In conclusion, in this bradycardic model, remodeling reverted toward control values after 8 days of physiological rate pacing. By day 2, both in vivo and cellular manifestations of remodeling reverted toward control values after 8 days of physiological-rate pacing. At specified time points after complete heart block induction and pacing initiation, steady-state QT interval measurements and variability as well as dynamic QT interval adaptation to abrupt heart rate acceleration were assessed in the absence and presence of isoproterenol. Rapidly ($I_{Kr}$) and slowly ($I_{Ks}$) activating delayed rectifier repolarizing $K^+$ current densities were evaluated using whole cell patch clamp in isolated right ventricular myocytes. Steady-state QT interval prolongation at both 2 and 8 days was associated with moderate $I_{Kr}$ reduction. $I_{Ks}$ downregulation was apparent by day 2 but more profound at day 8. Dynamic QT interval adaptation was impaired under baseline conditions at day 8 but only during isoproterenol administration at day 2. Both in vivo and cellular manifestations of remodeling reverted toward control values after 8 days of physiological-rate pacing. In conclusion, in this bradycardic model, $I_{Kr}$ downregulation ($I_{Kr}$) proceeds more gradually but more extensively than that of $I_{Ks}$ and 2) is most prominently associated with impaired dynamic QT interval adaptation to heart rate acceleration. Isoproterenol blunts the dynamic QT interval response in animals with partially downregulated $I_{Ks}$, consistent with stress-related phenomena in known $I_{Ks}$-impaired states. Relative early sparing of $I_{Ks}$ could explain the delay in the onset of lethal tachyarrhythmia predisposition in bradycardic electrical remodeling. Reversibility of remodeling supports the potential utility of preventive pacing intervention soon after bradycardia onset.

Bradycardia; ion channels; repolarization; ventricular arrhythmias

Ventricular repolarization abnormalities and predisposition to torsades des pointes ventricular tachycardia are established concomitants of chronic bradycardia in humans (10). Our laboratory (23) and others (27, 28, 30) have shown that downregulation of ventricular myocyte rapidly ($I_{Kr}$) and slowly ($I_{Ks}$) activating delayed rectifier repolarizing $K^+$ currents underlies electrocardiographic QT interval (QT) prolongation and predisposition to torsades des pointes following at least 1 wk of bradycardia in animal models of ventricular electrical remodeling.

In the present study, we explore the very early time course of $I_{Kr}$ and $I_{Ks}$ downregulation and its phenotypic manifestations during bradycardic remodeling in a rabbit complete heart block (CHB) model (22). The reversibility of these changes with short-term ventricular pacing at near-physiological rate is also demonstrated.

METHODS

This investigation conformed with the Guide to the Care and Use of Experimental Animals, published by the Canadian Council on Animal Care (2nd edition, 1993), and was approved by the Hospital for Sick Children Animal Care Committee. CHB model. Transcatheter radiofrequency atrioventricular node ablation and permanent right ventricular (RV) endocardial demand ($VVI$) pacing in healthy young (3.0–3.5 kg) adult male New Zealand White rabbits have been described previously (22). Animals undergoing atrioventricular node ablation were paced at 140 beats/min, approximately half of their physiological resting sinus heart rate (22). Bradycardic pacing was maintained for 2 days in 11 rabbits and for 8 days in 13 rabbits. Steady-state QT, and whole cell patch-clamp data from 8 of these 13 rabbits that were reported previously (23) are not included here.

An additional group of six animals was subjected to bradycardic pacing for 8 days, followed by 8 days of pacing at the near-physiological rate of 280 beats/min to assess the reversibility of remodeling changes induced by bradycardia. Control rabbits underwent intracardiac catheter manipulation without conduction system disruption, and an endocardial pacing system was implanted but not activated.

Steady-state QT, measurement and dynamic QT, adaptation to abrupt rate change. QT, measurements were obtained on day 0 and either on day 2 or on day 8 post-CHB induction from six-lead surface electrocardiograms recorded with a sampling interval of 1.2 ms in anesthetized animals (ketamine, 33 mg/kg; and acepromazine, 1.1 mg/kg iv) after at least 5 min of continuous pacing at 140 beats/min (23). Animals in the remodeling reversal protocol underwent ECG recording on days 0, 8, and 16. Steady-state QT, was measured manually off-line in blinded fashion using averaged values from three consecutive beats in the lead with the most readily discernible T-wave termination that was usually lead II. A modification of the approach described by Thomsen and colleagues (25) was used to assess short-term (beat-to-beat) QT, variability, applying the formula QT, variability = $\Sigma (QT_n - QT_{n+1})/10 \times 2^{1/2}$, where QT, and QT, are QT, values of 2 consecutive beats among a series of 10 consecutive beats recorded during steady-state pacing at 140 beats/min.

To assess dynamic QT, adaptation to abrupt rate change, a surface ECG was recorded continuously for 10 s before and 2 min after a pacing rate shift from 140 to 210 beats/min. Beat-by-beat QT, values measured manually off-line were plotted against time after rate shift.
and fitted with a single exponential decay equation to generate an adaptation time constant (τ).

Steady-state QT, recording and the QT, adaptation protocol were repeated after 5 min of intravenous isoproterenol infusion at 0.03 μg·kg⁻¹·min⁻¹. This infusion rate was chosen as the maximum at which the 140-beat/min pacing rate could be reliably maintained without isoproterenol-induced competition from ectopic foci.

Ventricular myocyte isolation. RV myocytes were isolated as described elsewhere (23). Briefly, the excised heart was retrogradely perfused for 10 min with nominally Ca²⁺ free HEPES-buffered saline (HBS). Perfusion was then switched to Ca²⁺ free HBS containing collagenase 1 mg/ml (Worthington Class 2, Lakewood, NJ) and protease 0.1 mg/ml (Sigma type XIV, St. Louis, MO) for 15–20 min, followed by 4 min of washout with Ca²⁺ free HBS. All perfusates were gassed with 100% O₂ and maintained at 37°C. Ventricles were removed and teased apart with forceps. After gauze filtration, dispersed myocytes were resuspended in sequentially higher Ca²⁺ concentrations (0.05, 0.1, 0.2, 1.0, and 1.8 mM Ca²⁺ HBS) and stored at room temperature for study within 10 h of isolation.

Cellular electrophysiologic recording. Conventional whole cell patch clamp was performed as previously described (2, 5, 23). Data analysis. Results are reported as means (SD). Curve-fitting and statistical procedures were performed by using Origin 7.0 (Microcal Software, Northampton, MA), yielding time constants and midpoint potentials for pooled data where appropriate. Statistical analysis was based on the paired or unpaired Student’s t-test or on one-way ANOVA followed by Bonferroni’s test for multiple pairwise comparisons. Differences were considered significant when P < 0.05.

RESULTS

Steady-state QT, prolongation. We previously observed significant steady-state QT, prolongation in rabbits paced for 8 days post-CHB induction at 140 beats/min but not at the near-physiological rate of 280 beats/min (23). In the present study, we found that steady-state QT, prolongation at day 2 had already proceeded essentially to the extent noted at day 8, with QT, values increasing from 203 (SD 12) on day 0 to 247 ms (SD 30) on day 2 (P < 0.01; Fig. 1).

Impaired dynamic repolarization response to abrupt rate change. We assessed the dynamic repolarization response to abrupt heart rate acceleration by measuring beat-to-beat QT, shortening over 2 min following a shift in the ventricular pacing rate from 140 to 210 beats/min. Representative QT, adaptation curves obtained on post-CHB induction days 0, 2, and 8 are shown in Fig. 2, A and B. The curve is flattened after 8 days of bradycardic pacing at 140 beats/min. Whereas the steady-state QT, duration had already shifted by day 2 of bradycardic pacing, the time course of QT, adaptation to abrupt rate change was still preserved at that time point, with τ on day 0 [35 s (SD 18)] showing no significant change by day 2 [38 s (SD 15), P = not significant; Fig. 2C]. In contrast, by day 8 there was marked slowing in QT, adaptation τ from 36 (SD 16) to 72 s (SD 42) (P = 0.01; Fig. 2D).

IKr and IKs current reduction. Bradycardic electrical remodeling extending over 8 days in this rabbit CHB model is associated with significant biventricular downregulation of both IKr and IKs but not of the inwardly rectifying (IK1) transient outward (Ito) K⁺ currents (23). We therefore assessed the expression of IKr and IKs in whole cell patch-clamp studies of RV myocytes isolated after either 2 or 8 days of bradycardic pacing. Interestingly, IKr at 2 days was reduced to an extent comparable with that seen after 8 days of bradycardic remodeling, whereas IKs expression at day 2 was intermediate between that observed on days 0 and 8 of pacing at 140 beats/min (Fig. 3).

Isoproterenol effects in remodeled myocardium. IKs is known to be autonomically modulated through a protein kinase A-dependent signaling pathway and is typically enhanced in the presence of the β-adrenergic receptor agonist isoproterenol (31). We assessed the effects of isoproterenol on repolarization parameters immediately after CHB induction and again after 2 days of continuous bradycardic pacing. There was no clearly discernible isoproterenol effect either on mean steady-state QT, or on the time course of dynamic QT, adaptation to abrupt pacing rate increase immediately after CHB induction. Interestingly, however, after 2 days of bradycardic remodeling, β-adrenergic stimulation had a pronounced and reproducible effect on dynamic QT, adaptation but not on the mean steady-state QT, in animals on the 2 day protocol, the time constant of adaptation increased from 38 (SD 15) to 77 s (SD 29) (P < 0.01; n = 7 animals), whereas steady-state QT, in the presence of isoproterenol [249 ms (SD 29)] did not differ significantly from that recorded in the absence of the drug [247 ms (SD 30)]. After 8 days of bradycardic pacing, QT, adaptation τ increased from 92 (SD 48) to 101 s (SD 58) during isoproterenol administration (P = not significant; n = 5), whereas steady-state QT, was similar in the presence and absence of the drug. The paired QT, adaptation τ data are illustrated graphically in Fig. 4.

Fig. 1. QT interval (QT,) during steady-state pacing at 140 beats/min increased from 203 (SD 12) on day 0 to 247 ms (SD 30) on day 2 (A), with comparable values of 244 ms (SD 34) recorded on day 8 (B). Two points joined by a line represent paired measurements recorded in one animal (N, number of animals).
Although isoproterenol had no discernible effect on mean steady-state QT\(_i\) values, it did increase the beat-to-beat variability of steady-state QT\(_i\) after both 2 and 8 days of bradycardic remodeling but not immediately after CHB induction (Fig. 5). Reversibility of remodeling changes with 8 days of near-physiological rate pacing. As shown in Fig. 6, there was an essentially complete reversal of in vivo bradycardia-induced remodeling changes when animals underwent ventricular pacing at 280 beats/min for 8 days. Both the steady-state QT\(_i\) and the time course of dynamic QT\(_i\) adaptation to abrupt rate change showed typical prolongation after 8 days of bradycardia, followed by reversion to prebradycardia values after a further 8 days spent at near-physiological heart rate. Interestingly, RV myocyte I\(_{Kr}\) recovery was not fully complete at the end of the reversal protocol, whereas I\(_{Ks}\) tail current densities did not differ significantly from those recorded in control animals (Fig. 3).

**DISCUSSION**

**Staged downregulation of repolarizing K\(^+\) currents.** This investigation extends our understanding of the earliest physiological and cellular manifestations of mammalian bradycardic...
ventricular electrical remodeling. We and others have previously observed similar degrees of bradycardia-mediated down-regulation of $I_{Kr}$ and $I_{Ks}$ after at least 1 wk of heart block in rabbits (23, 27, 28) as well as dogs (30). By advancing the earliest time point of study of bradycardic remodeling to just 2 days after induction of heart block, we now begin to see time-dependent differences in the downregulation of these delayed rectifier currents. $I_{Kr}$ reduction is fully manifest within 48 h of bradycardia onset in this model. In contrast, the initial diminution of $I_{Ks}$ is relatively modest but shows additional progression to the 8-day mark (23), when it is downregulated more substantially than $I_{Kr}$ and essentially to the extent previously observed by Tsuji et al. (27) in rabbits after 3 wk of unpaced bradycardia. It thus appears that stepwise regulatory change occurs during the first week after bradycardia onset, with $I_{Kr}$ downregulation reaching its full expression before that of $I_{Ks}$ and with $I_{Ks}$ reduction ultimately reaching more profound levels, in relative terms, than those attained by $I_{Kr}$.

Stengl and associates (21) recently used a novel serial myocardial needle biopsy approach to longitudinally characterize the early time course of $I_{Ks}$ and β-adrenergic receptor downregulation in their bradycardic canine CHB model. Of greatest relevance to the results we report here, their observations showed decreased levels of KCNQ1 mRNA and protein expression after 3 days of CHB, the earliest time point that they sampled. Thus it appears that transcriptional downregulation of $I_{Ks}$ likely begins almost immediately after bradycardia onset.

In marked contrast to early steady-state QT$_i$ prolongation, the physiological response of QT$_i$ shortening to abrupt heart rate acceleration is still preserved at day 2 of bradycardic pacing, whereas it is decidedly blunted by day 8. Interestingly, the reduction of $I_{Ks}$ is not yet fully developed by day 2, consistent with the proposed role of $I_{Ks}$ in the adaptive repolarization response to heart rate shifts (3, 15, 17, 20). Although $I_{Kr}$ likely plays a role in dynamic action potential heart rate adaptation (7), recent modeling studies by Choe and associates (4) seem to confirm the predominance of $I_{Ks}$ in the QT$_i$ adaptive response. This might be particularly relevant in species such as the rabbit where $I_{Ks}$ does not participate prominently in cardiac myocyte action potential repolarization under stable physiological conditions (11) and could assume additional importance in settings of $I_{Kr}$ impairment, as demon-

![Fig. 4](http://ajpheart.physiology.org/)

**Fig. 4.** Intravenous isoproterenol (Iso) administration (0.03 μg·kg$^{-1}$·min$^{-1}$) “unmasked” latent impairment of dynamic QT$_i$ adaptation to an abrupt pacing rate shift from 140 to 210 beats/min that was not apparent under baseline conditions at day 2 but did subsequently manifest by day 8 (see text and Fig 2). Iso had no consistent effect on steady-state QT$_i$ values obtained either immediately after CHB induction or following 2 days of bradycardic remodeling when the baseline QT$_i$ was already prolonged (not shown). Control indicates values obtained in the absence of Iso. Two points joined by a line represent paired measurements recorded in one animal.

![Fig. 5](http://ajpheart.physiology.org/)

**Fig. 5.** QT$_i$ beat-to-beat variability increases in response to Iso as bradycardic electrical remodeling proceeds. QT$_i$ variability was calculated from the formula

$$\text{QT}_i\text{variability} = \sum (\text{QT}_n - \text{QT}_{n+1})/10 \times 2^{1/2},$$

where QT$_n$ and QT$_{n+1}$ are QT$_i$ values of 2 consecutive beats among a series of 10 consecutive beats recorded during steady-state pacing at 140 beats/min. Under baseline conditions, a slight but statistically insignificant increase in short-term QT$_i$ variability was apparent after 8 but not after 2 days of bradycardic remodeling. However, Iso administration increased QT$_i$ variability at both time points of bradycardic remodeling ($P = 0.04$ at day 2 and 0.007 at day 8, Iso vs. control).
strated by Volders et al. (31) and as elegantly modeled by Silva and Rudy (20).

In this context, it is noteworthy that spontaneous torsades des pointes in bradycardic rabbits has been observed predominantly at least 1 wk after CHB induction (27). Moreover, these findings are consistent with clinical observations in humans affected by impaired Ik1 function in congenital long QT syndrome type 1, in whom baseline QT, is often indistinguishable from that of healthy individuals (24), but torsades des pointes is typically induced in settings of heart rate acceleration such as exercise (19).

Importance of β-adrenergic stimulation with reduced repolarization reserve. The responsiveness of cardiac myocyte Ik1 to β-adrenergic stimulation has been explored in a number of studies (14, 18, 31) and is presumably central to its function as a repolarizing current reservoir in settings of action potential prolongation. Sanguinetti and colleagues (18) showed that isoproterenol-mediated Ik1 augmentation could counter the action potential prolonging effect of pharmacological Ik1 blockade in guinea pig ventricular myocytes.

When Ik1 availability is reduced, the effect of β-adrenergic stimulation is to delay rather than accelerate cardiac myocyte repolarization. The combination of pharmacological Ik1 blockade and β-adrenergic stimulation prolongs the action potential in canine cardiac Purkinje cells (6) and enhances arrhythmogenicity in arterially perfused canine left ventricular wedge preparations (1). Repolarization abnormalities reminiscent of long QT syndrome were exacerbated by isoproterenol administration in a mouse knockout model lacking the Kcnn1 gene that encodes the pore-forming protein underlying Ik1 (9, 26).

Conversely, the combined pharmacological blockade of Ik1 and β-adrenergic receptors reduced ventricular tachyarrhythmia inducibility in a canine myocardial infarction model (13). Among patients with genotyped congenital long QT syndrome, the efficacy of β-blocker therapy is reportedly greatest in those with KCNQ1 mutations (long QT syndrome type 1) (8, 29).

In our bradycardic rabbit model, the effect of isoproterenol administration 2 days after induction of heart block is to “unmask” the abnormal QT, adaptive response seen at day 8 in the absence of isoproterenol. This speaks directly to the issue of repolarization reserve, suggesting that even moderate reduction of IKS availability against a backdrop of downregulated Ik1, could result in a severely impaired stress-induced repolarization response. When IKS is more substantially diminished, as at 8 days post-heart block induction in our model, the abnormal QT, adaptation to heart rate acceleration becomes fully manifest even in the absence of β-adrenergic stimulation.

Moreover, administration of isoproterenol after both 2 and 8 days of bradycardic electrical remodeling had the effect of destabilizing ventricular repolarization even under steady-state pacing conditions, as evidenced by increased short-term QT, variability. Thomsen and colleagues (25) correlated increased beat-to-beat QT, variability with the incidence of torsades des pointes among CHB dogs exposed to d-sotalol. Stengl et al. (21) found that canine bradycardic remodeling evolving from 3 to 30 days after CHB induction is associated with progressively greater isoproterenol effects on QT, instability.

Reversibility of early bradycardic electrical remodeling with physiological-rate pacing. Scent information is available with respect to the reversibility of bradycardic ventricular electrical remodeling. Peschar et al. (16) observed that QT, prolongation induced in dogs with CHB with 8 wk of profound, essentially unpaced, bradycardia persisted even after a subsequent 8-wk period of physiological-rate ventricular pacing, notwithstanding that bradycardia-induced structural changes showed evidence of regression. Among human CHB patients who had previously experienced torsades des pointes, Kuriya and colleagues (10) noted that evidence of delayed repolarization persisted even after at least 2 mo of appropriate permanent pacing. However, these clinical data are difficult to interpret because the duration of bradycardia before pacing was not specified and because other conditions associated with repolarization abnormalities such as congenital long QT syndrome were not systematically excluded.

In contrast to the foregoing evidence of remodeling irreversibility following prolonged, severe bradycardia, our findings suggest that significant reversal of repolarization abnormalities can be achieved when appropriate pacing intervention is instituted relatively soon after onset of bradycardic remodeling. While they raise interesting questions about mechanisms underlying remodeling and its reversibility, these observations

![Fig. 6. Reversibility of bradycardic electrical remodeling](http://ajpheart.physiology.org/Download.png)
could potentially impact upon clinical decision making with respect to the optimal timing of pacing intervention for brady-cardiac patients. However, the presence and nature of a “threshold” bradycardiac interval beyond which electrical remodeling becomes entrenched remain to be demonstrated in humans and further defined in our animal model.

Study limitations. Whereas the present study documents the early time course, in vivo manifestations, and reversibility of $I_{Kr}$ and $I_{Ks}$ downregulation in bradycardic rabbits, it does not specifically address mechanisms underlying the reduced expression of these currents. Reports published by others have essentially excluded shifts in voltage dependence of activation (27, 28) or deactivation kinetics (28) as major factors in either $I_{Kr}$ or $I_{Ks}$ downregulation in the rabbit with profound and prolonged bradycardia. Confirmatory evidence with respect to $I_{Kr}$ has come from the canine model (21). Transcriptional downregulation of pore-forming K+ channel subunits appears to play a significant role in each of these cases (21, 28). Intriguingly, Stengel and colleagues (21) did demonstrate reduced ventricular myocyte $\beta_1$-adrenergic receptor expression and reduced $I_{Ks}$ responsiveness to isoproterenol in addition to $I_{Kr}$ downregulation in their canine model of bradycardic electrical remodeling.

Although we previously observed no significant difference in bradycardic electrical remodeling between myocytes isolated from the right and left ventricles (23), our exclusive reliance on RV myocytes in the present study precludes a detailed regional or transmural evaluation of repolarizing current changes.

We have not assessed the inducibility of tachyarrhythmias through provocative testing in this model of early bradycardic electrical remodeling. Reversibility of less lethal manifestations of remodeling as demonstrated here might not encompass the predisposition to torsades des pointes observed in humans or animals with more developed or malignant variants of bradycardic remodeling.

In conclusion, in this rabbit CHB model of very early bradycardic ventricular electrical remodeling, $I_{Kr}$ downregulation proceeds earlier and to a smaller extent than that of $I_{Ks}$. The time course of steady-state QTc prolongation appears to correspond to that of $I_{Kr}$ reduction, at least with respect to the limited number of time points assessed in this investigation. This contrasts with the more gradual and profound downregulation of $I_{Ks}$ that is expressed phenotypically in impaired QTc adaptation to abrupt heart rate acceleration. Whereas this important physiological response is superficially preserved very early in the remodeling process when $I_{Kr}$ reduction is still relatively slight, isoproterenol administration can unmask significant adaptive impairment comparable with its effects in other settings of genetically and pharmacologically reduced $I_{Kr}$ availability. The reversibility of bradycardiac electrical remodeling with timely pacing intervention at physiological heart rates is clearly demonstrated for the first time.

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