Depressed pacemaker activity of sinoatrial node myocytes contributes to the age-dependent decline in maximum heart rate

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An inexorable decline in maximum heart rate (mHR) progressively limits human aerobic capacity with advancing age. This decrease in mHR results from an age-dependent reduction in "intrinsic heart rate" (iHR), which is measured during autonomic blockade. The reduced iHR indicates, by definition, that pacemaker function of the sinoatrial node is compromised during aging. However, little is known about the properties of pacemaker myocytes in the aged sinoatrial node. Here, we show that depressed excitability of individual sinoatrial node myocytes (SAMs) contributes to reductions in heart rate with advancing age. We found that age-dependent declines in mHR and iHR in ECG recordings from mice were paralleled by declines in spontaneous action potential (AP) firing rates (FRs) in patch-clamp recordings from acutely isolated SAMs. The slower FR of aged SAMs resulted from changes in the AP waveform that were limited to hyperpolarization of the maximum diastolic potential and slowing of the early part of the diastolic depolarization. These AP waveform changes were associated with cellular hypertrophy, reduced current densities for L- and T-type Ca^{2+} currents and the "funny current" (I_f), and a hyperpolarizing shift in the voltage dependence of I_f. The age-dependent reduction in sinoatrial node function was not associated with changes in β-adrenergic responsiveness, which was preserved during aging for heart rate, SAM FR, L- and T-type Ca^{2+} currents, and I_f. Our results indicate that depressed excitability of individual SAMs due to altered ion channel activity contributes to the decline in mHR, and thus aerobic capacity, during normal aging.

One of the most insidious aspects of growing older is an inevitable decline in maximum heart rate (mHR), which limits maximum aerobic capacity with advancing age (1–3). The decline in mHR proceeds at approximately the same rate for all individuals, without regard for lifestyle or physical fitness (4–8). For many otherwise healthy elderly people, it is the factor that ultimately restricts the ability to live independently (9, 10).

The decrease in mHR with age results primarily from a parallel age-dependent decline in "intrinsic heart rate" (iHR) (11–13), which is measured during autonomic blockade, and thus reflects the spontaneous pacemaker activity of the sinoatrial node of the heart. Although it is known that the intact sinoatrial node from aged animals contracts more slowly (14, 15) and contains fewer pacemaker myocytes (16), little is known about the functional properties of individual myocytes from the sinoatrial node of the aged heart.

Sinoatrial myocytes (SAMs) are highly specialized cells that serve a primarily electrical function as cardiac pacemakers via their production of spontaneous action potentials (APs). Sinoatrial APs are characterized by a spontaneous depolarization during diastole that drives the membrane potential to threshold, thereby triggering the subsequent AP. This "diastolic depolarization" (DD) phase of the sinoatrial AP results from the coordinated activity of numerous membrane conductances, including L- and T-type Ca^{2+} currents ($I_{Ca,L}$ and $I_{Ca,T}$, respectively) and the "funny current" (I_f), all of which contribute directly to the DD by conducting inward current at diastolic potentials (17–23). $I_{Ca,L}$ also contributes indirectly to the DD by stimulating Ca^{2+} efflux from the sarcoplasmic reticulum of SAMs (24), thereby activating the Na^+-Ca^{2+} exchange current (I_{NCX}), which is also known to be critical for normal pacemaker activity (25–29).

In this study, we determined the effects of aging on heart rates (HRs) and on spontaneous APs and membrane currents in acutely isolated SAMs. We observed age-dependent decreases in AP firing rates (FRs) in SAMs that corresponded to the age-dependent reductions in iHRs and mHRs. The slower AP FRs resulted from changes in the AP waveform that were associated with an increase in cell size and with alterations in $I_{Ca,L}$, $I_{Ca,T}$, and I_f. These findings indicate that changes in expression and/or regulation of ion channels in SAMs comprise part of the molecular program that limits mHR, and thus aerobic capacity, during normal aging.

Results

Similar Reductions in HR and SAM FR in Aged Mice. iHR and mHR were determined from ECGs recorded from awake, restrained mice of three age groups: 2–3, 21–24, and 32+ mo (corresponding to ~17–20, 65–69, and 87+ y in humans) (30, 31) (Fig. 1*A*). iHR was measured during autonomic blockade with atropine and propranolol, whereas mHR was induced by restraint stress [verified by administration of isoproterenol (ISO); Fig. S1]. Both iHR and mHR were significantly reduced in older mice compared with animals aged 2–3 mo ($P < 0.01$; Fig. 1*B* and Table 1). However, the mHR/iHR ratios were similar in each age group ($P > 0.05$; Table 1), demonstrating that the chronotropic

Significance

Maximum heart rate (mHR) declines with age, contributing to the reduced aerobic capacity of the elderly. A parallel reduction in "intrinsic heart rate" (which is measured during autonomic blockade) underlies the decline in mHR, and indicates that pacemaker function of the sinoatrial node is compromised during aging. In this study we demonstrate that the slower heart rates in the elderly result from depressed spontaneous activity of individual sinoatrial node myocytes (SAMs). Patch-clamp electrophysiology revealed that aged SAMs have slowed AP firing rates, altered AP waveforms, and changed properties of Ca^{2+} currents and the cardiac "funny current," I_f. Our findings demonstrate that age-dependent changes ion channel activity in SAMs are a major cause of the decline in mHR during aging.

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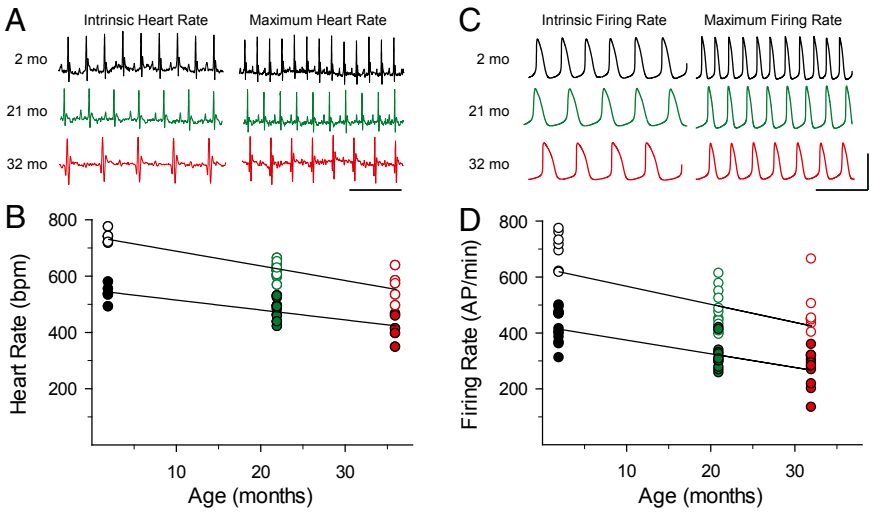

Fig. 1. Parallel age-dependent declines in HR and SAM AP FR. (*A*) Representative ECG recordings of iHRs and mHRs from mice aged 2–3 mo (black), 21–24 mo (green), and 32+ mo (red). (Scale bar: 250 ms.) (*B*) iHRs (filled circles) and mHRs (open circles) from mice of the three age groups. (*C*) Representative APs recorded from acutely dissociated SAMs from mice of different ages. (Scale bars: 250 ms, 70 mV). (*D*) iHR (filled circles) and mHR (open circles) AP FRs from SAMs isolated from mice of different ages. The lines in *B* and *D* are linear regressions to the data.

response to β-adrenergic receptor (βAR) stimulation is largely preserved during aging in mice, as it is in humans (11). In agreement with earlier studies (32–35), ECG intervals were also significantly prolonged or altered in the older mice (Table S1).

Because the spontaneous activity of pacemaker myocytes in the sinoatrial node determines HR, we next examined the effects of aging on the intrinsic excitability of individual SAMs. Spontaneous APs were recorded from acutely dissociated SAMs from mice of the three different age groups using the amphotericin perforated-patch technique (Fig. 1*C*). Stable intrinsic AP firing rate (iFR) was determined in the presence of 1 nM ISO (36), and maximum firing rate (mFR) was recorded in the same cells >2 min after wash-on of a saturating concentration of ISO (1 μM; Fig. S2). Both iFR and mFR were significantly reduced in cells isolated from the older animals ($P < 0.01$; Fig. 1*D* and Table 1), with age-dependent declines that were remarkably similar to the declines in iHR and mHR. As in the case of HR, the mFR/iFR ratio did not differ in cells from the three age groups ($P > 0.05$; Table 1), indicating that βAR responsiveness of individual SAMs did not change appreciably during aging.

Limited Changes in the AP Waveform in Aged SAMs. Age-dependent changes in AP waveform parameters were determined from perforated-patch current-clamp recordings using an analysis method adapted from Bucchi et al. (22). In these experiments, we found that the longer cycle length in aged SAMs was associated with substantial changes in only a subset of the AP parameters (Fig. 2 and Table S2). Specifically, aging hyperpolarized the maximum diastolic potential (MDP)and slowed the early part of the DD ($P < 0.05$; Table S2).In contrast, aging had little or no effect on the late phase of the DD, the AP upstroke velocity, the repolarization rate, or the AP duration ($P > 0.05$; Table S2). Intermediate effects, apparent only in cells from the oldest animals, were observed for the take-off potential and the AP amplitude (Table S2).

Reduced Ca^{2+} Current Densities and Increased Membrane Capacitance in Aged SAMs. Because $I_{Ca,L}$ and $I_{Ca,T}$ are important for the generation of spontaneous APs in SAMs (37–39), we next examined their properties in whole-cell voltage-clamp experiments in SAMs isolated from mice aged 2–3 mo, 21–24 mo, and 28 mo (older mice were not available at the time of these experiments). Total I_{Ca} was elicited by 200-ms depolarizing voltage steps from a holding potential of −90 mV. $I_{Ca,L}$ was then elicited in the same cells from a holding potential of −60 mV [where $I_{Ca,T}$ in SAMs is mostly inactivated (37, 39)]. $I_{Ca,T}$ was subsequently calculated for each cell as the difference current, $I_{Ca} - I_{Ca,L}$. We found that aging reduced the peak current densities for both $I_{Ca,L}$ and $I_{Ca,T}$ without alteration in the midpoint activation voltage ($V_{1/2}$) or the rates of inactivation (Fig. 3 and Table S3). These decreases in current density persisted in the presence of ISO and were associated with an age-dependent increase in cell size, as indicated by an increase in cellular capacitance [$P < 0.001$; Table S3; capacitance = 36.1 ± 1.0, 45.9 ± 1.2, and 48.2 ± 1.0 pF

Table 1. Age-dependent changes in HRs and SAM AP FRs

	Intrinsic			Maximum			βAR response (maximum/intrinsic)		
	2–3 mo	21–24 mo	32+ mo	2–3 mo	21–24 mo	32+ mo	2–3 mo	21–24 mo	32+ mo
HR, bpm	537.3 ± 14.3 (5)	478.7 ± 10.7* (12)	414.9 ± 21.6* (5)	737.3 ± 10.3 (5)	619.1 ± 7.4* (12)	562.9 ± 24.0* (5)	1.37 ± 0.04	1.30 ± 0.04	1.36 ± 0.05
FR rate, AP/min	414.3 ± 19.3 (10)	318.5 ± 14.7* (12)	268.6 ± 21.2* (10)	626.4 ± 41.7 (10)	478.4 ± 20.9* (12)	438.4 ± 33.1* (10)	1.51 ± 0.06	1.50 ± 0.03	1.46 ± 0.04

Data are averages (±SEM) of 30-s recording windows for HRs from ECG recordings or SAM FRs from perforated-patch recordings. Numbers of mice or cells are shown in parentheses. The βAR response is reported as the average ratio of maximum/intrinsic for both HR and FR.
*$P < 0.05$ vs. animals/cells aged 2–3 mo (one-way ANOVA with a Holm–Sidak posttest).

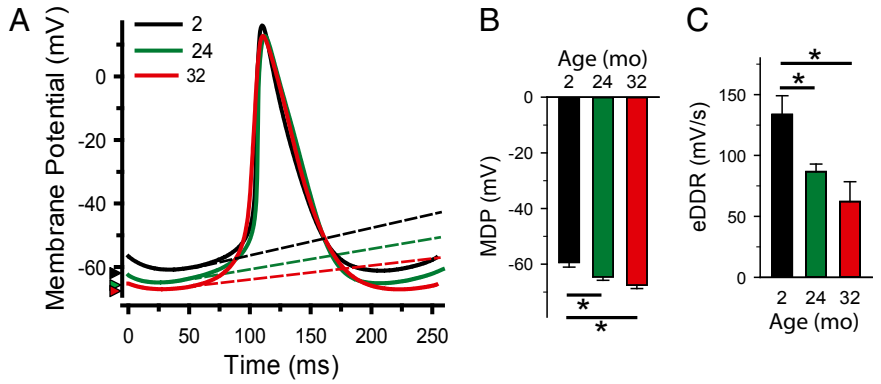

Fig. 2. Slower AP FRs in aged SAMs result from limited changes in the sinoatrial AP waveform. (*A*) Representative APs recorded from SAMs in mice aged 2–3 mo (black), 21–24 mo (green), and 32+ mo (red) superimposed at time of peak. Dashed lines indicate the slopes of the DD, and arrowheads mark the MDP. (*B*) Average (±SEM) MDP and early DD rate (eDDR) in SAMs from mice of different ages. *$P < 0.05$, one-way ANOVA with a Holm–Sidak posttest.

in cells from 2–3 mo, 21–24 mo, and 28+ mo, respectively]. The cellular hypertrophy appeared to account for at least some of the decreased density for $I_{Ca,L}$, $I_{Ca,T}$, and I_f (see below) because age-dependent differences were reduced when conductance was not normalized to cell size (Table S3). As in the case of the HR and FR, βAR responsiveness for both $I_{Ca,L}$ and $I_{Ca,T}$ was preserved during aging, as indicated by similar percentage increases in current density in response to ISO in SAMs of different ages ($P > 0.05$; Table S3).

Hyperpolarized Voltage Dependence of I_f in Aged SAMs. I_f is thought to be a major determinant of both the MDP and the early DD in SAMs (20–23). To evaluate age-dependent changes in the properties of I_f in SAMs, we applied 3-s hyperpolarizing voltage steps from a holding potential of −50 mV in whole-cell voltage-clamp recordings. As for $I_{Ca,L}$ and $I_{Ca,T}$, we found that the peak I_f density was substantially reduced in aged SAMs ($P < 0.05$; Table S3). Interestingly, the voltage dependence of activation of I_f was also altered by age. Specifically, there was a pronounced hyperpolarizing shift in the $V_{1/2}$ for I_f in aged SAMs ($P < 0.01$; Fig. 4 and Table S3). As for HR, FR, $I_{Ca,L}$, and $I_{Ca,T}$, βAR responsiveness of I_f was unchanged by age, as evidenced by the similar changes in the $V_{1/2}$ in the absence or presence of a saturating concentration of ISO (1 μM; Fig. S3 and Table S3).

Discussion

The major findings of this study are that (*i*) the age-dependent decline in mHR depends, at least in part, on a corresponding decrease in the spontaneous excitability of SAMs; (*ii*) the reduced SAM excitability results from changes in a limited set of AP parameters; and (*iii*) altered membrane currents contribute to the changes in the AP waveform and, as a consequence, to decreased SAM FR and HR.

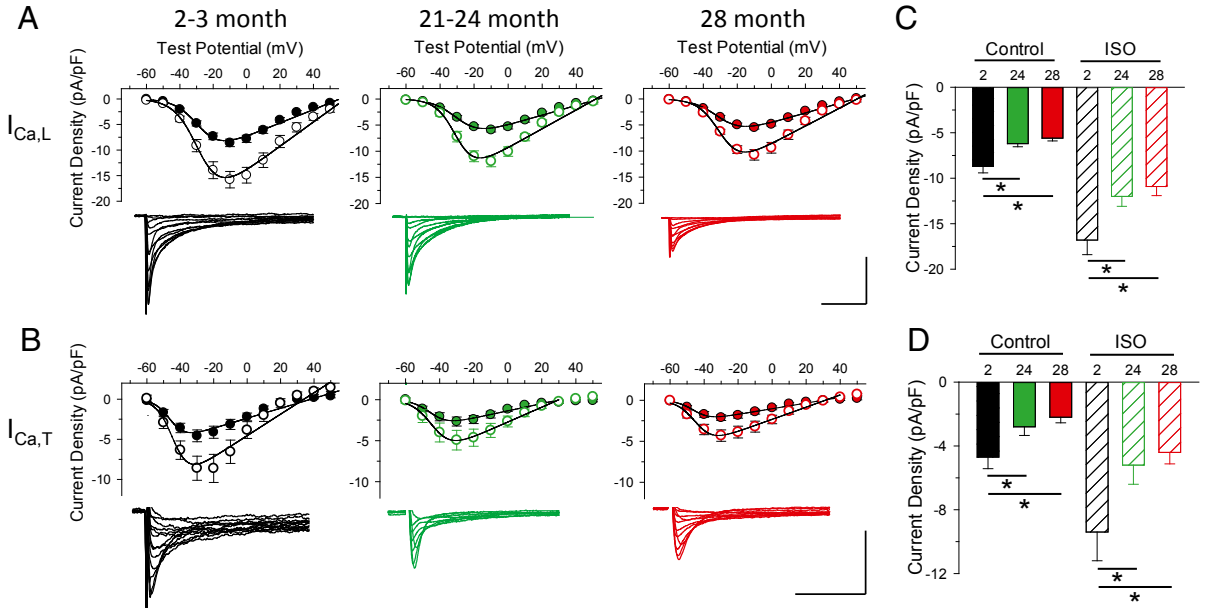

Fig. 3. Decreased $I_{Ca,L}$ and $I_{Ca,T}$ conductance densities in aged SAMs. (*A* and *B*) Average (±SEM) current-voltage relationships and representative currents for $I_{Ca,L}$ and $I_{Ca,T}$ in SAMs in mice aged 2–3 mo (black), 21–24 mo (green), and 28 mo (red) in the absence (filled circles) and presence (open circles) of 1 μM ISO. (Scale bars: 5 pA/pF, 25 ms.) (*Insets*) Representative current families. (*C* and *D*) Average (±SEM) peak $I_{Ca,L}$ and $I_{Ca,T}$ densities in SAMs in mice aged 2–3 mo (black), 21–24 mo (green), and 28 mo (red) in the absence (filled bars) and presence (hatched bars) of ISO. *$P < 0.05$, one-way ANOVA with a Holm–Sidak posttest.

Preserved βAR Response in the Aged Sinoatrial Node. Our observation that the chronotropic response to βAR stimulation was preserved in aged mice provides additional support for the long-standing idea that decreased iHR is the primary cause of the age-dependent decrease in mHR (11–13). We also found that aging did not alter βAR responsiveness of AP FR, Ca^{2+} current density, or the voltage dependence of I_f in SAMs. These preserved responses to βAR stimulation in aged SAMs differ from the reduced inotropic response to βAR stimulation in aged ventricular myocytes (16, 40), underscoring the high degree of specialization of myocytes from different regions of the heart. In SAMs, βAR-stimulated intracellular signaling pathways appear to be unaltered during aging, which, instead, targeted mechanisms responsible for basal pacemaker activity.

Depressed Excitability in Aged SAMs. Previous studies in intact sinoatrial node preparations have shown that aging reduces conduction velocity (32–35) and decreases the number of sinoatrial node myocytes (16). These changes are thought to contribute to the age-dependent declines in iHR and mHR by limiting the ability of the relatively small sinoatrial node to overcome the hyperpolarizing load of the much larger surrounding atrial tissue (41, 42). In addition to these mechanisms, a decrease in excitability of individual SAMs has been proposed as a potential factor that could contribute to age-dependent reductions in iHR and mHR (14, 15, 43). Our present data establish that aging does indeed depress the spontaneous activity of SAMs as a result of altered membrane properties. It will be important in future work to determine whether these changes in SAM properties arise from a preferential loss of a subpopulation of smaller cells that fire more rapidly and/or hypertrophy of individual myocytes.

Altered AP Waveform and Membrane Currents in Aged SAMs. It is noteworthy that aging slowed the FR of SAMs by changing only the MDP and the early DD, with little or no effect on other AP waveform parameters. This result indicates that the slower AP FR of older cells results from changes in the balance of ionic currents that are active during the early DD. As an initial description of candidate currents, we examined the properties of $I_{Ca,L}$, $I_{Ca,T}$, and I_f, all of which contribute to the DD.

$I_{Ca,L}$ in mouse SAMs is produced mainly by the $Ca_v1.3$ channel isoform (37), which activates at more negative potentials than $Ca_v1.2$ (44), the predominant L-type Ca^{2+} channel isoform in atrial and ventricular myocytes. The relatively hyperpolarized voltage dependence of $Ca_v1.3$ overlaps the voltage range of the DD (e.g., compare Figs. 2 and 3), and SAMs from $Ca_v1.3$ KO mice have prolonged cycle lengths (37) and slower DD (38). Thus, the reduced $I_{Ca,L}$ density we observed in aged SAMs would be expected to contribute to the age-dependent slowing of the DD, albeit with more substantial effects on the later part of the DD. In contrast, the voltage dependence of $I_{Ca,T}$ is considerably more negative than that of $I_{Ca,L}$ (e.g., Fig. 3), corresponding more closely to the early part of the DD. KO of the $Ca_v3.1$ T-type Ca^{2+} channel isoform nearly eliminates $I_{Ca,T}$ in mouse SAMs and reduces iHR and AP FR via slowing of the DD (39). Consequently, the reduced $I_{Ca,T}$ density we observed in aged SAMs would be expected to contribute to depressed pacemaker activity, although the relatively low availability of $I_{Ca,T}$ at diastolic potentials due to inactivation may limit its contribution.

Strong evidence supports a role for I_f in determining the FR of SAMs via effects on the DD and MDP (20–23). The exact mechanism(s) by which I_f contributes to spontaneous activity in SAMs remains enigmatic, given the apparent discrepancy between the voltage dependence of activation as determined by traditional voltage-clamp protocols (e.g., Fig. 4) and the voltage range of the DD. Possible explanations for this mismatch between the importance of the channels and their hyperpolarized voltage dependence include the production of voltage-independent leak current by hyperpolarization-activated, cyclic nucleotide-sensitive (HCN) channels (45, 46), hysteresis in the voltage dependence of activation (47, 48), and slow activation and inactivation that could contribute to persistent opening in response to the relatively rapid voltage changes in mouse SAMs. A precedent for activity of HCN channels at positive membrane potentials is provided by the recent report of a role for HCN3 in repolarization of ventricular myocytes (49).

Numerous molecular mechanisms likely conspire to produce all the age-dependent changes we saw in $I_{Ca,L}$, $I_{Ca,T}$, and I_f. However, the similar reductions in $I_{Ca,L}$, $I_{Ca,T}$, and I_f current densities and increase in membrane capacitance raise the intriguing possibility that these changes may be related. For instance, down-regulation of Ca^{2+} channels could contribute to the hypertrophy of older SAMs, because decreased Ca^{2+} flux via L-type channels is a known regulator of cardiac gene expression (50). Alternatively, there may be a common mechanism that controls channel expression in aged SAMs such that the number of Ca^{2+} and HCN channels per unit membrane does not increase sufficiently to compensate for the increased cell size. In this regard, the unchanged voltage dependence of activation and rate of inactivation of $I_{Ca,L}$ in aged SAMs strongly suggest that $Ca_v1.2$ and $Ca_v1.3$ are regulated in parallel during aging (because they activate at different potentials and have markedly different kinetics of inactivation). However, it is difficult to compare the reduced current densities in our study with previous studies of transcript expression in the aging rat sinoatrial node in which $Ca_v1.3$ and $Ca_v3.1$ transcripts were unchanged (51), $Ca_v1.2$ transcripts were increased (51), and HCN4 transcripts were either unchanged (51) or decreased (52) with age. These apparent discrepancies could result from species-dependent differences or from differential transcript expression in SAMs (as assayed in

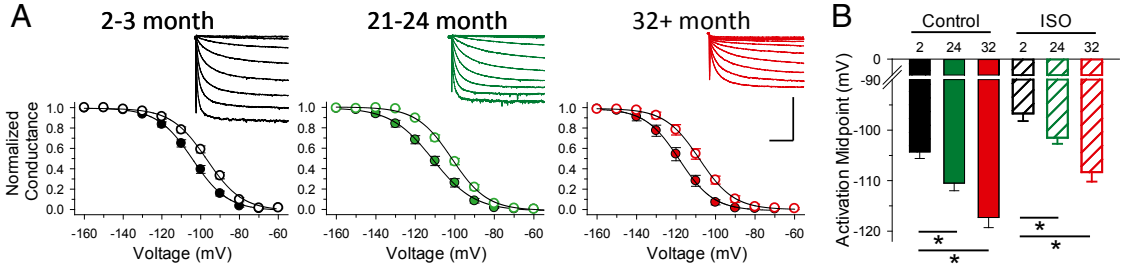

Fig. 4. Hyperpolarized activation midpoint for I_f in aged SAMs. (*A*) Normalized average (±SEM) conductance-voltage relationships for I_f in SAMs in mice aged 2–3 mo (black), 21–24 mo (green), and 32+ mo (red) in the absence (filled circles) and presence (open circles) of 1 μM ISO. (*Insets*) Representative I_f current families normalized to cellular capacitance. (Scale bars: 15 pA/pF, 750 ms.) (*B*) Average (±SEM) $V_{1/2}$ values for I_f in isolated SAMs from mice of different ages in the absence (filled bars) or presence (hatched bars) of ISO. *$P < 0.05$, one-way ANOVA with a Holm–Sidak posttest.

our patch-clamp experiments) vs. other cell types present in the entire sinoatrial node (as included in expression studies). Altered channel regulation could also contribute to the age-dependent reductions in current densities and shift in I_f voltage dependence in our studies. However, any such mechanism(s) could not depend on soluble cytoplasmic factors, because they persisted in whole-cell voltage-clamp studies in which soluble factors such as cAMP would be highly diluted. Finally, although age-dependent changes in HCN isoform expression are also possible, such changes are unlikely to account for the negative shift in the $V_{1/2}$ of I_f in aged SAMs, given that the activation of HCN2 is similar to that of HCN4 and that HCN1 and HCN3 activate at substantially more positive potentials (53).

Predicted Changes in Other Membrane Currents in Aged SAMs. Our observation that the AP upstroke, duration, and repolarization were largely preserved in aged SAMs indicates the there must be age-related changes in outward currents that are active during these phases of the sinoatrial AP. Without a decrease in net outward current, the age-dependent reduction in $I_{Ca,L}$ density would tend to decrease the action potential duration (APD), as it does in SAMs from $Ca_v1.3$ KO mice (38). Candidate outward currents that could offset the reduced $I_{Ca,L}$ density include the rapid delayed rectifier K^+ current (I_{Kr}) and the inward rectifier Cl^- current mediated by ClC-2, both of which influence MDP, APD, and repolarization in SAMs (36, 54). The decrease in I_f density in aged SAMs could also contribute to a reduction in repolarizing K^+ current, as it does in ventricular myocytes (49).

We also consider it likely that aging alters Ca^{2+} release from the sarcoplasmic reticulum (SR) in SAMs, a process that is known to be critical for pacemaker activity (27–29, 55). At very least, the age-dependent decrease in $I_{Ca,L}$ density would be expected to reduce the probability of SR Ca^{2+} release during diastole (24). Although Ca^{2+} release from the SR via ryanodine receptors and the ensuing inward I_{NCX} are thought to be associated with the later part of the DD (55), which was not altered in aged SAMs, the relationship between SR Ca^{2+} release and membrane voltage is complex and includes Ca^{2+}-dependent inactivation of L-type channels, regulation of cAMP production [which links Ca^{2+} levels to I_f activation in guinea pig SAMs (56)], and activation of large conductance Ca^{2+}-activated K^+ (BK) channels [which also contribute to pacemaking (57)].

Summary. Given that the spontaneous activity of SAMs determines HR, our central observation that pacemaker activity of individual SAMs is depressed during aging indicates that the slower AP FR of aged SAMs contributes to the age-dependent reductions in iHR and mHR (11–13). Furthermore, because the decrease in mHR with age is thought to be a major determinant of the age-dependent decrease in the maximum rate of oxygen consumption ($VO_{2\text{-max}}$) (1–3), our data support a model in which depressed excitability of SAMs underlies at least part of the reduction in aerobic capacity with age. This decline in $VO_{2\text{-max}}$ is a fundamental aspect of aging that has an enormous impact on individuals and on society. For athletes, the decrease in $VO_{2\text{-max}}$ is the main reason for the decline in exercise performance with age (1, 58). For many elderly individuals, a low $VO_{2\text{-max}}$ is the factor that limits functional independence by restricting the ability to perform daily activities (9, 10, 59). Elucidation of the molecular mechanisms responsible for the age-dependent reduction in SAM pacemaker activity may identify novel drug targets that could forestall the reduction in aerobic capacity during aging.

Materials and Methods

Animal procedures were approved by the University of Colorado Institutional Animal Care and Use Committee. ECGs were recorded from awake, restrained mice using the ECG Tunnel system (EMKA Technologies) and a Powerlab amplifier (AD Instruments), and they were analyzed offline using Labchart 7 Pro software (AD Instruments). SAMs were isolated from WT C57BL/6 male mice as previously described (60) and were patch-clamped at 35 ± 1 °C in current-clamp mode (for APs) and voltage-clamp mode (for membrane currents). Detailed methods are available in *SI Materials and Methods*.

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