Pacing-Induced Transient Depolarizations in Rabbit Atrial Myocardium

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Abstract. Transient afterdepolarizations (TD) were analyzed following a train of paced action potentials in isolated pectinate muscles bordering the crista terminalis in rabbit right atria. After cessation of a rapid drive TD peaked between 400 and 700 ms. The amplitude of TD were found to be pacing-dependent (maximum at 250 ms pre-drive pacing interval 12.9 ± 2.2 mV, $n = 27$). At pacing intervals longer than 1000 ms no TD could be observed but pacing intervals shorter than 250 ms provoked a triggered activity. Verapamil (13.2×10^{-6} mol . l^{-1}) completely blocked pacing-induced TD. TD could be described quantitatively using a model of the transient inward current.

Key words: Atrial myocardium — Transient depolarization — Pacing-dependence — Verapamil — Transient inward current

Introduction

Recent studies have suggested an important role of oscillatory afterpotentials in the genesis of arrhythmias (Cranefield 1975, 1977; Wit and Cranefield 1976, 1977; Wit et al. 1980; Hiraoka et al. 1981).

Under experimental conditions oscillatory or transient afterdepolarizations (TD) could be studied in ventricular strophantidin-intoxicated fibres (Ferrier et al. 1973). Voltage change seems to be caused by a transient inward current that occurs in intracellular Ca-overload (Lederer and Tsien 1976; Colquhoun et al. 1981; Hiraoka et al. 1981; Clusin et al. 1982; Karagueuzian and Katzung 1982; Kass and Tsien 1982; Lipsius and Gibbons 1982; Matsuda et al. 1982; Clusin 1983). We also could demonstrate pacing-induced TD in pectinate muscles bordering the crista terminalis in right atrial myocardium under physiological conditions (Nilius et al. 1983). Triggered activity could be provoked from TD's under physiological conditions resulting in a focal induced arrhythmia. In the present work properties of TD as a pacing-dependent phenomenon were studied. Since the described type of myocardial preparations does not allow voltage-clamping another goal was to

describe TD quantitatively by means of a mathematical model of the transient inward current. An analysis of afterhyperpolarizations following TD was presented in a previous paper (Nilius et al. 1984).

Materials and Methods

Preparation Electrical measurement

About 40 isolated pectinate muscles were investigated electrophysiologically Rabbits weighing 1—2 kg were sacrificed by neck blow The hearts were excised and after removing both the left atria and the ventricles right atrial preparations were pinned to a cork block Free running pectinate muscles bordering the crista terminalis were carefully isolated and fixed between two clips which were mounted in a superfusion chamber The diameters and lengths of the trabecular preparations were between 150—800 μm and 2—3 mm, respectively Transmembrane potentials were from the preparations fixed between the clips using the standard microelectrode technique The preparations were driven with extracellular electrodes connected to the clips (2 times threshold strength, pulse duration between 0 5 and 2 ms) Pacing intervals I, were varied between about 200 and 2000 ms Afterpotentials were measured after switching off the pacing ("pre-drive") The output of the microelectrode amplifier was displayed on a storage oscilloscope (OG 2—31, VEB Messelektronik Berlin, GRD) or on a pen-recorder (Endim 621 01, Schlotheim, GDR)

Solutions

The preparations were superfused (rate 10 ml/min, volume of the chamber 2 ml) with a control solution containing of (mmol l^{-1}) NaCl 137, KCl 2 7, $CaCl_2$ 2 5, $NaHCO_3$ 12, NaH_2PO_4 0 4, glucose 11, pH 7 3—7 4 The solution was pre-gassed with 95 % O_2, and 5 % CO_2 The temperature of the solution was kept constant at 30 ± 1 °C Verapamil (Knoll AG, Ludwigshafen FRG) was used in the concentration of $13\,2 \times 10^{-6}$ mol l^{-1} In high K solution the external K concentration was adjusted to 8 1 mmol l^{-1}

Numerical analysis of afterpotentials

A derivative free modification of the Levenberg-Marquardt algorithm for non-linear least square approximation (Brown and Dennis 1972) was used to model the measured time courses of afterpotentials This type of analysis was chosen since voltage clamping of mammalian atrial myocardium is extremely difficult (Ten Eick et al 1976) if possible at all Time course of afterpotentials was described by

$$\Delta U = a_1 e^{-a_2 t} + a_3 e^{-a_4 t} \quad (1)$$

where ΔU means the displacement from the maximum diastolic (post-drive) potential measured as a steady state value during rest after switching off the pre-drive

$$\Delta U = U - U_r \quad (2)$$

(U the actual transmembrane potential after switching off the predrive, U_r steady state transmembrane potential during rest, both in mV) The parameters a_1 a_4 could be obtained from the best approximation It is well established that the slowly changing transmembrane current, i_m, can be estimated by

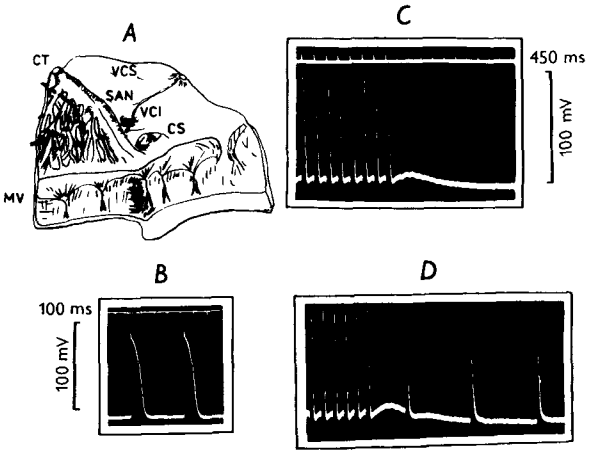

Fig. 1. Appearance of transient afterdepolarizations (TD) in rabbit atrial myocardium. *A*: The preparations were isolated from the area marked by the broken line just below the crista terminalis (CT). VCS: superior vena cava, VCI: inferior vena cava, SAN: sinus node region, CS: coronary sinus, MV: mitral valve. *B*: Isolated pectinate muscles show plateau-like depolarization. *C*: After cessation of a rapid drive TD was evoked. *D*: TD also appeared after changing the pacing to slower rates (the same calibration as in 1*C*).

$$i_m = -c_m \frac{d(\Delta U)}{dt} \tag{3}$$

(Boyett et al. 1980; Nilius et al. 1982) and therefore

$$i_m = c_m(a_1 a_2 e^{-a_2 t} + a_3 a_4 e^{-a_4 t}) \tag{4}$$

(i_m: net-transmembrane current in [nA . cm^{-2}] c_m: membrane capacity in [μF . cm^{-2}]. According to the results of Lederer and Tsien (1976); Kass et al. (1978a, b); Eisner and Lederer (1979); Tsien et al. (1979); Kass and Tsien (1982) the transmembrane current during the post-drive period is separated into

$$i_m = i_K + i_{ti} \tag{5}$$

$$i_K = g_K \Delta U \tag{6}$$

$$i_{ti} = \bar{i}_{ti} e^{-t/\tau_{ti}} \tag{7}$$

i_K may be interpreted as a background (K) current (Mc Allister et al. 1975; Boyett et al. 1980); i_{ti} is interpreted as the transient inward current described in the reports mentioned above. Therefore g_K means a background conductance (μS . cm^{-2}), $\bar{i}_{ti}$ the maximum instantaneous transient inward current (nA . cm^{-2}), τ_{ti} the inactivation time constant. $t = 0$ marks the predrive switching off. To separate i_{ti}, g_K can be obtained from

$$g_K = \lim_{t \to \infty} \frac{c_m d(\Delta U)/dt}{\Delta U} = c_m \cdot a_2 \tag{8}$$

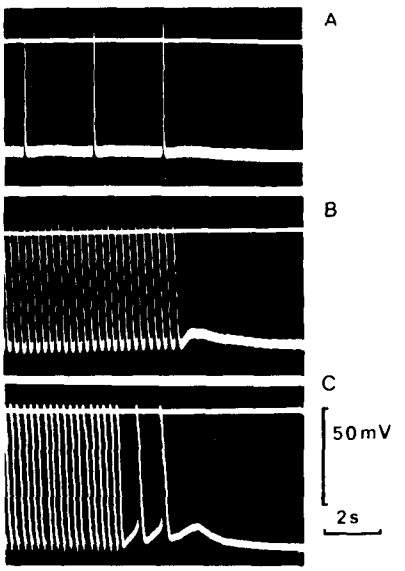

Fig. 2. Pacing-induced transient afterdepolarizations (TD) following a train of action potentials in isolated pectinate muscle from right rabbit atrium I_0 pre-drive pacing interval Note the two triggered action potentials after a pre-drive with $I = 220$ ms (2C) *A* $I_0 = 2.4$ s, *B* $I_0 = 245$ ms, *C* $I_0 = 220$ ms

as considered cases in all the $a_4 > a_3$ Therefore

$$\bar{i}_{ti} = c_m a_3 (a_4 - a_2) \tag{9}$$

$$\tau_{ti} = a_4^{-1} \tag{10}$$

In all the calculations c_m was fixed at 1.0 μF cm^{-2}

Results

Appearance of afterpotentials and triggered activity

Under physiological conditions fibres showing transient afterdepolarizations (TD) we found in right atrial preparations just below the crista terminalis (Fig. *1A*). Freely running pectinate muscles could be isolated and prepared for electrophysiological studies. All the preparations studied showed a plateau-like repolarization unlike the common atrial working myocardium (Fig. *1B*). TD could be observed after the pacing had been switched off (Fig. *1C*) as well as following decrease in the pacing frequency (Fig. *1D*).

If the pacing interval was shortened TD became accentuated. It disappeared

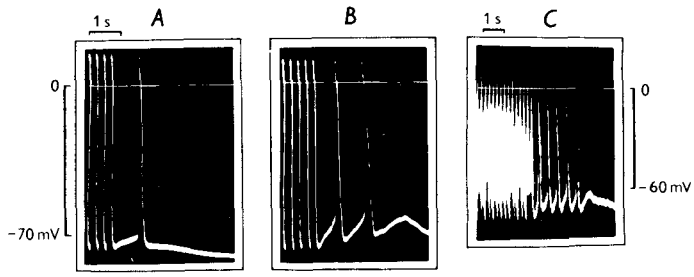

Fig. 3. Appearance of triggered activity due to an increase in the duration T of a high-frequent pre-drive. The preparation was isolated as described in the legend to Fig. 1 A. A: $I_o = 250$ ms, $T = 15$ s; B: $I_o = 250$ ms, $T = 30$ s; C: $I_o = 250$ ms, $T = 60$ s.

if the pacing interval I_o was prolonged to more than 1000 ms. At pacing intervals shorter than about 220 ms a spontaneous activity was triggered in preparations which did not show any signs of automaticity (Fig. 2). Triggering was found to be dependent on the duration of the train of conditioning paced action potentials: if the duration of the pre-drive was prolonged the number of triggered action potentials increased (Fig. 3).

Pacing dependence of TD

Appearance of TD was demonstrated to be a pacing-dependent phenomenon. Fig. 4 summarizes pacing-dependent properties of TD: Maximum TD (12.9 ± 2.3 mV) were observed at a pre-drive pacing interval I_o of 273 ± 51 ms ($n = 27$). At slow pacing rates no TD could be evoked. Maximum TD were measured between 400 and 700 ms after switching off the pacing. No pacing-dependent differences in peak time of TD could be demonstrated.

Analysis of TD

TD could be completely blocked by verapamil (13.2×10^{-6} mol . l^{-1}). Under these conditions only a hyperpolarizing afterpotential was generated by a rapid pre-drive (Fig. 5 *A*, *B*). No significant TD could be measured from eight time courses at 250 ms pre-drive pacing interval if the preparations were superfused with high concentrations of verapamil over 30 minutes. Fig. 6 shows an example of analysis of depolarizing afterpotentials. The voltage-time plot was obtained from averaging 4 afterpotentials following a pre-drive of 250 ms pacing interval. The enddiastolic potential after switching off the pacing at −73 mV was lowered to −68 mV (TD) but it declined during the rest to a steady state value of −82 mV. The time course was best approximated by the Equation (1) (see legend to Fig. 6). In terms of ionic

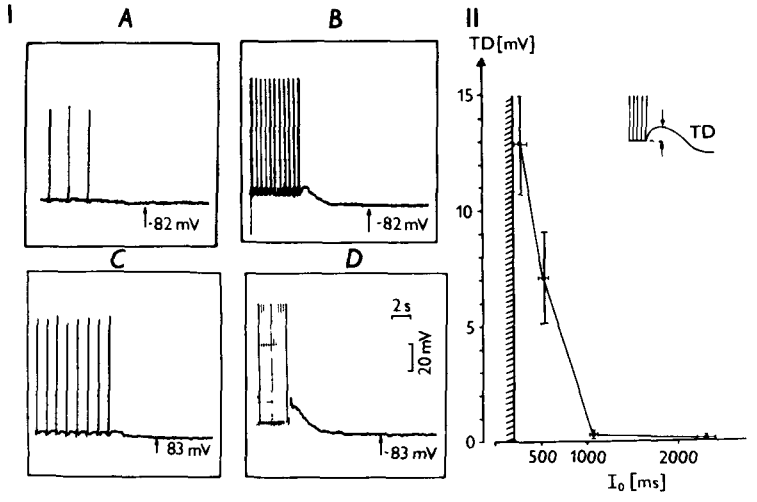

Fig. 4. Pacing-dependence of TD. I. Time courses of TD after cessation of the pre-drive $A: I_o = 2350$ ms, $B: I_o = 535$ ms; $C: I_o = 1030$ ms, $D: I_o = 270$ ms. II. Dependence of TD (measured as shown in the inset) on the pre-drive pacing interval I_o (abscissa). The hatched area represents the interval within which triggered activity was evoked.

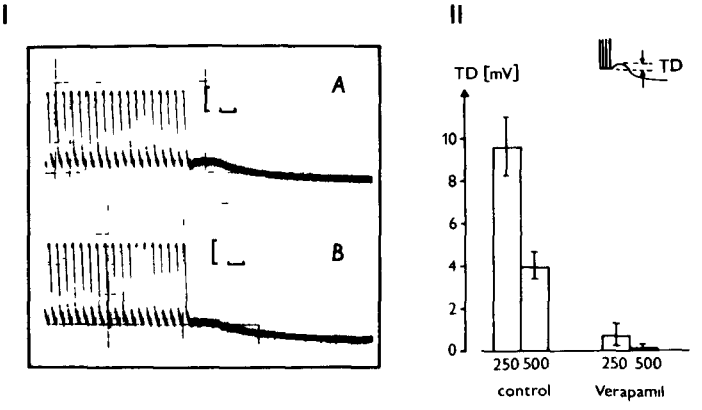

Fig. 5. Effects of verapamil (13.2×10^{-6} mol . l^{-1}) on TD. I. *A*: TD before verapamil administration *B*: TD 20 minutes after the administration of verapamil; calibration: 20 mV, 500 ms. Pre-drive pacing interval $I_o = 250$ ms. II. Synopsis of the effects of verapamil on TD. Abscissa: pre-drive pacing intervals, I_o. TD was measured as shown in the inset.

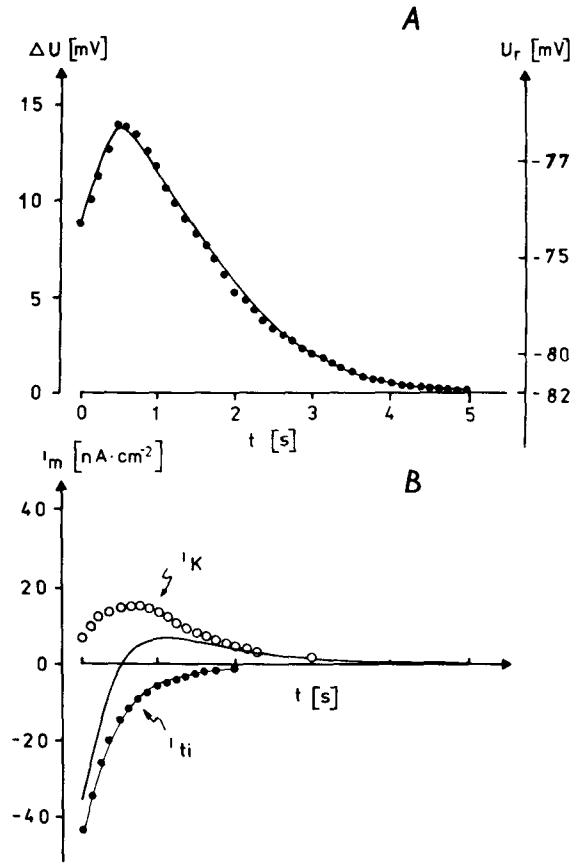

Fig. 6. Analysis of pacing-induced afterpotentials. U means the displacement of the actual transmembrane potential, U, from the steady state transmembrane potential U_r during the rest. $\Delta U = U - U_r$. *A*: analysis of ΔU; the points were obtained from four time courses of ΔU each; the smooth curve is best approximated by Eq. (1); $\Delta U = 32.01 \exp(-0.91\,t) - 25.10 \exp(-2.56\,t)$; *B*: estimation of net-transmembrane current, i_m, during the afterpotential (see Eqs. (3) to (10); $i_m = -c_m(\mathrm{d}(\Delta U)/\mathrm{d}t)$, $i_m = i_K + i_{ti}$ (i_K: background (K) outward current, i_{ti}: transient inward current), membrane capacity $c_m = 1.0\ \mu\mathrm{F} \cdot \mathrm{cm}^{-2}$. Following values were obtained from best approximation: $g_K = 0.91\ \mu\mathrm{S} \cdot \mathrm{cm}^2$; $\bar{i}_{ti} = -41.42\ \mathrm{nA} \cdot \mathrm{cm}^{-2}$; $\tau_{ti} = 391$ ms. Pre-drive pacing interval: $I_o = 250$ ms. Smooth curve: calculated i_m; filled circles i_{ti}; empty circles i_K.

currents using the model introduced in the Materials and Methods section afterpotentials are described as a result of overlapping of two ionic currents: an outward background (K) current, i_K, and a transient inward current i_{ti}. Fig. 6 illustrates the ionic currents used to explain afterpotentials.

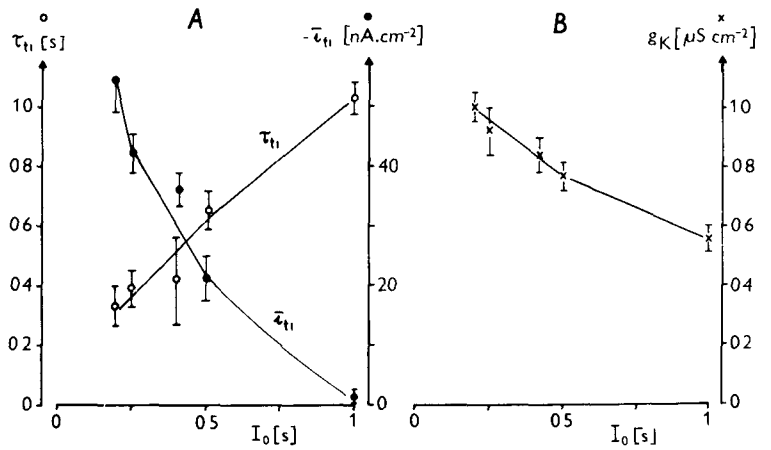

Fig. 7. Dependence of the estimated transmembrane currents on the pre-drive pacing interval. The parameters of i_{ti} and i_K were obtained from best approximation of ΔU according to Eqs. (8)—(10). *A*: Ordinate: inactivation time constant τ_{ti} in [ms] (empty circles), maximum instantaneous inward current $\bar{i}_{ti}$ in [nA . cm^{-2}] (full circles). *B*: Ordinate: background conductance g_K [μS . cm^{-2}]; abscissa: pre-drive pacing interval I_o in [ms]. Mean values ± S.D. from 4 experiments.

Three parameters required for the description of the ionic currents modelled (Eqs. (8), (9), (10)) could be obtained from the best approximation of afterpotentials using Equation (1). All the parameters were found to be dependent on the pacing interval, I_o of the pre-drive. If I_o was shortened the maximum instantaneous transient inward current, i_{ti}, become enlarged, whereas deactivation of i_{ti} become accelerated (Fig. 7). Based on best fits of TD, the background conductance, g_K, was also found to be pacing dependent: it increased with the shortening of the pre-drive pacing interval I_o (Fig. 7).

Discussion

Under certain conditions cardiac cells develop oscillatory depolarizing after-potentials (for a review, see Cranefield 1977; Wit et al. 1980). These conditions include high cardiac glycoside concentrations, high external Ca or low external Na concentrations, low external K concentration, low temperature and application of caffeine (Eisner and Lederer 1979; Karagueuzian and Katzung 1982; Kass and Tsien 1982; Clusin 1983). The importance of such transient afterdepolarizations (TD) is in their ability to reach threshold to fire off action potentials (triggered activity). Thus they may initiate ectopic extrasystoles and severe arrhythmias in the

heart muscle. In the present work we could demonstrate TD's under physiological conditions. Similar to the much better studied triggerable fibres found in the simian mitral valve and the coronary sinus of several animals (Cranefield 1977; Wit and Cranefield 1977; Wit et al. 1980; for a review, see Cranefield 1975) we found triggerable fibres showing afterdepolarizations in the pectinate muscles bordering the crista terminalis in the rabbit right atrium (Fig. 1). Similar afterpotentials were only qualitatively described in the same type of myocardium by Saito et al. (1978). In our experiments TD was elicited that the fibres studied were able to initiate triggered activity (Fig. 3).

Because of the clinical importance of this type of afterpotentials it is of interest to study the cellular mechanism of their origin. In general, TD may be generated (i) by a post-drive increase in the ratio of the sarcolemmal permeabilities to Na and $K(P_{Na}/P_K)$, (ii) by a post-drive Na-accumulation within restricted spaces at sufficiently high P_{Na} values, (iii) by Ca accumulation during the pre-drive phase in a membrane-near compartment and by an associated decrease in K conductance due to Ca depletion during the post-drive rest, (iv) by a K-increased K conductance and its decrease during K depletion, (v) by activation of a transient inward current due to a pacing-induced intracellular Ca overload. The last hypothesis seems to be the most plausible one (Lederer and Tsien 1976; Aronson and Gelles 1977; Kass et al. 1978a, b; Tsien et al. 1979; Kass and Tsien 1982), especially, since Ca_i-activated cation channels have been detected in patches of the myocardial sarcolemma (Colquhoun et al. 1981). In experiments on Purkinje fibres and on isolated ventricular cells it could be demonstrated that TD were evoked by intracellular injection of Ca^{++} and they disappeared following an intracellular injection of EGTA (Kass and Tsien 1982; Matsuda et al. 1982).

Any experimental conditions under which TDs develop seem to rise transiently the free intracellular Ca concentration above its normal diastolic value after a train of action potentials (Kass et al. 1978a, b; Eisner and Lederer 1979; Kass and Tsien 1982; Matsuda et al. 1982). An increase in pacing rate may also be a suitable tool to overload myocardial cells with Ca. Under our experimental conditions TD may be due to pacing-induced Ca overload in cells with a weak Ca-sequestration. We could show that verapamil prevents the appearance of TD (Fig. 5). This means that verapamil would block TD by preventing the pacing-induced Ca overload. A Ca-triggered transient inward curcent (i_{ti}) has been found to be responsible for TD in Purkinje fibres treated with toxic concentrations of strophantidin (Lederer and Tsien 1976; Kass et al. 1978a, b). However, the kinetics of this Ca-mediated current has remained unknown despite the efforts to analyse it from single channel currents in inside-out or cell-attached patches (Colquhoun et al. 1981).

If this hypothesis is valid transient inward current, i_{ti}, may be estimated from best approximation of pacing-induced TD (Fig. 6). It was found that the faster the pre-drive the larger the activated transient inward current, and the slower the

predrive the more delayed the decay of i_{ti} (Fig. 7).

Another finding obtained from best fits may be of functional importance. It could be shown that the background conductance g_K, is increased due to an acceleration of pacing. Such a pacing-dependent increase in g_K may counteract TD. It may be also mediated by a pacing-induced increase in Ca_i (Isenberg 1977).

From this analysis it seems likely that high pacing rates favoure TD by activation of a transient inward current and that an accelerated decay of i_{ti} and a pacing-induced increase in g_K counteract the depolarizing effects of i_{ti}.

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Received March 14, 1983/Accepted September 8, 1983