Pacing-interval Dependent Properties of the Repolarization Phase in the Mammalian Atrial Myocardium

B. NILIUS, W. BOLDT and K. SCHÜTTLER

Julius Bernstein Institute of Physiology, Martin Luther University Halle-Wittenberg, Leninalle 6, 4020 Halle (Saale), GDR

Abstract. Action potentials were recorded from small right atrial trabeculae of the rabbit heart using a conventional microelectrode technique and analyzed by phase-plane plots. The polarization of an action potential following a prolonged pacing interval showed a) an initial rapid repolarization (IR), followed by b) a plateau or secondary depolarization (SD), and c) a retarded terminal repolarization (TR). IR disappeared in low Cl^--solutions and was diminished by 4-aminopyridine. SD was accentuated in high Ca solutions, after application of 4-aminopyridine or ouabain. It disappeared in low Ca solutions or after verapamil. TR was slowed down after a more rapid prepause drive, in high Ca solutions, after application of ouabain, but accelerated in low Ca solutions and after application of acetylcholine or verapamil. It is discussed that IR is generated upon activation of the positive dynamic outward current and that both SD and TR are due to an unmasked slow inward current, a decreased extracellular K accumulation and decreased late outward currents.

Key words: Atrial myocardium — Phase-plane-analysis — Repolarization — Pacing-dependent electrical properties

Introduction

The repolarization phase of all types of cardiac preparations is found to be highly sensitive to changes in the pacing interval (Carmeliet 1977; Boyett and Jewell 1980). Especially, the repolarization phase of the rabbit atrial myocardium changes its configuration distinctly after prolonged pacing intervals (Tanaka et al. 1967; Saito 1972; Nilius and Boldt 1980a; Nilius et al. 1980). However, our knowledge of the mechanisms which are responsible for the pacing-related variations of the repolarization process in the myocardium is very limited. In contrast, the study of pacing-related properties enables a deeper insight into both recovery of ionic currents and phenomena of accumulation on either side of the sacrolemma. The aim of the present paper is to evaluate changes in the repolarization process of the atrial myocardium after prolonged pacing intervals. Specific inhibitors of trans-

membrane currents and a phase-plane method were used to analyse pacing-dependent mechanisms in terms of ionic currents.

Material and Methods

Preparation. Electrical measurements.
43 trabeculae from the left atrial myocardium (2—5 mm in length, 200—400 µm in diameter) from rabbit hearts were used. The preparations were placed in a perfusion chamber and were superfused with a solution described below. Stimulation was performed by means of extracellular field electrodes (twice the treshhold strength, pulse duration 0.5—1.0 ms). This overall stimulation of the trabecular preparations may be convenient to fulfil sufficiently the space clamp condition $\partial U/\partial x \equiv 0$ in a multicellular preparation (for further details see Nilius and Boldt 1979). Transmembrane potentials were recorded using a convential microelectrode technique. The output of the microelectrode amplifier was displayed on a storage oscilloscope (OG-2-31, VEB Messelektronik Berlin) and stored on a tape (Analog 7, Philips). All the potential values during the repolarization phase were expressed as displacement from the resting potential. We only considered action potentials which arised from the same resting potential during the „pre-pause" and „post-pause-phase".

The following pacing programm was used (Fig. 2): the steady state pacing (pacing interval I_O: from 0.25 to 1.5 s) was interrupted to produce one prolonged pacing interval I_P (or pacing pause) lasting 5 to 300 s.

Solutions.
Control solution (rate of superfusion 10 ml/min, volume of the preparation chamber 1.2 ml) contained (in mmol/l): NaCl 137, KCl 2.7, $CaCl_2$ 2.5, $NaHCO_3$ 12, NaH_2PO_4 0.4, glucose 11, gassed with 95% O_2 and 5% CO_2 (pH 7.2, temperature 30 ± 0.5 °C). The Ca-concentration of the solutions was changed between 0.25 and 7.5 mmol/l, the K-concentration between 2.7 and 8.1 mmol/l. The only applied concentration of verapamil (Knoll AG, Ludwigshafen) was 13.2 µmol/l which was sufficient to block the slow inward current (Kohlhardt et al. 1972; Kass and Tsien 1975; Fleckenstein 1977; Ehara and Kaufmann 1978). In order to block the potassium conductivity of the membrane (Kenyon and Gibbons 1979 a, b; Freeman 1979) a modified "cut-end" method (Imanaga 1974; Ochi and Nishiye 1974; Ito and Surawicz 1977) was used to apply 4-aminopyridine (4-APy, E. Merck, Darmstadt) intracellularly. For this purpose the preparations were cut from the fixed endings and were incubated for 15 min in a Ca free depolarizing solution containing in mmol/l: 4-APy 5, KCl 10.8, NaCl 137, $NaHCO_3$ 12, NaH_2PO_4 0.4, Glucose 5.5. Preparations were then fixed in the perfusion chamber and superfused with the control solution again. Preparations which satisfied the following criteria were only included into analysis: 1. a distinct prolongation of the steady state action potential at 25% repolarization, 2.development of this effect in about 10—15 min after reincubation, 3.almost unchanged resting potential after 10 min superfusion with the control solution (for further details see Nilius and Boldt 1980 b).

To obtain a Cl^--deficient solution NaCl was replaced by Na-glutamate, KCl by K_2SO_4, and 2.5 mmol/l $CaCl_2$ by 7.5 mmol/l Ca-thiosulfate to compensate for the decrease in the external Ca-activity (Kenyon and Gibbons 1977). Ouabain (g-strophantin, VEB Jenapharm, Jena) and acetylcholine (ACh, VEB Berlin-Chemie, Berlin) were used in concentrations of 1 µmol/l and 2 µmol/l, respectively.

Estimation of membrane currents.
A phase-plane analysis was used to estimate the membrane currents during the repolarization phase. It is well established that the slow repolarizing membrane currents can be approximated (see Mc Allister

et al. 1975; Beeler and Reuter 1977; Šimurdová et al. 1977; Nilius 1980; Boyett et al. 1980) by

$$I_m = -c_m \frac{dU}{dt} \tag{1}$$

(I_m: total transmembrane current in $\mu A.cm^{-2}$, c_m: membrane capacity in $\mu F.cm^{-2}$, U: displacement from the actual resting membrane potential in mV). In accordance to other authors (Beeler and Reuter 1977; Mc Allister et al. 1975) we fixed c_m to 1.0 $\mu F.cm^{-2}$. The exact values were found to be between 0.89 and 1.75 $\mu F.cm^{-2}$ for the same preparation (Bonke 1973). By numerical differentiation of the repolarization phase for each measured action potential we obtained a phase-plane plot which was approximated by means of the least square method using equation (1) and

$$I_m = \sum_{i=1}^{n} a_i U^i, \quad 1 \leq n \leq 5 \tag{2}$$

(a_i: conductivity in $mS.cm^{-2}.mV^{1-i}$). To test the validity of the approximation the coefficients of correlation r between the measured ($-c_m.dU/dt$, see (1)) and the modelled (I_m, see (2)) current values were calculated. If $r<0.9$, then the degree of the polynom n was increased (starting with 1). This $I_m - U$ polynomial of minimal degree n was considered for further calculations which fulfils the condition $r \geq 0.9$. As an additional test of accuracy of the polynomial approximation the measured repolarization phase was approximated by means of the numerical solution of the initial value problem

$$\frac{dU}{dt} = -c_m^{-1}.I_m, \quad U(O) = U^o \tag{3}$$

(for I_m see equation (2), U^o means the maximum overshoot potential). The numerical solution was compared with the measured repolarization phase. If the coefficient of correlation between these two time series r was found to be <0.9 then we tried to improve the approximation (increase in n, changing c_m). If $r \geq 0.9$, then the calculated membrane current I_m was used for further estimations (Fig. 1).

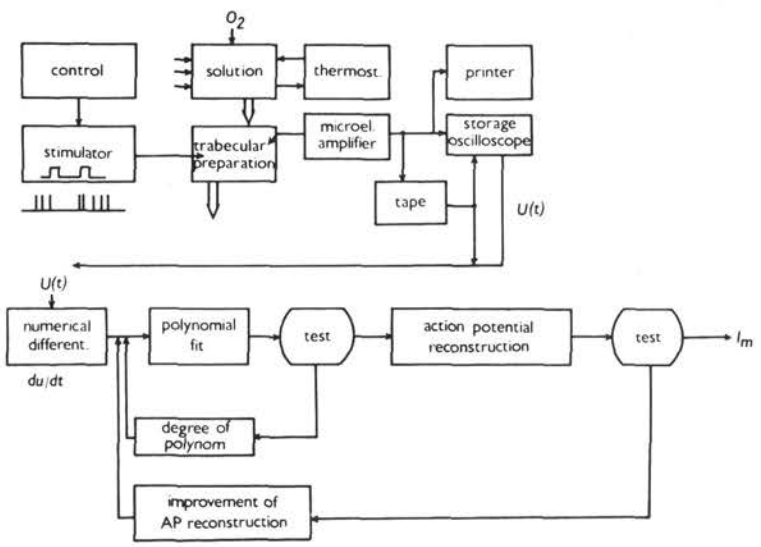

Fig. 1. Scheme of the experimental method and the numerical analysis of the measured repolarization phases $U(t)$.

Applying channel blockers as pharmacological tools (e. g. verapamil for the slow inward current) to separate a current I_i this current was estimated by a simple substraction of the control and the test current (after application of the drug) at the same potentials U

$$I_i(U) = I_{m,\,control}(U) - I_{m,\,test}(U) \tag{4}$$

Henceforward, the currents calculated in this way are termed „verapamil-sensitive current", „4-APy-sensitive current". For this type of analysis the ionic currents are considered to be voltage-dependent only. The time-dependent gating has been neglected in the following sense: 1. The activation of the slow inward current may be instantaneous. 2. The inactivation of this current and processes of accumulation are much slower than changes in the voltage during the repolarization, hence, the inactivation is of lesser importance than the decline of the voltage and is disregarded. 3. Delayed outward currents show very large time constants of activation and deactivation compared to the duration of the action potential. These currents were believed to be voltage dependent only. Because of the deactivation during the long lasting pacing pause there are different pre- and post-pause values. Considering these uncertainties we emphasize that the currents estimated from one repolarization phase are not identical with ionic currents in terms of the voltage clamp analysis (for further explanations see Nilius 1980; Nilius and Boldt 1980 b, c). All calculations were performed using an EMG 666 or a Wang 2200 computer.

For statistical analysis a parameter free test was used (Wilcoxon-test). The values of the above parameters are expressed as mean values ± S. D.

Results

The repolarization phase after prolonged pacing intervals. The steady state action potential (AP) showed a very smooth repolarization without a marked initial phase or a well developed plateau (Fig. 2). After prolonged pacing intervals (pacing pauses) the first AP was characterized by an initial rapid repolarization (IR) followed by a plateau-like phase sometimes showing a secondary depolarization (SD), and a distinct delayed terminal repolarization (TR). After the pacing pause

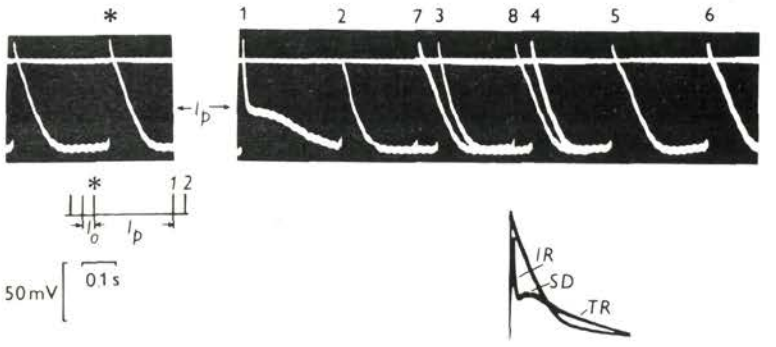

Fig. 2. Top: Post-pause action potentials in the atrial myocardium. Note the rapid initial repolarization (IR) followed by a plateau or secondary depolarization (SD), and a prolonged terminal repolarization (TR). The post-pause action potentials are numbered. * marks the steady state action potential. Bottom: pacing programme and denotations. $I_o = 0.28$ s, $I_p = 30$ s.

the regular shape of the repolarization was restored after about the tenth AP.

Analysis of the initial repolarization, IR. Both low Cl^- solutions (Fig. 3) and 4-APy load reduced the slope of IR. To estimate the membrane current which is believed to be involved in IR the repolarization was differentiated numerically during the first fifteen ms. Because of the linear semilogarithmic plot the following formula is obtained as the time course of IR

$$U = U^0 e^{-\lambda t} \tag{5}$$

and by using equation (1)

$$I_m = c_m . \lambda . U = g_s . U \tag{6}$$

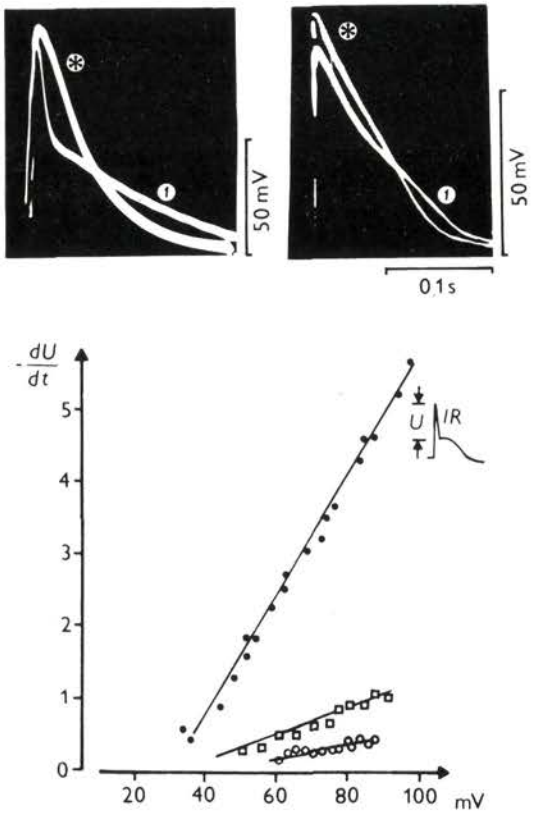

Fig. 3. Analysis of the initial repolarization (IR). Above: variation of the post-pause action potentials in low Cl^--solutions (denoted by 1). Left: 145 mmol/l-Cl^-; right; 10 mmol/l-Cl^-. $I_o = 0.5$ s, $I_p = 20$ s. Bottom: estimation of the transmembrane current during IR. If c_m is assumed to be 1.0 $\mu F . cm^{-2}$ the phase-plane plot of IR is equivalent to the current-voltage relationship. Filled circles: control, squares: 4-APy containing solution, hollow circles: low Cl^- solutions (10 mmol/l).

The values of λ were obtained by approximating IR using equation (5). Assuming that during the repolarization the membrane capacity was constant (1.0 μF.cm^{-2}) λ represents a slope conductance, g_s, of 0.12 ± 0.1 mS.cm^{-2} under control conditions. In both, 4-APy containing and Cl^--deficient solutions, g_s was decreased (0.02 ± 0.01 mS.cm^{-2} in 4-APy; 0.01 ± 0.008 mS.cm^{-2} in 10 mmol/l Cl^- solution; mean values ± S. D., four experimetnts). A more detailed analysis of IR by means of 4-APy is demonstrated in Fig. 4.4-APy prolonged the duration of the steady state action potentials, especially in the earlier phases of repolarization. The post-pause AP showed a delayed IR. The phase-plane plots for estimating the 4-APy sensitive (blocked) current showed that during steady state pacing only a very small fraction of the outward current is influenced by 4-APy, after a pacing pause, however, a large fraction of the outward current which is responsible for IR was found to be 4-APy sensitive.

It was possible to approximate both the 4-APy and Cl^--sensitive currents by a linear current-voltage relationship (dashed lines in Fig. 4, see also Fig. 3). Two

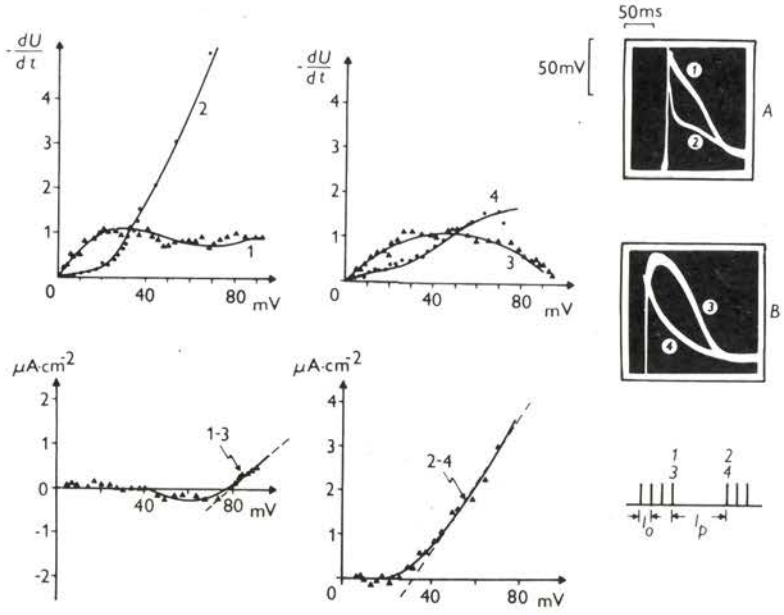

Fig. 4. Evaluation of the 4-aminopyridine (4-APy) sensitive current (5 mmol/l). Records on the right: 1,3- steady state, 2,4- post-pause action potentials. A: controls; B: in 4-APy.
$I_o = 0.5$ s, $I_p = 5$ s. Top left: phase-plane plots of the repolarization phases of the action potentials (1,2) demonstrated in A. The smooth curves were calculated according equation (2). Bottom left: evaluation of the 4-APy sensitive current by subtracting (1—3) the voltage-current relationship in 4-APy from the control curves ($c_m = 1.0$ μF.cm^{-2}, I_m is expressed as μA.cm^{-2}). The records in B are evaluated similarly at the right. Note the increased 4-APy sensitive outward current after pacing pauses (2—4).

quantitative data were obtained from this analysis to characterize IR in terms of an ionic current: 1. the slope conductance of the calculated current-voltage relationship and 2. the intercept of the abscissa for zero current (may be an estimate of the reversal potential) (Table 1). From this type of experiments it might be concluded

Table 1. Analysis of both the 4-aminopyridine (4-APy) and Cl^--sensitive outward currents by linearization of the outward current-voltage plots during IR. g_s: slope conductance in $\mu S.cm^{-2}$ assuming $c_m = 1.0\ \mu F.cm^{-2}$; U_R: potential for zero-current of the 4-APy and Cl^--sensitive outward current.

	4-aminopyridine; $I_o = 500$ ms		
group No.	pacing	g_s [$\mu S.cm^{-2}$]	U_R [mV]
1	steady state	39.2 ± 12.3	74.3 ± 11.1
2	I_p = 5 s	65.0 ± 15.1	31.7 ± 9.8
3	I_p = 30 s	93.5 ± 20.2	20.5 ± 3.2
	10 mmol/l Cl^-; $I_o = 500$ ms		
group No.	pacing	g_s [$\mu S.cm^{-2}$]	U_R [mV]
4	I_p = 5 s	48 ± 14.1	36.3 ± 17.9
5	I_p = 30 s	143 ± 21.7	27.7 ± 15.2
Significance			
i/k		1/3, 1/5, 4/5	1/2, 1/3 1/4, 1/5

Notes: mean values of 5 preparations ± S. D.; significance i/k means that the i-th and the k-th groups are separated significantly; tests of significance were performed by the Wilcoxontest; $P < 0.05$.

that after prolonged pacing intervals the IR arises upon activation of both the 4-APy and Cl^--sensitive current.

Analysis of the plateau phase or secondary depolarization, SD. Following IR a distinct plateau marked the post-pause action potential (see Fig. 2, also Fig. 7). After pauses longer than 20 s the majority of the recorded action potentials showed a secondary depolarization. For quantitative description the AP were differentiated numerically to evaluate the minimum current during SD. This current is negative (inward current) if a secondary depolarization takes place.

Fig. 5 demonstrates current-time plots for evaluating the minimum current during SD. SD was never observed in 1 mmol/l Ca^{2+} solutions. The minimum current during the plateau was found to be positive (outward current). The same results were obtained after application of 13.2 µmol/l verapamil (see also Fig. 5, 6, 7). After application of ouabain (1 µmol/l), increased external Ca concentrations and 4-APy respectively, the minimum currents were negative (inward currents).

Because SD was found to be highly sensitive to changes in the external Ca concentration (Fig. 5, 7; see also Nilius and Boldt 1980a) we tried to estimate the involvement of the slow inward current mediated mainly by Ca in both the SD and TR. Fig. 6 shows the effects of verapamil in concentrations which are known to block the slow inward current (13.2 μmol/l) upon the post-pause action potential: the notch after IR disappeared as a consequence of the loss of SD; TR was found to be accentuated, the verapamil-sensitive current was shifted to less positive poten-

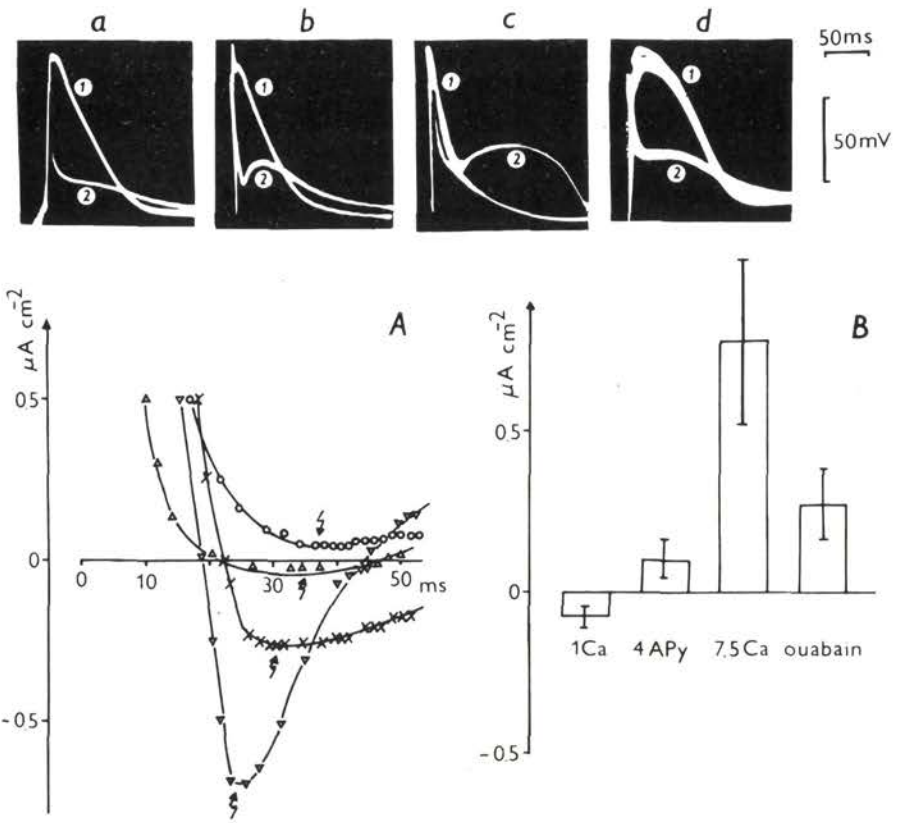

Fig. 5. Analysis of the secondary depolarization, SD. Above: steady state (1) and post-pause action potentials (2) under different experimental conditions. a: 1 mmol/l—Ca^{2+}; b: 7.5 mmol/l—Ca^{2+}; c: 1 μmol/l-ouabain; d: 5 mmol/l—4-APy. For all records $I_o = 500$ ms, $I_p = 30$ s. Below: A: current-voltage plot obtained from the numerical differentiation of the post-pause repolarization during SD ($I_m = -c_m \, dU/dt$; $c_m = 1.0$ μF.cm^{-2}). The minimum currents during SD are marked by arrows. Hollow circles: 1 mmol/l-Ca; downwards triangles: 7.5 mmol/l-Ca; upwards triangles: 5 mmol/l—4APy; crosses: 1 μmol/l-ouabain. B: dependence of the minimum current upon the external Ca concentration (1 mmol/l, 7.5 mmol/l), 4-APy and ouabain (1 μmol/l) (mean values ± S. D. from seven experiments; the columns represent values showing significant differences; Wilcoxon test, $P < 0.1$).

tials and, the slope conductance which was obtained by linearization of the initial inward part of this current (dashed line in Fig. 6) was increased (see Table 2). In

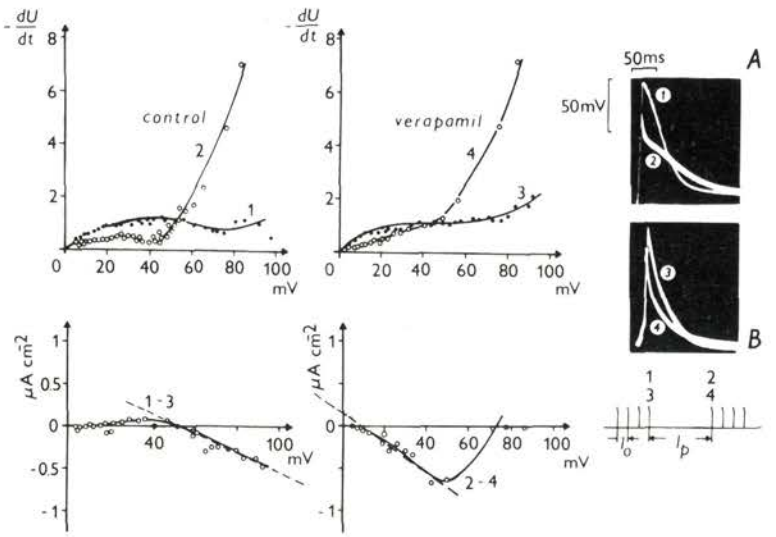

Fig. 6. Analysis of the effects of verapamil on both SD and TR. Records on the right: 1,3-steady state; 2,4-post-pause action potentials. A: controls; B: in 13 μmol/l. 4-aminopyridine for 30 min. $I_o = 0.5$ s, $I_p = 10$ s. The numbers of the action potentials are identical with those on the current-voltage plots. Top left: steady state and post-pause phase-plane plots of the repolarization phases shown on the right before (A) and after application (B) of verapamil (dU/dt in [$V.s^{-1}$]). Bottom left: evaluation of the steady state and post-pause verapamil-sensitive currents by means of the indicated substraction (1—3) of the phase-plane plots (I_m in $\mu A.cm^{-2}$). The dotted lines mark the linearized initial part of the current voltage relationship from which the slope conductance g_s and the threshold potential U_S (see Table 2) were calculated. The records in B are evaluated similarly at the right.

Table 2. Properties of the verapamil-sensitive current obtained by phase-plane analysis. g_s describes the slope conductance of the linearized initial parts of the current-voltage plots in $\mu S.cm^{-2}$ ($c_m = 1.0\ \mu F.cm^{-2}$) U_S is the threshold potential (intercept of the abscissa for zero current) in mV (see Fig. 7).

group No.	pacing	g_s [$\mu S.cm^{-2}$]	U_S [mV]
1	steady state	12.37 ± 4.79	49 ± 11
2	I_p = 5 s	13.34 ± 4.93	17 ± 6
3	I_p = 10 s	17.50 ± 5.50	5 ± 4
4	I_p = 30 s	21.11 ± 6.11	7 ± 3
Significance		1/4	1/2, 1/3 1/4, 2/4

Note: For the test of significance see Table 1; mean values ± S. D. from 6 experiments.

some cases also a verapamil-sensitive outward current at more positive potentials was apparent refering to a possible effect of verapamil on the current responsible for IR.

Analysis of the terminal repolarization, TR. The post-pause action potential showed a retardation of the terminal repolarization TR which has been expressed in the prolongation of the post-pause action potential compared to the steady state AP at 90% repolarization (Fig. 7). This prolongation was accentuated by decreasing the pre-pause pacing interval, increasing the external Ca concentration, and

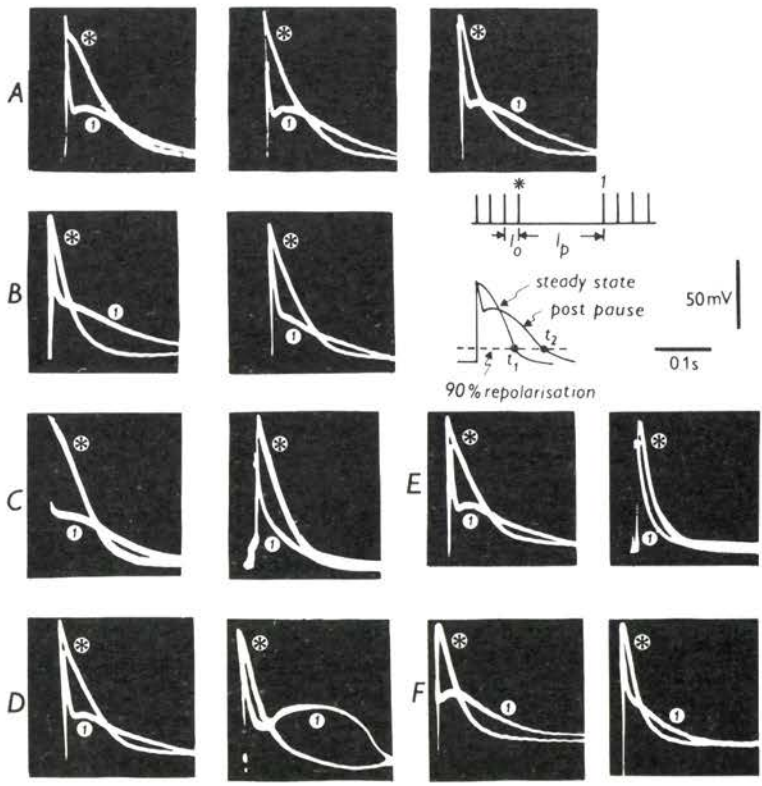

Fig. 7. The influence of various experimental conditions upon TR. A: a variation in the pre-pacing interval I_o (right: 250 ms; middle: 500 ms; left: 1000 ms); B: variation in the external Ca concentration (left: 7.5; right: 0.25 mmol/l), $I_p = 10$ s, $I_o = 0.5$ s; C: effects of verapamil (13.2 mmol/l), $I_p = 30$ s, $I_o = 0.5$ s, left: control solution ; D: changes in SD and TR 10 min after application of 1 µmol/l ouabain, $I_p = 30$ s, $I_o = 0.6$ s; left: control solution; E: effect of acetylcholine (2 µmol/l) upon post-pause repolarization; $I_p = 30$ s, $I_o = 0.5$ s; left: in control solution; F: variation in external K concentration (5 and 8.1 mmol/l respectively); $I_p = 30$ s, $I_o = 500$ ms; $I_p = 30$ s.

Table 3. Analysis of the late terminal repolarization (TR). Δt in ms means variation of the post pause action potential at 90% repolarization compared to the steady state action potential before the pause (see inset Fig. 8). All the values are obtained from pacing pauses lasting $I_p = 30$ s. Negative Δt means a shortening of the post-pause action potential. g_c and g_p were obtained by approximation of the last 20 mV of the repolarization by means of $U = U_o \times e^{-at}$. If $-dU/dt = c_m^{-1} . I_m$ then $g = a . c_m$ means a background conductivity (g_c: steady state, control, g_p: post-pause) ($c_m = 1.0\ \mu F . cm^{-2}$). n: number of experiments.

group No.	pacing I_o [ms]	exp. conditions	t [ms]	g_p/g_s	n
1	250	control	119.7 ± 12.4	0.30 ± 0.07	21
2	500	control	71.3 ± 9.2	0.34 ± 0.06	49
3	1000	control	32.7 ± 4.7	0.46 ± 0.06	19
Significance			1/2, 1/3, 2/3	1/3, 2/3	
4	500	Ca: 7.5 mmol/l	112.1 ± 10.9	0.24 ± 0.03	7
5	500	Ca: 0.25 mmol/l	−9.2 ± 4.7	1.07 ± 0.09	7
Significance			4/5, 2/4, 2/5	4/5, 2/4, 2/5	
6	600	ouabain, 1 μmol/l	127.3 ± 27.9	1.09 ± 0.11*	6
7	500	verapamil, 13.2 μmol/l	5.0 ± 4.1	1.05 ± 0.08	12
8	500	acetylcholin, 2 μmol/l	11.1 ± 1.7	1.05 ± 0.08	19
9	270	K: 8.1 mmol/l	41.3 ± 4.1	0.69 ± 0.07	6
Significance			2/6, 2/7 2/8, 1/9	2/6, 2/7, 2/8 1/9	

Note: For explanation of significance see Table 1 (* g_p/g_s changes opposite to Δt).

after application of ouabain (1 μmol/l) respectively. Both SD and TR were suppressed by acetylcholine (2μmol/l). Verapamil (13.2 μmol/l) and high external K concentrations (8.1 mmol/l) accelerated TR (Fig. 7, Table 3). A prolonged TR was found in solutions containing increased Ca-concentrations and after increasing the pre-pause pacing frequency.

In contrast, an increased pre-pause pacing interval, Ca-poor solutions or application of verapamil shortened TR. Similar to the analysis of IR (equation (5) and (6)) the terminal repolarization was analyzed for the last 20 mV. From TR a linear semilogaritmic voltage-time plot was obtained. Assuming a constant membrane capacity (1.0 $\mu F.cm^{-2}$) the rate constant of TR was equivalent to a conductivity which describes a "background conductivity" g. If the pacing pause was prolonged, this conductance was found to be decreased significantly (Fig. 8). 4-APy diminished the steep, pacing dependent decline of the background conductivity indicating that a potassium conductance may be involved in the generation of TR (see points marked by arrow in Fig. 8). To test possible mechanisms which may be involved in TR we quantified the changes in the post-pause background conductivity. g_P/g_C in Table 3 marks the ratio between the post-pause (g_P) and

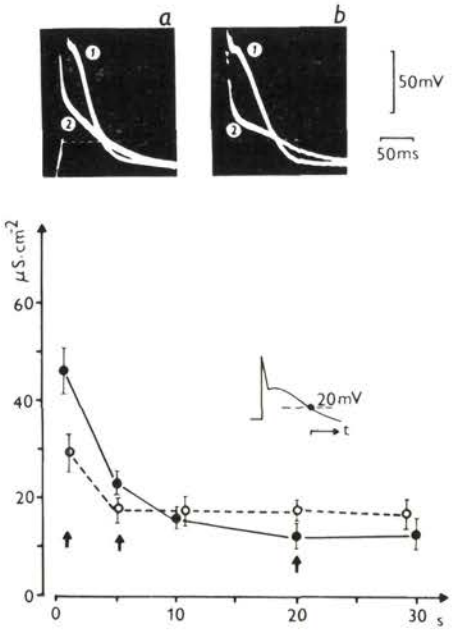

Fig. 8. Analysis of TR, evaluation of the background conductivity. Dependence of the „background" conductivity $g = c_m . a$ ($c_m = 1.0$ $\mu F.cm^{-2}$) upon the duration I_P of the pacing pause in control solution (filled circles) and after application of 4-APy (hollow circles). Records *a*, *b*: $I_P = 5$ s (*a*) or 30 s (*b*). $I_o = 500$ ms.

steady state or pre-pause (g_C) "background conductivity" obtained from the last 20 mV of the repolarization. If the pre-pause pacing frequency is increased then g_P/g_C decreases. Verapamil, ouabain, acetylcholine and low Ca solutions did not change the post-pause background conductivity significantly, whereas the ratio is decreased in Ca-rich solutions. Increased external K^+ concentrations lessened the decline of g_P.

Fig. 9 summarizes both the changes in the background conductivity in the steady state and after a 30 s lasting pacing pause. Absolute values of the

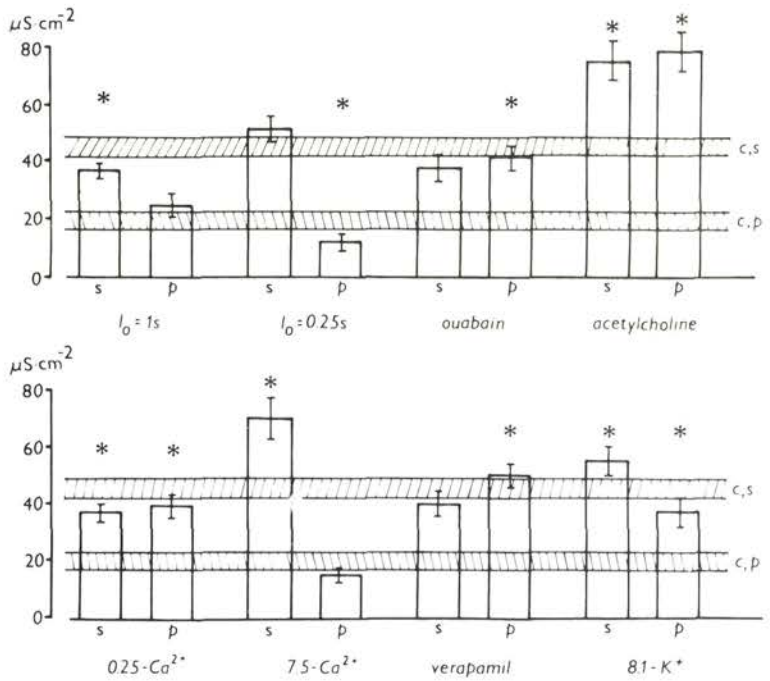

Fig. 9. Synopsis of both steady-state and post-pause changes in the "background conductivity" g under various experimental conditions. g (in[$\mu S.cm^{-2}$]) was obtained from the same analysis indicated in Table 3; c, s means the steady state control value (46.7 ± 2.9 $\mu S.cm^{-2}$), c, p the post-pause value of g (19.6 ± 2.9 $\mu S.cm^{-2}$). s denotes the steady state, p the post-pause values. * indicates that the differences as compared to c, s or c, p respectively are significant (Wilcoxon test, $P<0.05$).

background conductance indicate that ouabain, acetylcholine, verapamil, high K^+ and low Ca solutions all failed to decrease the post-pause conductance and that rapid pre-pacing and Ca-excess did accentuate this decrease.

Discussion

In the present study, the following phases of action potential after prolonged pacing intervals were investigated: the rapid initial repolarization (IR), followed by the plateau phase or secondary depolarization (SD), and the delayed terminal repolarization (TR, see Fig. 2).

IR was found to be Cl^--sensitive and decelerated by an intracellular 4-APy load. The slope conductance of the outward current during IR reconstructed by phase-plane analysis was increased if pacing pauses were prolonged. The current almost disappeared at steady state pacing intervals shorter than 1 s. These findings suggest a contribution of the so-called positive dynamic outward current I_{qr} (Mc Allister et al. 1975) in the genesis of IR. This current has been described in the Purkinje fibre (Dudel et al. 1967; Fozzard and Hiraoka 1973; Hiraoka and Hiraoka 1975; Siegelbaum et al. 1977; for review see Carmeliet 1977; Boyett and Jewell 1980), but never in the atrial myocardium. The current might be inactivated during the depolarization but its recovery from inactivation is very slow at the normal resting potential (Peper and Trautwein 1968; Fozzard and Hiraoka 1973; Siegelbaum and Tsien 1980).

A recovery time constant of about 15 s was calculated from the dependence on the duration of the pacing pause of the slope conductance g_S (Table 1, Fig. 2, 3, 4). This recovery time constant of I_{qr} is much higher in the atrial myocardium (about 15 s) than in the Purkinje fibre (about 1 s).

Because of the blocking action of 4-APy this current may be a K^+ current in accordance to the findings in Purkinje fibres (Siegelbaum et al. 1977; Isenberg 1978; Kenyon and Gibbons 1979 a, b; Siegelbaum and Tsien 1980).

In high Ca solutions and after application of ouabain the secondary depolarization of the post-pause action potential was found to be accentuated (increase in the minimum current during SD see Fig. 6), whereas all interventions which are believed to decrease the Ca influx (low Ca solutions, verapamil, see Fig. 6—8) diminished SD. Ouabain increases the slow inward current I_{si} (Weingart et al. 1978) which could accentuate SD; the latter could be also accentuated by unmasking the slow inward current due to inhibition of the electrogenic Na pump resulting in the decrease of the pump current (Isenberg and Trautwein 1974; Glitsch 1979; Gadsby and Cranefield 1979).

The accumulation could result in a rise of the internal Na concentration during the rapid drive (Lüllmann and Peters 1979). A fall in the internal Na concentration during the pacing pause could diminsh the outward current which is generated by the electrogenic Na pump. This mechanism would contribute to a decrease in the post pause outward currents and an inhibition of the Na pump by ouabain could thus accentuate SD. Also, processes of accumulation may be involved in the increase of the slope conductance of the verpamil-sensitive current after pacing

pause and in the shift of the threshold potential to less positive values (Fig. 7, Table 2). The decreased verapamil-sensitive current after pacing pauses may be due to the overlapping blocking effect of verapamil on both I_{qr} and I_{si}. Similar decrease of I_{si}, when the pacing interval was decreased, was reported by Ehara and Kaufmann (1978) in a voltage-clamped ventricular preparation of the cat. According to our analysis SD arises after prolonged pacing pause due to 1. an increased slope conductance of I_{si} near the treshold potential, 2. a shift in the treshold potential of I_{si} to less positive potentials, and 3. an unmasking of I_{si} by decreased outward currents.

The distinct prolongation of the late repolarization (TR) could reflect changes in both the potassium and sodium concentrations on either side of the membrane. It was found that after a pre-drive with shorter pacing intervals TR was accentuated stronger than after a slower pre-drive (Fig. 7, Table 3) and that also TR was accelerated in high K^+ solutions (Table 3). If the pre-pause pacing interval is decreased the K^+ accumulation within extracellular clefts would be increased (Boyett and Jewell 1978, 1980; Kline and Morad 1978; Kunze 1977; Baumgarten and Isenberg 1977; Kline et al. 1980). During the pause a K depletion takes place. This depletion causes a diminution in the K conductivity in accordance to the constant field formalism (Weidmann 1956; Kline and Morad 1978; Cleeman and Morad 1979; Di Francesco and Mc Naughton 1979; Noma et al. 1979); therefore, the post-pause repolarization is prolonged. This hypothesis is supported by the effects of prolonged pacing intervals upon the background conductivity (Fig. 9). Prolongation of the pacing pauses yields a distinct background conductivity. The decrease is less accentuated after 4-APy load refering to the potassium conductivity. A further finding which supports the hypothesis that TR may be generated by a decrease in the potassium conductivity is the disappearance of both TR and SD if the potassium conductivity is increased (after application of 2 μmol/l acetycholine, Table 3, Fig. 7). During the pacing pause a deactivation of delayed outward currents could take place. As a consequence, also a decreased outward current would result.

Finally, it should be mentioned that high Ca solutions prolong TR, but low Ca solutions and verapamil accelerate TR. A Ca-sensitive potassium conductivity might be involved in the generation of TR (Kass and Tsien 1975; Bassingthwaighte et al. 1976; Isenberg 1977).

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