Why T waves change: A reminiscence and essay

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The following article is a personal reflection on my study of a subject which has long interested me. The subject is the T wave, and especially the T wave changes occurring as a marker of cardiac memory. My interest evolved over coffees that Mauricio Rosenbaum and I used to share at the Hotel Algonquin during his frequent trips from Buenos Aires to New York. There is something about the Algonquin, whose scarred wooden tabletops carry the imprints of Robert Benchley, Dorothy Parker, and the 1920’s New York literati, and there was something about Mauricio—clinician-scientist, friend, and raconteur extraordinaire—that made his repeated challenges to me to “look at cardiac memory before you begin losing your own” irresistible. So began my personal voyage into trying to understand the T wave. My guideposts were the experiments of Wilson and Finch,1 the astute observations of a host of investigators who followed, and Mauricio’s iconoclastic insights. The story is far from over . . . I doubt I’ll see the end of it in my lifetime. But if the beauty of discovery is in the voyage, then it has been – for me - a memorable trip.

KEYWORDS Cardiac memory; Ventricular pacing; Ion channel trafficking; Ion channel gene transcription; Transient outward potassium current

ABBREVIATIONS 4-AP = 4-aminopyridine; AP-1 = activator protein-1; CREB = cyclic adenosine monophosphate response element binding protein

In 1923, Wilson and Finch1 performed a simple experiment in which ice water was ingested and an ECG was recorded. The result led them to describe T-wave changes as either primary, arising “from disturbances in the function of fairly large regions of ventricular muscle,” or “secondary to changes in the form of the QRS complex” and persisting as long as the QRS changes were present. Knowledge of primary T-wave changes has expanded over the years to incorporate those induced by structural alterations, such as hypertrophy, as well as pharmacologic and electrolytic influences and, more recently, channelopathies. Examples of secondary T-wave changes include those induced by conduction abnormalities, ventricular arrhythmias, and myocardial infarction.

However, every story has caveats. One for the categorization of T-wave changes as primary or secondary was noted by several groups of individuals and formalized by Mauricio Rosenbaum2 (Figure 1). The caveat was a change in the T wave that appeared at first to be secondary (as it was initiated by a change in the QRS complex) but persisted long after the QRS had normalized, such that it mimicked a primary change. Rosenbaum and associates2 referred to such T waves as “pseudoprimary” and—given the property of the persistent T wave to follow the vector of the inciting QRS complex called the phenomenon “cardiac memory” (Figure 2 [1b]). They performed animal and human experiments on cardiac memory and concluded—although not definitively—that altered electrotonic coupling among cells might provide the mechanism. Studies of Langendorff-perfused rabbit hearts in Michael Franz’s laboratory gave further credence to this idea.3 Because we recently summarized our own experiments and those of other groups that bear on repolarization and cardiac memory,4 my goal in this article is to provide a more personal description of the events that have occurred since my coffees with Mauricio. The process has been one of growth and education. I started as a cardiologist who viewed electrophysiology as a be-all and end-all. Electricity was sacrosanct . . . it was the property of cables and batteries, and I tried to simplify everything I saw biologically to fit that context. However, exploring the machinations of the T wave has led to collaborations with colleagues in molecular biophysics, molecular biology, and signal transduction, and together we have explored the mechanisms determining the electrical output (the T wave) of an electrical signal generator (an electronic cardiac pacemaker). Although this has been a personal voyage, it has not been a solitary one. The research throughout has been performed in partnership with Peter Danilo and Ira Cohen, and along the way there have been significant contributions by Susan Steinberg, Rich Robinson, and Penny Boyden.

Our adventure, coupled with parallel discoveries by colleagues in other laboratories with interests in remodeling and in channelopathies, has led me to marvel at the complexity of events leading to seemingly simple changes in
electrical output while I despair of ever learning all the answers. In any event, in querying “why T waves change,” I offer the following summary of what we have learned and how we learned it. Figure 2 provides an annotated roadmap of the experimental results.

Starting off . . . the T-wave change

Ughetta del Balzo and I began with a variant on the Rosenbaum model of memory, pacing dogs from the ventricle for 20 to 30 minutes at about 5% faster than their sinus rate, and interspersing this with sinus rhythm or atrial pacing (Figure 2 [1a and 1b]). Following the Rosenbaum lead, we referred to this as short-term memory, and based on the literature we hypothesized that the T-wave change resulted from an altered T-wave gradient consequent to altered transmural dispersion of $I_{\text{to}}$ (Figure 2 [6]). To test a potential role for $I_{\text{to}}$ in short-term memory, we infused dogs with 4-aminopyridine (4-AP), to nonselectively block $I_{\text{to}}$. In the presence of 4-AP, memory could not be induced. Subsequently, Christoph Geller created a memory model in isolated, paced ventricular epicardial and endocardial slabs. Here, the “T wave” change (the result of processing the epicardial and endocardial action potentials through a difference amplifier) was prevented by 4-AP.\(^5\)

Why did ventricular pacing elicit these changes? Was it the electrical shocks . . . the altered activation . . . the resultant alterations in myocardial stretch (Figure 2 [2])? Sadoshima and Izumo\(^7,8\) previously showed that altering stretch in myocyte–fibroblast cultures results in angiotensin II synthesis and release. Philippe Ricard\(^9\) hypothesized that if altered myocardial activation in situ resulted in altered stretch and angiotensin II availability, then interfering with angiotensin II synthesis or binding to its receptor should prevent memory from evolving (Figure 2 [3]). He was right. He could not produce short-term memory in the presence of an angiotensin-converting enzyme inhibitor, an AT-1 receptor blocker, or a tissue chymase inhibitor. That a tissue chymase inhibitor prevented memory induction was consistent with angiotensin II being synthesized in the heart rather than carried from other sites via the circulation. The same experimental series also demonstrated that tissue chymase attenuates the evolution of short-term memory, consistent with a role for Ca\(^2+\) in the process (Figure 2 [5a]).

The next step was to develop a model for long-term memory. Joris de Groot and I performed a series of mapping experiments to document the magnitude and consistency of T-wave changes induced by test pacing at multiple epicardial sites. This provided Alexei Shvilkin with a template for ventricular pacing of chronic dogs that incorporated ease of lead implantation and a T-wave change of sufficient magnitude for consistent study. Shvilkin et al\(^10\) then demonstrated that about 3 weeks of pacing could induce long-term memory that persisted for weeks in the absence of coronary flow changes, failure, or hypertrophy, and that evolution of long-term memory was delayed but not prevented by AT-1 receptor blockade or calcium channel blockade (Figure 2 [5b]). As part of these experiments on long-term memory, we studied left ventricular epicardial and endocardial transmembrane action potentials and found an altered transmural gradient, with epicardial potentials lengthening more than endocardial, while the action potential notch diminished.\(^10\)

How did these changes at the cellular level play out in the heart in situ? Giel Janse, Ruben Coronel, and Tobias Opthof joined us to study the T-wave changes in short-term memory (Figure 2 [7a]). The experimental plan included long days of mapping and long nights at Peter Lugger’s Steakhouse. Under control conditions (during the days), we found a left ventricular apicobasal gradient with the shortest repolarization times anterobasally and the longest repolarization times posterobasally. There was no significant transmural gradient in atrial-paced controls or after 2 hours of ventricular pacing.\(^11\) Because both repolarization time and monophasic action potential durations shortened during induction of short-term memory, we proposed that the deep T wave of short-term memory might be explained by the steeper phase 3 of repolarization. We then studied long-term memory (Figure 2 [7b]) and found no transmural gradient in the control setting.\(^12\) However, a gradient appeared during long-term memory, with epicardial repolarization being longer than endocardial.

This contrasts with the finding of David Rosenbaum’s laboratory\(^13\) that long-term memory is associated with disproportionate and localized action potential prolongation of late-activated myocardial segments, but without changes in transmural action potential duration gradients. However, their study design was different because it centered on isolated wedge preparations from dogs in long-term memory. Using yet another study, design, and species, Yoram Rudy’s group\(^14\) used ECG imaging to study patients undergoing radiofre-
frequency ablation for Wolff-Parkinson-White syndrome. These patients had long activation-recovery intervals and large apex-to-base dispersion in preexcited regions of epicardium that required about 1 month to resolve postablation.

To sum up these pharmacologic and electrophysiologic experiments, short-term memory was prevented by interfering with angiotensin II synthesis and binding and by calcium channel blockade. Long-term memory was delayed by these interventions but was not prevented. The key factors appeared to be altered activation, angiotensin II, calcium, and Ito.9,10

As for repolarization gradients, they are clearly important in memory. The only study of short-term memory in situ shows the gradient is apicobasal.11 In long-term memory, one study showed the change to be establishment of a transmural gradient, and two studies found different results using different techniques.12–14 However, given that the long-term studies were done in two different species using three different methods to induce and record memory, they may not be contradictory. We simply have three pieces of information from what appears to be a complex puzzle.

**Is it all a matter of activation?**

From early in our studies, we questioned whether it was simply a change in activation or a change in myocardial stretch induced by altered activation that was responsible for the increase in angiotensin II and possibly for gene transcription (the role of which will be detailed later). A wealth of information pointed to the importance of altered wall motion and stretch, including the studies of Prinzen and associates15,16 on activation and dyssynergy of contraction, the work of Sadoshima and Izumo7,8 showing that stretch in cell culture results in altered angiotensin II synthesis and release, and the work of Max Lab and associates17 showing that altered stretch without altered activation increases c-fos and c-jun levels within about 30 minutes, consistent with the initiation of transcription.

More recently, David Rosenbaum’s laboratory13 and Sosunov et al18 indicated that altered stretch is the “missing link” in the path between activation and memory (Figure 2 [2]). The Rosenbaum laboratory performed studies in intact dogs, and Sosunov et al studied isolated, perfused rabbit hearts. Both laboratories reached the same conclusion regarding stretch. Moreover, the latter studies showed that altered activation without altered stretch induced no memory, and altered stretch without altered activation still induced memory.

**Digging deeper: How do memory, angiotensin II, and Ito interrelate?**

Yu et al19 noted in long-term memory a reduction in epicardial Ito density and altered kinetics as well as reduced...
mRNA for Kv4.3, the genetic determinant of the pore-forming unit of the channel carrying Ito. A study by Plotnikov et al reinforced the importance of a gradient for Ito by showing that, in the absence of Ito and a related repolarization gradient, there is no expression of short-term memory. Based on the reduced mRNA for Kv4.3, we opined that the change in Ito for long-term memory is transcriptional. Left open was not only the mechanism for gene transcription but how Ito might be altered in the setting of short-term memory, before transcription has occurred. I shall return to these issues later.

What of angiotensin II? Susan Steinberg had initially suggested that we pursue this signaling pathway aggressively, and Yu et al studied the role of angiotensin II in modulating Ito. We isolated epicardial or endocardial ventricular myocytes in test tubes, exposed them for 2 to 96 hours to angiotensin II, and found that angiotensin II had the same effect on epicardial Ito in the test tube as did pacing to induce long-term memory, a reduction in the current and altered kinetics. This effect was blocked by losartan, thus confirming the role of the AT-1 receptor in the pathway. Very importantly, mRNA for Kv4.3 was unchanged, arguing against gene transcription playing a role in this setting.

The sum of these studies suggests a link between angiotensin II synthesis and release and expression of Ito that contributes to the occurrence of short-term memory. Whereas long-term memory is associated with altered Ito density and kinetics that appears transcriptional, angiotensin II affects Ito over the short term, but via a nontranscriptional pathway.

Short-term memory: Induced by ion channel trafficking?

Doronin and colleagues explored the possibilities of a nontranscriptional pathway for angiotensin II–dependent Ito reduction (Figure 2 [4]). They transfected a cell line with Kv4.3, with KChIP2 (an accessory subunit important to Ito density and kinetics), and with the AT-1 receptor. They found that the three components formed a macromolecular complex within the cell membrane and that angiotensin II binding to the receptor resulted in internalization of the complex and reduction in the current (Figure 2 [8]). They also found that in myocytes the AT-1 receptor and Kv4.3 co-immunoprecipitate, and that in cell lines the two reside within 100 Å of one another. Taken together, these observations offer an explanation for the nontranscriptional evolution of short-term memory induced by pacing the ventricle. This idea of internalization of the macromolecular complex awaits testing in situ.

Long-term memory and gene transcription

The initial suggestion that gene transcription might be important to long-term memory came from two sources. One was the work of Kandel and associates on long-term potentiation in Aplysia californica, showing that the cyclic adenosine monophosphate response element binding protein (CREB) is important to transcription when electrical shocks are delivered to Aplysia. The other insight came from Lab and associates, who showed that altering stretch for brief periods without altering activation in pig heart recruited changes in c-fos and c-jun.

Niels Patberg and Nazira Özgen have studied gene transcription in cardiac memory. Patberg et al initially focused on the molecular mechanisms underlying the loss of the action potential notch and prolongation of epicardial action potential duration in long-term memory. We used a 2-hour pacing protocol to test whether ventricular pacing to induce memory alters CREB production in heart and found that CREB is reduced within the 2-hour period (Figure 2 [9]). The reduction is maximal near the pacing site and is prevented by angiotensin II receptor blockade or calcium channel blockade. We also noted the cyclic adenosine monophosphate response element present in the promoter region of KChIP2 and demonstrated a reduction in KChIP2 protein parcelling that in CREB (Figure 2 [10]).

Patberg et al next asked whether reduced CREB levels in the absence of pacing might still reduce Ito and alter repolarization, thereby establishing cause and effect between CREB and Ito reduction. We injected a CREB antisense virus into a region of the canine left ventricular epicardium. After several days, monophasic action potential recordings in the region showed no phase 1 notch, whereas the notch was prominent in control regions. Myocytes disaggregated from the injected regions had no Ito, and Ito was normal in the uninjected sites.

Taken together, these findings suggested that CREB is a transcription factor for KChIP2 and is involved in Ito regulation in initiating long-term memory. Additional transcription factors likely are involved in memory induction. For example, preliminary data from Özgen et al suggest activator protein-1 (AP-1) is important for the ether-a-gogo-related gene (ERG) that determines Ikr.

Other studies by Özgen et al have focused on transduction of events between angiotensin II binding to its receptor and the occurrence of CREB reduction. The preliminary data show that ventricular pacing increases malondialdehyde levels, consistent with the occurrence of oxidative stress, implying a role for H2O2 production (Figure 2 [11]). The outcome appears to be CREB phosphorylation and its proteasomal degradation (Figure 2 [12]). We now are exploring whether reactive oxygen species regulate CREB in cardiac myocytes. Preliminary results suggest that H2O2 down-regulates CREB content via mitogen-activated protein (MAP) kinase and protein kinase C/protein kinase D (PKC/PKD) regulated pathways (Figure 2 [13]).

Is it all a matter of Ito?

No, it is much more complicated (Figure 2 [6]). Plotnikov and colleagues showed that Ical kinetics are altered in long-term memory in a way that can explain the prolongation seen in the action potential plateau. In trying to understand the determinants of the change in Ical, Thomsen et al reinforced not only the role of KChIP2 as an accessory protein for Kv4.3, the pore-forming unit of Ito, but that it
serves the same purpose for the $\alpha$ subunit of $I_{Ca,L}$ (Figure 2 [5b]).

However, $I_{Ca,L}$ is not the only other ion channel involved. Obrechtikova et al$^{32}$ studied $I_{Kr}$ in long-term memory. In controls, $I_{Kr}$ density and mRNA and protein of its $\alpha$ subunit, ERG, display a transmural gradient such that current density and ERG expression are greater in epicardium than in endocardium. In long-term memory, the transmural gradients of current density and ERG mRNA and protein are reversed.

There are also important changes in the connexins that facilitate cell-to-cell communication. Patel and colleagues$^{33}$ showed that in long-term memory there is diminished connexin43 density, especially near the pacing site. In addition there is lateralization of connexin43. These changes jibe well with Mauricio Rosenbaum’s initial idea that altered electrotonus may contribute to memory. However, cell-to-cell conductance has not yet been reported in memory.

**Clinical updates**

In his initial studies, Mauricio Rosenbaum described the clinical patterns of cardiac memory in detail and also noted the extent to which memory might be confused with ischemic patterns on ECG.$^{2,34}$ Twenty-five years later, Shvilkin et al$^{35}$ described the means for distinguishing the ECG patterns of cardiac memory and ischemic precordial T-wave inversion. A combination of a positive T wave in aVL with a positive or isoelectric T in lead I and maximal precordial T waves in lead I>lead III was 92% sensitive and 100% specific for cardiac memory, discriminating it from ischemic precordial T-wave inversion. Shvilkin and colleagues$^{36}$ also described memory occurring consequent to the QRS widening induced by propafenone toxicity, showing a clinical corollary to the earlier work by Plotnikov et al$^{37}$ on the interaction of memory and antiarrhythmic drugs in a canine model. Finally, Wecke and colleagues$^{38,39}$ systematically explored the clinical expression of cardiac memory in paced human subjects, detailing which vectorcardiographic measurements most consistently reflect the onset and offset of memory as well as the time course of accumulation and resolution.

**Is cardiac memory really memory, and why bother with it anyhow?**

Borys Surawicz$^{40}$ painted a provocative image of cardiac memory as a form of forgetting, and likely he is right. Whereas the neuronal memory expressed as long-term potentiation is a synthetic process involving the strengthening of protein links,$^{23}$ memory in heart involves two processes: the “forgetting” of a current state of repolarization and the “remembering” of a neonatal repolarization pattern. This was uncovered by Plotnikov et al$^{20}$ in a study of neonatal, young, and adult dogs. The neonate has no $I_{to}$, no action potential notch, and no pacing-induced cardiac memory. At approximately 6 weeks of age, $I_{to}$, the notch, and inducible memory evolve, and with advancing age and a larger $I_{to}$, memory is ever more inducible. The memory pattern in the adult is, in many ways, reminiscent of a neonatal heart with no $I_{to}$.

Given the apparent benignity of cardiac memory, is its study much ado about little? I think not. True, we are always impatient to understand the causality of events that are at least discomforting and at worst life-threatening, and memory hardly fits this range of descriptors. Yet, given its associations with angiotensin II and reactive oxygen species, memory may reflect the beginning of a series of changes leading ultimately to hypertrophy, necrosis, and worse. If this is true, then we shall come to view these T-wave changes as early signs of pathologic processes at a time when their reversal is still possible. We have some inklings of this, already, in the atrium. Herweg et al$^{41}$ and Chandra et al$^{42}$ studied atrial memory: the changes in the atrial T wave induced by pacing from ectopic sites. Interestingly, the changes seen with memory were accompanied by an increased propensity to atrial tachyarrhythmias and fibrillation. This sequence and outcome fit well with the demonstration by Allessie’s group that atrial fibrillation begets atrial fibrillation.$^{43}$

A final thought: One thing a career in research affords us, if we are lucky, is the opportunity to study natural phenomena in their own right simply because they are interesting. Although this is against the grain of applied research that seeks cures for diseases, the observation of Comroe and Dripps$^{44}$ some 30 years ago has, to my knowledge, never been refuted: that advances in medicine derive foremost from research that asks, “why is the grass greener,” that asks the question simply in its own right because of the curiosity of the investigator. Although it is undeniable that applied research is essential, at the roots of knowledge has always been the fundamental desire to know for the sake of knowing. In this context, is cardiac memory too “simple” a system, too devoid of pathologic meaning to warrant study? I think not. I believe that only by understanding the complexity of that which appears simple can we begin to appreciate the depth of those mysteries that appear to be complex.

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