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Correlation between changes in morphology, electrical properties, and angiotensin-converting enzyme activity in the failing heart

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Abstract

Evidence is available that morphologic and electrophysiologic abnormalities are present in the failing heart. In the present work, the progressive changes in electrical properties and morphology of the failing heart of Syrian cardiomyopathic hamsters (TO2) were investigated at different stages of the pathological process, and the possible role of the renin–angiotensin system was studied. Cardiomyopathic hamsters 2 and 11 months of age were used. Age-matched normal hamsters (F1B) were utilized as controls. Measurements of membrane potential, conduction velocity and refractoriness were made with conventional intracellular electrodes connected to a high impedance DC amplifier. Serum and cardiac angiotensin-converting enzyme (ACE) activities were measured in controls and cardiomyopathic animals. The results indicated that interstitial fibrosis and calcification were present in the heart of 2-month old Syrian cardiomyopathic hamsters. Measurements of the resting potential performed in the isolated right ventricle of 2-month old Syrian cardiomyopathic hamsters indicated an average value of -66.7 ± 0.96 mV ($n = 25$); in the controls of the same age was -78.5 ± 1 mV ($n = 25$, $P < 0.05$); and in 11-month old cardiomyopathic hamsters was -67.8 ± 0.83 mV ($n = 10$). The duration of the action potential measured at 50 and 90% of repolarization in 2-month old hamsters was well above the controls. The conduction velocity measured in the isolated right ventricle of 2-month old Syrian cardiomyopathic hamsters (44.2 ± 1.6 cm/s, $n = 12$) was not different from the control (43.7 ± 1.1 cm/s, $n = 7$, $P > 0.05$) but was significantly larger than that recorded from the ventricle of 11-month old animals (37.8 ± 2.9 cm/s, $n = 11$, $P < 0.05$). ACE activity was 0.26 ± 0.01 nmol/mg × min in the heart of controls at 2 months of age and did not change with age. Although in the 2-month old cardiomyopathic hamsters the enzyme activity (0.28 ± 0.04 nmol/mg × min) was not different from the controls ($P > 0.05$), in myopathic animals at 11 months of age, the enzyme activity (0.56 ± 0.027 nmol/mg × min) was greater than controls ($P < 0.05$). The ACE activity in plasma followed the same pattern. The conclusion from these experiments is, that some parameters like resting potential, action potential duration, and morphological abnormalities appeared quite early in the failing process. The decline in conduction velocity, however, appeared later on, concurrently with the activation of plasma and cardiac renin–angiotensin systems.

Keywords: Electrical property; Renin–angiotensin system; Heart failure; Cardiomyopathic hamster

1. Introduction

Previous studies from our laboratory indicated that severe morphologic and electrophysiologic abnormalities are seen in the ventricle of cardiomyopathic hamsters (TO2) at the late stage of heart failure (De Mello et al., 1997).

The cardiomyopathic hamster, which is a well-described model of cardiac failure (Bajusz, 1969; Jasmin and Eu, 1979; Jasmin and Proschek, 1984), presents ventricular hypertrophy followed by progressive cardiac dilatation and death by congestive heart failure (Bajusz, 1969; Gertz, 1992). The pathological changes resemble those found in a Ca^{+2}-induced necrotic process with myolitic and coagulative type of cell degeneration (Jasmin and Eu, 1979). Recently, evidence has been provided that cell coupling is greatly impaired in the failing heart (De Mello, 1996) and that the fall in gap junction (gj) conductance seen in 11-month old cardiomyopathic hamsters is, in part, related to the activation of the plasma and cardiac renin–angio-

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tensin systems (De Mello, 1996). Angiotensin II, for instance, added to the bath, caused cell uncoupling in myopathic cell pairs characterized by a low value of gj, and the intracellular dialysis of angiotensin I abolished cell com-

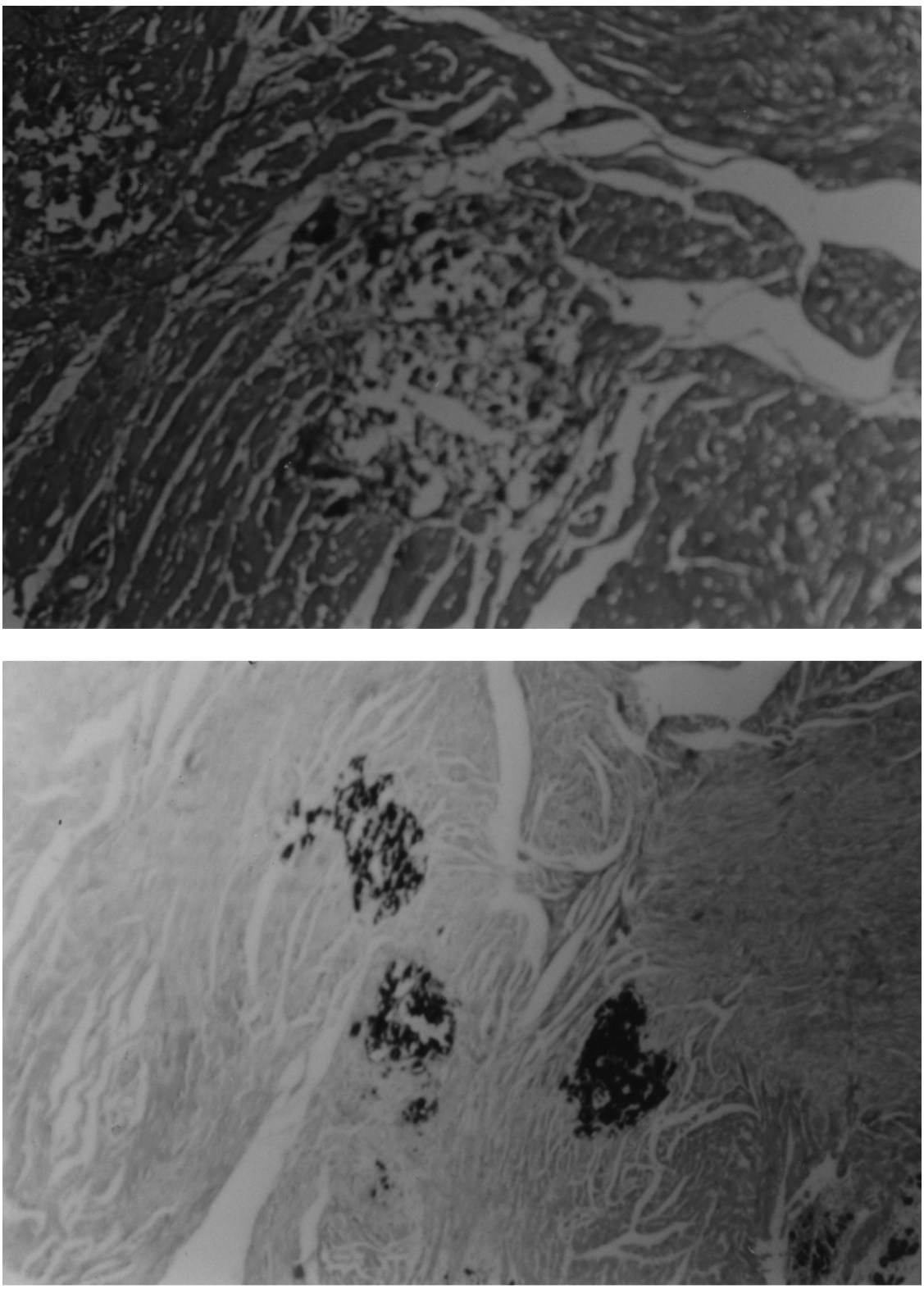

Fig. 1. Top: Histopathology of the right ventricle of cardiomyopathic hamster (2 months old) showing interstitial fibrosis with Masson trichrome (original magnification ×50). Bottom: von Kossa's stain of the left ventricle of cardiomyopathic hamster (2 months old) showing calcium deposits and fibrosis (original magnification ×50).

munication — an effect suppressed by the addition of enalapril to the cytosol (De Mello, 1996).

The conduction velocity, which was reduced in the ventricle of 11-month old animals, was appreciably enhanced by acute administration of enalapril to the extracellular fluid (De Mello et al., 1997). Interstitial fibrosis, necrosis, and calcification seen in the ventricle of these animals lead to rupture of cell contacts and profound alteration of ventricular structure (Cherry and De Mello, 1996) that contributes to the deterioration of the ventricular function.

Two major questions arise from these observations. (a) Are the impairment of impulse conduction and the morphologic abnormalities present in the heart of these animals at an early stage of the disease? (b) If this is the case, how involved is the activation of the plasma and of the cardiac renin–angiotensin system in the generation of these alterations? In the present work, this problem was investigated in cardiomyopathic hamsters at different stages of the pathological process. Some of these results were presented in a preliminary form (Crespo et al., 1997; Crespo and De Mello, 1999).

2. Materials and methods

2.1. Experimental animals

Twenty-five 2- and 11-month old adult male cardiomyopathic hamsters (TO2 from Biobreeders, Fitchburg, MA) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The heart was removed and immersed in oxygenated Krebs solution (37°C). The wall of the right ventricle was dissected and transferred to a bath through which normal Krebs solution (37°C) flowed continuously. The 2-month old cardiomyopathic hamster presented no signs of heart failure, and the aspect of the heart by visual inspection was normal except for a few small sites of calcification. The composition of the Krebs solution was as follows (mM): NaCl 150; KCl 5.1; $CaCl_2$ 1.8; $MgCl_2$ 1; Hepes 5; glucose 11 (pH 7.3). This solution was saturated with 100% O_2. Age-matched normal hamsters (F1B) from Biobreeders

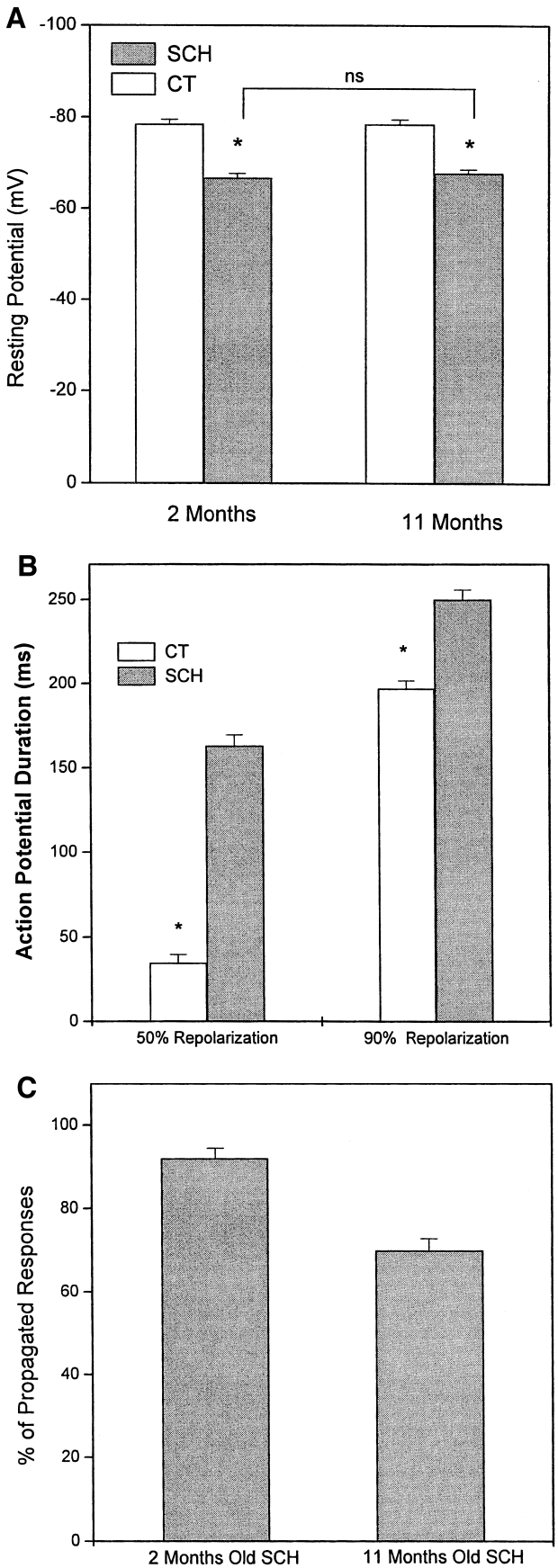

Fig. 2. Top: resting potential of control (CT) and myopathic (SCH) ventricular fibers of 2- and 11-month old animals. Each bar is the average of 25 experiments for 2-month old animals, CT and SCH, and of 10 experiments for 11-month old hamsters. An average of seven impalements was used to determine the electrical parameters for each animal. Vertical line at each bar, S.E.M. Center: action potential duration of controls (CT) and 2-month old cardiomyopathic hamsters (SCH) recorded at 50 and 90% repolarization. Each bar is the average of 12 experiments. An average of four measurements was made in each animal. Vertical line at each bar, S.E.M. Bottom: incidence of propagated responses recorded in the isolated ventricle of 2-month old cardiomyopathic hamster (A) and in the isolated ventricle of 11-month old myopathic animal (B). Each bar is the average of 20 experiments. An average of four measurements was made in each animal. Vertical line at each bar, S.E.M.

were used as control subjects. All the animals were maintained at the animal facilities under controlled temperature and humidity and received healthy animal food and water ad libitum.

2.2. Electrical measurements

Three molar KCl microelectrodes were used to measure the transmembrane potentials. The voltage-recording micropipette was connected to a high impedance DC amplifier (WP Instruments, New Haven CT). The ventricular muscle was stimulated with a fine platinum electrode (0.3 mm in diameter) by using rectangular current pulses generated by an electronic stimulator and isolation unit (Grass Instruments, Boston). The intensity of the current pulses was twice the threshold, and the pulse duration was 2 ms. A bipolar stimulation was used and the rate of stimulation was 0.6 Hz.

After 30 min of equilibration in normal Krebs solution, the membrane potential was recorded from superficial endocardial fibers located near the center of the right ventricular wall. In some experiments, an electrophysiologic exploration of the whole ventricular wall was made.q

The conduction velocity was measured with two micropipettes impaled at a fixed distance (about 10 mm) apart. The values of conduction velocity measured in the ventricular wall represent approximations of the real values. Measurements of action potential duration were made at 50 and 90% of repolarization.

To investigate the possible changes in refractoriness, strength–interval curves were obtained by applying a second pulse of variable intensity but constant duration (2 ms) at different moments of the action potential. The minimal current intensity required to elicit a propagated response was determined for each interval (Brooks et al., 1955; De Mello et al., 1997). The strength of the effective current was then plotted against the interval, and strength–interval curves were obtained. The determination of the stimulus strength was achieved by amplifying the voltage drop across a 10-MΩ resistor placed between the muscle and the ground. Voltage calibration was determined by injecting known voltages between the Krebs solution and the ground. Changes in membrane potential and current were displayed on an oscilloscope and photographed.

2.3. Histological techniques

Right and left ventricles were fixed in formalin and embedded in paraffin. Five-micrometer sections were stained with Masson trichrome and von Kossa's calcium stain and then photographed.

2.4. Determination of serum and tissue angiotensin-converting enzyme (ACE) activity

To measure the vascular and cardiac ACE activity, a fluorimetric assay previously described (Cushman and Cheung, 1991) was used. In this method, ACE activity is correlated with the rate of generation of His–Leu from Hip–His–Leu substrate. Heart homogenates were prepared with 1 g of tissue weight per 10 ml of ice-cold potassium phosphate buffer (50 mM, pH = 7.5). An aliquot (100 μl) of the homogenate was added to 100 μl Hip–His–Leu (12.5 mM), and a final volume of 250 ml was achieved by the addition of 50 ml of water. The reaction was allowed to proceed for 10 min at 37°C, after which it was stopped with 1.45 ml of NaOH (280 mM). Following this procedure, 1% phthalaldehyde (100 μl) was added and the mixture was incubated for 10 min at room temperature. Two hundred microliters of 3 N HCl were added, and the mixture was incubated for another 30 min at room temperature. The fluorescence at 486 nm was determined using an excitation wavelength of 364 nm. Plasma ACE activity was also determined by using plasma samples and phosphate buffer at 1:10 concentration ratio. To determine ACE activity, a calibration curve was performed using variable concentration of His–Leu (0 to 3 nM). A Shimadzu RF5000U spectrofluorometer was used to perform these measurements.

2.5. Statistical analysis

Data are presented as mean ± S.E.M. The Student's *t*-test was used to determine the statistical significance, defined as $P < 0.05$.

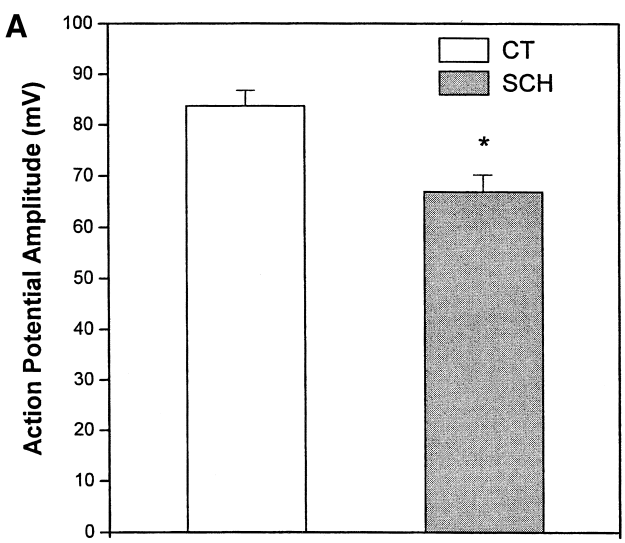

Fig. 3. **A)** action potential amplitude recorded from controls (CT) and from cardiomyopathic hamsters (SCH) at 2 months of age. Each bar is the average of 12 experiments. An average of four impalements was made in each animal. Vertical line at each bar, S.E.M. **B)** action potential recorded from single ventricular fiber of control (A), cardiomyopathic hamster at 2 months (B), and 11 months of age (C). Vertical calibration in (A), 30 mV. Time calibration in (B), 10 ms. Speeds in (A), (B), and (C) are the same. Resting potential in (A), −78 mV; in (B), −60 mV; and in (C), −56 mV.

B

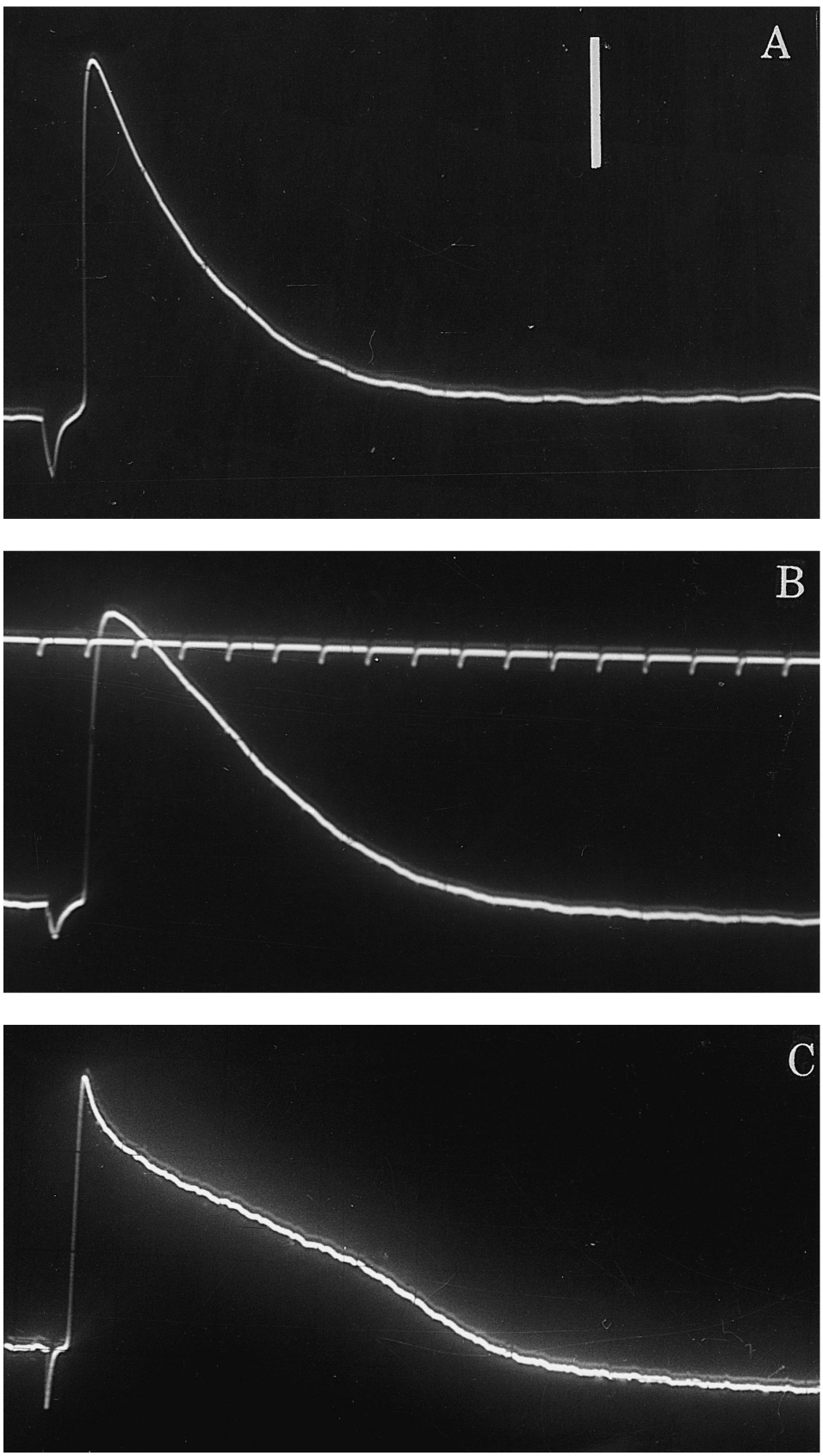

Fig. 3 (continued).

3. Results

Histological studies performed on the right and left ventricles of 2-month old cardiomyopathic hamsters showed interstitial fibrosis, necrosis and calcification (see Fig. 1). The lesions, however, were of small size and distributed in limited spots. These findings contrast with those described in the ventricular wall of 11-month old cardiomyopathic hamsters in which the damaged areas are of large dimensions and widely distributed, occupying sometimes 30–35% of the preparation (De Mello et al., 1997). In these animals, severe edema and ascitic fluid indicate advanced heart failure.

Measurements of the resting membrane potential performed with intracellular microelectrodes on the central area of the right ventricular wall of 2-month old cardiomyopathic hamsters indicated an average value of -66.7 ± 0.96 mV ($n = 25$), which was smaller than that recorded in the controls of the same age (-78.5 ± 1 mV, $n = 25$, $P < 0.05$) but not different from the values recorded in the same area of 11-month old animals (-67.8 ± 0.83 mV, $n = 10$, $P > 0.05$) (see Fig. 2). The action potential amplitude was already reduced at 2 months of age, and the duration of the action potential measured at 50 and 90% repolarization in the ventricle of 2-month old myopathic hamsters was well above the controls ($P < 0.05$) (Figs. 2 and 3).

The conduction velocity measured with two intracellular microelectrodes at the center of the right ventricular wall showed an average value of 44.2 ± 1.6 cm/s ($n = 12$), which was not different from the controls (43.7 ± 1.1 cm/s, $n = 7$, $P > 0.05$) but significantly larger than that found in the same area of the right ventricle of 11-month old cardiomyopathic animals (37.8 ± 2.9 cm/s, $n = 11$, $P < 0.05$) (see Fig. 4).

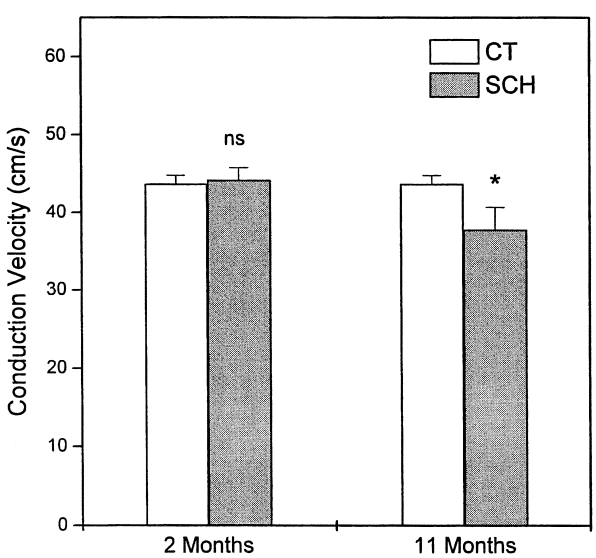

Fig. 4. Conduction velocity (cm/s) recorded from the right ventricular wall of control (CT) and cardiomyopathic hamsters at 2 months ($n = 12$) and at 11 months of age ($n = 11$). An average of four measurements was made in each animal. Vertical line at each bar, S.E.M.

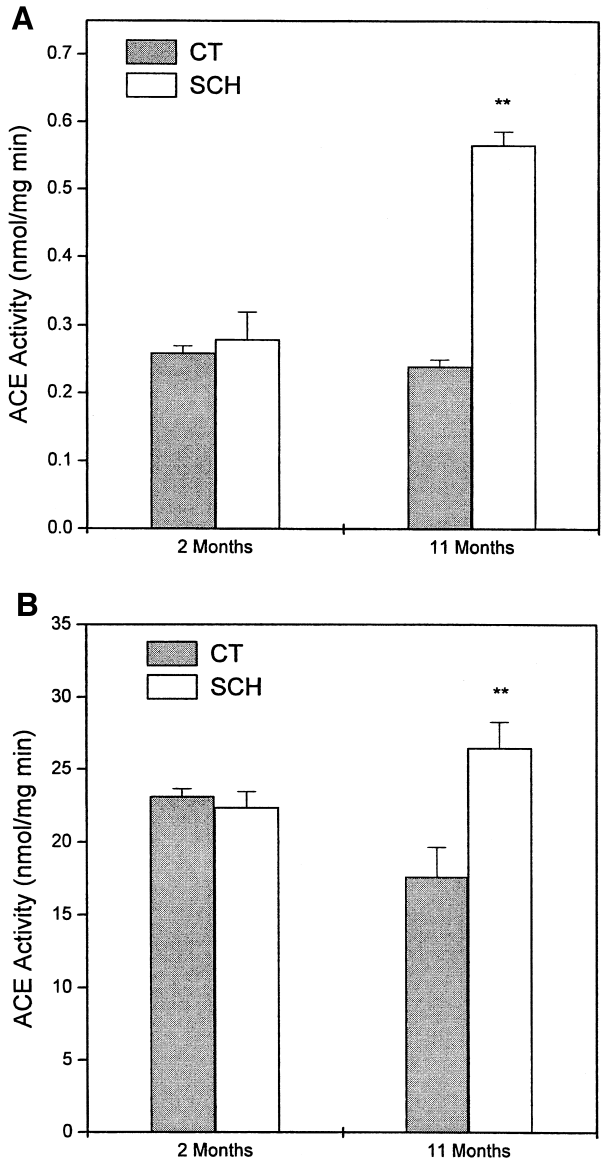

Fig. 5. Top: ACE activity (nmol/mg×min) in ventricular muscle of controls (CT) and cardiomyopathic (SCH) hamster at 2 and 11 months of age. The values represent the means ± S.E.M. for eight animals (CT and SCH) with 2 months of age and 20 animals for 11 months of age. No significant difference was found between controls and cardiomyopathic animals at 2 months of age ($P > 0.05$). The enzyme activity was significantly increased at 11 months of age ($P < 0.05$). Bottom: ACE activity (nmol/mg×min) in plasma of control hamsters (CT) and cardiomyopathic animals at 2 and 11 months of age. Each bar represents the means ± S.E.M. of eight animals. No significant difference was found between controls at different ages and between controls and cardiomyopathic hamsters (SCH) at 2 months of age. At 11 months of age, the differences were significant ($P < 0.05$).

The electrophysiologic exploration of the whole right ventricle wall of 2- and 11-month old cardiomyopathic hamsters demonstrated a larger incidence of propagated responses ($92\% \pm 2.5$, $n = 15$) in the young animal compared with $70\% \pm 2.8$ ($n = 15$, $P < 0.05$) in the ventricle

of 11-month old cardiomyopathic hamsters (see Fig. 2). In old animals, action potentials with different shapes and sizes, as well as nonpropagated responses, were recorded throughout the right ventricle (not shown). Because the activation of the renin–angiotensin system is involved in the decline in gj conductance seen in cardiomyopathic hamsters (De Mello, 1996), we decided to determine the ACE activity at different moments of the failing process and compare with changes in electrical properties. The results indicated that at 2 months of age, the ACE activity in the myocardium (0.28 ± 0.04 nmol/mg × min) was not different from the controls (0.26 ± 0.01 nmol/mg × min) (see Fig. 5). However, in animals with 6 months of age, the enzyme activity was already increased (not shown), reaching a maximal value at 11 months of age (0.56 ± 0.027 nmol/mg × min, $P < 0.05$) when an overt heart failure was seen (Fig. 5). Moreover, the ACE activity measured in the plasma of these animals was not increased in 2-month old hamsters, but the values recorded at 11 months of age showed an appreciable increase in enzyme activity (26.11 ± 1.87 nmol/mg × min in cardiomyopathic vs. 18.65 ± 1.77 nmol/mg × min from age-matched controls, $P < 0.05$) (see Fig. 5).

4. Discussion

The present results indicate that morphologic and electrophysiologic abnormalities are observed in 2-month old cardiomyopathic hamsters when no signs of heart failure, like peripheral edema, ascitic fluid or enlargement of the left ventricle, are found. Furthermore, no increment of ACE activity is seen in these young animals, whereas in 6-month old hamsters, the enzyme activity (0.41 ± 0.05 nmol/mg × min) (Crespo, 1999) is well above the controls. These animals at 6 months of age are in a compensatory stage of the disease because no signs of overt heart failure are found despite the presence of extensive areas of necrosis and calcifications in the right and the left ventricles (De Mello and Crespo, unpublished). Although the ACE activity values in plasma and heart muscle are not increased in 2-month old cardiomyopathic hamsters, evidence is available that in 25-day old animals, when no histological lesions are seen, the density of angiotensin AT_1 receptors is increased by 90% (Lambert et al., 1995). These observations support the possibility, that with the overexpression of angiotensin AT_1 receptors, the responsiveness of the myopathic cells to normal plasma levels of angiotensin II is increased, leading to changes in morphology and electrical properties even before the rise in ACE activity. Other studies indicated that the synthesis of angiotensin II is increased in the myocardium of cardiomyopathic hamsters with 3 months of age (Nakamura et al., 1994) prior to the development of cardiac failure, supporting the view that the overactivity of the cardiac renin–angiotensin system precedes the appearance of heart failure.

It is not known whether the fall in membrane potential found at the early stage of the failing process is due to a

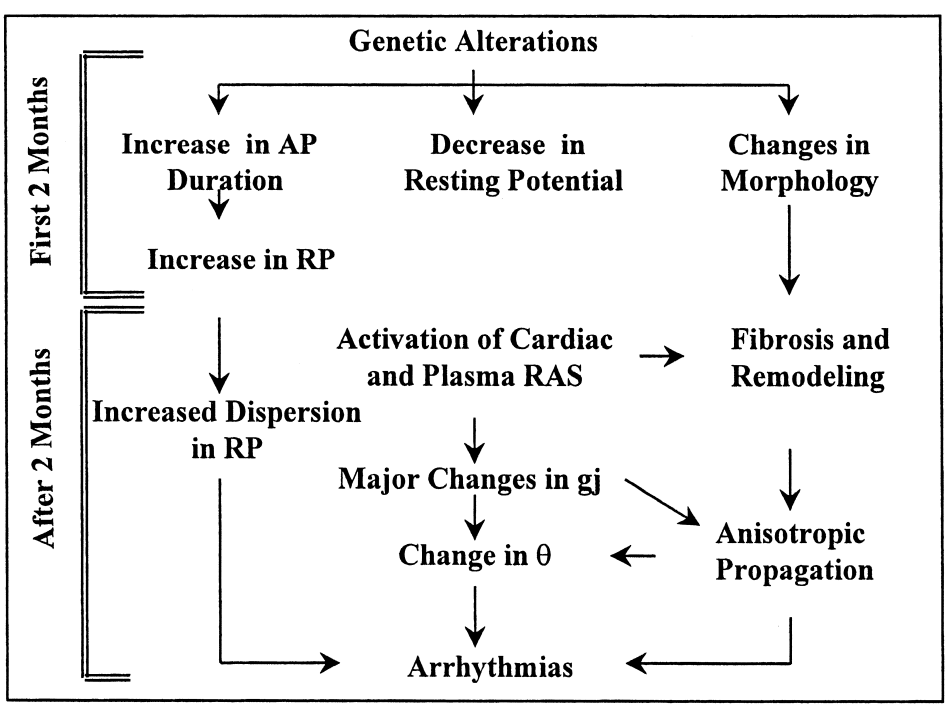

Fig. 6. Diagram illustrating the sequence of changes in electrical properties and morphology of the failing heart of cardiomyopathic hamsters at an early stage of the disease and beyond 2 months of age. θ: conduction velocity; RAS: renin–angiotensin system; RP: refractory period; AP: action potential; gj: gap junction conductance.

reduced activity of the Na^+, K^+-ATPase (Sole and Liew, 1988), to an ischemic process (Jasmin and Proschek, 1984), or to both. On the other hand, the increase in duration of the action potential seen in 2-month old cardiomyopathic hamsters (Thuringer et al., 1996) as well as in other models of heart failure (Kaab et al., 1996) could be related to the enhanced effect of angiotensin II due to overexpression of angiotensin AT_1 receptors. Evidence is indeed available that angiotensin II increases the duration of the action potential in cardiomyopathic hamsters (De Mello, unpublished).

With the ulterior increase of ACE activity in plasma and heart muscle found during more advanced stages of heart failure, the electrophysiologic alterations are accentuated. Indeed, a decline in conduction velocity, in part related to a fall in junctional conductance (De Mello, 1996), is certainly involved in the generation of reentrant circuits and the generation of cardiac arrhythmias. The further deterioration of the ventricular morphology induced by the activation of the renin–angiotensin system leads to ventricular hypertrophy and remodeling (Weber and Janicki, 1989; De Mello et al., 1997) as illustrated in Fig. 6. Because the angiotensin II content of the cardiomyopathic hamsters ventricle is also increased (Nakamura et al., 1994), it is reasonable to conclude that the activation of the cardiac renin–angiotensin system contributes to the abnormalities in morphology and electrical properties described above.

Acknowledgements

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References

Bajusz, E., 1969. Dystrophic calcification of myocardium as conditioning factor in genesis of congestive heart failure: an experimental study. Am. Heart J. 78, 202–209.

Brooks, C.M.C., Hoffman, B.F., Suckling, E.E., Orias, O., 1955. Excitability of the Heart. Grune Straton, New York.

Cherry, R., De Mello, W.C., 1996. Confocal microscopy and intercellular communication in the failing cardiomyopathic heart. FASEB J. [Abstract 2275].

Crespo, M.J., 1999. Vascular alterations during the development and progression of experimental heart failure. J. Card. Failure 5 (1), 55–63.

Crespo, M.J., De Mello, W.C., 1999. Correlation between ACE activity and impulse propagation in the failing heart. Cardiovasc. Drug Ther. 19 (1), A3.

Crespo, M.J., Escobales, N., Altieri, P.I., Quidley, J., 1997. Heart failure in the cardiomyopathic hamster model; possible role of angiotensin II. J. Mol. Cell. Cardiol. 29, A53.

Cushman, D.W., Cheung, H.S., 1991. Concentrations of angiotensin-converting enzyme activity in tissues of the rat. Biochim. Biophys. Acta 250, 261–265.

De Mello, W.C., 1996. Renin–angiotensin system and cell communication in the failing heart. Hypertension 27, 1267–1272.

De Mello, W.C., Cherry, R., Manivannan, S., 1997. Electrophysiologic and morphologic abnormalities in the failing heart; effect of enalapril on the electrical properties. J. Card. Failure 3, 53–61.

Gertz, E.W., 1992. Cardiomyopathic Syrian hamster: a possible model of human disease. Prog. Exp. Tumor Res. 16, 242–247.

Jasmin, G., Eu, H.Y., 1979. Cardiomyopathy of hamster dystrophy. Ann. N.Y. Acad. Sci. 317, 46–58.

Jasmin, G., Proschek, L., 1984. Calcium and myocardial cell injury: an appraisal in the cardiomyopathic hamster. Can. J. Physiol. Pharmacol. 62, 891–900.

Kaab, S., Nuss, H.B., Chiamvimonvat, N., O'Rourke, B., Park, P.H., Kass, D.E.A., Marban, E., Tomaselli, G.F., 1996. Ionic mechanism of action potential prolongation in ventricular myocytes from dogs with pacing-induced heart failure. Circ. Res. 78, 262–273.

Lambert, Massillon, Y., Meloche, S., 1995. Upregulation of cardiac angiotensin II AT1 receptors in congenital cardiomyopathic hamsters. Circ. Res. 77, 1001–1007.

Nakamura, F., Nagano, M., Kobayashi, R., Higaki, J., Mikami, H., Kawagushi, N., Onishi, S., Ogihara, T., 1994. Chronic administration of angiotensin II receptor antagonist TCV-116 in cardiomyopathic hamsters. Am. J. Physiol. 267, H2297–H2304.

Sole, M.J., Liew, C.C., 1988. Catecholamines, calcium and cardiomyopathy. Am. J. Cardiol. 62, 20G–24G.

Thuringer, D., Coulombe, A., Deroubaix, E.M., Coraboeuf, E., Mercadier, J.J., 1996. Depressed transient outward current density in ventricular myocytes from cardiomyopathic Syrian hamsters of different ages. J. Mol. Cell. Cardiol. 28, 3387–3401.

Weber, K.T., Janicki, J.S., 1989. Angiotensin and the remodeling of the myocardium. Br. J. Clin. Pharmacol. 28, 141S–150S.