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Cardiac Memory Evolves With Age in Association With Development of the Transient Outward Current

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Background—Calcium-insensitive transient outward current (I_{to}) is important to the development of cardiac memory (CM), which itself reflects the capacity of the heart to remodel electrophysiologically. We used cardiac pacing to test the hypothesis that CM evolution can be explained by developmental maturation of I_{to} .

Methods and Results—Acutely anesthetized dogs from 1 day old to adult were paced from the left ventricle (VP, n=29) or left atrial appendage (AP, n=12) to induce CM. T-wave vector displacement (TVD) obtained during VP was greater than with AP (adults, 0.39 ± 0.06 mV; neonates, 0.04 ± 0.01 mV; $P < 0.05$). TVD began to increase at ≈ 40 days of age, reaching adult levels by ≈ 200 days. Microelectrode studies performed in 18 dogs (ages 3 to 94 days) after completing the CM protocol and 20 additional dogs (1 day old to adult) revealed that the epicardial action potential notch was absent in neonates, became apparent in the young, and was deepest in adults. The relationship between TVD and epicardial notch was such that as notch magnitude increased, TVD increased ($r = -0.65$, $P < 0.05$). KChIP2 and Kv4.3 mRNA (measured via reverse transcription–polymerase chain reaction) also increased with age.

Conclusions—The inducibility of CM gradually increases with age in association with evolution of the epicardial action potential notch and mRNA expression for KChIP2 and Kv4.3. This suggests that the capacity of the heart to remodel electrophysiologically and to manifest memory during development depends in part on evolution of the determinants of I_{to} . (*Circulation*. 2004;110:489-495.)

Key Words: electrocardiography ■ electrophysiology ■ ion channels ■ genes ■ pacing

Cardiac memory is a T-wave change on the ECG that can be induced by ventricular pacing (VP) or arrhythmia, such that the T wave persists in following the vector of the abnormal QRS complex even after normal sinus rhythm and normal ventricular activation have returned.¹⁻³ As is the case for memory in the central nervous system, cardiac memory is a specialized, nonpathological form of remodeling induced by electrical stimulation.^{4,5} We previously have demonstrated that blockade of the transient outward potassium current, I_{to} , suppresses cardiac memory both in situ³ and in vitro.⁶ We and others have shown that developmental maturation of I_{to} occurs in canine heart,^{7,8} such that the current is not manifested before ≈ 2 months of age. Given these findings, we now hypothesized that cardiac memory would not be demonstrable at birth in canine heart and would evolve concordantly with the epicardial action potential notch, a surrogate for I_{to} . If the hypothesis was verified, it would suggest that cardiac memory cannot occur until the evolution of normal developmental modeling has determined the myocardial substrate.

We performed experiments in intact animals and isolated ventricular tissues to test the evolution of memory and its

concordance with the action potential notch, inscription of which depends on I_{to} .⁹ We also measured mRNA for Kv4.3 and KChIP2, the molecular correlates of I_{to} in canine heart.¹⁰⁻¹²

Methods

All experiments were performed according to protocols approved by the Columbia University Institutional Animal Care and Use Committee.

Surgical Preparation

Male and female mongrel dogs, ages 3 days to adult, were anesthetized with thiopental 17 mg/kg IV, intubated, and ventilated with isoflurane 1.5% to 3.0% and oxygen. A heating pad was used to maintain body temperature within physiological limits.

A thoracotomy was performed at the fifth left intercostal space, and the heart was suspended in a pericardial cradle. Platinum bipolar electrodes were sewn to the epicardium of the left atrial appendage (LAA) and the inferolateral wall of the left ventricle. Animals were equilibrated during LAA pacing (AP) at cycle length (CL)=400 ms for 15 minutes (4 animals ages 1 to 4 days were not atrially paced because the LAA was too small for electrode placement). Twenty-nine dogs, ages 3 days to adult, were paced for 2 hours from the left

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TABLE 1. Baseline ECG Characteristics

ECG	Neonates	Young	Adults
Heart rate, bpm	138±4	147±3	149±0
PR, ms	94±9	117±7	123±8
QRS, ms	58±4	55±1	65±2*
QT, ms	253±15	228±2	236±8
QTc, Bazett	380±14	357±5	373±16
QTc, Van de Water	301±13	279±3	288±8
QTc dispersion	15±2	18±1	21±3

*P<0.05 vs young.

ventricular (LV) site at CL=400 ms (VP) to induce cardiac memory. After 2 hours of pacing, AP was reinstated to estimate evolution of cardiac memory. Twelve control dogs, ages 6 days to adult, were paced from the LAA for the same time interval and at the same CL.

ECG Recordings

ECGs were recorded and frontal plane vector images plotted by use of Dr. Vetter PC-EKG software. ECG intervals were measured from at least 5 consecutive complexes at each experimental time point and averaged values analyzed. QT intervals were rate-corrected by use of the methods of Van de Water [QTc=QT-0.087×(RR-1000)]¹³ and Bazett¹⁴

$$\left(QTc = \frac{QT}{\sqrt{RR}} \right)$$

All ECG intervals were calculated in milliseconds. Cardiac memory was quantified as T-wave vector displacement (TVD), expressed as distance between frontal plane T-wave vector peaks recorded during AP before onset of VP and at intervals thereafter (see Plotnikov et al¹⁵ for details).

QTc dispersion was calculated with PC-EKG software as the difference between average minimal and maximal QT intervals across 7 ECG leads (6 precordial leads plus V₁₀) during 5 consecutive beats by use of the Bazett equation.

Isolated Tissue Studies

Hearts were removed from 18 dogs ages 3 to 94 days and adult after the end of the pacing protocol in the intact animals. Twenty additional dogs of ages 1 to 180 days and adult that had not been paced were anesthetized with sodium pentobarbital (30 mg/kg IV or 40 mg/kg IP). Hearts were removed through a left lateral thoracotomy and immersed in cold Tyrode's solution equilibrated with 95%

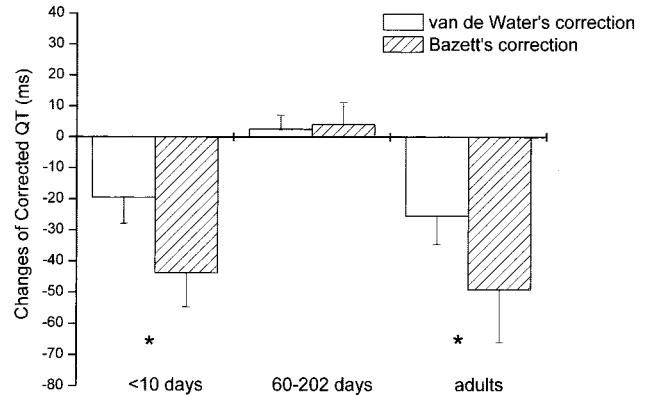


Figure 1. Comparison of changes in QTc interval in each age group after 2 hours of pacing calculated by use of Bazett's and Van de Water's equations. In adults and in neonates <10 days old, QTc intervals were significantly longer when calculated by use of Van de Water's equation. *P<0.05 vs 2 correction methods.

O₂/5% CO₂ and containing (mmol/L) NaCl 131, NaHCO₃ 18, KCl 4, CaCl₂ 2.7, MgCl₂ 0.5, NaH₂PO₄ 1.8, and dextrose 5.5. Epicardial strips were filleted with surgical blades parallel to the surface of the LV free wall. The preparations were placed in a tissue bath and superfused with control Tyrode's solution (37°C, pH 7.35±0.05). Solution was pumped at 12 mL/min, changing chamber content 3 times per minute. The bath was connected to ground via a 3-mol/L KCl/Ag/AgCl junction.

Preparations were impaled with 3 mol/L KCl-filled glass capillary microelectrodes having tip resistances of 10 to 20 MΩ. The maximum upstroke velocity of the AP (\dot{V}_{max}) was obtained by electronic differentiation with an operational amplifier. The electrodes were coupled by an Ag/AgCl junction to an amplifier with high input impedance and capacity neutralization. Transmembrane action potentials and \dot{V}_{max} were digitized with an analog-to-digital converter (D-210, DATAQ Instruments Inc) and stored to a personal computer for subsequent analysis. Preparations were driven at cycle lengths from 4000 through 250 ms in sequence by use of standard techniques to deliver square-wave pulses 1.0 ms in duration and 1.5 times threshold through bipolar Teflon-coated silver electrodes. Each frequency was maintained for 3 minutes before data were collected. Experiments began after 3 hours of equilibration in control Tyrode's solution. To study the effects of I_o blockade, 4-aminopyridine (4-AP, 3×10⁻⁵, 10⁻⁴, and 10⁻³ mol/L) was added to control Tyrode's solution, and measurements commenced after 30 minutes of equi-

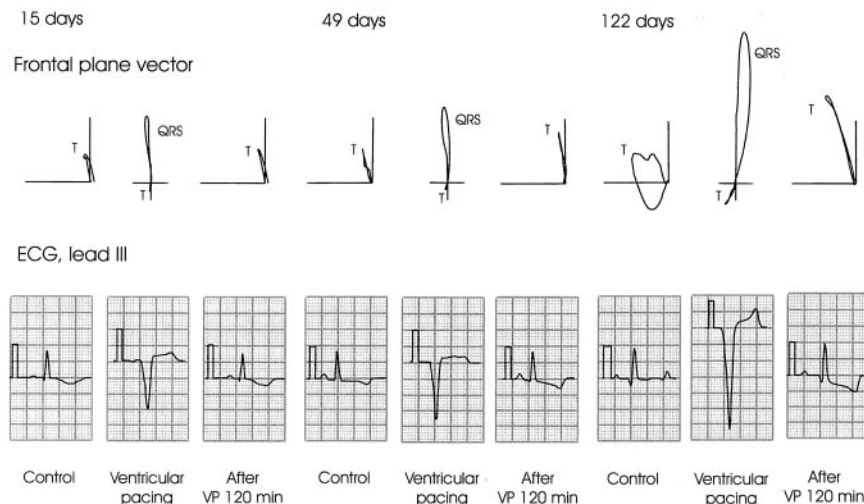


Figure 2. Developmental evolution of cardiac memory in 15-, 49-, and 122-day-old dogs. Top, Frontal plane T-wave vector projection and paced QRS vector; bottom, ECG. For each animal, left column shows control measurements during AP; center, paced QRST complex and its vector; and right, AP after 2 hours of VP. T indicates T-wave vector; QRS, paced QRS vector.

bration at each concentration. Only data from impalements maintained throughout an experiment were used for analysis.

mRNA Studies

In 14 additional dogs of ages <10 days, 40 to 50 days, and adult, LV epicardial tissue samples were collected as for the microelectrode studies. Total RNA was extracted by use of the RNeasy midi kit from Qiagen. First-strand cDNA was generated from 2 μg of total RNA by use of Superscript II (Invitrogen). Real-time polymerase chain reaction (PCR) was performed in duplicate by use of a light cycler (Roche) with a reaction mixture composed of 2 μL of the cDNA mixture, 2 μL of LightCycler–Faststart DNA master SYBR Green I (Roche), 4 mmol/L Mg^{2+} , and 0.5 $\mu\text{mol/L}$ of each primer in a final volume of 20 μL . The oligonucleotides used were KChIP2 (accession number, AF458385; amplicon size, 164 bp; 60°C); forward primer, 5'-GCTGGTTTGTCTGGTGA; reverse, 5'-CGGAGTG-CAGGATACG; and Kv4.3 (accession number, AF049887; amplicon size, 153 bp; 60°C); forward, 5'-AGAAGTCCTTCGCTGT; reverse, 5'-CTCACTGCCCTGGATG. The following reaction conditions were used: an initial denaturation at 95°C, 10 minutes, followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at 60°C for 5 seconds, and extension at 72°C for an amplicon-size–dependent annealing time (calculated: amplicon size/25) with a single fluorescence acquisition point. This was followed by a melting curve program from 65°C to 95°C with continuous fluorescence acquisition. Product sequences were verified by sequencing (DNA facility, Columbia University, NY).

For each target, relative differences in samples were calculated by use of light cycler analysis software (Roche). A standard curve was generated from a serially diluted adult sample.

Statistics

Data were analyzed by use of 1- and 2-way repeated-measures ANOVA to estimate effect and duration of pacing between the AP and VP groups. Subsequent analysis used the Bonferroni test when variances were equal and Games-Howell when variances were unequal. A polynomial regression analysis was conducted to evaluate the relationship between T-wave vector displacement and age. Real-time PCR data were analyzed with 1-way ANOVA. All data are presented as mean \pm SEM. A value of $P < 0.05$ was considered significant.

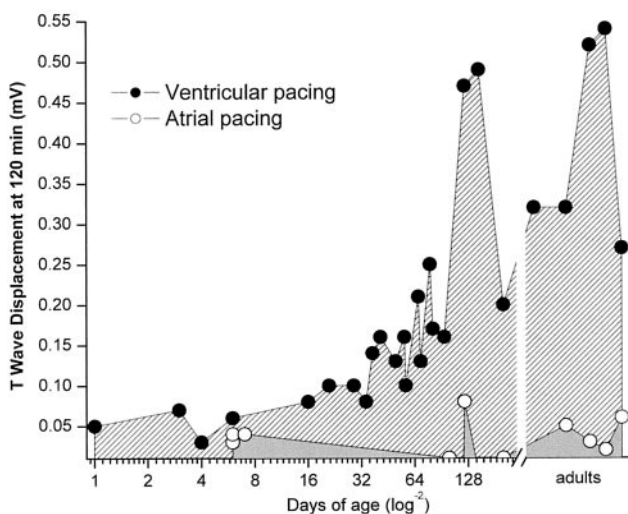


Figure 3. Cardiac memory represented as T-wave vector displacement in dogs of different ages after 2 hours of VP or AP. Horizontal axis is age plotted on a \log^{-2} scale. At all ages, AP induced minimal displacement. Conversely, VP induced minimal displacement in dogs of 1 to 40 days and a gradual increase of displacement thereafter. There is comparable development of cardiac memory in 3-month-old dogs and adults.

Results

Intact Animal Study

Control ECG data are presented in Table 1. Regardless of the QT correction method, no significant developmental change in QT intervals was seen. Changes in the rate-corrected QT intervals after AP or VP and a comparison between 2 different methods of rate correction are shown in Figure 1. Note that the change in QTc was always greater with the Bazett than with the Van de Water formula.

Figure 2 shows representative ECGs and vectorcardiograms of 15-day-old, 49-day-old, and 122-day-old dogs. At 15 days, there is little change in T wave or T-wave vector during the course of the cardiac pacing protocol, whereas at 49 days, modest changes are evident, and at 122 days, markedly increased vector amplitude and displacement occur, as is typical of cardiac memory. Table 2 summarizes all vectorcardiogram data for the study, and Figure 3 details T-wave vector displacement in each animal. Although significant changes in T-wave vector displacement occur only in the adult, the beginnings of substantive change are seen in the vector amplitude and angle of the young (ie, 50-day age range) animals. The T-wave vector displacement recorded during VP was significantly greater than that with AP (in adults, 0.39 ± 0.06 versus 0.04 ± 0.01 mV, respectively, $P < 0.05$).

Isolated Tissue Studies

Representative action potentials for neonatal, young, and adult LV epicardium are shown in Figure 4A. Maximum diastolic potential and action potential duration increased

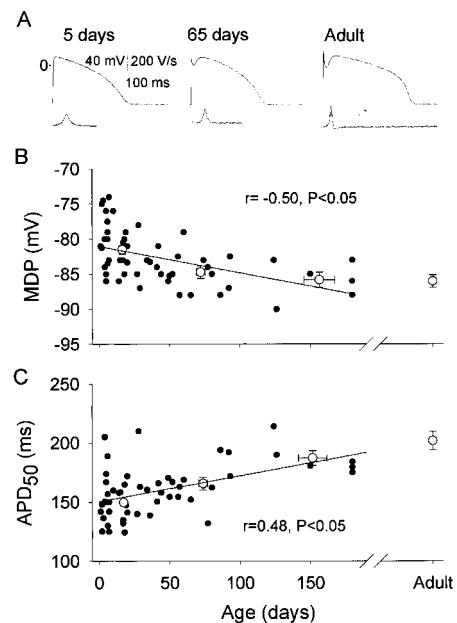


Figure 4. A, Representative transmembrane potentials recorded from LV epicardium of 5-day-old, 65-day-old, and adult dogs at $\text{CL} = 1000$ ms. Top traces show action potentials and bottom, V_{max} . Vertical calibration is for action potentials and V_{max} ; horizontal for action potentials. B and C, Plots showing correlations between age and maximum diastolic potential (B) or action potential duration to 50% repolarization (C) of LV epicardial action potentials at $\text{CL} = 1000$ ms.

TABLE 2. Vectorcardiogram Characteristics

	Neonates	Young	Adults
T-vector displacement after VP 60 minutes	0.06±0.01	0.12±0.03	0.29±0.05‡§
T-vector displacement after VP 120 minutes	0.07±0.00	0.18±0.02	0.39±0.06‡§
T-vector amplitude before VP	0.21±0.05	0.14±0.02	0.46±0.11
T-vector amplitude after VP 60 minutes	0.21±0.03	0.24±0.04*	0.73±0.16*‡§
T-vector amplitude after VP 120 minutes	0.19±0.04	0.29±0.03*†	0.83±0.16*‡†
T-vector angle before VP	-89.67±13.84	-128.80±11.91	-111.00±2.92
T-vector angle after VP 60 minutes	-93.33±12.17	-108.00±5.30*	-100.80±1.50
T-vector angle after VP 120 minutes	-88.33±16.68	-102.20±1.93*	-95.20±9.18

* $P<0.05$ vs before VP.† $P<0.05$ vs VP 60 minutes.‡ $P<0.05$ vs neonates.§ $P<0.05$ vs young.

significantly with age (Figure 4, B and C). There were no age-dependent changes in action potential amplitude (data not shown), whereas \dot{V}_{\max} increased with age: at CL=1000 ms, it was 111 ± 5 V/s in neonates (n=28 preparations) and 221 ± 15 V/s in adults (n=18 preparations, $P<0.05$).

The action potential notch was absent in neonates, became apparent in the young, and was deepest in adults (Figure 4A) over a wide range of cycle lengths. Moreover, the age-related increase in notch amplitude was significant ($P<0.05$) at all cycle lengths from 250 to 4000 ms, with r values ranging from 0.88 to 0.92. The correlation between the changes in

notch potential of isolated tissues and in T-wave vector displacement on the ECG is explored in Figure 5. Note the concordant evolution of both variables. Moreover, there was a significant relationship between TVD and epicardial notch potential (see inset in Figure 5) such that as the notch attained more negative potentials, TVD increased ($r=-0.65$, $P<0.05$).

In a subset of epicardial preparations, we examined the effects of the I_{to} blocker 4-AP. In neonates, 4-AP induced only action potential prolongation, whereas at older ages, it decreased notch amplitude while exerting no effect on action

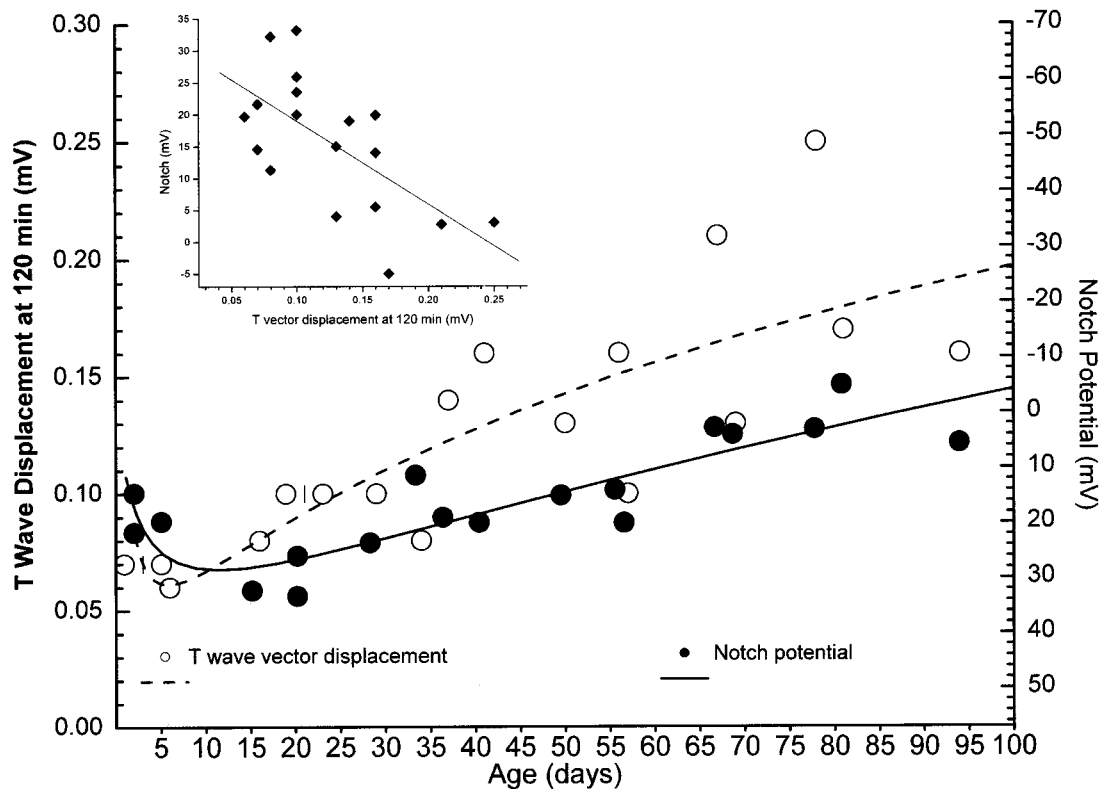


Figure 5. Developmental changes of epicardial notch potential recorded during pacing at CL=1000 ms and evolution of T-wave vector displacement in dogs ages 1 to 95 days. Small positive notch recorded in younger animals becomes deeper (ie, more negative) with age, and magnitude of cardiac memory expressed as T-wave vector displacement (TVD) gradually increases with age. A scatterplot of TVD and epicardial notch potential from same animals (inset) indicates that 2 variables are linearly related ($r=-0.65$, $P<0.05$).

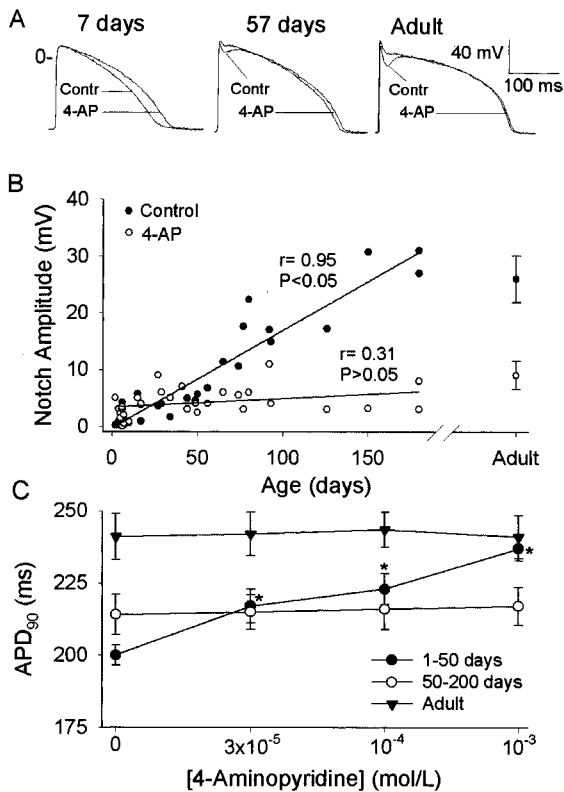


Figure 6. A, Representative experiments illustrating effects of 10^{-3} mol/L 4-AP on LV epicardium of 70-day-old, 57-day-old, and adult dogs. B, Correlations between notch amplitude of LV epicardial action potential and age before (control) and after application of 10^{-3} mol/L 4-AP. Corresponding adult values ($n=12$) are shown on right. C, Concentration-dependent effects of 4-AP on action potential duration at 90% repolarization in 1- to 50-day-old ($n=19$), 50- to 200-day-old ($n=12$), and adult ($n=12$) dogs. CL=1000 seconds. * $P<0.05$ vs respective control (zero drug concentration).

potential duration (Figure 6A). The strong correlation between age and notch potential disappeared in the presence of 4-AP (Figure 6B). Summary data for the effects of 4-AP on action potential duration in the 3 age groups are shown in Figure 6C.

mRNA for Molecular Determinants of I_{to}

Figure 7 shows representative real-time PCR curves by use of Kv4.3 (A) and KChIP2 (B) specific primers. The summarized data, shown in the insets, demonstrate significant increases in mRNA levels for both targets with increasing age ($P<0.05$). The magnitude of change appears larger for KChIP2.

Discussion

In earlier studies, we described the role of altered stress/strain relationships associated with pacing in inducing cardiac memory,¹⁶ the roles of angiotensin II and calcium in initiating memory and of calcium in sustaining it, and the changes in I_{to} ¹⁷ and in $I_{Ca,L}$ ¹⁸ that appear to be associated with the memory process. In other studies, we have shown that I_{to} is reduced in density and that I_{to} and $I_{Ca,L}$ kinetics are altered in ventricular myocytes from hearts of dogs with long-term memory.^{17,18}

Moreover, $I_{Ca,L}$ - and I_{to} -blocking agents prevent the induction of memory induced by 1 to 2 hours of pacing.^{3,18} Finally, preliminary data^{18a} have suggested that an inverted transmural gradient of I_{Kr} also plays a role. This work has led us to understand that the mechanism for memory resides in a complex signal transduction program that feeds into several downstream targets.

A critical aspect of the expression of memory derives from an alteration in the transmural gradient for repolarization,² to which I_{to} is a major contributor.⁹ The transmural gradient for I_{to} , such that current is large epicardially and minimal endocardially, most likely reflects a transmural KChIP2 gradient. We state this because the transmural gradient for the current is paralleled by a comparable gradient for KChIP2 mRNA.^{12,19} KChIP2 protein levels have been reported to show the same transmural gradient,¹² although there is controversy here.¹⁹ Despite this controversy regarding protein levels, the parallel between the gradients in I_{to} and in KChIP2 mRNA argues strongly in favor of an association between the two.

These observations led to our hypothesis that in the absence of I_{to} and a transmural gradient for repolarization (as in the neonatal heart), memory would not be seen. Our present results bear out the hypothesis. Neonatal animals, which previously have been shown not to express I_{to} ,^{7,8} in fact showed a minimal and statistically insignificant response to pacing. Although some memory may reside in the neonatal heart, clearly it was below our level of detection. I_{to} is expressed at 50 to 60 days of life in dogs.⁷ Studies in the rat suggest that developmental changes in I_{to} are the result of a signal provided by the evolving sympathetic innervation of the ventricle.²⁰ It is at the same time that I_{to} evolves that an increase in expression of memory is seen (Figure 3).

In addition to the notch of the epicardial action potential, we used the evolution of its response to 4-AP and the evolution of KChIP2 and Kv4.3 mRNA as additional surrogates for I_{to} . The fact that increases in both mRNAs (more notably the former) occurred as memory evolved is an indicator of the age-related evolution of the current they encode. As for 4-AP, this had no effect on phase 1 repolarization in the neonate but did prolong repolarization. As age increased, 4-AP increasingly suppressed the notch and had a diminishing effect on repolarization. The age-dependent effects of 4-AP on action potential duration can be explained as follows: neither I_{to} nor I_{Ks} is expressed in neonatal canine ventricle.^{7,8,21} The probable basis for prolongation of action potential duration by 4-AP in the neonate is that it blocks I_{Kr} ²² as well as I_{to} . At older ages, at which I_{to} is present, the effect of 4-AP to block this current shifts the plateau positively. This most likely speeds activation of I_{Kr} , in part counteracting any I_{Kr} inhibition by 4-AP. The result would be little to no change in action potential duration, as we in fact have seen.

In a previous publication,¹⁷ we showed that in the setting of memory, I_{to} is reduced. However, in that article, the influence of age was not considered. The present article clearly demonstrates that before I_{to} evolves, memory cannot be expressed. In other words, until the normal developmental remodeling of the heart has advanced to a critical juncture, the imposition of a pacing- or arrhythmia-induced altered activation pathway

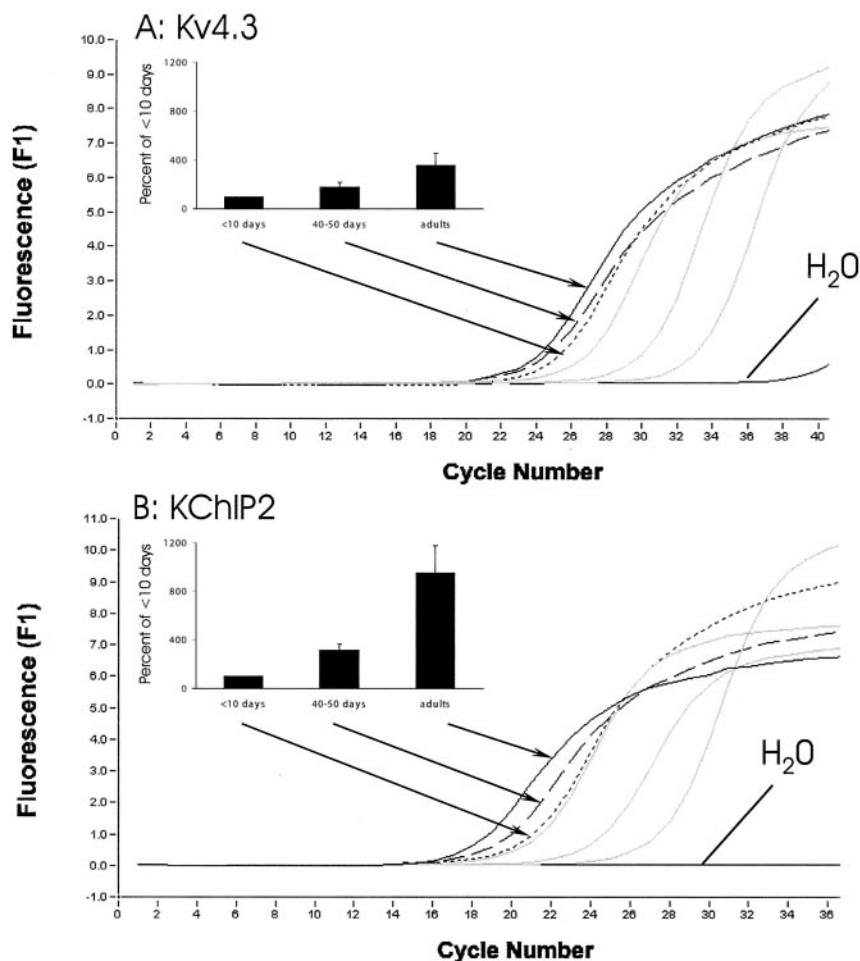


Figure 7. Representative PCR curves and summary data for Kv4.3 (A) and KChIP2 (B) for adult, 40- to 50-day-old, and <10-day-old dogs. A, Gray curves represent a dilution series (1:10, 1:100, 1:1000) from adult samples to generate a standard curve to calculate relative differences among samples. H₂O marks a control reaction that contained 2 μ L H₂O instead of cDNA. No amplification occurs in this reaction. Analyses of summary data in insets indicate developmental increases in Kv4.3 and KChIP2 mRNA (both $P < 0.05$, ANOVA).

cannot lead to the evolution of the altered repolarization characteristic of cardiac memory. Within this context, the neonatal heart cannot evolve a memory pattern in response to pacing because it is not yet sufficiently differentiated to do so.

Also within this context, the primary memory pattern of the normal heart (ie, the normal ST segment and T wave) would be interpreted as imposed by the expression of sinus rhythm during growth and development. When a second pacing site alters activation, the memory pattern that is recruited would not necessarily be the memory of something new but rather might reflect the return to a pattern that previously (in the neonate) had been dominant and diminished with development. The heart would be, in effect, forgetting one pattern and relearning an earlier one. If this is the case, then neural tissues for which the process of forgetting is integral to the expression of memory might be looked to as a further paradigm for heart.

Finally, one technical aspect of the study bears comment. In measuring the QTc interval, we used both the Bazett and Van de Water methods for rate correction. We did so because we and others had used primarily the Bazett correction in most of our earlier work, and the recent literature¹³ has suggested that the correction factor of Van de Water is more accurate in the dog. As noted in Figure 1, the differences between the two were modest at best, with Bazett showing a greater magnitude of change than Van de Water. This

information should be taken into account in designing future studies that quantify QT-interval measurements and changes associated with pharmacological and other interventions.

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