The "Pacemaker" Function of the Transient Outward Current in the Rabbit Myocardium

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Abstract. The single sucrose gap technique was employed to study the electrically induced automaticity in rabbit papillary muscles. When the potential was clamped at the level of the "maximum diastolic potential" following the first spike of automaticity an initial decline of the outward ionic current with subsequent activation of the delayed potassium current was observed. The initial decline was potential-sensitive with a maximum at approximately −2 mV; it diminished when the rate of stimulation increased and was abolished with 4-aminopyridine plus Sr^{2+}. It is suggested that the transient outward current determines the development of the "pacemaker potential" after the first spike of electrically induced automaticity in rabbit papillary muscles.

Key words: Transient outward current — Pacemaker current — Double responses — 4-aminopyridine

Introduction

Transient outward currents may influence the configuration of action potentials in rat cardiomyocytes (Josephson et al. 1984), rabbit ventricular myocardium (Kukushkin et al. 1983), and rabbit cardiomyocytes (Giles and van Ginneken 1985). It has been suggested that a transient outward current may be involved in the generation of abnormal electrical responses in mammalian ventricular muscle (Saxon 1984). The hypothesis concerned the so-called double responses that had been observed in electrically depolarized rabbit papillary muscles (Gainullin and Kukushkin 1977; Kukushkin and Gainullin 1979). Two lines of evidence supported the hypothesis.

(a) The secondary calcium spike of the double responses disappeared in the presence of 4-aminopyridine (Saxon 1984). Though 4-aminopyridine is not a selective blocker of transient outward currents it decreased the transient outward currents in sheep Purkinje fibers (Kenyon and Gibbons 1979; Coraboeuf and Carmeliet 1982) and rabbit papillary muscles (Kukushkin et al. 1983).

(b) The double responses were highly rate dependent, they disappeared after a period of stimulation at high rate (Gainullin and Kukushkin 1977; Kukushkin and Gainullin 1979). This is in agreement with a very slow recovery of the transient outward current in rabbit papillary muscles (Kukushkin et al. 1983) and in sheep Purkinje fibers (Boyett 1981). Anyhow the recovery is very rapid in rat single ventricular cells (Josephson et al. 1984).

The purpose of the present work was to investigate the currents responsible for the "pacemaker" potential before the secondary calcium spike of the double responses.

Materials and Methods

Papillary muscles were excised from the right ventricles of New Zealand rabbits weighing 1.5—2.0 kg. The diameter of the papillary muscles ranged from 0.3 to 0.9 mm and the length of the muscles was 0.5—4.0 mm. The experimental procedure was described in detail elsewhere (Kukushkin et al. 1983). Briefly, rabbit papillary muscles were mounted in three-compartment chamber for the single sucrose gap technique. The temperature of the bathing solution was 31°C. The quality of isolation was estimated by comparing the amplitudes of action potentials recorded intracellularly with those of action potentials recorded extracellularly across the sucrose gap. The ratio of the amplitudes was 1.05—1.20 within an experiment.

In "current" clamp mode, a relatively high resistance in series with the preparation (0.2—1.0 MΩ) ensured rectangular waveform of the direct current pulses.

In order to avoid the slow shift of the membrane parameters due to a very slow factor of uncertain nature (Kukushkin et al. 1982; Boyett and Felida 1984) the experimental protocol was arranged in cycles, each cycle including 4—6 action potentials at 0.3 Hz, a pause of 7 seconds and a voltage clamp step. Seventeen preparations survived well and were used in voltage clamp experiments.

Glass microelectrodes filled with 2.5 mol $.l^{-1}$ KCl were used (their resistance was 10—30 MΩ). Routinely a microelectrode was inserted into a cell approximately 0.1 mm from the partition. The traces were photographed from the screen of an oscilloscope (Tectronix 5111).

The bathing solution contained (in mmol $.l^{-1}$): NaCl 137, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 1.8, $NaHCO_3$ 10.0. The isotonic sucrose solution contained 0.36 mmol $.l^{-1}$ $CaCl_2$. All NaCl was substituted with KCl in the depolarizing solution used in the single sucrose gap technique. The solutions (pH was 7.2—7.4) were saturated with 96%O_2—4%CO_2 gas mixture.

Results

Any hypothesis concerning the "pacemaker" potential is bound to explain the reversal and the development of the net membrane current in the inward direction. Obviously, a direct current alone cannot account for these events; a time-dependent ionic current must be present. The latter may be an outward current that declines with time; together with an appropriate constant inward current it may yield the reversal of the net membrane current. The decline of the

delayed potassium current was described as the main cause of automaticity in electrically depolarized myocardium (Katzung and Morgenstern 1977; Meier and Katzung 1981). This was not exactly the case in rabbit papillary muscles.

A pulse of direct current initiated automaticity in the "current clamp" mode (Fig. 1*A*). The first two spikes (i.e. the "double response") are shown above the current recordings. The membrane potential was clamped at the level of the "maximum diastolic potential" after the first spike (in the next cycle of the experimental protocol). When the voltage clamp circuit was connected the "current clamp" was released. As a result the net membrane current minus the direct current, i.e. the time-dependent ionic current was recorded.

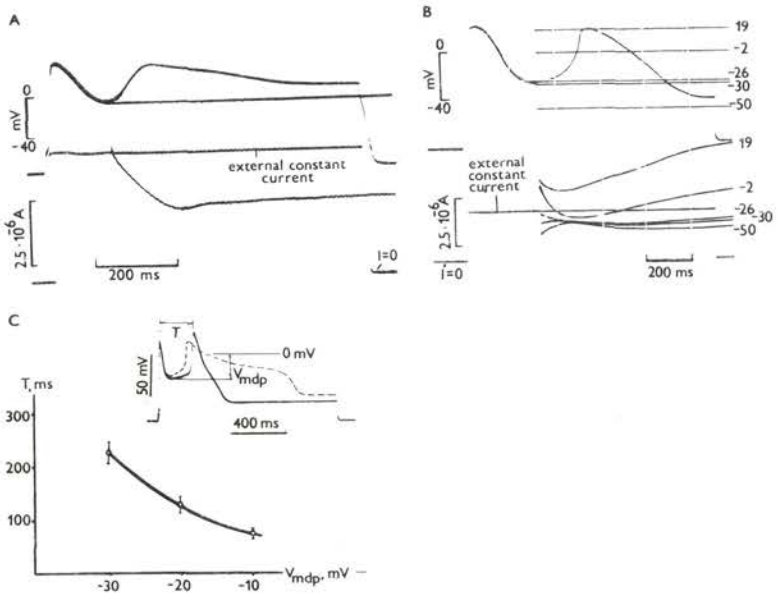

Fig. 1. The pacemaker current in the rabbit papillary muscle. Upper traces are intracellular recordings of the membrane potential; lower traces are the corresponding currents. *A* — the double response was initiated in the first cycle of the protocol. In the next cycle, the membrane potential was clamped at the level of the "maximum diastolic potential". The direct depolarizing current was not exactly constant because the resistance in series with the muscle (200 kΩ) could not ensure the true current clamp mode. *B* — the membrane potential was clamped at different levels after the first spike. The visible amplitude of the "pacemaker" current (i.e. the initial decline of the time-dependent outward current) was the largest at −2 mV and became smaller at both higher and lower potentials. Such behaviour is characteristic of the transient outward current. *C* — the dependence of the delay (*T*) of the second spike on the "maximum diastolic" potential (*V*mdp), *t*°C = 37. Direct current pulses of stepwise increasing amplitude were delivered at a rate of 2 per minute and induced double responses (Kukushkin and Gainullin 1979). The inset shows how the measurements were done.

The time-dependent current was always outward and changed in two phases: an initial decline that lasted approximately 170 ms at −9 mV, and a final increase that was comparatively slow. Obviously the latter had nothing to do with the net current reversal and development in the inward direction. This slow increase was difficult to attribute otherwise but to the development of the delayed potassium current (McDonald and Trautwein 1978). The increase of the delayed potassium current was even more rapid and large at higher membrane potentials (Fig. 1*B*). Clearly the developing outward current could not account for the reversal of the net membrane current; it was the initial decline of the time-dependent current that made the net current inward.

Probably, the transient outward current underlied the initial decline of the time-dependent outward current. The results of further experiments did not contradict this idea.

The voltage dependence of the initial decline (Fig. 1*B*) was qualitatively similar to that of the transient outward current (Kukushkin et al. 1983). The initial decline was very small at −50 mV, it was the largest at approximately −2 mV and diminished at 19 mV. It is rather interesting that at −50 mV and at −26 mV a brief initial increase of the time-dependent current was seen. It was probably the effect of the slow inward current deactivation (McDonald and Trautwein 1978).

There was a general agreement between the slope of the pacemaker potential and the initial decline. The slope (estimated by the delay of the second spike) was steeper for more positive potentials (Fig. 1*C*).

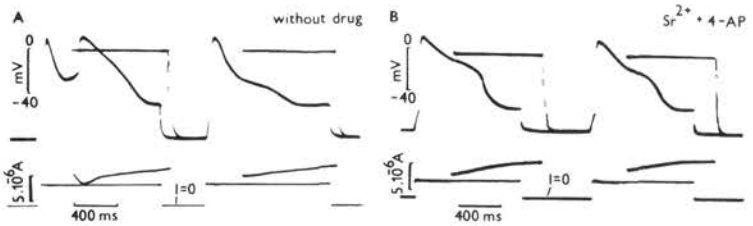

Fig. 2. The influence of rate and 4-aminopyridine plus Sr^{2+} on the double responses and currents. *A* — control Tyrode. Two closely spaced responses were initiated with direct current pulses after a pause of 7 seconds in the first cycle. The first of the two was the double response, the second was a single action potential. In the next cycle, the potential was clamped at −5 mV after the first spike. The initial decline of the time-dependent ionic current was relatively small in the second response. *B* — the same protocol repeated in the presence of 4-aminopyridine (100 mg/litre) with Sr^{2+} in the bathing solution substituted for Ca^{2+}.

The initial decline displayed the same rate-dependence as the transient outward current. An example is shown in Fig. 2*A*. A pair of equal direct current pulses initiated electrical responses after 7 seconds of rest. The first response had two spikes; the second one was a usual single action potential. This finding is consistent with the results reported earlier (Kukushkin and Gainullin 1979). The initial decline of the time-dependent current developed when the potential was clamped at −5 mV in the course of the first response and was virtually absent in the second response. In a similar experimental protocol, the transient outward current was large in the first response and very small in the second one (Kukushkin et al. 1983; see also Fig. 3).

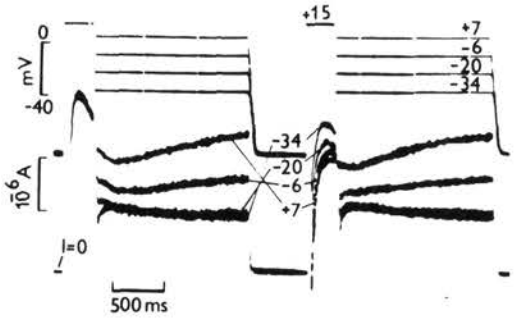

Fig. 3. Tail currents on repolarization after a prepulse. Two identical voltage clamp steps with prepulses were switched on after a pause of 7 seconds. The prepulses imitated the first spikes of double responses. The procedure was repeated for different levels of potential on repolarization. The second of the two equal prepulses always activated lower "humps" of the transient outward current. The respective tails of the transient outward current were small or absent for the second voltage clamp step.

The transient outward current could account for the rate dependence of the initial decline of the time-dependent current. A model experiment confirmed this hypothesis (Fig. 3). The same experimental protocol was reproduced with voltage clamp prepulses to 15 mV substituted for the first spikes of automaticity. The first prepulse in the pair yielded a higher peak of the transient outward current than did the second prepulse. The respective tail currents (at 7; −6; and −20 mV) showed much smaller decline of the transient outward current in the second step than in the first one. A simple comparison of Fig. 3 and 2*A* reveals a close resemblance of the rate-dependent changes in the tails of the transient outward current and the initial decline of the time-dependent current responsible for the "pacemaker" potential.

The initial decline of the time-dependent outward current displayed the

same drug sensitivity as the transient outward current did. It was inhibited by a combination of 4-aminopyridine and Sr^{2+} ions substituting for Ca^{2+} in the bathing solution (Fig. 2*B*). In the presence of both 4-aminopyridine and Sr^{2+}, no second spike and no signs of the initial decline of the time-dependent current remained (the same preparation as in Fig. 2*A*). An equivalent result was described earlier for the transient outward current (Kukushkin et al. 1983).

These results seem to establish a correlation between the transient outward current, the initial decline of the time-dependent outward current, and the "pacemaker" potential of the double responses.

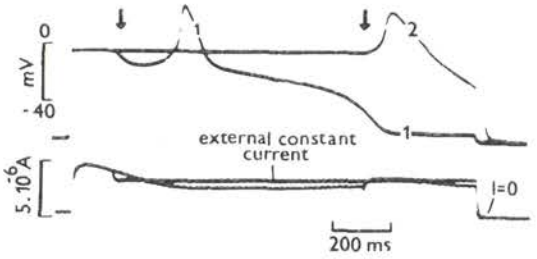

Fig. 4. The "pacemaker" potential induced by the transient outward current. First, the voltage clamp pulse activated a typical transient outward current. At the end of the pulse when the delayed outward current was developing the direct current was switched on and the voltage clamp was released. The slow spike (*1*) was recorded immediately after the switch from the voltage clamp to the "current clamp" mode. In the next cycle, the voltage clamp pulse was interrupted and the "current clamp" switched on at the early stage of the transient outward current decline. This caused the appearance of the "pacemaker" potential before the slow spike (*2*).

In addition, the following experiment confirmed the ability of the transient outward current to induce the "pacemaker" potential (Fig. 4). A voltage clamp step to −7 mV activated the transient outward current. When the transient outward current started declining the voltage clamp was interrupted and immediately the direct current was applied. A typical phase of diastolic depolarization ensued. In this case, one can hardly doubt that it was the decline of the transient outward current that caused the reversal in the course of the membrane potential changes from repolarization to depolarization. No diastolic depolarization was seen when the voltage clamp was prolonged until the delayed potassium current started developing. In this case, a slow upstroke started developing immediately after the direct current had been applied after the end of the voltage clamp step.

Discussion

Two currents are primarily responsible for the "pacemaker" potential before the second spike of the electrically induced automaticity in rabbit papillary muscles.

They are (1) the direct depolarizing current and (2) the time-dependent ionic current that develops in the inward direction when the potential is clamped after the first spike (Fig. 1*A*). Obviously, the direct current may flow through any channel available at a given moment (the constant current source generates a variable potential that changes the driving force equally for all ions). As to the second current, the results of the present work permit to identify it tentatively as the transient outward current. In fact, pharmacological interventions, variations of potential and rate, induced changes of the initial decline of the time-dependent outward current that were characteristic of the transient outward current. The new "pacemaker" function proposed in the present paper extends the list of the physiological functions of this current system (Boyett 1981; Josephson et al. 1984).

The delayed potassium current was described as the main "pacemaker" current for the electrically induced automaticity in mammalian myocardium (Katzung and Morgenstern 1977; Meier and Katzung 1981). In rabbit papillary muscles, the situation seems to be somewhat different. The available data suggests that in the course of the first spike repolarization, the transient outward current begins declining. The decline of the transient outward current together with the direct depolarizing current makes the net current inward with subsequent change from repolarization to depolarization in the course of the membrane potential changes. Finally, the calcium current activates and yields the second spike (the secont spike of automaticity could be suppressed with Ca-channel blockers — see Kukushkin and Gainullin 1979). The proposed tentative scheme raises a new problem of the relative significance of the two "pacemaker" currents: the transient outward and the delayed potassium one. The rate-dependent inactivation of the transient outward current (see Fig. 3) makes the involvement of this current in the cycles of automaticity beyond the first few ones improbable. In the following cycles, the delayed potassium current probably determined the course of the "pacemaker" potential.

In comparison with many repetitive action potentials that follow it, the double response in rabbit papillary muscle may seem a minor event, unworthy of consideration. Moreover, small depolarizing currents never induced any double responses in many other preparations: frog atrial trabecula (Brown and Noble 1969), cat ventricular muscle (Saxon et al. 1975), guinea-pig ventricular muscle (Katzung 1974; Imanishi and Surawicz 1976). Probably the double response in electrically depolarized rabbit papillary muscles would have remained a sheer curiousity if it was not for the close resemblance of its properties to those of the double responses in human atrial muscle (Sleator and deGubareff 1964; Fabiato and Fabiato 1971). Therefore, elucidation of the mechanisms of the rabbit double responses may help in understanding the nature of abnormal action potentials in human heart.

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