1. Introduction

Cardiac memory was characterized by Rosenbaum et al. in 1982 as ST-T wave changes induced by ventricular pacing or arrhythmia that persist long after normal ventricular activation has resumed [1]. Rosenbaum’s work enlarged upon the earlier studies of Chatterjee et al. [2] who reported persistent postpacing changes in T wave morphology. The key aspects of cardiac memory were that the magnitude and persistence of the postpacing T wave changes increased with the duration of abnormal ventricular activation (referred to as ‘accumulation’) (Fig. 1), and the subsequent normalization of the T wave occurred slowly, requiring hours to months, depending on the duration of the inciting stimuli.

Exploration of the mechanisms for cardiac memory is important clinically as the phenomenon (1) is induced not only by pacing, but by the occurrence of spontaneous ventricular premature depolarizations or tachycardias [3–8]; (2) impacts on the determinants of ventricular repolarization and refactoriness [1] and, therefore, potentially on the expression (or prevention) of arrhythmias; (3) can be confused with the ST-T wave changes of ischemia [1] and (4) induces changes in ion channel physiology that are also seen with cardiac failure [9] or that follow an ischemic insult [10].

In this review we shall focus on the mechanisms underlying cardiac memory. Our intent is to demonstrate not only that the heart is endowed with memory but that the mechanisms for induction and maintenance of cardiac memory involve second messenger cascades, which at least in part mimic the events that underlie memory in the central nervous system. We shall first consider the terminology related to cardiac memory and hypotheses relating to its causality, then means for its induction and evaluation in the intact heart, its signal transduction cascades, its expression at the level of the action potential and the ion channel, and finally, its implications with respect to cardiac rhythm.

2. Terminology related to cardiac memory and hypotheses concerning its genesis

As shall be detailed below, ‘cardiac memory’ refers to a change in repolarization induced by an altered pathway of activation. The T wave change that characterizes cardiac memory is of the type often referred to as ‘secondary;’ i.e., reflecting conduction abnormalities such as those occurring in bundle branch block, the Wolff-Parkinson-White Syndrome, and ventricular ectopy. As such, it differs in etiology from primary T wave changes whose origin, as summarized by Katz [11], may be (a) structural (reflecting hypertrophy, myocyte destruction or fibrosis), (b) metabolic and either physiologic (autonomic transmitter-induced), pharmacologic (antiarrhythmic drug-induced), or pathologic (electrolytic abnormalities), or (c) molecular, as in T wave evolution after myocardial infarction.

A key aspect of cardiac memory is that the T wave change persists for a variable period (minutes to months) after the QRS complex has returned to normal. This is a critical point because it indicates cardiac memory is neither a typical secondary T wave change (defined by Wilson and Finch [12] as one secondary to a change in form of the QRS complex), nor a typical primary T wave change (defined by Wilson and Finch [12] as one that is independent of concurrent changes in the QRS complex).
Moreover, as shall be demonstrated below, in the discussion of short-term memory, there are settings in which the T wave change can occur without any change in the QRS complex. This situation – albeit the less common for cardiac memory – would be considered a primary T wave change.

Hence, the phenomenology of cardiac memory falls ‘between the cracks’ of Wilson and Finch’s [12] initial definition of terms. The unusual characteristics of cardiac memory suggest that to understand the T wave requires thinking beyond electricity and incorporating information regarding the molecular, biochemical and biophysical determinants of repolarization (see, for example, reference 11). Other factors characterizing cardiac memory are that it is independent of changes in cardiac structure, or the presence of cardiac failure or decreased perfusion. However, as shall be described below, the proposed molecular mechanisms for cardiac memory involve signal transduction pathways that also influence gene expression and can lead to structural changes. As such, it may be that cardiac memory is not an isolated event but is, rather, a relatively early change in electrophysiologic function that – while completely reversible – also augurs more persistent structural and functional changes to come if the inciting stimuli for memory are not reversed. Hence, it is likely that cardiac memory is an essential component of what is generally referred to as ‘electrical remodeling’ of the heart.

Although the term ‘cardiac memory’ might arguably be replaced by ‘electrical remodeling’, we prefer ‘cardiac memory’ in part for reasons of history (i.e. the nomenclature applied by Rosenbaum et al. [1] and in part because the term focusses our attention on similarities between events in the heart and the memory that has been described in other organ systems such as the CNS (see below). Indeed, the understanding that even highly differentiated organ systems share common signal transduction systems has provided (and should continue to provide) clues for investigation and understanding of the specialized functions of individual organ systems like the heart.

Rosenbaum et al. [1] suggested that cardiac memory results from altered electrotonic interactions among adjacent segments of ventricular myocardium that change the timing of repolarization of these regions of the ventricle. Based on the work of Wilson et al. [13] it was assumed that (1) if adjacent muscle fibers differ in their state of activity there must be an electromotive force across and normal to the plane that separates them, (2) the extent to which the electromotive force can change action potential duration of adjacent fibers depends on the passive electrical properties of the tissue, and (3) these electrical changes will influence T wave configuration. This explanation has the advantage of simplicity as it considers cardiac memory to result largely from electrotonus. However, in this and any system that offers to explain cardiac memory the T wave changes have to appear relatively rapidly and yet dissipate slowly following the respective onset and offset.

Fig. 1. (A) T wave changes in a human subject in complete heart block before and after siting a ventricular pacemaker, and then with the pacemaker turned off after 15 minutes and 3, 8 and 15 days after the onset of pacing. Note the progressive evolution of the T wave change (reprinted from reference [2], by permission). (B) Cardiac memory evolution over 21 days of ventricular pacing (VP). Depicted are leads I and aVR from one dog during control, during VP, and at the end of 1 hour after pacing was discontinued on days 7, 14, and 21. In both leads, evolution of the T wave is such that it tracks the vector of the paced QRS complex (reprinted from reference [15], by permission). (C) Frontal plane vectorcardiographic depiction of cardiac memory from the same dog presented in Figure 1B. A shows P, QRS, and T wave vectors in control, before the onset of pacing. B: The QRS and T vectors during ventricular pacing. C: The T wave vector alone (note enlarged scale as well as dotted line indicating the change in vector) during sinus rhythm in control (identical to A) and on days 14 and 21. Note the increase in amplitude of the vector as well as the shift in vector angle from that in control to one that approximates the QRS vector during pacing (B). D: A record made within 1 hour of returning to sinus rhythm on day 21 (reprinted from reference [15], by permission).
of an inciting stimulus such as pacing. A problem inherent in the assumption that electrotonus is the inciting factor is that there is no reason to assume a slow offset of the pacing-induced change. In other words, the persistence of T-wave changes well beyond the cessation of pacing suggests that a process other than or in addition to electrotonus is responsible. Neither can accumulation and depletion of extracellular ions (e.g., K⁺) be considered a unique cause of cardiac memory, as these, too, have a limited duration of effect on the ST segment and T wave.

Ischemia or local tissue injury induced by electrical stimulation [14] have been suggested to produce memory. However, Rosenbaum et al. noted [1] and we have recently affirmed [15] that cardiac memory occurs in settings characterized by normal myocardial perfusion and no cardiac failure. Moreover, Rosenbaum et al. noted [1] that cardiac memory could not be induced in the setting of ischemia (which, in fact, manifests T wave changes similar to those of cardiac memory). As shall be enlarged on later, this observation of Rosenbaum’s may be explained by the fact that similar changes in ion channels such as Iᵦ occur following an ischemic insult [10] and in the setting of memory [15].

Another hypothesized determinant of cardiac memory is pacing-induced activation of autonomic nerves. Geschwind et al. [16] reported that closely coupled premature ventricular beats increase coronary sinus plasma norepinephrine levels, an effect thought to result from post-extrasystolic potentiation [17]. Herre and Thames [18] found that programmed electrical stimulation of the ventricles of anesthetized dogs increased sympathetic nerve activity, which was abolished by cardiac denervation; and Welch et al. [19] reported that single ventricular beats could increase sympathetic nerve activity in humans beyond the normal background activity during sinus rhythm. Whether in similar fashion, prolonged cardiac pacing can produce sustained sympathetic stimulation which might contribute to memory is, to our knowledge, unknown. We also do not know to what extent this stimulation might alter the function of adrenergic receptors and post-receptor regulatory mechanisms. The possibility that pacing, even in the absence of an effect on sympathetic input, might induce cardiac memory via altered mechanoelectrical feedback also has been suggested [20].

3. Memory in the CNS: a template for the heart?

We have hypothesized that molecular mechanisms for memory in the heart are likely to parallel, at least in part, the mechanisms that determine learning and memory in neuronal systems. Paraphrasing Kandel’s question regarding the biological basis of memory [21] we ask “Does the heart utilize new biological mechanisms based on principles not found in other cells of the body, or does the heart simply use old biological tricks in a novel way?” The enormous strides that have been made toward understanding the molecular representation of memory in neurons should provide a particularly useful guide for studies of memory in the heart. For example, it is well established that memory in CNS has at least two forms, a short-term, labile form which lasts seconds to minutes and depends on covalent modification of preexisting proteins, and a long-term, consolidated form which lasts days to weeks or longer and requires transcriptional activation and new protein synthesis. Noting that stimuli that lead to short-term memory in CNS increase cAMP [22], early studies focused on the role of this second messenger pathway. It was found that cAMP-dependent activation of protein kinase A (PKA) induces short-term memory via the direct phosphorylation of K⁺ channels and other proteins and via enhanced neurotransmitter release [23]. However, the measured increases in cAMP are small and transient. Therefore, a mechanism that would lead to the persistent activation of PKA and play a role in the consolidation of memory even at basal cAMP concentrations seemed likely. Such a mechanism was identified and involves the selective loss of the regulatory subunits of PKA without any change in its level of catalytic subunits [24]. The resulting decrease in the ratio of regulatory to catalytic subunits is key in that it permits continued phosphorylation of target proteins by PKA even after cAMP returns to basal levels. This persistent phase of PKA activation and the consolidation of memory requires synthesis of new protein.

The importance of a cAMP/PKA pathway in the neuronal changes leading to long-term memory does not exclude the participation of other second messenger pathways. Rather, there is mounting evidence that other signaling cascades influence memory. This did not come as a surprise to those who recognized that the storage of memory is not a single process, but rather distinct processes with likely distinct molecular bases (for example, the conscious recall of facts vs the unconscious performance of a learned skill or a habit). In this context, it is noteworthy that the concept that protein kinases become modified so as to remain active at low concentrations of intracellular second messenger molecules has been generalized to include protein kinase C (PKC). To date, evidence from two laboratories [25,26] is consistent with translocation of PKC from the soluble to the particulate fraction in the induction phase of long term potentiation (LTP) in the hippocampus. This is followed by the accumulation of a persistently active form of the kinase in the maintenance phase of LTP [25].

Yet additional signaling mechanisms have been shown to influence memory in CNS. For example, other serine/threonine kinases such as mitogen-activated protein kinase (MAPK) [27,28], and calcium/calmodulin-dependent protein kinase [CaMK] [29,30] target to nuclear sites where they phosphorylate one or more cyclic AMP response element-binding proteins (CREBs) [31]. Although initial studies focused on positive regulatory effects of CREB1,
4. Means for the induction and evaluation of cardiac memory

Critical to Rosenbaum et al.'s definition of cardiac memory was that the T wave change occurs in an otherwise normal heart [1]. In fact, they considered cardiac memory a normal physiological variant whose significance lay, in part, in the extent to which it could be confused with the T wave changes of a variety of pathological conditions. For example, in patients with intermittent left bundle branch block inverted T waves are often present during conduction of sinus impulses with narrow QRS complexes [33–35], and these are sometimes misdiagnosed as indicators of ischemic heart disease. This was demonstrated by Denes et al. [36] and Engel et al. [37] who found angiographically normal coronary arteries and no evidence of coronary disease in patients with intermittent left bundle branch block and T wave abnormalities during normal A-V conduction. That pacing can give rise to a similar phenomenon was shown by Chatterjee et al. [2] who noted marked precordial T wave inversions on cessation of chronic right ventricular pacing. Moreover, they found a positive correlation between the duration of pacing and the time required for regression of the repolarization abnormalities. Gould et al. [38] described prominent, symmetric T wave inversions on ECG of patients on return to sinus rhythm after temporary right ventricular pacing. The T wave inversions, while most conspicuous in precordial leads [33,36,37] are also seen on limb leads [36,38]. Other conditions producing a similar ECG pattern on resumption of sinus rhythm include ventricular preexcitation [39], ventricular tachycardias [3,6] and repeated uniform extrasystoles [6,40].

In summary, clinical studies have described a phenomenon incited by a variety of events that themselves transiently alter depolarization and repolarization and subsequently have profound and long lasting effects on repolarization. Whereas the studies in human subjects clearly have provided the impetus for identifying cardiac memory, it is largely work in the experimental animal laboratory that is systematizing our understanding of the means by which memory is induced and the mechanisms for its evolution. These are summarized next, under short- and long-term memory.

4.1. Short-term memory

Recent animal research on short-term cardiac memory has employed intact animal, isolated heart or isolated tissue models. Del Balzo and Rosen, in 1992 [41], studied a canine model similar to that used earlier by Rosenbaum et al. [1] that permitted quantification of the T wave changes induced by pacing as well as the accumulation of these changes over sequential periods of recovery from pacing (Fig. 2), thereby fulfilling Rosenbaum et al.'s. [1] criteria. To put this model into perspective it is important to understand a critical aspect of the Chatterjee et al. [2] and the Rosenbaum et al. [1] studies. In both, the T wave axis of cardiac memory (i.e. on return to sinus rhythm after cessation of pacing) was concordant with the QRS axis that had occurred during pacing. This implied an obligatory concordance of the 'remembered' T wave with the paced QRS, and thereby implied a specific relationship between the pathway of activation (reflected in the QRS) and the change in repolarization (reflected in the T wave). Del Balzo and Rosen [41] selected pacing sites in the ventricle such that the 'remembered' T wave maintained a pattern reflecting the paced T wave, and not the paced QRS. This establishment of independence of the T wave of cardiac memory and the paced QRS complex led to two conclusions: first, that the complexity of cardiac memory was sufficiently great that patterns of repolarization might be expected to vary depending on pacing site, and second (and as a result of the first) that in a generic sense the term cardiac memory might be generalized to imply the persistence of any altered pattern of repolarization, in an otherwise normal heart, long after the return of its control pattern of repolarization might have been anticipated.

Another finding in the del Balzo and Rosen study was that IV administration of 4-aminopyridine (4-AP), a blocker of the cardiac transient outward current, \( I_{\text{to}} \), prevented the induction of cardiac memory [41]. This suggested that block of an ion channel that contributes to the transmural gradient for repolarization in the canine heart would prevent memory from occurring (Fig. 2). That non-specific alterations of repolarization induced by administration of drugs would not have the same effect as 4-aminopyridine was tested using IV lidocaine: after administration of lidocaine and demonstration of its attendant effects on repolarization, memory still could be elicited (Fig. 2) [41].
One of the critical aspects in evaluating this study, as well as any studies on cardiac memory, is to ensure the pacing protocol does not induce myocardial ischemia or failure. This was not done by del Balzo and Rosen, leaving open the possibility of some embarrassment of ventricular function. However, as reviewed below, subsequent experiments using long-term pacing to induce cardiac memory demonstrated the absence of ischemia or failure [15].

Because there is a gradient for the transient outward current $I_{to}$ across the canine ventricular myocardium (with density greatest in the epicardium) the success of 4-aminopyridine in abolishing memory in an isolated tissue model. Thin slabs of epicardium and endocardium were isolated from the canine ventricle and placed in a tissue bath. Pacemaker wires were affixed such that each slab could be paced parallel to (mimicking normal activation) or perpendicular to (mimicking the activation seen during ventricular pacing) the long axis of the myocardial fibers. Action potentials recorded from epicardial and endocardial slabs were recorded individually and in addition were channeled into a difference amplifier with the resultant waveform modeling the ECG (Fig. 3). The same pacing protocol and pacing cycle lengths used by del Balzo and Rosen [41] in the intact animal were used here, as well. In brief, it was found that pacing at two different cycle lengths from one site (simulating atrial pacing) did not induce any memory pattern on the simulated ECG; but pacing alternately from the two sites, simulating rapid ventricular pacing interspersed with normal sinus rhythm, induced a pattern consistent with memory. The gradients for repolarization were determined, and 4-AP and lidocaine were studied as in the intact animal. Again, 4-AP altered the gradient between the tissues (Fig. 4), whereas lidocaine did not (Fig. 5) [42].

Insights into the relationship between the cardiac activation pathway and repolarization were provided by Costard-Jäckle et al. [43]. They paced the right atrium of a Langendorff-perfused rabbit heart for a 45 min control period, paced the right ventricle for 120 min, and then returned to right atrial pacing for another 60 min. Monophasic action potential (MAP) recordings were made from right and left ventricular epicardium during the entire pacing sequence. The critical measurements made were of activation time to the various MAP recording sites and the duration of the MAP. During control atrial pacing, MAP
Fig. 3. Transmembrane action potentials from endocardial (ENDO, upper trace) and epicardial (EPI, middle trace) 1 cm x 1 cm x 2 mm tissue slabs and the resulting difference signal recording (DIFF, bottom trace) during a stimulation protocol incorporating changes in both stimulation rate and activation sequence. A. Stimulation from the basal border of the tissue slab (simulating normal activation) at a basic cycle length (BCL) of 650 milliseconds. The difference in activation time between epicardium and endocardium was 10 milliseconds in this experiment. Thus, the endocardial fiber is activated slightly before the epicardial one, resulting in a positive depolarizing deflection (QRS-like) in the difference recording. Because of the longer action potential duration (APD) in endocardium, repolarization of epicardium precedes that of endocardium, and the repolarization deflection in the difference recording is also positive. B. Both action potentials and the resulting difference signal recording during simulated ventricular pacing (St1) performed at lateral margin of preparation, perpendicular to the long axis of the myocytes. BCL = 450 milliseconds. In contrast to A, during ventricular pacing, activation occurs slightly earlier in epicardium, resulting in a negative depolarization ('QRS') wave in the difference recording, and APD is decreased more in endocardium than in epicardium, so that the repolarization (T) wave in the difference recording is inverted. This simulates secondary T wave changes. C. The action potentials and their difference signal recording 30 minutes after return to normal activation at BCL of 650 milliseconds. The picture at the end of the first recovery (R1) period is very similar to that in A. D. The end of the second 20-minute period of ventricular pacing at a BCL of 450 milliseconds (St2). As in B, a negative depolarizing and repolarizing waveform in the difference signals results from the changes in the action potentials. E. The action potentials and their difference signals 30 minutes after normal pacing rate and activation are resumed (R2). The depolarizing wave in the difference signal recordings is again upright (due to earlier activation of endocardium) but in contrast to A and C, the negative repolarizing wave has persisted. The third ventricular pacing period (St3), depicted in F, shows changes similar to those in B and D. G. The third recovery period (R3) after 30 minutes of normal activation at a BCL of 650 milliseconds. The amplitude of the persisting inversion of the ‘T wave’ in the difference signal is higher than in E, such that in the difference recording, progressive and accumulating ‘T wave’ inversions are demonstrated (reprinted from reference [42], by permission).

duration was inversely related to activation time: i.e., sites that were activated earlier repolarized later. When ventricular pacing was initiated, the inverse correlation between MAP duration and activation time was lost, a result that would suggest increased dispersion of repolarization. With the passage of further time during ventricular pacing, an inverse relationship was again established between activation time and MAP duration. Finally, on return to atrial

Fig. 4. Effect of 4-aminopyridine (4-AP) on transmembrane action potentials recorded from an epicardial (EPI, A) and an endocardial (ENDO, B) slab during normal activation. 4-AP decreases the phase 1 notch, plateau height, and APD_{90} in epicardium and decreases APD_{30} and APD_{90} in endocardium. The bottom half of the figure shows the superimposition of epicardial and endocardial action potentials. The electrical gradient between epicardium and endocardium during control (C) is greatly diminished by 4-AP (D). Co indicates control; APD_{30}, APD_{90}; action potential duration at 30% and 90% of repolarization, respectively (reprinted from reference [42], by permission).

Fig. 5. Effect of lidocaine (Lido) on transmembrane action potentials recorded from an epicardial (EPI, A) and an endocardial (ENDO, B) slab during normal activation. Lidocaine does not affect notch height or plateau amplitude in either tissue and reduces action potential duration more in endocardium than in epicardium. Superimpositions of epicardial and endocardial action potentials during control (C) and lidocaine superfusion (D) are depicted, and little change in the ventricular gradient between epicardium and endocardium occurs during lidocaine superfusion. Co indicates control (reprinted from reference [42], by permission).
Pacing there was again a loss of the inverse relation which, by the end of 60 min of atrial pacing, was reestablished.

In evaluating this work it is essential to understand that Costard-Jäckle et al. [43] were not studying cardiac memory, per se, which is a T wave change conditioned by pacing or arrhythmias. However, they were studying an important factor underlying the T wave change, that is, the effect of activation on repolarization. They mention that “the inverse relation between activation time and APD appears to be the result of a very slow mechanism that conditions ventricular repolarization according to the sequence of ventricular activation.” This process is very slow in light of many electrophysiologic studies which often see changes occurring on a beat to beat basis. However, that the changes of memory require minutes is certainly not a slow process in light of the biochemical and metabolic events thought to influence and control memory. Moreover, as noted by Costard-Jäckle et al. [43] it may be that the activation change that occurs is associated with an alteration in current flow through gap junctions. They state, “It is conceivable that repeated current flow through these junctions may decrease their resistance, thereby amplifying the electrotonic effect. If conditioning of cellular electrical communication through repeated uniform use is indeed responsible for the slow modulation of repolarization (and its retention) myocardium can be thought to possess some form of rudimentary ‘memory’ [43].”

The findings of Costard-Jäckle et al. [43] presuppose that activation pathway influences repolarization via a mechanism that involves transmission of information, presumably electrical, through gap junctions. One aspect of this view bears further elaboration: that is, not only electrical information, but chemical signals are transmissible through gap junctions. Among the molecules that can be passed from cell to cell is cAMP [44], which is increased in amount at times of sympathetic neural stimulation, which, in turn, can be induced by electrical stimulation of the myocardium. Moreover, cAMP is one of the second messengers that lead to long term memory in the central nervous system [31]. This is consistent with the hypotheses that cAMP or other second messenger levels are locally elevated at the time of cardiac pacing and via gap junctional transmission establish a concentration gradient across distances in the myocardium, thereby phosphorylating channels and influencing electrical gradients.

4.2. Long-term memory

The work described above in the intact heart, the isolated perfused heart and isolated cardiac tissues provides methods for studying and a framework for interpreting certain of the mechanisms underlying cardiac memory. It establishes, as well, that short-term cardiac memory is influenced importantly by an altered pathway for activation which modifies transmural voltage gradients for repolarization. The latter may result from effects on a specific ion channel or channels (it is difficult if not impossible to ascribe the action of 4-AP uniquely to an effect on \( I_{Na} \), given its actions on cardiac nerves and on other repolarizing channels). However, the experiments reviewed do not address the mechanisms that underlie long-term memory. Moreover, the brief, unstable nature of short-term memory limits our opportunity to investigate potentially important ion channel or molecular determinants. The latter are more amenable to study in a setting where memory is sufficiently established that the disaggregation of cardiac myocytes necessary for identification of functional and structural alterations in channels can be achieved without concern over a change in cellular properties during the interval needed for cell preparation.

For the above reasons, as well as a primary interest in the mechanisms of long term cardiac memory, a conscious canine model for the latter was developed [15]. Animals were instrumented with a ventricular pacemaker and ECG recording leads, permitted to recover from surgery for 2 to 3 weeks to ensure a stable ECG, and then paced for weeks at 10 to 15% faster than sinus rate. Several times a week the pacemaker was turned off to observe the T wave in sinus rhythm, and once a steady state change in the T wave occurred, pacing was permanently discontinued. At this time the intact animal experiment was either terminated to permit study of ventricular myocytes or continued such that the resolution of the memory process could be determined.

Approximately three weeks of pacing were required to induce a steady state change in the T wave vector [15]. This change was seen in two ways; firstly, as an alteration in the T wave axis to approximate that of the paced QRS complex. This criterion is precisely that described earlier by Rosenbaum et al. [1] and by Chatterjee et al. [2]. Secondly, the T wave increased in amplitude to a new steady state. This pattern of T vector change was elicited regardless of the site of ventricular pacing: the ‘memorized’ T wave always followed the paced QRS (Fig. 6). Even though a steady state was reached in about three weeks, the longer the pacing persisted beyond three weeks, the longer the memory that was induced persisted.

We emphasize that the measurements of time required for onset, steady state and resolution of memory reflect a protocol for pacing the ventricles at a rate only 10–15% greater than sinus. Based on earlier work [45] we assume that the faster the pacing rate, the more rapidly will a steady state be established; i.e., the process directly reflects the number of paced beats that have occurred. However, maintenance of a relatively slow pacing rate is necessary to ensure that the means used to induce memory do not induce either cardiac ischemia or failure. That such pathology does not eventuate with this protocol was evidenced by microsphere studies which have shown no decrease in myocardial flow; hemodynamic and echocardiographic studies showing no evidence of congestive failure; studies of cell capacitance, reflecting myocyte size,
showing no differences between control dogs and those with cardiac memory [15,46]; and histological studies showing no myocardial edema or cell size abnormalities (P. Danilo, preliminary data).

Hence, it is possible to induce cardiac memory, with its typical changes in the T wave, in the absence of any alteration in myocardial function. Moreover, resolution of the memory process long outlasts the termination of pacing, suggesting persistent retention of information. It is reasonable to hypothesize that such retention reflects new protein synthesis, as appears to be the case for the central nervous system [22–24]. If we accept the view that long-term neuronal memory involves new protein synthesis, then in applying this view to heart, we would surmise that some level of new – or altered – protein synthesis would occur here, as well, and might be occasioned by the change in activation pathway.

The work of Karpawich et al. [47] in which the ventricles of neonatal dogs were chronically paced provides evidence that a change in activation pathway can modulate protein synthesis in heart. Structural study of ventricular myocytes from paced hearts demonstrated altered orientation of the myofibrillar arrays of individual myocytes, reflecting the pacemaker-induced activating wavefront for the ventricle. Given this demonstration of altered myofibrillar orientation visible structurally (consistent with a change in synthesis of myofibrillar proteins), it is not difficult to envision that periods of pacing sufficient to induce cardiac memory might alter the synthesis and orientation of other proteins as well (such as those of ion channels) thereby contributing to the phenomenon of memory. Moreover, although protein synthesis inhibitors like cyclohexamide which suppress long-term memory in CNS [48] are non-selective drugs, administration of cyclohexamide delays the onset and magnitude of cardiac memory in dogs [15] giving credence to the hypothesis that altered protein synthesis is important.

5. Signal transduction and cardiac memory

In the setting of ventricular pacing or arrhythmia, the altered activation pathway of part or all of the ventricle can be assumed to alter the mechanical pathway of contraction and, with this, to alter the direction of stretch and relaxation on individual myocytes. In effect, there should be a significant change in deformation of myocytes with every systole. Moreover, Katz [11] has suggested that many conditions leading to cardiac memory are associated with regional overdistention and myocyte deformation. These, in turn, activate multiple intracellular signal transduction pathways and can lead to altered expression of genes determining structure and function of ion channels. It is clear, as well, that functional and – ultimately – structural changes in the myocardium occur as a consequence of ventricular pacing as well as of pathophysiologic
events that alter myocardial activation. For example, epicardial pacing of the left ventricular apex of anesthetized, open-chested dogs at rates of ~110 bpm for consecutive 15 minute periods changes epicardial fiber strain to ~23% of control in regions close to the pacemaker electrode and to ~250% of control in late-activated regions of the left ventricle [49]. In studies of conscious dogs paced for three months from the left ventricular epicardium, wall thickness of the areas of earliest activation were reduced by about 20%, a finding that was paralleled in patients with left bundle branch block, in whom the early-activated septum was thinner than the late-activated posterior wall of the left ventricle [50]. The point made here is that in settings of pacing-induced or pathology-induced altered ventricular activation there are resultant, profound mechanical changes expressed at the level of the fiber in particular and the myocardium, in general.

The following discussion considers pathways that are activated by altered mechanical stretch and that might influence repolarization. Mechanical stretch has been linked to the accumulation of cAMP, activation of PKA and accelerated rates of protein synthesis and ribosome formation in perfused adult rat hearts [51] (although no cAMP elevation is observed in stretched, cultured neonatal myocytes) [52]. In this way the cAMP second messenger system so important to memory in CNS can be brought into play.

Dilatation and stretch also stimulate phosphoinositide hydrolysis in isolated perfused atrial and ventricular myocardium [53] and induce modest and transient increases in inositol phosphate accumulation in cultured neonatal rat ventricular myocytes [52,54,55]. The effect of mechanical stretch of cardiac myocytes to stimulate phospholipase C and phospholipase D activity results in a sustained increase in diacylglycerol content and activation of PKC [48]. Other signaling molecules activated by mechanical stretch in cultured neonatal myocytes include tyrosine kinases [52,56], p21 

Important, serine/threonine protein kinases mobilized during mechanical deformation of cardiac myocytes phosphorylate and functionally modify numerous critical target proteins which could lead to important acute changes in the electrophysiologic properties of the heart. Among these are proteins involved in the regulation of intracellular calcium homeostasis (phospholamban and the cardiac isoform of the ryanodine receptor [59–62]), and plasma membrane ion channels (including the voltage-sensitive calcium channel [63]). Recent studies also indicate that stretch-induced activation of signaling cascades could lead to the modulation of gene expression and thereby induce relatively stable alterations in the cardiac electrophysiologic phenotype. For example, in the adult intact ventricle, hemodynamic overload leads to induction of proto-oncogenes and re-expression of fetal isoforms of contractile proteins as well as other genes normally expressed in the ventricle only during perinatal life [64–66] and static stretch of cultured neonatal myocytes causes hypertrophy and induces expression of multiple members of the early immediate gene program [67,68].

Which of the many signal transduction pathways operative in heart are important to the memory process is uncertain. One candidate is the local cardiac angiotensin system, which plays a critical role in acquisition of a hypertrophic phenotype. This system is of interest as well in that angiotensin II increases and saralasin suppresses memory in brain [61]. In the heart, Sadoshima and Izumo [69,70] demonstrated that angiotensin II increases protein synthesis and induces many early immediate genes as well as several ‘late’ markers for cardiac hypertrophy. Angiotensin II increases intracellular levels of inositol triphosphate, diacylglycerol, phosphatidic acid, and arachidonic acid suggesting activation of phospholipase C, phospholipase D and phospholipase A2. Results of studies with various pharmacologic agents suggested that phospholipase C and PKC are linked to c-fos gene expression. Izumo’s laboratory recently established that angiotensin II is a critical mediator of stretch-induced hypertrophy by demonstrating that (1) stretch induces the release of angiotensin II and increases angiotensinogen gene expression, (2) the stretch response involves an autocrine or paracrine mechanism since stretch conditioned medium, when transferred to non-stretched myocytes mimics the effects of stretch to induce c-fos expression and activate ERK, and (3) stretch-induced hypertrophy is blocked by angiotensin II-specific receptor antagonists [71]. These observations provide a framework to consider the role of the cardiac angiotensin system as a critical mediator of cardiac myocyte responses to mechanical stimuli and one of particular interest in light of our own preliminary observations that the angiotensin II receptor blocker, saralasin, the angiotensin enzyme inhibitor, captopril, and the tissue chymase inhibitor, chymostatin, all prevent short-term cardiac memory [72].

6. Cardiac memory at the level of the action potential and ion channel

It is likely that more than one ion channel is involved in the induction of cardiac memory. Much of the work done on ion channels to date, however, has focussed on the transient outward current, I_o, which is represented more in epicardium than endocardium. I_o is responsible for the phase 1 notch of the action potential, such that cells having the current (such as epicardial myocytes) manifest a typical ‘spike and dome’ configuration, and cells largely lacking
the current (such as endocardial myocytes) manifest little or no notch (e.g. [73, 74]). A key aspect of $I_{\text{Na}}$ is the dependence of its expression on stimulus rate: at rapid rates (i.e., greater than 120–130 bpm), even cells having large $I_{\text{Na}}$ manifest a minimal phase 1 notch; at slow rates (i.e., less than 40–60 bpm) the notch is large. Our initial hypothesis for the genesis of short- and long-term cardiac memory was that the pacing and altered activation protocols used to evoke memory diminish $I_{\text{Na}}$, thereby contributing to an alteration in the transmural gradient for repolarization and the expression of the T wave. As described above, the hypothesis evolved and was tested, initially, in animal [41] and isolated tissue [42] models of short-term memory.

However, protocols for inducing long-term memory offer a more definitive means for studying the role of ion channels. In one such study, we paced hearts for three weeks to induce memory, discontinued pacing and within 10 hours thereafter, removed the heart, disaggregated left ventricular anterior free wall epicardium and studied myocytes via voltage clamp [45, 46]. This protocol demonstrated that long-term memory is associated with profound changes in $I_{\text{Na}}$, as follows: the threshold for activation of $I_{\text{Na}}$ was shifted above approximately 15 mV positive to control and the recovery from inactivation of $I_{\text{Na}}$ was delayed by over an order of magnitude (i.e., from ~35 to ~600 msec). Moreover, the peak current density measured for $I_{\text{Na}}$ was reduced by approximately 1/3. An interesting corollary to these observations concerning $I_{\text{Na}}$ was that message levels for Kv 4.3, the $I_{\text{Na}}$ channel protein in canine heart, were diminished by approximately 1/3: i.e. showing a decrease in mRNA comparable to that in current density [46]. Therefore, it would appear that the channel protein is altered in the setting of cardiac memory. Such a result is consistent with the hypothesized alteration in protein synthesis.

These characteristic changes in $I_{\text{Na}}$ occur in a situation where the heart has been paced from the ventricle for approximately three weeks and has then returned to sinus rhythm. The changes in $I_{\text{Na}}$ function persist despite the return to sinus rhythm, but are not seen in settings where the ventricle is paced for comparable periods and memory is not induced. Nor are the T wave changes characteristic of cardiac memory inducible in hearts paced atrially at the same rates and for the same periods [15]. Finally, in hearts in which $I_{\text{Na}}$ current is not expressed, e.g. in the dog less than about 60 days of age, cardiac memory is not inducible using short-term pacing protocols (P. Danilo, unpublished data).

The alterations in the action potential that accompany the changes of $I_{\text{Na}}$ in long term memory have been studied using epicardial and midmyocardium at a local site in the left ventricular free wall. Accompanying memory are a loss of the notch and prolongation of action potential duration in epicardial muscle. Yet, there is comparable prolongation of repolarization in endocardium, and there is no significant effect on duration of repolarization in midmyocardium (Fig. 7) [15]. Given the prolongation of repolarization in epicardial and endocardium with no change in midmyocardium we have suggested that the transmural gradient for phases 2 and 3 of repolarization that determine T wave axis and amplitude would be altered in memory [15]. Moreover, with the notch being lost on the epicardial action potential and repolarization being prolonged in both epicardium and endocardium (the latter having little $I_{\text{Na}}$) [73, 74] and with the minimal change in repolarization in midmyocardium (which has a large $I_{\text{Na}}$) it is likely that ion channel(s) in addition to $I_{\text{Na}}$ are (are) involved importantly in the memory process [15]. Although not yet studied, likely candidates are reduced $I_{K_{\text{C}}, I_{K_{\text{C}}}}$ and/or $I_{K_{\text{Ca}}, I_{K_{\text{Ca}}}}$ and/or increased $I_{K_{\text{Ca}}, I_{K_{\text{Ca}}}}$, late $I_{Na}$, as well as up-regulation of the Na/Ca exchanger.

7. A unifying concept for cardiac memory

Integrating this information, it is possible to hypothesize mechanistic pathways for the generation of both short- and long-term memory (Fig. 8). During ventricular pacing, the activation pathway of the heart is altered. The result is a change in the pattern of stretch on cardiac myocytes and on cardiac sympathetic nerves. As stated above, altered stretch on cardiac myocytes in culture induces activation of their angiotensin (and other signaling) systems. If the same is true in the heart paced from the ventricle, one would expect that the accumulation of short-term memory would be attenuated by interfering with the tissue angiotensin II cascade which, in fact, does occur (see above). A role for the sympathetic nervous system can also be proposed here; based on a variety of experiments demonstrating association of ventricular pacing with increased sympathetic neural activity, (e.g. [16–19]) and based on the role of cAMP in neural memory [24, 31]. Also demonstrated in Fig. 8 is initiation of new protein synthesis contributing to genetically-based alterations in channel structure and/or current density and in channel function. These latter events would contribute to long-term memory.

8. Implications of cardiac memory with respect to cardiac rhythm

The implications of memory with respect to clinical arrhythmias have received little attention. Certainly, to the extent that cardiac memory alters the configuration of the T wave and the duration of the Q-T interval, one might expect changes in the effective refractory period to accompany memory. In preliminary studies we have found these to occur in the setting of short-term memory. However, there is no specific pattern to these changes, i.e. refractoriness increased in some experiments, suggesting protection from the propagation of premature beats, and decreased in
Fig. 7. Action potential duration to 50% (APD$_{50}$) and 90% (APD$_{90}$) of repolarization measured from epicardium (Epi) and endocardium (Endo) of LV in a control dog (A) and a dog with cardiac memory (B). Preparations were paced over a range of cycle lengths (CL: horizontal axis) from 2000 to 400 ms. Three to five minutes was required for equilibration at each cycle length. Recordings were made at 10 minutes. Three sets of preparations were used per heart, and multiple impalements were made per preparation. The shaded areas are those inscribed between APD$_{50}$ and APD$_{90}$ of the epicardial and endocardial preparations. In A at almost all cycle lengths, almost all values for endocardium differ from those at corresponding cycle lengths in epicardium ($P<0.05$ for all). In B, statistical significance ($P<0.05$) was seen only for APD$_{90}$ at the two longest cycle lengths. Hence, with the occurrence of memory there is a marked change in the gradient between the two sites (reprinted from reference [15], by permission).

Fig. 8. Schematic for short- and long-term cardiac memory evolution. See text for discussion.

Allessie et al. on atrial fibrillation, indicating that ‘fibrillation begets fibrillation’, and its corollary, that sinus rhythm begets sinus rhythm, both suggest that a pattern held in memory by the heart can facilitate further patterning [75,76]. Allessie and associates have demonstrated very clearly that in the setting of pacing to induce fibrillation there is shortening of the effective refractory period of the atrium which increases the susceptibility to further bouts of fibrillation. However, the interpretation of refractory period shortening as indicative of atrial memory is premature in light of current information, for at least two reasons. First, the return of the effective refractory period to control values in Allessie’s experiments was a matter of only days despite the long period of pacing needed to induce them. This is not consistent with long-term memory, although it might be consistent with the short-term or intermediate processes. Second, a study of the isolated, perfused rabbit heart [77] using a variation on the model initially employed by Costard Jäckel et al. [43] was unable to demonstrate even short-term memory in the rabbit atrium. Moreover, it must be emphasized that the key change in atrium is a shortening of action potential duration and the effective refractory period, in association with reduced $I_{Ca,L}$ [78].

However, given the ubiquity of memory in other tissues including the ventricles, it is possible that memory in atrium is simply expressed differently than that in ventri-
References


