Chronic heart rate reduction remolds ion channel transcripts in the mouse sinoatrial node but not in the ventricle

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Leoni, Anne-Laure, Céline Marianneau, Sophie Demolombe, Sabrina Le Bouter, Matteo E. Mangoni, Denis Escande, and Flavien Charpentier. Chronic heart rate reduction remolds ion channel transcripts in the mouse sinoatrial node but not in the ventricle. Physiol Genomics 24: 4–12, 2005. First published October 11, 2005; doi:10.1152/physiolgenomics.00161.2005.—We investigated the effects of chronic and moderate heart rate (HR) reduction on ion channel expression in the mouse sinoatrial node (SAN) and ventricle. Ten-week-old male C57BL/6 mice were treated twice daily with either vehicle or ivabradine at 5 mg/kg given orally during 3 wk. The effects of HR reduction on cardiac electrical activity were investigated in anesthetized mice with serial ECGs and in freely moving mice with telemetric recordings. With the use of high-throughput real-time RT-PCR, the expression of 68 ion channel subunits was evaluated in the SAN and ventricle at the end of the treatment period. In conscious mice, ivabradine induced a mean 16% HR reduction over a 24-h period that was sustained over the 3-wk administration. Other ECG parameters were not modified. Two-way hierarchical clustering analysis of gene expression revealed a separation of ventricles from SANs but no discrimination between treated and untreated ventricles, indicating that HR reduction per se induced limited remodeling in this tissue. In contrast, SAN samples clustered in two groups depending on the treatment. In the SAN from ivabradine-treated mice, the expression of nine ion channel subunits, including Navβ1 (−25%), Cav3.1 (−29%), Kir6.1 (−28%), Kvβ2 (−41%), and Kvβ3 (−30%), was significantly decreased. Eight genes were significantly upregulated, including K+ channel α-subunits (Kv1.1, +30%; Kir2.1, +29%; Kir3.1, +41%), hyperpolarization-activated cation channels (HCN2, +24%; HCN4, +52%), and connexin 43 (+26%). We conclude that reducing HR induces a complex remodeling of ion channel expression in the SAN but has little impact on ion channel transcripts in the ventricle.

NEW GENOMICS APPROACHES, based on high-throughput gene expression profiling, have just begun to provide information on the transcriptional consequences of cardiac therapies, i.e., pharmacological remodeling (37, 40, 42). In this context, we have recently shown that chronic amiodarone induces a complex remodeling of ion channel transcript expression in the mouse ventricle (20). Ionic remodeling related to amiodarone could not be explained by the hypothyroid syndrome induced by the drug, because it markedly differed from that produced by hypo-thyroidism alone in the same mouse model (19). Because a remarkable feature of amiodarone was also to produce a moderate heart rate reduction, we wondered whether heart rate reduction per se could impact ion channel expression in the heart.

While the effects of tachycardia on ion channel gene expression are well described (1, 17, 31, 34, 48), much less is known about the consequences of bradycardia. In the context of atrial fibrillation, a moderate heart rate reduction with β-blockade was shown to prevent or reverse cardiac electrical remodeling (47). In this latter situation, however, direct regulation of gene expression by decreased β-adrenergic input rather than heart rate reduction could explain the results. Strong bradycardia, such as in complete atrioventricular block, is known to produce hyptertyph and electrical remodeling (45). Schoenmakers et al. (39) showed that, in the atrioventricular block dog model, electrical remodeling precedes ventricular hypertrophy. In this model, however, heart rate is profoundly reduced.

The only way to chronically and moderately reduce heart rate in the mouse model is to use a pharmacological agent. Ivabradine (S 16257-2) is a selective inhibitor of the cardiac pacemaker hyperpolarization-activated current (I f) current (2, 4, 43), which dose dependently reduces heart rate in humans and animals without altering the atrioventricular or intraventricular conduction or contractility (11, 28, 36, 41). Most importantly, a feature of ivabradine is that it does not target the ventricle, whereas other bradycardic agents such as β-blockers, digitalis, or Ca2+ channel inhibitors do. We thus used ivabradine to investigate the effects of moderate and chronic heart rate reduction on ion channel transcript expression in the mouse. Our results demonstrate that chronic heart rate reduction only slightly affects the expression of ion channels in the ventricle, thus ruling out the possibility that amiodarone effects on ion channel expression are caused by bradycardia. Inversely, our data demonstrate that lowering heart rate induces a complex remodeling of ion channel expression in the mouse sinoatrial node (SAN).

METHODS

Animals and Ivabradine Treatment

Animal protocols were approved by our Institutional Animal Care and Use Committee and performed in compliance with European directives (86/609/CEE). Ivabradine hydrochloride was supplied by the Institut de Recherches Internationales Servier (Courbevoie, France). The drug was diluted in sterile water at a concentration of 0.5 mg/ml free-base ivabradine. Fresh drug solution was prepared weekly.

After a 2-wk acclimatization period on a 12:12-h light-dark cycle (with light on at 8:30 AM), 48 10-wk-old C57BL/6 male mice (Charles River, L’Arbresle, France) were randomized to receive either...
vehicle or 5 mg/kg ivabradine given orally twice daily (total daily dose of 10 mg/kg) for 3 wk. Administrations were performed by gavage between 8:30 and 9:00 AM and 8:30 and 9:00 PM. The last administration was performed at days 23–24 in the morning.

At least 1 wk before the treatment period, a subgroup of nine mice were implanted with a telemetric device, allowing the measurement of electrocardiogram (ECG) parameters and activity. Animals were anesthetized with an intraperitoneal (ip) injection of etomidate (35 mg/kg). Local anesthesia was obtained with lidocaine (1%) injected subcutaneously at the sites of electrodes and transmitter implantation. A midline incision was made on the back along the spine to insert a telemetric transmitter (TA10EA-F20; Data Sciences International, St. Louis, MO) into a subcutaneous pocket with paired wire electrodes placed over the thorax (chest bipolar ECG lead). Five of these mice were randomly assigned to the ivabradine group and four to the vehicle group.

**ECG Recordings**

In conscious mice. Telemetric ECG signals were computer recorded with the use of a telemetry receiver and an analog-to-digital conversion data acquisition system for display and analysis by Dataquest A.R.T. (Data Sciences International) and ECG auto 1.5.11.26 (EMKA Technologies, Paris, France) software. Data recordings were obtained for 48 h after each week of treatment [on day 7 (D7) and D8, D14 and D15, D21 and D22] starting 30 min before the morning administration.

In anesthetized mice. In both vehicle and ivabradine groups, one-lead ECG recordings (lead I) were obtained with 25-ga subcutaneous electrodes on a computer through an analog-digital converter (IOX 1.585; EMKA Technologies) for later analysis and measurements with ECG auto 1.5.7 (EMKA Technologies). ECG recordings were obtained 2 min after anesthesia with etomidate (30 mg/kg ip) and 10 min after subsequent injection of atropine sulfate (0.5 mg/kg ip) and propranolol (1 mg/kg ip). The ECG recordings were obtained 24 h before the first administration and then on D8–D9, D15–D16, and propranolol (1 mg/kg ip). The ECG recordings were 10 min after subsequent injection of atropine sulfate (0.5 mg/kg ip) and propranolol (1 mg/kg ip). The ECG recordings were obtained 24 h before the first administration and then on D8–D9, D15–D16, and D21–D22 after the first administration. They were performed 3–4 h after the morning administration.

Criteria used for measuring RR, PR, QRS, and QT intervals as well as P wave duration have been reported previously (18). QT intervals were corrected (QTc) for heart rate using the formula, QTc = QT/(RR/100)1/2, with QT and RR measured in milliseconds (27).

**RNA Preparation**

Under general anesthesia with etomidate (30 mg/kg ip), mice were killed by cervical dislocation and the beating hearts quickly excised. A thin strip of SAN tissue (~1 × 0.8 mm), limited by crista terminalis, atrial septum, and orifices of the venae cavae, was cut from the right atrium as previously described (24, 26) and flash frozen in liquid nitrogen for further RNA isolation. Both right and left ventricles were dissected and frozen together. Total RNA from individual ventricular preparations and from pools of two SANS was isolated and DNase treated using the RNasey Fibrous Tissue Mini and Micro Kit (Qiagen), respectively. The quality of total RNA was assessed by microelectrophoresis on acrylamide gel (Agilent 2100 Bioanalyzer). Lack of genomic DNA contamination was checked by PCR amplification of total RNA samples without prior cDNA synthesis.

**TaqMan Real-Time RT-PCR**

TaqMan Low Density Arrays (Applied Biosystems) were used in a two-step RT-PCR process to characterize the ion channel gene expression patterns of live pools of SANS and six individual ventricular preparations from the vehicle group and six pools of SANS and six ventricular preparations from the ivabradine-treated group. First strand cDNA was synthesized from 220 ng of total RNA using the High Capacity cDNA Archive Kit (Applied Biosystems). PCR reactions were then carried out in Low Density Array using the AB PRISM 7900HT Sequence Detection System (Applied Biosystems). The 384 wells of each array were preloaded with 96 × 4 predesigned TaqMan probes and primers (TaqMan Gene Expression Assays, Applied Biosystems). The probes were labeled with the fluorescent reporter dye 6-carboxyfluorescein (FAM, Applied) on the 5′-end and with nonfluorescent quencher on the 3′-end. The genes selected for their cardiac expression encode 68 ion channel α- and auxiliary subunits, 9 proteins involved in calcium homeostasis, 5 transcription factors, specific markers of cardiac regions, vessels, neuronal tissue, fibroblasts, inflammation and hypertrophy, and 4 endogenous control genes used for normalization. Two nanograms of cDNA combined with 1× TaqMan Universal Master Mix (Applied Biosystems) were loaded into each well. The Low Density Arrays were thermal cycled at 50°C for 2 min and 94.5°C for 10 min, followed by 40 cycles of 97°C for 30 s and 59.7°C for 1 min. Data were collected with instrument spectral compensations by the Applied Biosystems SDS 2.1 software and analyzed using the threshold cycle (Ct) relative quantification method (23). Data were normalized using an internal control gene. Genes with Ct >32 were eliminated for lack of reproducibility. The outliers were excluded using a robust statistical modified z-score method based on the median of absolute deviation (26). The nonexcluded values (n = 4–6) were used for the 2−ΔΔCt calculation; 2−ΔΔCt corresponds to the ratio of each gene expression vs. internal control gene.

The expression of genes encoding hyperpolarization-activated cyclic nucleotide-gated ion channel (HCN)4 and Kir3.4 was quantified by individual TaqMan real-time PCR (TaqMan Gene Expression Assays were not available for Low Density Array experiments). PCR reactions were performed on 10 ng of cDNA using TaqMan Gene Expression Assay designed by Applied Biosystems. Amplifications were performed in duplicate. Data were normalized and expressed as described above.

Two-way hierarchical agglomerative clustering was applied to the gene expression matrix consisting of the 23 tissue samples and 79 analyzed genes. The input consisted of the ∆ΔCt for each gene and sample. We applied average linkage clustering with uncentered correlation using the Cluster program (10). Clusters were visualized using the TreeView program.

**Assay of Ivabradine and S 18982 Plasma Levels**

On the day of the last administration (D23–D24), blood samples were collected to assess plasma levels of ivabradine and its main active metabolite S 18982 at six different times: just before the administration (i.e., 12 h after last gavage) and 15 min, 30 min, 1 h, 4 h, and 8 h postadministration. For each sampling time, venous blood from four mice was collected. Levels of ivabradine and S 18982 were evaluated using a validated method involving liquid chromatography with tandem mass spectrometry detection.

**Statistical Analysis**

Plasma levels of ivabradine and S 18982 are expressed as means ± SD. All other data are expressed as means ± SE. Statistical analysis was performed with Student’s t-test and one- or two-way analysis of variance completed by a Tukey’s test when appropriate. A value of P < 0.05 was considered significant.

**RESULTS**

On the basis of our previous study (20), we planned to evaluate ion channel expression under experimental conditions leading to a moderate (i.e., 15–20%) decrease in heart rate. ECG recordings on freely moving mice showed that ivabradine at 5 mg/kg twice daily increased the mean RR interval over a 24-h period by 16% (from 109 ± 4 ms in the vehicle group to 127 ± 2 ms in the ivabradine group; P < 0.01, n = 4 and 5, respectively) after 8 days of treatment. This effect was constant.
throughout the 3-wk period of the study with RR intervals of 110 ± 4 ms and 112 ± 4 ms after 8 and 22 days with vehicle, respectively, and 128 ± 5 ms (P < 0.01 vs. vehicle; +16%) and 129 ± 4 ms (P < 0.05; +15%) after the same periods with ivabradine.

The effects of ivabradine on heart rate in freely moving mice are shown in Fig. 1. Administration of ivabradine induced a progressive prolongation of the RR interval, which was maximal at about 1 h and remained stable for 4–6 h (see Fig. 1A). This correlated with the plasma levels of ivabradine, which reached 172 ± 55 ng/ml (~0.35 μM) 1 h after administration (after a transient peak to 450 ± 34 ng/ml) and then declined progressively to 41 ± 16 ng/ml (~0.08 μM) and 14 ± 5 ng/ml (~0.02 μM) 4 and 8 h after administration, respectively. Ivabradine’s active N-demethylated metabolite S 18982 showed a similar pattern with a maximum concentration of 47 ± 4 ng/ml. As shown in Fig. 1B, the effects of ivabradine on heart rate measured during 12-h light or dark periods were consistently more pronounced during nighttime over the 3-wk administration; the mean RR interval over 12 h in the ivabradine group was 16–21% longer than in the vehicle group during nighttime and 11–13% longer during daytime.

Under anesthesia and after blockade of the autonomic nervous system, the heart rate of ivabradine-treated mice was 30–40% lower than in the vehicle group (Fig. 2 and Table 1; data obtained 3–4 h after morning administration). Ivabradine had no significant effect on atrial, atrioventricular, and ventricular conduction parameters. Similarly, ventricular repolarization was not affected, since the corrected QT interval was not modified (Table 1). We did not observe any altered expression of genes encoding for brain natriuretic peptide, skeletal and cardiac α-actin, or the β-myosin heavy chain, demonstrating the absence of even mild ventricular hypertrophy.

An overview of gene expression data is provided by a two-way hierarchical clustering analysis of genes and cardiac tissues (Fig. 3). In this global analysis, the samples are linked to a tree. The size of the tree branches indicates the distance between the clusters: the higher the branch size, the more significant the separation between the clusters. Inversely, small branch size indicates low-significance clustering. Hierarchical clustering revealed a clear separation between transcript expression pattern of ventricular samples and SANs, confirming our previous findings (26). This analysis also showed an absence of discrimination between ventricular samples from the vehicle and ivabradine-treated groups, indicating that heart rate reduction had little effect on ventricular ion channel transcripts. In contrast, SAN samples clustered separately in two distinct groups depending on the treatment, although the degree of discrimination was mild as indicated by the small size of the tree branches.

Figures 4–6 show the effects of sustained moderate heart rate reduction on the expression of genes encoding ion channel subunits and Ca²⁺ homeostasis regulators in the SAN. Among the voltage-gated Na⁺ channel subunits, the β-subunit Navβ1
(Scn1b) was negatively regulated (Fig. 4). Similarly, only one Ca\(^{2+}\) channel subunit, Cav3.1 (Cacnalg), was downregulated. The gap junction protein expressed in the mouse SAN, Cx43 (Gjai), was upregulated. Most of the remodeling concerned K\(^+\) channel gene families and their regulators (Fig. 5). Among the α-subunits, only one voltage-gated channel, Kv1.1 (Kcnal1), was regulated in a positive way. One of the α-subunits forming the pore of the inward rectifier K\(^+\) channel \(I_{K1}\), Kir2.1 (Kcnj2), was upregulated. Kir2.2 (Kcnj12) expression was also moderately, albeit not significantly, increased. Similarly, Kir3.1 (Kcnj3), which generates the acetylcholine-activated K\(^+\) (ACh, was upregulated. Two other K\(^+\) channels α-subunits, Kir6.1 (Kcnj11) and SK2 (Kcnj11), were downregulated. Among the K\(^+\) channel regulatory proteins, the β-subunits Kvβ2 (Kcnab2), Kvβ3 (Kcnab3), and minK (Kcnel1) were downregulated, whereas SAP97 (Dlg1), a membrane-associated guanylate kinase (MAGUK) protein known to regulate voltage-dependent and inward rectifier K\(^+\) channels, was upregulated. Hyperpolarization-activated cation channels (HCN) are key elements of the SAN automaticity. Among the three HCN channels expressed in mouse SAN, HCN2 and HCN4 were significantly upregulated by 25 ± 7 and 52 ± 6%, respectively. Finally, chronic heart rate reduction also affected regulators of Ca\(^{2+}\) homeostasis including the type 2 ryanodine receptor (RyR2) and the type 2 inositol 1,4,5-trisphosphate receptor RIP3-2 (Itp2; Fig. 6).

Heart rate reduction-induced ionic remodeling was much less extensive at the ventricular level, and only six genes were significantly up- or downregulated in mice treated with ivabradine. For these genes, the variations were similar to those observed in SAN tissue. The expression of Cav3.1 was downregulated by 41 ± 9%, Sk2 by 19 ± 3%, and Kvβ3 by 39 ± 8%, whereas SAP97, Kir2.2, and Caco282 (Cacna2d2) were upregulated by 15 ± 1, 22 ± 2, and 27 ± 9%, respectively. For Kir2.2 and Caco282, variations in the SAN were comparable but did not reach significance (respectively, +14 ± 3 and +23 ± 8%; see Figs. 4 and 5).

**DISCUSSION**

In the present study, we demonstrate that moderate chronic heart rate reduction as produced specifically by a drug that does not

**Table 1. ECG parameters in anesthetized mice from vehicle and ivabradine groups before and after treatment**

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<tr>
<td>RR, ms</td>
<td>150±4</td>
<td>164±5</td>
<td>13±1</td>
<td>14±0</td>
<td>39±1</td>
<td>40±1</td>
<td>12±0</td>
<td>13±0</td>
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<tr>
<td>P, ms</td>
<td>171±11</td>
<td>221±3‡</td>
<td>14±1</td>
<td>12±0</td>
<td>42±2</td>
<td>41±1</td>
<td>14±0</td>
<td>13±0</td>
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<tr>
<td>PQ, ms</td>
<td>161±3</td>
<td>228±8‡</td>
<td>13±1</td>
<td>12±0</td>
<td>40±1</td>
<td>41±1</td>
<td>13±0</td>
<td>14±0</td>
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<tr>
<td>QRS, ms</td>
<td>155±5</td>
<td>202±6‡</td>
<td>13±1</td>
<td>13±0</td>
<td>40±1</td>
<td>38±1</td>
<td>14±0</td>
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<tr>
<td>QT, ms</td>
<td></td>
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<td></td>
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<td>67±2</td>
<td>71±1</td>
<td>55±2</td>
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<tr>
<td>QTc</td>
<td></td>
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<td></td>
<td>76±2</td>
<td>83±2*</td>
<td>58±3</td>
<td>56±1</td>
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Data are means ± SE. Electrocardiogram (ECG) parameters in anesthetized mice from vehicle (Veh; \( n = 6 \)) and ivabradine (Iva; \( n = 9 \)) groups before treatment (baseline) and after 7, 14, and 21 days of treatment. ECGs were recorded 10 min after blockade of the autonomic nervous system with ip injection of atropine sulfate (0.5 mg/kg) and propranolol (1 mg/kg). RR, RR interval; P, P wave duration; PQ, PQ interval; QRS, QRS complex duration; QT, QT interval; QTc, corrected QT interval. *\( P < 0.05 \), †\( P < 0.01 \), and ‡\( P < 0.001 \) vs. vehicle group.
not target the ventricle induces a complex ionic remodeling in the SAN but has only a slight impact on ion channel expression in the ventricle. To our knowledge, this is the first report of pharmacological ionic remodeling induced by a specific ion channel inhibitor.

Comparison With Previous Studies

In the present study, the effects of ivabradine on heart rate were in the same order of magnitude as in other experimental models using comparable doses (8, 29) and, more importantly, corresponded to the therapeutic heart rate reduction obtained in patients (3, 25). Ivabradine treatment had no effect on atrial, atrioventricular, and ventricular electrical activity as previously described (11, 28, 41, 43). There was also no sign of even mild ventricular hypertrophy.

Heart Rate Reduction-Induced Ionic Remodeling in the Ventricle

In a recent study, we have shown that chronic amiodarone treatment induces a complex ionic remodeling in the mouse ventricle (20) that differed markedly from that produced by hypothyroidism in the same model (19). Because amiodarone induced a moderate bradycardia, we hypothesized that part of the ventricular ionic remodeling could be attributable to slowing of the heart rate. Our new data rule out this hypothesis. Indeed, we have now shown that ivabradine modified the expression of only six transcripts and also that the change in the expression pattern differed from that observed in the amiodarone situation. As an example, the expressions of Cav3.1 and Kir2.2 were not affected by amiodarone. Moreover, SK2 expression, which was downregulated under ivabradine treatment, was inversely upregulated by amiodarone. It is therefore unlikely that bradycardia participates to the ventricular ionic remodeling induced by chronic amiodarone. In this latter situation, ionic remodeling is more likely a mixed consequence of the cardiac hypothyroid syndrome, the direct effect of amiodarone/desethylamiodarone on gene promoters and the primary physiological changes induced by the inhibition of the multiple sarcolemmal targets of this nonspecific drug (16, 20).

Although limited, both in the number of affected genes and in the amplitude of the variations, some degree of ionic remodeling was observed in the mouse ventricle, suggesting that a moderate decrease in heart rate can affect cardiac gene expression. This remodeling was actually too small to be detected with two-way hierarchical clustering, a method sensitive enough to discriminate different mouse strains based on their ventricular ion channel gene expression profiles (6). Strong bradycardia, as in complete atrioventricular block, was previously shown to induce ventricular electrical remodeling (45). However, complete atrioventricular block also induces ventricular hypertrophy associated with ionic and electrical remodeling (see Ref. 32 for review). In complete atrioventricular block, ionic remodeling was recently proposed to be the primary consequence of heart rate reduction and not a secondary effect of ventricular hypertrophy (39). In addition, strong bradycardia induces prolonged repolarization, altered $\text{Ca}^{2+}$

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**Fig. 3.** Two-way hierarchical clustering applied to 79 genes (vertical axis) and to 5 pools of sinoatrial nodes (SANs) and 6 ventricles from the vehicle group (SAN and V, respectively) and 6 pools of SANs and 6 ventricles from the ivabradine group (Iva SAN and Iva V, respectively). The input consisted of ΔCt for each preparation and gene. Each gene is represented by a single row of colored boxes and each pool of SANs or each ventricle by a single column. Each color patch represents gene expression level, with a continuum of expression levels from dark green (lowest) to bright red (highest). Gray boxes correspond to missing values. Ct, threshold cycle.
homeostasis, and arrhythmias. Conversely, moderate heart rate reduction induced by ivabradine does not alter ventricular function. Several studies suggest that, in pathological situations such as heart failure, heart rate reduction could even prevent or reverse cardiac remodeling (22, 29, 30).

Heart Rate Reduction-Induced Ionic Remodeling in the SAN

Heart rate reduction-induced ionic remodeling was more extensive in the SAN. Among the regulated genes are genes encoding channels directly involved in automaticity, such as HCN2 and HCN4. Interestingly, HCN2 and HCN4 channels are the targets for ivabradine. Upregulation of these pacemaker channels could be interpreted as a tentative compensatory mechanism, although this did not translate, in terms of heart rate, in a loss of efficacy of ivabradine, since a similar heart rate reduction was maintained over the 3-wk administration of a constant dose. HCN genes are specifically regulated in the SAN and not in the ventricle, where they have no assigned role despite their high expression (26). One could therefore speculate that upregulation of HCN genes involves a positive-feedback pathway. Positive- or negative-feedback mechanisms involving in part transcriptional regulation are well described for seven-transmembrane domain receptors such as β-adrenoceptors (33, 35, 46). To our knowledge, the only description of such a regulation process for ion channels concerns cardiac Na⁺ channels: in rats, blockade of Na⁺ channels with mexilitine results in the upregulation of Na⁺ channel transcription (9). The fact that HCN1 expression was not altered illustrates the specificity of gene transcription regulation (38).

Another important gene in the regulation of the pacemaker activity is Kir2.1. The impact of its upregulation by chronic heart rate reduction is more difficult to comprehend. Because the amplitude of the inward rectifier K⁺ current in mouse SAN cells is low (5), a 30% increase in Kir2.1 expression would possibly lead to a small hyperpolarization, which could either decrease sinus rate, by counterbalancing diastolic inward currents, or inversely, increase sinus rate because of a larger activation of pacemaker and T-type Ca²⁺ channels. To this respect, the observation that HCN2 and HCN4 channel transcripts are also upregulated suggests the existence of a transcriptional cross talk between HCN and Kir2 channel expression. This hypothesis is supported by the fact that Kir2.1 expression, like HCN2 and HCN4 expression, is not regulated by heart rate reduction in the ventricle, indicating tissue-specific regulation in the SAN. Such a mechanism can contribute to keep the proper setting of the voltage range in which the diastolic depolarization develops. The increased expression of SAP97 further complicates the situation, since this MAGUK protein was shown to interfere with Kir2 channels in a macromolecular complex (21). Finally, the gene encoding the type 2 ryanodine receptor is also specifically, though moderately, upregulated in the SAN. This could also compensate for the inhibition of If current, since ryanodine receptors were reported to play a role in the late phase of diastolic depolarization in SAN (14, 44).

The gene encoding the T-type Ca²⁺ channel Cav3.1 was downregulated by heart rate reduction. The contribution of T-type Ca²⁺ channels to cardiac automaticity has long been
controversial (7, 13), but recent results in the Cav3.1 knockout mouse model (15) demonstrate that this channel participates to SAN pacemaker activity (Mangoni ME, unpublished observation). A decreased expression of Cav3.1 is therefore expected to slightly decrease the sinus rate. Similarly, the upregulation of Kir3.1, one of the acetylcholine-activated K⁺ channel subunits, should potentiate heart rate reduction under vagal stimulation. Interestingly, these two genes were regulated in the SAN but also in the ventricles, where their normal expression is much lower than in the SAN (26). This suggests that their regulation might be a consequence of chronic heart rate reduction rather than a direct consequence of HCN channel inhibition, as observed for Kir2.1 and Ryr2 channels.

Fig. 5. Ivabradine effects on the expression of K⁺ and hyperpolarization-activated cation channels (HCN ch) in SAN. Bar graphs show the relative quantification vs. internal control gene (y-axis) of selected genes (x-axis) in mice treated with vehicle (open bars) or ivabradine (solid bars). Bottom: low-expressed genes. Data are means ± SE from 4–6 pools of SAN. *P < 0.05, #P < 0.01, and §P < 0.001 vs. vehicle group.

Fig. 6. Ivabradine effects on the expression of Ca²⁺ homeostasis regulators in SAN. Bar graphs show the relative quantification vs. internal control gene (y-axis) of selected genes (x-axis) in mice treated with vehicle (open bars) or ivabradine (solid bars). Bottom: low-expressed genes. Data are means ± SE from 4–6 pools of SAN. *P < 0.05 vs. vehicle group.
From our results, it appears that two types of ionic remodeling are induced by ivabradine. One, limited to the SAN, can be interpreted as a direct adaptive consequence of HCN channel inhibition. The second, observed in both SAN and ventricle, is more likely a secondary consequence of the new physiopathological state of the myocytes under heart rate reduction. Whatever the mechanism of ionic remodeling in SAN, it does not seem to have deleterious consequences. For instance, one might speculate that the upregulation of HCN channels would lead to increased heart rate on termination of ivabradine treatment. Experimental and clinical studies published so far show inversely an absence of such a rebound phenomenon (3, 29).

Message levels quantified in the present study are not necessarily correlated with protein expression, as with posttranscriptional and/or translational adaptation. However, in contrast to the recent progress in mRNA quantification that allows work with small tissues, even for genes expressed at low levels (as illustrated by the present study; see also Ref. 26), Western blotting needs a considerable amount of protein, which restrains its use with small specialized tissues such as sinus nodes.

In conclusion, the present study shows that reducing heart rate moderately with the If inhibitor ivabradine induces a complex ionic remodeling in the SAN. There was little impact on the ventricle in accordance with the absence of effects of the drug at the ventricular level. This result rules out our hypothesis that amiodarone-induced ventricular ionic remodeling could be partly due to heart rate reduction. Whether the remodeling observed in our study is a specific consequence of ivabradine inhibition of HCN channels or a more general consequence of long-term heart rate reduction should be further investigated in genetically engineered mice with conditional deletion of key pacemaker genes.

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