Transient Outward Current, $I_{to1}$, Is Altered in Cardiac Memory

Hangang Yu, PhD; David McKinnon, PhD; Jane E. Dixon, PhD; Junyuan Gao, PhD; Randy Wymore, PhD; Ira S. Cohen, MD, PhD; Peter Danilo, Jr, PhD; Alexei Shvilkin, MD, PhD; Evgeny P. Anyukhovsky, PhD; Eugene A. Sosunov, PhD; Motoki Hara, MD; Michael R. Rosen, MD

**Background**—Cardiac memory refers to a change in the T wave induced by and persisting for minutes to months after cessation of a period of altered ventricular activation. Cardiac memory” refers to a change in the T wave induced by and persisting for minutes to months after cessation of a period of altered ventricular activation.1-4 Canine studies have shown that short-term T-wave memory is prevented by intravenous 4-aminopyridine (4-AP), suggesting that the memory might depend on pacing-induced changes in the transient outward current ($I_{to}$). Studies of an isolated tissue model of cardiac memory supported this view, demonstrating that an altered epicardial-endocardial voltage gradient contributes to memory and that 4-AP abolishes it.6 The expression of 4-AP-sensitive $I_{to}$ is heterogeneous through the canine ventricular wall, with current density higher in epicardial than endocardial myocytes.7,8 We hypothesized that the 4-AP–sensitive $I_{to}$ might be altered during the evolution of cardiac memory and used a chronic canine model to study it. The persistence of memory long after cessation of pacing permitted us to perform our experiments without fear that decay of the memory phenomenon would occur. We focussed on the 4-AP–sensitive $I_{to}$ in normal canine epicardial myocytes and those from animals with “long-term memory.” For convenience, we use the term $I_{to}$ instead of $I_{to1}$ throughout.

**Methods**

Mongrel dogs weighing 20 to 26 kg premedicated with propofol 5 mg/kg IV were anesthetized with 3% isoflurane and subjected to a left thoracotomy under sterile conditions. A Medtronic 5069 screw-in pacemaker (contact diameter, 0.020 inches) was inserted transepicardially into the anteroapical left ventricle (LV) and attached to a Medtronic 8340 programmable pacemaker embedded in a subcutaneous pocket. Animals recovered for 2 to 3 weeks, during which time ECGs were recorded every 2 to 3 days with the animal resting comfortably in a sling. Within 3 weeks, all postsurgical ECG changes had disappeared, and stable, reproducible recording of standard limb leads was demonstrated over a period of 1 week. LV pacing was then initiated at 120 bpm and maintained for 23 hours of each day. Holter monitoring was performed for 2 to 3 days of the pacing period. In no animal was there <75% capture. While pacing was discontinued, the ECG was recorded continuously to permit determination of ST-T waves in sinus rhythm. After 20 to 22 days of pacing, we performed terminal studies, which began within 3 hours of discontinuation of ventricular drive.
**TABLE 1. Effects of Chronic-Pacing on the ECG**

<table>
<thead>
<tr>
<th>Heart Rate, bpm</th>
<th>P-R Interval, ms</th>
<th>QRS Duration, ms</th>
<th>Q-T Interval, ms</th>
<th>Q-Tc Interval, ms</th>
<th>T-Wave Amplitude, mV</th>
<th>T-Wave Vector, °</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>93±3</td>
<td>106±3</td>
<td>58±2</td>
<td>234±3</td>
<td>285±22</td>
<td>0.12±0.18</td>
</tr>
<tr>
<td>After pacing</td>
<td>83±6</td>
<td>111±9</td>
<td>56±3</td>
<td>231±6</td>
<td>271±16</td>
<td>-0.34±0.21†</td>
</tr>
</tbody>
</table>

*All data were recorded during sinus rhythm before and 1 to 4 hours after termination of long-term ventricular pacing at 20 to 22 days. Data are mean±SEM.

†P<0.05 vs control (paired t test).

**Isolated Tissue and Single Myocyte Studies**

Animals were anesthetized with pentobarbital sodium 30 mg/kg IV, and the heart was rapidly excised and placed in ice-cold Tyrode’s solution, containing (in mmol/L) NaCl 131, KCl 4, CaCl 2 2.7, NaHCO 3 18, MgCl 2 0.5, NaH 2 PO 4 1.8, and dextrose 5.5, gassed with 95% O 2/5% CO 2. For microelectrode studies, strips of LV free wall epicardium obtained 1 cm below the mitral ring and measuring ~1 cm×1 cm×0.5 to 1.5 mm were filleted from control dogs and those studied at 20 to 22 days of pacing and having cardiac memory. Tissues were placed in a chamber perfused with Tyrode’s solution at 37±0.1°C and stimulated by standard techniques via bipolar silver electrodes insulated with Teflon. The tissue bath was connected to ground with a 3 mol/L KCl-Ag-AgCl bridge. Glass microelectrodes filled with 3 mol/L KCl were used to impale the tissues. Standard means for calibration and recording were used.‡ Single cells were isolated from control hearts and those with cardiac memory. The free-wall epicardial layer (1 to 3 mm) near the LV base, ≥2 cm from the pacemaker, was excised as above and subjected to trituration as reported previously.§ Details of the transport solution and disaggregation have been described.¶ Disaggregated cells were kept in KB medium at room temperature for 1 hour before electrophysiological experiments.

To avoid Ca 2+ current contamination, I o was recorded by the whole-cell patch-clamp technique in modified Tyrode’s solution containing (in mmol/L) NaCl 137.7, NaOH 2.3, MgCl 2 1, glucose 10, HEPES 5, KCl 5.4, CaCl 2 1.8, MnCl 2 2, and MgCl 2 0.2, pH 7.4. Pipettes were filled with solution containing (in mmol/L) NaCl 6, potassium aspartate 130, MgCl 2 5, EGTA 11, HEPES 10, Na 2-ATP 2, and Na-GTP 0.1; pH was adjusted to 7.2 by KOH. Pipette resistance was 2 to 4 MΩ. The pH of the Tyrode’s solution containing 4-AP was titrated to 7.4 with HCl. Temperature was maintained at 30°C to 32°C. An Axopatch 1B amplifier (Axon Instruments, Inc) was used.

We used Tyrode’s solution containing NaCl 137.7 mmol/L to facilitate comparison with action potential studies. Because I o was not blocked, we performed control experiments to determine whether the overlap of I o distorted our estimate of I C threshold. No change in threshold occurred (ie, in 5 control myocytes, I o threshold was −24±2 mV in both Na 140 and 10 mmol/L. At the same [Na+] i, the threshold in all 3 myocytes from memory animals was −10 mV.)

Data were collected by FM recording (Hewlett-Packard Co; 3964a, speed 7/8 in s-1, 600-Hz bandwidth) and pClamp software (Axon Instruments, Inc).
potential of 0 mV (see protocol, Figure 4, inset). Results and a Boltzmann 2-state model fit to the data are provided in Figure 4, and midpoint values and slope factors are in Table 3. There is an 8-mV positive shift of the inactivation-versus-voltage curve in myocytes from hearts with memory, and no change in slope factor. Six additional experiments were performed with memory myocytes, with 2-second prepulses. No change in midpoint for inactivation voltage occurred (−35±3 mV, n=6).

Kinetic Properties of Inactivation. Figure 5 shows results from a protocol demonstrating the inactivation kinetics of \( I_{to} \) from normal and memory myocytes. Each cell was held at −65 mV and depolarized to the values indicated on the figure. There were no significant differences in inactivation time constants, although a positive shift in their voltage dependence was observed (for average values for time constants of inactivation at +20 mV, see Table 3).

Despite the lack of significant difference in inactivation kinetics in memory, recovery from inactivation was dramatically altered (see Figure 6). From a holding potential of −65 mV, we depolarized to +5 mV in control and +20 mV in memory (to maintain approximately equal activation), returned to the holding potential for a variable time, and again depolarized to the test potential. The ratio of \( I_{to} \) amplitude in the second pulse compared with the amplitude in response to the first was plotted against time along with the best fit to a monoeponential function in Figure 6C. In Figure 6, the time constant of recovery was 33 ms in control and 531 ms in memory. As summarized in Table 3, the recovery time constant is more than an order of magnitude slower in cells from animals with memory.

Studies of hearts from several species have shown that \( I_{to} \) recovery from inactivation proceeds more rapidly with hyperpolarization.14 We investigated whether a similar voltage dependence exists for control and memory myocytes (Figure 6D). Although \( I_{to} \) recovery from inactivation is an order of magnitude slower in memory than control, there is still the same speeding of recovery with hyperpolarization.

\( I_{to} \) Conductance

The changes in activation threshold and inactivation recovery kinetics provide a potential explanation for the reduction in the action potential notch recorded during cardiac memory. We also asked whether \( I_{to} \) conductance was reduced. Ideally, to estimate this conductance, one would saturate activation, measure tail currents at the same voltage, and normalize the tail currents to the capacitance of the cells. This was not possible because we could not fully activate the current at depolarized potentials. We therefore quantified \( I_{to} \) amplitude at each test potential and then divided current magnitude by the driving force to estimate the conductance at each poten-

### Table 2. Epicardial Recordings From 2 Control Animals and Three 20-Day LV-Paced Animals

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>MDP, mV</th>
<th>Overshoot, mV</th>
<th>Phase I Notch, mV</th>
<th>APD_{50}, ms</th>
<th>APD_{90}, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>81±1</td>
<td>14±2</td>
<td>−1±1</td>
<td>127±6</td>
<td>184±6</td>
</tr>
<tr>
<td>20-day paced</td>
<td>22</td>
<td>82±1</td>
<td>11±2</td>
<td>4±2*</td>
<td>156±6*</td>
<td>203±5</td>
</tr>
</tbody>
</table>

MDP indicates maximum diastolic potential. Preparations were equilibrated for 3 hours at a basic cycle length of 650 ms.

*P<0.05 vs control.
The above-described work was performed in models of short-term cardiac memory, lasting minutes. Such models are of limited value in evaluating ion channel contributions to memory, because the electrophysiological changes do not persist long enough to ensure a stable substrate for myocardial disaggregation and whole-cell study. The use of a long-term memory model that we have demonstrated to be limited in value in evaluating ion channel contributions to memory, because the electrophysiological changes do not persist long enough to ensure a stable substrate for myocardial disaggregation and whole-cell study. The use of a long-term memory model that we have demonstrated to be
unassociated with cardiac failure or hypertrophy (cell capacitance, reflecting cell size, is unchanged) provides long-term T-wave changes that render the study of disaggregated myocardial myocytes practicable. Moreover, we have previously shown that the statistically insignificant variations in sinus rate occurring before and after pacing cannot be the cause of these T-wave changes.

The action potentials we recorded are entirely consistent with results from earlier isolated tissue studies in that the notch of the epicardial action potential in T-wave memory is smaller than in controls. In short-term pacing, however, the epicardial APD did not manifest the prolongation seen with long-term pacing and cardiac memory in our present or previous study. The fact that the APD is not significantly prolonged in a memory model of short duration suggests that in the long-term setting, repolarizing currents other than $I_{la}$ may be involved (possibly $I_{caL}$, $I_{K}$, Na/K pump current, $I_{K1}$). This possibility is also suggested by the study of Shvilkin et al. Here, epicardial, endocardial, and midmyocardial action potentials were studied with microelectrodes in animals paced for ≥20 days. Significant prolongation of epicardial and endocardial action potentials as well as reduction of the difference in duration between the 2 types of action potentials occurred, such that epicardial action potentials prolonged to a greater extent than endocardial. In contrast, APDs of midmyocardial cells were unchanged. Hence, with memory, there was a reorientation of the interrelationships of APDs of each myocardial tissue. A prolongation of endocardial action potentials, which have a small notch and little $I_{la}$, and no change in duration of M cell action potentials, which have significant $I_{la}$, provides a further argument that currents other than $I_{la}$ are involved in the memory process.

Kinetics of $I_{la}$

Despite the need to expand our studies to include other ion channels, our observations concerning $I_{la}$ provide an understanding of the fundamental alterations in one ion channel that contribute to the memory phenomenon. Specifically, the shift in long-term memory $I_{la}$ activation toward more positive potentials by almost +20 mV (Table 3) is consistent with the absence of a notch in ventricular epicardial action potentials from dogs with cardiac memory. $I_{la}$ activation at relatively positive potentials will contribute reduced outward current to phase 1 repolarization. In the physiological voltage range, the memory-induced positive shift of $I_{la}$ activation along the voltage axis in epicardium mimics the activation of $I_{la}$ in endocardium and therefore would decrease the voltage gradient from epicardium to endocardium.

A significantly longer time course of $I_{la}$ recovery from inactivation was found in memory myocytes. This phenomenon is an additional contributor to the loss of the action potential notch recorded in epicardial myocytes from animals with cardiac memory; eg, at a ventricular pacing rate 20% greater than sinus rate, the basic cycle length was ≈500 ms. Here, a 531-ms time constant of recovery from inactivation will not allow $I_{la}$ to recover to a significant degree (even if it is activated at the end of phase 0).

A major question relates to the identity of the signaling mechanism responsible for the change in $I_{la}$. Our work in progress and the published literature suggest that altered stress-strain relationships induced by ventricular pacing activate the endogenous cardiac renin–angiotensin II system; this protein kinase C–linked signaling cascade both alters channel phosphorylation (perhaps explaining the changes in $I_{la}$ in short-term memory) and induces the immediate early gene program, which alters new protein (channel) synthesis. Although we hypothesize that new gene expression and protein synthesis are involved in long-term memory, we do not know which genes are expressed and which new proteins are synthesized. Even though the sensitivity to 4-AP is not significantly changed in long-term memory myocytes,
this result does not completely rule out the possibility of an altered isoform, which remains to be investigated.

**Clinical Importance**

The clinical importance of cardiac memory initially was said to lie in its ECG patterns, mimicking those of ischemia. However, the potential importance of the memory phenomenon appears to be greater, as follows: first, any event that alters repolarization might alter refractoriness as well, and changes in the effective refractory period can be permissive of or suppress arrhythmias. Second, in the setting of tachycardia-induced CHF when heart rate is also rapid, abnormalities in $I_{to}$ and in the T wave occur. The possibility exists that the rate- and activation-induced changes in $I_{to}$ induced by the memory phenomenon are important contributors to the T-wave anomalies in such pathological conditions. Third, recent studies by Allessie et al have demonstrated pacing-induced atrial fibrillation in the goat. This, too, is associated with accelerated repolarization and loss of the action potential notch. Here, an atrial analog to the memory phenomenon might be considered. The statement by Wijffels...
et al that “fibrillation begets fibrillation” leads us to suggest that any change in rhythm that alters activation pathway and rate might alter the action potential and render persistence/re-occurrence of the arrhythmia more likely. Similarly, maintenance of sinus rhythm and normal activation and/or interventions that maximize $I_{to}$ might turn out to be antiarrhythmic. Finally, these initial results demonstrate that this stable canine model for cardiac memory can provide a “window” through which the alterations of ion channel characteristics that underlie altered repolarization and their relationship to signal transduction pathways can be investigated intensively.

Acknowledgments

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Figure 9. $Kv4.3$ mRNA expression declines in cardiac memory. A, RNase protection analysis of $Kv4.3$ expression in RNA samples from LV muscle of control and memory animals. Cyclophilin (Cyc) probe is internal control. B, Histogram of mean values for $Kv4.3$ mRNA expression in control and memory animals expressed in arbitrary units (a.u.), normalized to a mean control value of 100%. Average $Kv4.3$ mRNA expression in long-term memory is 65% ± 7% of control ($P<0.05$, n=6).

References

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