Pacing Dependence of the Slow Inward Current in Frog Atrial Myocardium

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Abstract. By means of an improved double sucrose gap technique the dependence of the slow inward current in small frog atrial trabeculae on the activation pattern was studied. Increasing the frequency of depolarizing voltage steps (pacing) the steady state slow inward current (TTX resistent and blocked by verapamil) was decreased. After a 300 s resting interval the slow inward current was increased but declined due to reactivation with frequencies greater than 1 Hz. If the duration of the first post pause depolarization was prolonged the slow inward current activated by a second depolarization was decreased. The voltage-steady state inactivation relationship of the slow inward current was shifted to more negative potentials due to increase in the pacing rate. In contrast to the onset of inactivation the recovery from inactivation was found to be insensitive to variation in the pacing pattern. The onset and extent of inactivation was pronounced by increasing the pacing rate. The strong pacing dependence of the slow inward current seems to be mediated by a pacing dependent inactivation.

Key words: Voltage clamp — Sucrose gap technique — Heart muscle — Slow inward current — Pacing dependence

Introduction

In contrast to the electrical activity in nerve and skeletal muscle, the repolarization process in all types of cardiac muscle is highly sensitive to changes in rate and rhythm of activation (pacing). However, the pacing dependent properties of ionic currents have been neither well nor often studied (for review see Carmeliet 1977; Boyett and Jewell 1980). The only preliminary considerations on mechanisms of pacing dependent changes of ionic currents in heart muscle fall in two groups: 1. incomplete recovery of ionic currents due to repetitive activation, 2. depletion or

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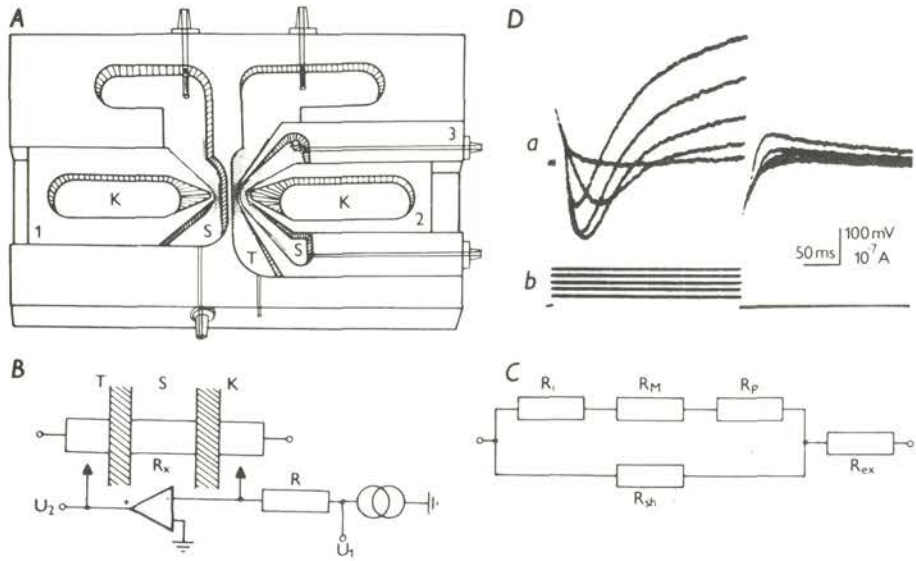

Fig. 1. Diagram of the double sucrose gap method. A: Perspex chamber with 3 movable parts (1, 2, 3). K, S, T are the depolarizing, sucrose and test compartments respectively. The solution flows in by gravity, and out by suction. B: measurement of the input resistance R_x. R is a known resistor (1 to 10 MΩ). C: equivalent circuit of the resistance R_x, R_i, R_M: internal and membrane resistance; R_p: series resistance due to the preparation; R_{sh}: shunt resistance in parallel with the preparation; R_{ex}: external series resistance. D: Membrane currents (a) activated by 5 depolarizing voltage steps; (b): inward currents are downward traces. Holding potential -50 mV. TTX: 1.2×10^{-5} mol/l. $R_x = 1.2$ MΩ.

accumulation of ions on either side of the cell membrane due to changes in the pattern of activation (Boyett and Jewell 1980).

In this paper we examine pacing dependent properties of the functional important slow inward current (I_{si}) in frog atrial myocardium. The aim of this study was to investigate the nature of the evident pacing sensitivity of this current. Our results indicate that the inactivation of I_{si} is the main determinant of its pacing dependence. A preliminary report on the results has already been presented (Nilius and Henček 1981).

Material and Methods

Ionic currents were recorded from single auricular trabeculae of *R. esculenta*. The diameters of the preparations were between 55 and 165 μm (mean value ± S.E. 103.6 ± 3.6 μm, 17 preparations). Experiments were performed at temperatures between 17° and 21 °C. A new double sucrose gap chamber was developed for voltage clamping (Fig. 1A). The main advantage of the new chamber are three movable parts, which allow to adjust the three gap widths according to the requirements of the preparation geometry. The test membrane area („node") was adjusted to about 1.6 times the diameter of the fibre but the sucrose gaps were enlarged to more than twice the diameter of the preparation

(Attwell and Cohen 1977). Therefore, the widths of the node were between 100 and 220 μm (mean 166 ± 37 μm), whereas the width of the sucrose gap were adjusted to between 180 and 230 μm (197 ± 49 μm). The diameters of the seals were between 200 and 250 μm.

As control solution a frog Ringer's solution was used (mmol/l) NaCl 110, KCl 2.5, $CaCl_2$ 4, $NaHCO_3$ 2.38, K_2HPO_4 0.08, glucose 5.5, pH 7.13. In three cases a Na-current inactivating solution was chosen (mmol/l): NaCl 85, KCl 25, $CaCl_2$ 8, $NaHCO_3$ 2.38, K_2HPO_4 0.08, glucose 5.5, pH 7.13. For electrical isolation of the outer compartments, filled with a depolarizing solution (isotonic K asparaginate), an isotonic sucrose solution (Merck, BRD) was used. The walls separating the sections of the perfusion chamber were covered with a special vaseline-silicone grease mixture layer.

Low resistance extracellular Ag/AgCl electrodes with agar bridges were used to record the trans-gap potential and to supply current to the preparation.

The amplifiers and the voltage clamp circuit were described elsewhere (Henček and Zachar 1977). Command signals were provided by a programmable pulse generator. The chosen impulse programme was applied repetitively. The interval T_0 between the impulse programmes varied between 0.5 and 10 s to test the pacing dependence of ionic currents. Transmembrane currents and the clamp programme were displayed on a storage oscilloscope and photographed.

The input resistance of the preparation was measured as indicated in Fig. 1B. Test voltage U_1 was applied via a known resistor R to the depolarizing compartment K and the potential drop U_2 was measured via an extracellular electrode in the test node T. The input resistance R_x was then obtained as U_2R/U_1 and was between 0.5 and 6.3 MΩ (mean value 2.6 ± 2.0 MΩ). The resistance R_x (see Fig. 1C) represents a sum of internal resistance R_i in series with the membrane resistance R_M and the series resistance of the preparation R_p (due to intracellular clefts, presence of connective tissue etc.) all in parallel to the shunt resistance R_{sh}. In series with this circuit a series resistance R_{ex} arises in the chamber and electrodes. The whole input resistance R_x can be expressed as

$$R_x = \frac{R_{sh}(R_p + R_i + R_M)}{R_{sh} + R_p + R_i + R_M} + R_{ex}$$

Furthermore we tried to estimate the so-called access resistance R_{ac}. The R_{ac} was obtanied by dividing a small subthreshold voltage clamp step U by the maximum non-compensated capacity current (Connor et al. 1975; Jakobsson et al. 1975). R_{ac} would represent the sum of R_p and R_{ex}. R_{ac} was estimated to be about 0.3 MΩ (between 0.1 and 0.85 MΩ). From the geometry of the preparation R_M was estimated to be 0.6 to 8 MΩ, but R_i varied between 2—6 kΩ (data from Chapman and Fry 1978). In a preliminary series of experiments, the transmembrane potential in the test node during current at voltage clamp was measured with conventional glass microelectrodes and compared with transmembrane potential records obtained using the clamping circuitry. By means of this method it was possible to measure the voltage drop in the test node and to correct the applied clamping voltage. The spatial homogenity of the clamp was found to be sufficiently good.

Results

Membrane currents under steady-state pacing

Fig. 1D demontrates the membrane currents in frog atrial trabeculae recorded in Ringer's solution containing 12 μmol/l tetrodotoxin (TTX) under voltage clamp conditions. The slow activated inward current was insensitive to TTX (1.2×10^{-5} mol/l) but blockable by verapamil (2 μmol/l). The outward currents were completely abolished by application of 0.5 mmol/l 4-aminopyridine.

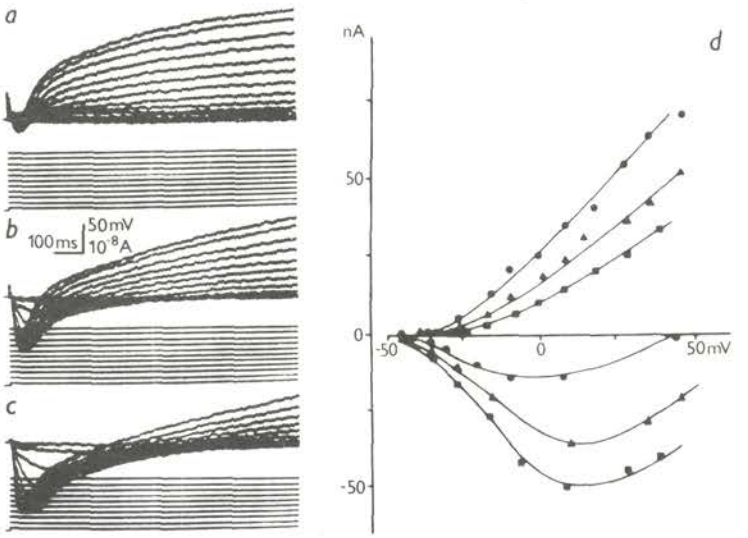

Fig. 2. Membrane currents under steady-state pacing *a*, *b*, *c*: families of voltage-current traces at three different pacing intervals T_o (1 s, 5 s, 10 s). All the single records were obtained under steady-state conditions. *d*: current-voltage plots for peak inward current and maximal outward current (measured 1 s after the start of depolarization). Holding potential -50 mV, pulse duration 1.4 s. The duration of T_o in s is: ● 1, ▲ 5, ■ 10.

The slow inward current was found to be highly sensitive to variations in the activation pattern. Increasing the frequency of depolarizing voltage steps the peak of slow inward current (I_{si}) decreased (Fig. 2.). In contrast to the decrease in I_{si} the outward currents after 1 s lasting depolarization increased but to a much smaller extent in comparison with the decrease in I_{si}. As compared with the peak inward current at $T_o = 10$ s the I_{si} was inhibited by 64.7 ± 7.8 % (7 experiments) at $T_o = 1$ s.

Effect on I_{si} of repetitive activation following resting intervals

The experiments described above confirmed that I_{si} is dependent on the steady state pacing rate. The next step was to examine the slow inward current under non-steady state conditions. Fig. 3 shows the result of an experiment in which 300 ms voltage clamp pulses were applied repetitively at intervals $T_o = 10, 5, 1$ and 0.5 s respectively after a constant resting interval of 300 s. At intervals shorter than 1 s between the repetitive activating pulses a declining inward current was observed after a rest. The largest decrease of I_{si} was observed after the first activation. This effect was found to be dependent on the duration of the depolarization. Despite a constant activation interval T_o, the second I_{si} was reduced after a 300 s resting

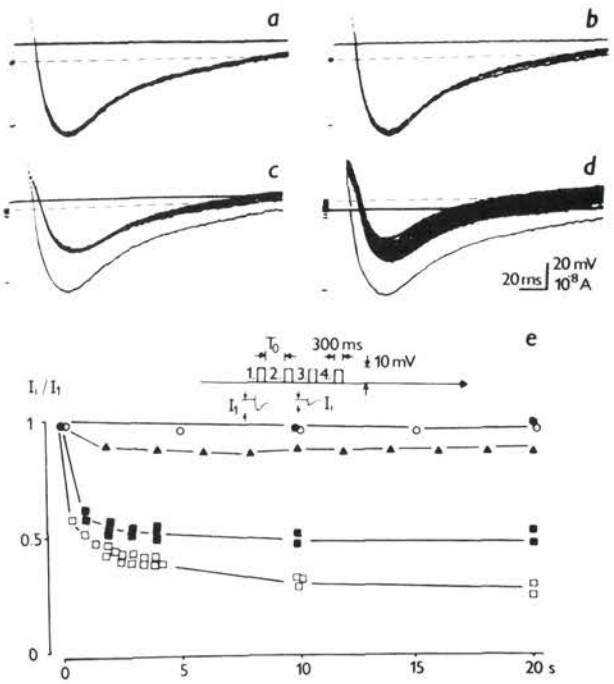

Fig. 3. Repetitive activation of slow inward currents after a constant resting interval lasting 300 s. The pacing interval T_o was: 10 s(*a*), 5 s(*b*), 1 s(*c*), 0,5 s(*d*). Superposition of the first 10 records is shown in *a* and *b*; that of 20 records in *c* and of 40 records in *d*. Inward currents after 300 s rest at pacing intervals lasting 10, 5, 1, 0.5 s. Holding potential −50 mV, duration of voltage clamp pulses 300 ms; depolarization to +10 mV. *e*: Time course of the decrease in I_{si} due to repetitive activation after a 300 s resting interval. The testing inward currents I_t were normalized (see inset) to the first current after the rest (I_1). Abscissa: time after the 300 s resting interval. Ordinate: relative peak I_{si}. ○, ●: $T_o = 10$ and 5 s; ▲: $T_o = 2$ s; ■: $T_o = 1$ s; □: $T_o = 0.5$ s.

interval due to the prolongation of the first depolarizing voltage clamp pulse, as demonstrated in Fig. 4.

Dependence of steady state inactivation

Using a conventional double step programme (500 ms prepulse, 300 ms test-pulse to 0 mV) the steady state inactivation of I_{si} was estimated (Fig. 5). The term f_∞ was calculated as the ratio between the maximal inward current and the actual test current. The measured data were approximated using the equation:

$$f_\infty = (1 + \exp((U - U_s)/S))^{-1},$$

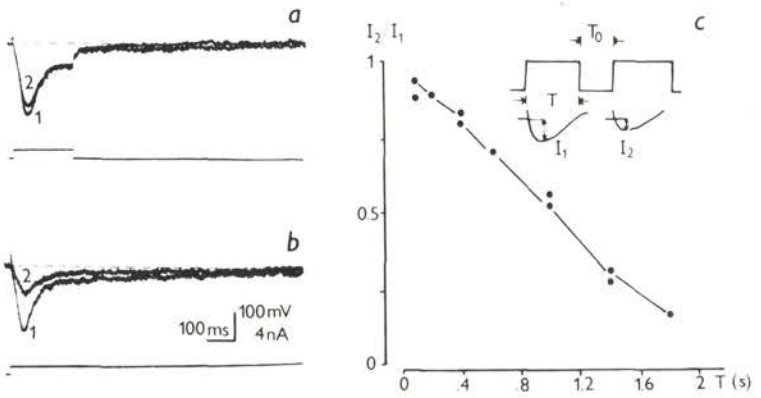

Fig. 4. Dependence of the testing membrane current I_{si} on the duration (T) of the conditioning depolarization to −20 mV. Records: First (1) and second (2) (smaller) I_{si} after a 300 s resting interval, holding potential −50 mV, a: $T=0.2$ s; b: $T=1.4$ s; Graph: Dependence of the normalized I_{si} (see inset) on the duration of the depolarizing voltage clamp step: T_0 (pacing interval) was chosen to 5 s for all measurements. Ordinate: normalized peak I_{si}, abscissa: duration of the pacing pulse.

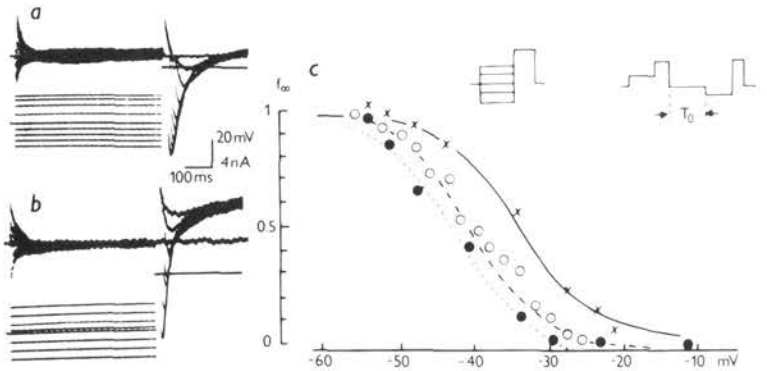

Fig. 5. Dependence of steady state inactivation on the frequency of activation. a, b: Slow inward currents activated by a test pulse to 0 mV, holding potential −40 mV, duration of the test pulse 300 ms. Before each test pulse a 500 ms lasting prepulse was applied. The double step programme was supplied repeatedly with the pacing interval $T_0=10$ s(a) and $T_0=2$ s(b) c: steady state inactivation curve of I_{si}. The measured f_∞ — values were fitted by $f_\infty=(1+\exp((U-U_s)/S))^{-1}$. The calculated slope parameter S was 4.6. ● pacing interval $T_0=2$ s, $U_s=40.5$ mV; ○: $T_0=5$ s, $U_s=-43$ mV; x: $T_0=10$ s, $U_s=-34$ mV. Inset figure: U — variable prepulse amplitude and constant duration (500 ms); the test pulse amplitude and duration were constant (depolarization from −40 to 0 mV; 300 ms).

where U is the test depolarization and U_s is the membrane potential at 0.5 f_∞. Without a significant change in the slope parameter S (4.6 ± 0.9); mean $\pm$ S.D. at shorter activation intervals U_s was shifted to more negative values (-34 mV at 10 s; -43, -40.5 at 5 and 2 s, respectively). Therefore, increasing the activation frequency favours the inactivation.

Recovery from inactivation

During a hyperpolarizing prepulse recovery of I_{si} from inactivation occurs. The time course of the recovery from inactivation has been examined by varying the duration of this prepulse (Fig. 6). The time constant of recovery was found to be independent on the interval of repetitive activation. It varied from 0.35 to 0.40 s at pacing intervals between 1 and 10 s.

Onset of inactivation

The time course of the onset of inactivation has been tested using a simple two-step programme. The inactivation of I_{si} starts during the depolarizing prepulse and can be tested by a following test pulse. Increasing the duration of the prepulse the extent of inactivation of I_{si} is increased. For the same prepulse the onset of inactivation is accellerated by increasing the frequency of activation (Fig. 7).

Discussion

Our findings clearly show that the slow inward current is highly sensitive to changes in the activation pattern. With increasing activation rate the inward current decreased but the steady state outward current increased to a much smaller extent than the peak inward current (Fig. 2). Such a rate-dependent decrease of I_{si} was also observed in the mammalian ventricular myocardium without substantional changes in the instantaneous outward current (Payet et al. 1981). Our results concerning the decrease of the peak inward current by increasing the pacing frequency agree with the findings of a diminished slow inward current due to repetitive stimulation in the mammalian myocardium (Reuter 1973; Šimurda at al. 1976). In contrast, in experiments with atrial myocardium of the bullfrog *R.catesbeiana,* Noble and Shimoni described a decreased inward current during repetitive stimulation after short periods of rest after twin pulse stimulation (Noble and Shimoni 1981 a, b; Shimoni 1981). Such changes in the slow inward current could however be due to a possible increase in the longitudinal resistance with increasing frequencies. Our results presented in Fig. 2 were obtained from steady state measurements. Noble and Shimoni demonstrated a tri-phasic response of the slow

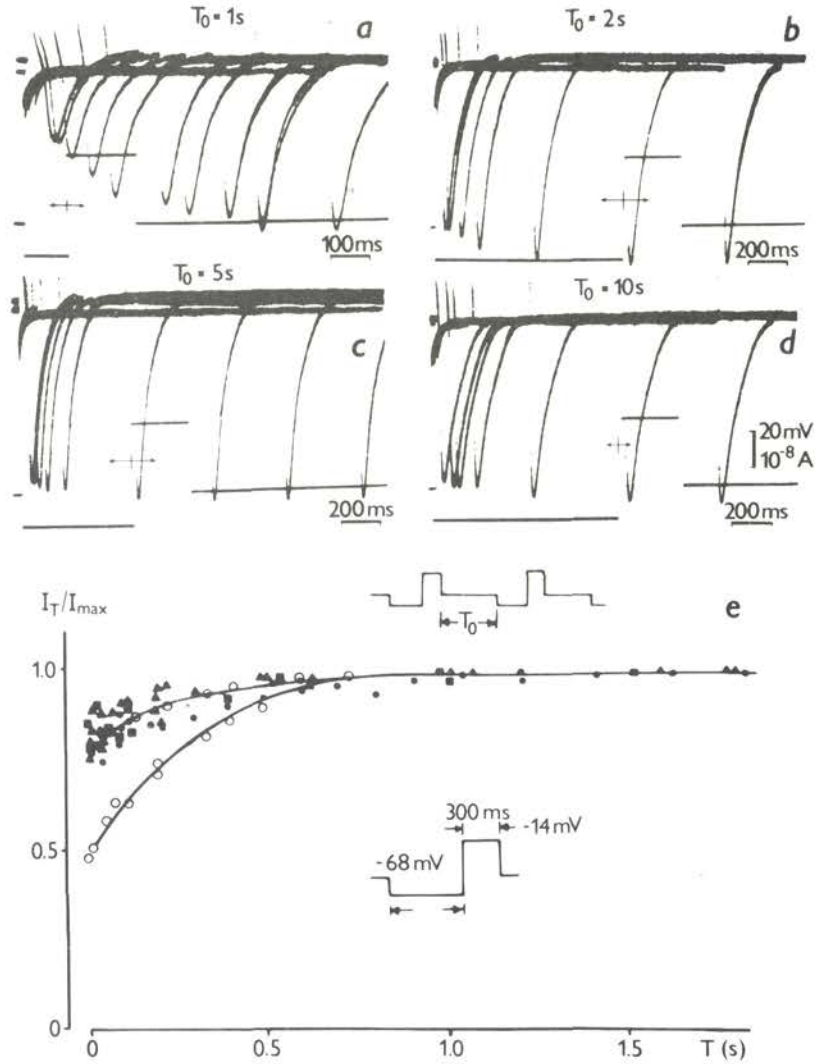

Fig. 6. Recovery from inactivation at different pacing intervals. Records ($a-d$): families of current-voltage records at different durations of the hyperpolarizing prepulse (double step programme, see inset). T_o is the interval between the voltage clamp double steps during repetitive activation. Graph e: abscissa-duration T of the hyperpolarizing prepulse preceeding a 300 ms lasting test step to -14 mV. Ordinate-normalized peak slow inward current T_o was 10 s (●, n = 3); 5 s (▲, n = 3); 2 s (■; n = 2); 1 s (○, n = 3). n: number of measurements.

inward current during repetitive stimulation after periods of rest which were not in a steady state: an initial dip followed by increased currents and a delayed decrease (Noble and Shimoni 1981 a, Fig. 3). In our experiments the second phase could not

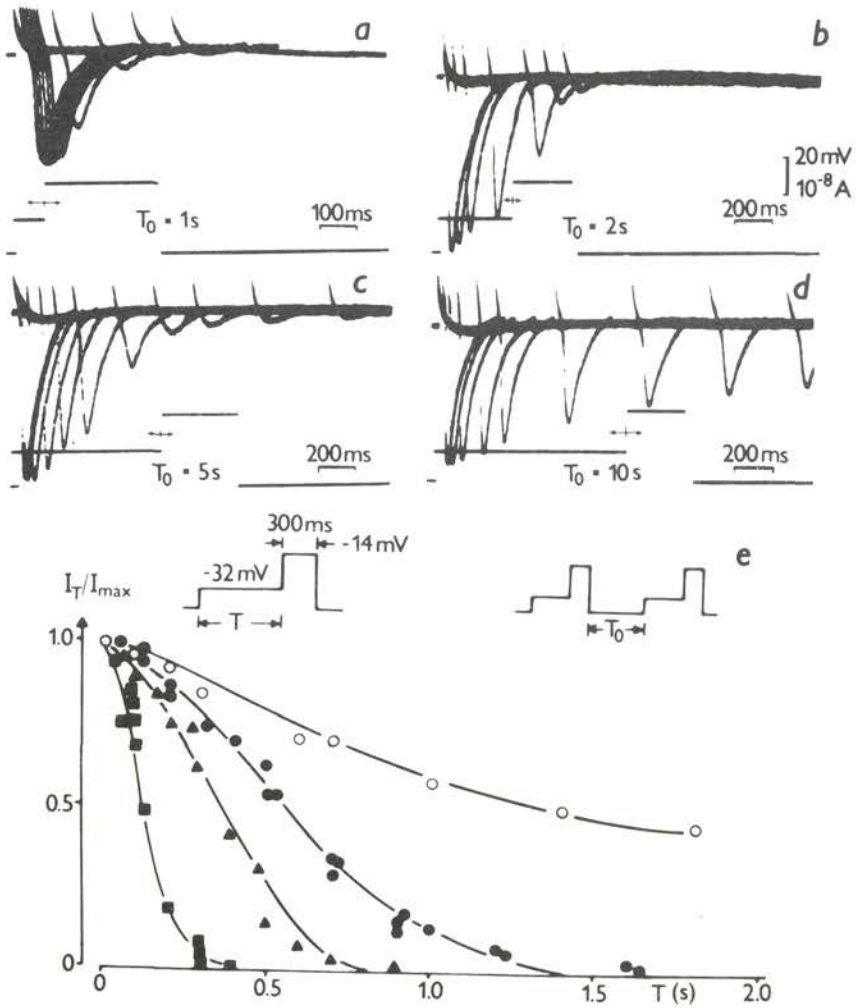

Fig. 7. Onset of inactivation at different pacing intervals. Records ($a-d$): families of current-voltage traces at different pacing intervals. A depolarizing prepulse precedes the test pulse (see inset). T_o represents the interval between the double step voltage clamp pulses. Graph *e*: abscissa-duration of the depolarizing prepulse before a 300 ms lasting test step to −14 mV. Ordinate-normalized peak slow inward current. T_o was 10 s (○; n=3); 5 s (●; n=5); 2 s (▲, n=2); 1 s (■, n=4). n: number of measurements.

be observed. Also, it should be mentioned, that these authors found the pacing dependence to be voltage-sensitive (Noble and Shimoni 1981b). We only tested the pacing dependence of the slow inward current for different steps from a holding potential of −50 or −40 mV. The same result as demonstrated in Fig. 3 was observed by Noble and Shimoni using voltage steps from −85 to −45 mV (Noble

and Shimoni 1981b, Fig. 1). A direct comparison was therefore not possible. Considerable variations in the supposed pacing-dependent outward currents (Shimoni 1981) may represent another source of problems. At present, the mechanism of the modification of I_{si} as the activation rate increases is difficult to explain. An increase in the outward currents due to increased rate of activation may unlikely reflect the apparent decrease in I_{si}. The current-voltage relationship (Fig. 2) demonstrates clearly that the pacing-dependent shift of the maximum inward current is much larger than the shift in the outward current. It is also unlikely that the pacing-dependent decrease of I_{si} is generated by a decrease in $\bar{g}_{si}$. The positive slope of the I_{si} — voltage relationships did not change significantly in every experiment (Fig. 2). This would contrast with the above assumption. A further explanation of the activation-dependent decrease of I_{si} might be a decrease in the driving force mediated by an intracellular accumulation of Ca. Two arguments are against this hypothesis: a) injection of Ca into sheep Purkinje fibres was reported to produce an increase in I_{si} (Isenberg 1977), b) at positive potentials the variations in I_{si} were found to be comparable with the variations in the outward current, the shift in the apparent reversal potential would thus reflect the pacing dependent increase in the outward current (Fig. 2). There is no experimental evidence for such a pacing-dependent shift in the true reversal potential of I_{si}. Only at very high activation frequencies (interval between two pulses shorter than 1 s) a very slow decrease in I_{si} might be mediated by Ca ions accumulation. In all other cases the changes in I_{si} due to abrupt changes in the pacing pattern were completed during the first one or two currents (Fig. 3). A further explanation of rate-dependent changes in I_{si} might be looked for in the incomplete recovery of I_{si} between two activating pulses. However, such an explanation had been ruled out by the experimental finding of the independence of recovery of I_{si} on the pacing rate under the our conditions (Fig. 6). The most plausible explanation of the pacing dependence of I_{si} seems to be an activation-dependent inactivation. If the pacing rate was increased, the steady state inactivation curves were shifted to more negative potentials than it was the case during slow pacing. The slopes S of the inactivation curves was not changed significantly but the voltage U_s for half-maximum inactivation was shifted to more negative potentials at increased pacing rates (Fig. 5). The same finding would be expected if the density of fixed negative surface charges was increased (Brown 1974), or, equivalent to it, if the Ca concentration near the membrane surface was lowered. The shift of the steady state inactivation curve could reflect a surface charge effect: increasing the pacing rate unmasks negative binding sites or yields Ca depletion near the Ca channels. It has recently been suggested that Ca channel inactivation may differ from Na inactivation the former being dependent on Ca entry. Ca-dependent inactivation was originally described in *Paramecium* (Brehm and Eckert 1977), *Aplysia* neurones (Tillotson 1979), *Helix* neurones (Standen 1981),

embryonic chick sensory neurones (Dunlap and Fischbach 1981) and also supposed in skeletal muscle (Ashcroft and Stanfield 1981) and the atrial myocardium of frog (Fischmeister at al. 1981). An increased Ca influx due to increase in the pacing rate or a subsarcolemmal accumulation of Ca would accellerate the inactivation by such a Ca-dependent mechanism. The experiments showed clearly that the onset of inactivation was accellerated when the activation intervals were shortened (Fig. 7). It is possible that an accumulation of intercellular Ca may be operative in this pacing-dependent inactivation. Such accumulation may be too small to alter the Ca-equlibrium potential but is sufficient to inactivate Ca channels. These findings also agree with the discussed hypothesis of a pacing dependent change in the surface charge or a pacing-dependent Ca depletion near the membrane surface. Another experiment which supports this hypothesis was demonstrated in Fig. 4. If at the same pacing intervals the duration of the depolarizing steps had been prolonged the inward current activated by a second pulse was found to be distinctly decreased.

Thus, explanations concerning rate-dependent changes in the electrical activity of the heart have hitherto been searched for in: a) incomplete recovery of ionic currents between activation pulses, b) changes in ion concentrations on either site of the sarcolemma (Carmeliet 1977; Boyett and Jewell 1980; Attwell at al. 1981). The present experiments showed that there is some evidence for the existence of a pacing-dependent inactivation of the slow inward current as a further factor for determining the electrical activity in the heart muscle following changes in heart rate.

References

Ashcroft F. M., Stanfield P. R. (1981): Calcium dependence of the inactivation of calcium currents in skeletal muscle fibres of an insect. Science **213**, 224—226

Attwell D., Cohen I. (1977): The voltage clamp of multicellular preparations. Progr. Biophys. Mol. Biol. **31**, 201—245

Attwell D., Cohen I., Eisner D. A. (1981): The effects of heart rate on the action potential of guinea-pig and human ventricular muscle. J. Physiol. (London) **313**, 439—461

Boyett M. R., Jewell B. R. (1980): Analysis of the effects of changes in rate and rhythm upon electrical activity in the heart. Progr. Biophys. Mol. Biol. **36**, 1—52

Brehm P., Eckert R. O. (1977): Calcium entry leads to inactivation of calcium channels in Paramecium. Science **202**, 1203—1206

Brown R. Jr. (1974): Membrane surface charge: discrete and uniform modelling. Progr. Biophys. Mol. Biol. **28**, 343—370

Carmeliet E. (1977): Repolarisation and frequency in cardiac cells. J. Physiol. (Paris) **73**, 903—923

Chapman R. A., Fry C. H. (1978): An analysis of the cable properties of frog ventricular myocardium. J. Physiol. (London) **283**, 263—282

Connor J. A., Barr L., Jakobsson E. (1975): Electrical characteristics of frog atrial trabeculae in the double sucrose gap. Biophys. J. **15**, 1047—1067

Dunlap K., Fischbach G. D. (1981): Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurons. J. Physiol. (London) **317**, 519—535

Fischmeister R., Mentrard D., Vassort, G. (1981): Slow inward current inactivation in frog heart atrium. J. Physiol. (London) **320**, 27P—28P

Henček M., Zachar J. (1977): Calcium currents and conductances in the muscle membrane of the crayfish. J. Physiol. (London) **268**, 51—71

Isenberg G. (1977): Cardiac Purkinje fibres: The slow inward current component under the influence of modified Ca_i^{2+}. Pflügers Arch. **371**, 61—69

Jakobsson E., Barr L., Connor J. A. (1975): An equivalent circuit for small atrial trabeculae of frog. Biophys. J. **15**, 1069—1085

Nilius B., Henček M. (1981): Voltage-clamp-Untersuchungen von Membranströmen des Myokards bei Variation der Treibfrequens. Ref. 5. Gem. Tagung Ges. Exp. Med. DDR, Teil 1, P 91

Noble S., Shimoni Y. (1981a): The calcium and frequency dependence of slow inward current "staircase" in frog atrium. J. Physiol. (Lond.) **310**, 57—75

Noble S., Shimoni Y. (1981b): Voltage-dependent potentiation of the slow inward current in frog atrium. J. Physiol. (Lond.) **310**, 77—95

Payet M. D., Schanne O. F., Ruiz-Ceretti E. (1981): Frequency dependence of the ionic currents determining the action potential repolarization in rat ventricular muscle. J. Mol. Cell. Cardiol. **13**, 207—215

Reuter H. (1973): Time- and voltage-dependent contractile responses in mammalian cardiac muscle. Europ. J. Cardiol. **1**, 177—181

Shimoni Y. (1981): Parameters affecting the slow inward channel repriming process in frog atrium. J. Physiol. (London) **320**, 269—291

Šimurda J., Šimurdova M., Braveny P., Šumbera J. (1976): Slow inward current and action potentials of papillary muscles under non-steady state conditions. Pflügers Arch. **362**, 209—218

Standen N. B. (1981): Ca channel inactivation by intracellular Ca injection into Helix neurones. Nature **293**, 158—159

Tillotson D. (1979): Inactivation of Ca conductance dependent on entry of Ca ions in molluscan neurons. Proc. Nat. Acad. Sci. USA **70**, 1497—1500

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