4} seem to support this view. On the other hand, bradycardia induced by the I\textsubscript{f} blocker zatebradine was reportedly associated with an increase in heart rate variability (HRV) and baroreflex sensitivity (BRS), both interpreted as signs of vagal prevalence.\textsuperscript{5} Direct information on the effect of pure bradycardic agents on neurally recorded sympathetic activity is required to solve this apparent discrepancy.

The present study addresses two separate, but related questions.

The first one, of practical relevance, concerns the effect of selective I\textsubscript{f} inhibition on cardiac sympathetic activity. The second, more fundamental, relates to the significance of indirect indexes of autonomic variability by ivabradine.

Bradycardic agents are currently used in the treatment of angina and heart failure; direct information on their effects on cardiac sympathetic nerve activity (SNA) may be relevant to their chronic use. The present study evaluates the effect of pacemaker inhibition on SNA; direct nerve recordings and indirect autonomic indexes are compared.

Experiments were performed in 18 anaesthetized rats. SNA (direct nerve recording) and heart rate variability (HRV) indexes were evaluated in parallel. All parameters were recorded 10 min before to 60 min after administration of the I\textsubscript{f} blocker ivabradine (IVA; 2 mg/kg, i.v.; n = 8) or vehicle (VEH; n = 5). IVA-induced RR interval (RR) prolongation (at 60 min +15.0 ± 7.1%, P < 0.01) was associated with decreased diastolic arterial pressure (DAP; −17.3 ± 8.4%, P < 0.05) and increased SNA (+51.1 ± 12.3%, P < 0.05). These effects were accompanied by increased RR variance (RR\textsuperscript{2}), which showed strong positive correlation with RR. Frequency-domain HRV indexes (in normalized units) were unchanged by IVA. After baroreceptor reflexes had been eliminated by sino-aortic denervation (n = 5), similar IVA-induced RR prolongation (at 60 min +14.3 ± 5.9%, NS vs. intact) was associated with a larger DAP reduction (−30.9 ± 4.1%, P < 0.05 vs. intact), but failed to affect SNA.

(i) IVA-induced bradycardia was associated with increased SNA, resulting from baroreceptor unloading; if this applied to chronic IVA use in humans, it would be of relevance for therapeutic use of the drug. (ii) Whenever mean HR is concomitantly changed, time-domain HRV indexes should not be unequivocally interpreted in terms of autonomic balance.

Ivabradine • Bradycardic agents • Sympathetic nerve activity • Heart rate variability

| Aims | Bradycardic agents are currently used in the treatment of angina and heart failure; direct information on their effects on cardiac sympathetic nerve activity (SNA) may be relevant to their chronic use. The present study evaluates the effect of pacemaker inhibition on SNA; direct nerve recordings and indirect autonomic indexes are compared. |
| Methods and results | Experiments were performed in 18 anaesthetized rats. SNA (direct nerve recording) and heart rate variability (HRV) indexes were evaluated in parallel. All parameters were recorded 10 min before to 60 min after administration of the I\textsubscript{f} blocker ivabradine (IVA; 2 mg/kg, i.v.; n = 8) or vehicle (VEH; n = 5). IVA-induced RR interval (RR) prolongation (at 60 min +15.0 ± 7.1%, P < 0.01) was associated with decreased diastolic arterial pressure (DAP; −17.3 ± 8.4%, P < 0.05) and increased SNA (+51.1 ± 12.3%, P < 0.05). These effects were accompanied by increased RR variance (RR\textsuperscript{2}), which showed strong positive correlation with RR. Frequency-domain HRV indexes (in normalized units) were unchanged by IVA. After baroreceptor reflexes had been eliminated by sino-aortic denervation (n = 5), similar IVA-induced RR prolongation (at 60 min +14.3 ± 5.9%, NS vs. intact) was associated with a larger DAP reduction (−30.9 ± 4.1%, P < 0.05 vs. intact), but failed to affect SNA. |
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balance (HRV and BRS) under the specific condition of direct pharmacological modulation of sinus-node pacemaking. The results obtained show that bradycardia caused by acute administration of a selective I:\ blocker was indeed associated with a reflex increase in the activity of sympathetic efferent fibres. However, the latter was paralleled by an increase in the variance of RR intervals \( \text{RR}_{\text{var}} \), i.e. a change opposite to that commonly expected from sympathetic dominance, but consistent with an intrinsic dependency of HRV on pacemaker cycle length.\(^6\)^\(^7\)

### 2. Methods

#### 2.1 Experimental model

Experiments were performed on male normotensive Sprague–Dawley rats (350–450 g) from the Animal Facility of the Department of Clinical and Biomedical Sciences, University of Milan, Milan, Italy. At the end of the experiments, the animals were euthanized by cervical dislocation under continuing urethane anaesthesia (see below). All experimental procedures were in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition, published by the US National Research Council in 2011 and were submitted to and approved by the Commission for Ethics in the Use of Animals in Research of University of Milan.

#### 2.2 Experimental preparation

Rats were anesthetized with urethane (1.2 g/kg, i.p.), allowing for spontaneous breathing with a respiratory rate between 56 and 120 cycles/min and an end-tidal CO\(_2\) between 3.5 and 5.5%. Central temperature was monitored and maintained at 37°C by a thermostated pad.

A midline cervical incision was performed. The trachea was cannulated using a T-tube (PE 120), which was connected on one side to an end-tidal CO\(_2\) analyzer (Capstar, Stoelting Co., Ardmore, PA, USA). Polyethylene catheters (PE 50) were inserted into the right common carotid artery and the right jugular vein for measurements of systolic (SAP) and diastolic (DAP) arterial pressures (AP) and drug infusion, respectively. The surface ECG was recorded for the measurement of cycle length (RR interval).

After a xifo-umbilical incision, the aorta and the inferior vena cava were isolated and pumatic cuffs were placed around each vessel to induce controlled increases (inflation of the aortic cuff) and decreases (inflation of the vena cava cuff) in AP.

#### 2.3 Baroreceptor denervation

Sino-aortic denervation (SAD) was performed in five animals according to Krieger’s technique,\(^8\) i.e. by bilateral section of the aortic depressor and superior laryngeal nerves, associated with stripping of the carotid sinus regions. Denervation was confirmed by the absence of reflex changes in sympathetic nerve activity (SNA) and heart rate during positive and negative AP changes, induced by inflation of aortic and caval cuffs, respectively.\(^9\)

#### 2.4 Recordings of efferent SNA

The left cervical sympathetic trunk, containing sympathetic efferent fibres directed to the heart, was isolated from the surrounding tissues and peripherally cut. The nerve sheath was removed and the trunk was split at the pre-ganglionic level to isolate a small number of fibres, which were placed on a bipolar recording electrode immersed in a vaseline-filled cradle for isolation. Signals were pre-amplified in an AC mode (Grass RPS 107, Quincy, MA, USA) analogically filtered using a 30–3000 Hz bandwidth, digitized at a sampling rate of 12 KHz, and stored for offline analysis.

Efferent cardiovascular sympathetic activity was identified by its response to the baroreceptor reflex, namely by its decrement and increment during induced hypertension and hypotension, respectively (inflation of aortic and caval cuffs).\(^10\)

#### 2.5 Experimental protocol

Animals were divided into three experimental groups: (i) neurally intact animals to be treated with IVA (INTACT + IVA; \( n = 8 \)); (ii) neurally intact animals to be treated with drug vehicle (INTACT + VEH; \( n = 5 \)); and (iii) animals after SAD to be treated with IVA (SAD + IVA; \( n = 5 \)).

After nerve filament isolation, the preparation was allowed to stabilize for 1 h. SNA, ECG, pulsatile AP, and end-tidal CO\(_2\) were monitored from 10 min before to 1 h following an intravenous bolus injection of 2 mg/kg of IVA (dissolved in 1 mL/kg of VEH 0.85% saline solution) or its VEH. The same protocol was applied to all experimental groups.

#### 2.6 Signal analysis

All variables were analysed at 1 min before and 1, 5, 15, 30, 45, and 60 min after IVA or VEH administration.

##### 2.6.1 Nerve activity

Neural spikes were automatically detected and counted offline from nerve recordings by a dedicated software,\(^5\)^\(^11\) the detection threshold was adjusted for optimal rejection of background noise. Autonomic nerve activity is often expressed in spike per cardiac cycle to account for its phasic variation. However, when RR intervals are shorter than 1 s, RR prolongation might increase the time over which nerve activity is integrated within each cycle; thus, expressing nerve activity in spikes/cycle might lead to overestimate its increment during IVA infusion. Thus, we chose to measure nerve activity in spikes/s instead; to highlight IVA-induced effects, text and figures report percent changes in spikes/s from baseline values. SNA was also calculated in spike/cycle and found, as predicted, to magnify IVA-induced changes.

##### 2.6.2 HRV indexes

ECG was processed with a customized software that determines beat-by-beat RR values at 1 min before and 1, 5, 15, 30, 45, and 60 min after i.v. IVA or VEH administration. RR variability measurement was performed on segments of 250–300 cycles; linear segment detrending was applied to eliminate the effect of progressive changes in RR interval. HRV in the ‘time domain’ was evaluated as total RR variance (\( \text{RR}_{\text{var}} \)), whereas in the ‘frequency domain’ it was assessed by autoregressive spectral analysis as described elsewhere.\(^12\)–\(^18\) Briefly, a modelling of the oscillatory components present in RR time series was calculated based on the Levinson–Durbin recursion, with the order of the model chosen according to Akaike’s criterion. This procedure allows an automatic quantification of the centre frequency and power of each relevant oscillatory component present in the time series. The oscillatory components were labelled as low (LF) or high frequency (HF) when their central frequency was located in bands of 0.25–0.75 and 0.75–2.50 Hz, respectively.\(^19\) In addition, the LF/HF ratio was also calculated as an index of autonomic balance independent of total RR variance.

##### 2.6.3 Baroreflex sensitivity

BRS was monitored throughout the infusion period by correlating spontaneous changes in SAP and RR, according to the sequence method described by Bertinieri et al.\(^15\) and validated for rats.\(^11\)^\(^16\) Briefly, at 1 min before and 1, 5, 15, 30, 45, and 60 min after i.v. IVA or VEH administration, series of at least three progressively incremental (or decremental) SAP values were automatically detected using a customized software (BRS software) and stored along with the simultaneous RR values. The recorded series were used for BRS analysis if (i) SAP and RR changes exceeded 1 mmHg and 1 ms, respectively; (ii) SAP and RR changes had the same sign, as expected from a ‘baroreflex’ sequences; and (iii) the correlation coefficient of linear RR vs. SAP fitting was \( > 0.8 \). Spontaneous cardiac BRS (in ms/mmHg) was calculated from each series as the regression coefficient (slope) of linear RR vs. SAP fitting. Spontaneous BRS was finally obtained at each measurement time point by averaging slopes values from baroreflex sequences.\(^11\)^\(^15\)^\(^16\)
2.7 Statistical analysis

Data are expressed as absolute changes from baseline values and reported as mean ± SEM. Effects over time between treatment groups (IVA vs. VEH or INTACT vs. SAD) were compared by two-way ANOVA for repeated measures: means of baseline values were compared by one-way ANOVA. In both cases, post hoc comparisons between individual means were performed by Tukey’s test. All statistics were performed using the software SPSS 7.5 for Windows (SPSS, Inc., Chicago, IL, USA). Differences were considered significant when P < 0.05.

3. Results

3.1 Neurally intact animals

Baseline values of all variables measured 1 min before were similar between VEH and IVA treatment groups (Table 1). VEH administration failed to modify RR, DAP, SAP, and SNA throughout the 60 min observation period (Figure 1).

IVA caused a progressive increase in RR, which started shortly after administration and achieved steady state in 20–30 min (Figure 1A). Steady-state RR change (at 60 min) corresponded to a 15.5% of reduction in mean heart rate. While SAP was unchanged (Figure 1C), DAP was significantly reduced (at 60 min −17.3 ± 8.4%; P < 0.05; Figure 1D); DAP time course roughly matched that of RR.

A sample recording of SNA, ECG, AP, and respiration during IVA infusion is shown in Figure 2. Following IVA infusion, SNA monotonically increased from its baseline value (summarized in Table 1) throughout the recording period (at 60 min +51.1.1 ± 12.3%; P < 0.05); SNA became significantly different from that recorded in the VEH treatment group at 30 min (+19.8 ± 4.9%), i.e. in the presence of a 15.8 ± 6.6 mmHg DAP reduction (Figure 1B and D). While SNA did not achieve a steady state during the 60 min observation period, it probably contributed to level off drug-induced RR and DAP changes after 30 min.

IVA-induced bradycardia and SNA augmentation were associated with a clear-cut increment in RRr2, which, albeit of variable magnitude, amounted to an average six-fold change at 60 min (+639%; P < 0.05; Figure 3). Thus, contrary to what commonly expected from sympathetically activation, HRV significantly increased after IVA administration (Figure 3A). Within each animal, during IVA infusion, RRr2 was non-linearly related to RR (Figure 4); such a relationship was present also for mean values (Figure 4, inset; R² = 0.72; P < 0.05), even if likely distorted by the RRr2 inhomogeneity between animals. As expected, the absolute variance under LF (Figure 3B) and HF (Figure 3C) RR spectral components was also increased. However, the LF/HF ratio was unaffected by IVA (Figure 3D). All time- and frequency-domain indexes of HRV were unaffected by VEH infusion (Figure 3).

Neither VEH nor IVA affected BRS significantly at all time points during the observation period (Figure 5A).

3.2 Animals with SAD

In the SAD group, baseline SNA and DAP were higher (+57 and +28%, respectively) and BRS lower (−72%) when compared with animals with intact innervation (Table 1). Although basal RRr2 appeared to be lower in the SAD group (Figure 1A), the difference did not achieve significance because of the large scatter of values. All other baseline values were similar between SAD and intact animals.

Early response of mean RR to IVA was somewhat blunted in SAD animals, but it approached that of neurally intact animals at later times (Figure 6A). While SAD abolished IVA effect on SNA (Figure 6B), it increased RRr2 (ΔRRr2 at 60 min +2.38 ± 1.42 ms²; P < 0.05), as it did in neurally intact animals (Figure 3A, ΔRRr2 at 60 min +3.87 ± 2.58 ms²; NS vs. SAD). SAP was unchanged by IVA (Figure 6C); however, as expected from loss of baroreceptor control, the drop in DAP accompanying RR prolongation was slightly more pronounced than in neurally intact animals (Figure 6D). As in neurally intact animals, IVA did not change BRS (Figure 5B).

4. Discussion

The experiments performed show that IVA-induced RR prolongation increased RR variance, reduced DAP, and increased efferent sympathetic activity. IVA-induced sympathetic activation, but not the changes in RR and its variance, was abolished by baroreceptor denervation.

4.1 Haemodynamic effects

As expected from its pharmacological profile,17 IVA reduced heart rate. The magnitude of heart rate reduction (15–20%) was comparable to that achieved in clinical studies and considered desirable for the therapeutic action of bradycardic agents.2,18 In the present experimental setting, bradycardia was associated with a significant decrease in DAP, with a time course roughly parallel to RR, and no change in SAP (increased

Table 1 Baseline values (i.e. prior to IVA administration) in the three experimental groups

<table>
<thead>
<tr>
<th></th>
<th>INTACT + VEH (n = 5)</th>
<th>INTACT + IVA (n = 8)</th>
<th>SAD + IVA (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR (ms)</td>
<td>165 ± 6</td>
<td>160 ± 6</td>
<td>163.6 ± 9.0</td>
</tr>
<tr>
<td>RRr² (ms²)</td>
<td>0.60 ± 0.43</td>
<td>0.63 ± 0.21</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td>LF (ms²)</td>
<td>0.10 ± 0.08</td>
<td>0.06 ± 0.05</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>HF (ms²)</td>
<td>0.39 ± 0.28</td>
<td>0.26 ± 0.08</td>
<td>0.21 ± 0.08</td>
</tr>
<tr>
<td>L/F-HF</td>
<td>0.15 ± 0.08</td>
<td>0.24 ± 0.013</td>
<td>0.15 ± 0.07</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>80.0 ± 5.1</td>
<td>82.5 ± 4.9</td>
<td>105.6 ± 4.5*</td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>120 ± 3</td>
<td>123 ± 6</td>
<td>118 ± 4</td>
</tr>
<tr>
<td>SNA (spike/s)</td>
<td>96.6 ± 1.2</td>
<td>100.4 ± 1.3</td>
<td>157.5 ± 14.5*</td>
</tr>
<tr>
<td>BRS (ms/mmHg)</td>
<td>1.20 ± 0.33</td>
<td>1.1 ± 0.26</td>
<td>0.3 ± 0.23*</td>
</tr>
</tbody>
</table>

INTACT + VEH, vehicle with intact innervation; INTACT + IVA, ivabradine with intact innervation; SAD + IVA, ivabradine with sino-aortic denervation.

*P < 0.05 vs. INTACT + IVA.
pulse amplitude). While a drug-induced decrease in peripheral resistance would be expected to affect SAP to some extent, a selective reduction of DAP might be accounted for by prolongation of diastolic interval alone. Indeed, the latter would allow for aortic recoil to get closer to steady state, thereby reducing the elastic force developed during terminal diastole (Windkessel effect). IVA-induced DAP change was larger than that commonly observed in humans; several mechanisms, related to the experimental model, might account for this difference. Aortic recoil is reportedly faster in smaller animals; thus, recoil force, responsible of DAP, may more steeply decay when diastolic interval is prolonged. Secondly, adrenergic vasoconstrictor response might be partly blunted in the present setting by anaesthesia, thus leading to less complete compensation of bradycardia-induced haemodynamic changes. Nevertheless, the larger drop in DAP induced by IVA in SAD animals indicates that reflex neural compensation was still significant, at least for what concerns vascular resistance.

In animals with intact sino-aortic nerves, IVA-induced bradycardia was associated with a progressive increase in efferent activity of cardiac sympathetic nerves which, at 60 min from drug administration, exceeded basal activity by ~50%. Such a change, which has implications of potential clinical relevance, is opposite to what previously reported for another β-blocker by a study based on HRV analysis only. While sympathetic activity continued to increase throughout the infusion period, DAP achieved a steady state at ~30 min in neutrally intact animals, but not in SAD ones. Thus, late DAP time course likely reflected a balance between haemodynamic changes and increased vasomotor tone. SAD removes neural input from carotid and aortic baroreceptors, whose activity normally supports vagal contribution to the autonomic balance. After SAD, basal sympathetic activity and DAP were higher, as expected from removal of tonic baroceptive restraint and the resulting vasoconstriction. Under this condition, IVA-induced haemodynamic changes were not associated with a further increment of sympathetic discharge. This suggests that the SNA response to IVA, observed with intact innervation, was largely mediated by a baroreceptor reflex. On the other hand, SAD unexpectedly failed to affect basal RR and its response to IVA. This points to weak sympathetic modulation of sinus rate in this preparation, also consistent with the lack of IVA effect on the LF/HF ratio (see below).

### 4.2 Effects on SNA and HRV

In the present study, for the first time, nerve activity, RR variance (RR²), and BRS were simultaneously measured during pharmacological modulation of SAN function, thus allowing a comparison between direct measurement and indirect estimates of autonomic activity.

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**Figure I** Mean values (± SEM) of IVA- (closed circles, n = 8) or VEH-induced (open circles, n = 5) changes in RR (A), SNA (B), and SAP (C) and DAP (D) from animals with intact baroreceptor innervation. IVA or VEH administration started at time 0. Changes in sympathetic activity were expressed as % of the activity (in spikes/s) recorded under baseline conditions. *P < 0.05 vs. control VEH group.
IVA increased SNA and RR$^2$ at the same time (Figures 1 and 2), i.e. opposite to what expected from the widely accepted assumption that an increase in RR variability (also referred to as HRV) reflects parasympathetic dominance. SAD abolished IVA-induced sympathetic activation, but the RR$^2$ increment persisted, thus showing that the two variables were not causally related. This can be simply interpreted by considering that RR$^2$ may intrinsically depend on mean RR, which was directly prolonged by IVA. Such a dependency, which results from the non-linear relation between sino-atrial diastolic depolarization rate (dV/dt) and cycle length, implies that RR response to perturbations (including neurally induced ones) is bound to be proportional to mean RR, irrespective of changes in the autonomic balance. This interpretation is supported by the presence of a close relationship between RR$^2$ and mean RR (Figure 4), which is non-linear, as expected from the mathematical relationship between diastolic depolarization rate and RR.7 Such a relationship has been recently confirmed to hold true for humans, as well as for several in vivo and in vitro animal preparations, and interpreted essentially as we did in our previous work.6,7 The increase in absolute LF and HF powers likely reflects the increment in RR$^2$, which represents the sum of individual spectral components.

The observation that IVA-induced bradycardia was associated with increased RR$^2$ is largely consistent with previous reports on the effect of $I_f$ blockers in animals and humans.24 An exception is represented by a small study in which zatebradine was found to reduce RR$^2$ in humans, an effect attributed to loss of SAN responsiveness to autonomic modulation upon $I_f$ blockade.25 It is fair to stress that dependency on mean RR may amplify RR$^2$ response to perturbations, but it cannot be a source of perturbation on its own. This concept is highlighted by the observation that, in conscious rats, removal of the main perturbation source through autonomic blockade (atropine + propranolol) prevented the increment in RR$^2$, otherwise induced by $I_f$ blockade.26 Furthermore, its intrinsic dependency on mean RR does not rule out that RR$^2$ may be also modulated by neural influences, as is probably the case under many conditions of genuine autonomic imbalance (e.g. heart failure, autonomic receptor blockade etc.).27 Nevertheless, the results of the present study lend strong support to the notion that, whenever accompanied by consensual changes in mean RR, changes in RR$^2$, or time-domain variability indexes in general, should not be interpreted as independent measures of autonomic balance.7

Theoretical considerations suggest that RR$^2$ and BRS may be similarly biased by mean RR.7 Consistent with this expectation, $I_f$ inhibition was previously reported to increase BRS, evaluated by phenylephrine infusion.5 Nevertheless, IVA-induced bradycardia was not associated with BRS increment in the present study. $I_f$ is an important effector of cholinergic pacemaker modulation; therefore, its blockade might obscure the effects of spontaneous changes in vagal activity, which are conceivably smaller during spontaneous pressure fluctuations than when baroreceptor activation is induced by infusion of vasoconstrictor agents. Furthermore, RR$^2$ dependency on mean RR may be less obvious over a narrower range of RR values. Thus, failure of RR prolongation to affect BRS might simply be the consequence of the measurement method adopted in the present study.

A substantial body of evidence indicates that sympathetic predominance is associated with an increase in the LF/HF ratio.12,13 LF/HF was surprisingly insensitive to IVA-induced sympathetic activation, indisputable in the present case because directly measured (Figures 2 and 3). This observation should not be overinterpreted to argue against the value of LF/HF as a reporter of autonomic balance; indeed, other findings in this study (see above) point to poor sensitivity of sino-atrial pacemaker (but not of vascular tone) to sympathetic modulation in the present experimental setting.
4.3 Study limitations

Anaesthetized animal models have substantial limitations in the evaluation of autonomic activity, even if care is taken to avoid unnecessary perturbations. In the present study, urethane anaesthesia was chosen for its minor of interference with autonomic activity and spontaneous breathing, respiration, and temperature were monitored. Despite this, the lack of RR sensitivity to SAD and of LH/HF ratio to sympathetic activation point to subnormal response of sino-atrial pacemaker to autonomic modulation. On the other hand, robust sympathetic activation by (IVA-induced) DAP decrement, as well as unveiling of the latter by SAD, indicates that central baroreceptive integration and vascular responsiveness were at least partially preserved.

Whatever its cause, pacemaker unresponsiveness suggests caution in translating some of the findings to conscious animal physiology. For instance, had pacemaker responsiveness been preserved, sympathetic activation might have limited the extent of IVA effects on RR and DAP. At any rate, loss of pacemaker responsiveness cannot account for the main findings of the study, i.e. IVA-induced sympathetic activation and its coexistence with RR$\sigma^2$ increment.

**Figure 3** Effect of IVA (closed symbols, $n = 8$) or VEH (open symbols, $n = 5$) on RR variance (RR$\sigma^2$, A), its LF (B), HF (C) components, and LF/HF ratio (D) in animals with intact baroreceptor innervation. Spectral components are expressed in absolute spectral power units. IVA or VEH administration was started at time 0. *P < 0.05 vs. control VEH group.

**Figure 4** Relationship between variance of RR$\sigma^2$ and mean RR in an individual animal during IVA infusion. Data are fitted with arbitrary function (exponential). The inset shows the same relationship for sample mean values ($n = 8$). R, correlation coefficient computed from the fitted function.
Construction of HRV time course required measurements to be performed on short RR series. The factors underlying short-term RR are different from those underlying the same parameter measured over 24 h, which include circadian variations, physical activity, etc. This should be considered when comparing the present results with HRV measurements obtained from Holter recordings.
4.4 Practical and theoretical implications

‘Bradycardic agents’ (β blockers) have been recently introduced in the therapeutic toolkit for stable coronary artery disease and heart failure. The major benefits expected from prolongation of diastole in both these conditions, and empirically observed with β-blocker therapy, conceptually justify the pursuit of pharmacologically induced bradycardia. Nevertheless, by breaking the pressure homeostatic loop, β-blockade is expected to cause reflex sympathetic activation, an expectation confirmed by the present findings and consistent with the previously reported effect on blood catecholamine levels.3 4

It should be stressed that, for technical reasons, the present experiments were limited to the first hour of drug infusion; thus, we cannot rule out that sympathetic activation may subside at later times due to ‘adaptation’ of the reflex.29 However, that β blockade may indeed promote long-term myocardial remodelling is suggested by the finding that, while reducing overall infarct size and mortality, zatebradine increased myocardial catecholamine content and facilitated ventricular dilatation at 8 weeks after coronary ligation in rats.4 Nevertheless, also the latter evidence comes from studied rodents; therefore, direct extrapolation to the clinical setting may be unwarranted. On the other hand, if it occurred in humans, persistent bradycardia-induced sympathetic activation might have detrimental effects; this would be a concern particularly with β blockers which, unlike β-blockers, do not interfere with activation of adrenergic receptors. This, along with other consequences of rate limitation, such as the larger diastolic volume required to support a given cardiac output, should be weighed against the benefits expected from bradycardia in the specific condition to be treated. A recent study reported that IVA, in spite of its significant antiaginal effect, failed to reduce the incidence of major cardiac events in patients with preserved contractile function.29 Whether such unexpected outcome may be related to the present findings remains to be established. At any rate, the present findings may suggest that IVA use in the absence of β-blockers should be considered with particular caution.

The present results also provide a robust in vivo confirmation to the notion that HRV and mean HR are intrinsically related.6 7 This reinforces the view that, in the presence of concomitant changes in mean HR, time- and frequency-domain HRV indexes may not be unequivocally interpreted to indicate changes in autonomic balance. A large body of previous literature is potentially affected by this conclusion.

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