Anomalous Response of Rabbit Papillary Muscles to Depolarising Current: The Possible Role of the Transient Outward Current. A Pharmacological Analysis

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Abstract. Rabbit papillary muscles under current depolarization generate an anomalous double action potential (AP) instead of a usual repetitive activity characteristic of myocardial fibres of different mammalian species. The mechanism of the double AP consisting of a spike-like and a delayed slow component was analysed using pharmacological approach. No changes in the anomalous double AP were observed in the presence of Cs ions. This contrasted with the inhibitory action of 4-aminopyridine (4-Apy). High sensitivity of the phenomenon to 4-APy suggests a contribution of the transient outward current, previously postulated for rabbit working myocardial fibres, to account for the double AP.

Key words: Rabbit myocardium — Anomalous depolarizing automaticity — 4-Aminopyridine — Double AP

Introduction

The phenomenon of current-induced automaticity has been observed in cardiac preparations from different mammalian species and analysed in several laboratories (Katzung 1974; Katzung and Morgenstern 1977; Hume and Katzung 1980; Imanishi and Surawicz 1976; Imanishi et al. 1978; Arita et al. 1976; Saikawa et al. 1978; Zintsadze et al. 1973; Saxon et al. 1975, 1976, 1981). This phenomenon raised much interest as an electrophysiological model of primary ectopic centers in a border zone of myocardial infarction (Janse et al. 1980). Of interest in this regard is the unusual pattern of current-induced response seen in rabbit ventricular myocardium. Instead of standard repetitive firings rabbit papillary muscles generate an anomalous double response prior to showing automaticity (Kukushkin and Gainullin 1978). Double response may also be recorded by passing a constant current through a sucrose gap chamber (Kukushkin and Gainullin 1978, 1979). Pharmacological data indicate that ionic processes underlying the two components of the double AP are different: the first sodium

component could be blocked by TTX, the other one by calcium antagonists (Kukushkin and Gainullin 1979). However, the reason for such an anomalous behaviour of depolarized rabbit ventricular fibres has not yet been elucidated. We have analysed this phenomenon in more detail taking into account certain specificities of membrane properties of the rabbit myocardium. As shown by recent pharmacological and voltage-clamp data this species exhibits the fast or transient component of the outward current (I_t) which is quite unusual for working myocardium fibres (Saxon and Safronova 1982; Nilius et al. 1982; Kukushkin et al. 1984). Activation of I_t seems to contribute to another anomalous electrical pattern in rabbit myocardium, namely AP plateau loss at low (< 1 Hz) stimulation rate (Saxon and Safronova 1982). In order to get a deeper insight into the nature of the double AP, the role of the outward current system was analysed pharmacologically. Some preliminary aspects of the study were presented previously in an abstract form (Saxon 1982).

Materials and Methods

New Zealand rabbits and guinea pigs were used. Hearts were rapidly excised and dissected in a warm continuously oxygenated physiological solution.

Unbranched papillary muscles from the right ventricles were prepared. Preparations of 0.7 mm – 1.3 mm in diameter and 2.5—3.5 mm in length were pulled through a hole in a tightly fitted thin plate dividing the tissue chamber into 2 compartments. The plate was vaseline coated to obtain electrical insulation of the compartments. To depolarise papillary muscles, currents of different intensity (10^{-5} A—3×10^{-5} A) and duration (1—5 s) were applied across the two compartments using spiral Ag—AgCl electrodes, placed in each compartment. The polarising current was kept almost constant during its flow through a resistor (30 kOhm). This two-chamber method of stimulation was first introduced by Kamiyama and Matsuda (1966) and has been widely used in experiments (Katzung 1974; Imanishi 1971; Imanishi and Surawicz 1976; Imanishi et al. 1978; Arita et al. 1976).

Membrane potentials were measured using two microelectrodes, placed inside and outside the fibre, respectively. The electrodes were filled with 2.5 mmol/l KCl and had a resistance of 20—30 MΩ. Data presented herein were obtained from single impalements maintained throughout both the control and drug perfusion period.

During a one-hour equilibration period, the preparation was stimulated at 1 Hz with 3 ms square pulses. The driving stimuli (1 Hz) were turned off before the current application. Current steps (1—5 s) of varying intensities were repeated at 1 min intervals. Both compartments were perfused with standard Tyrode's solution (3 ml/min) containing (in mmol/l): Na^+ 150.8; K^+ 4.0; Ca^{2+} 2.7; Mg^{2+} 1.0; HCO_3^- 12; $H_2PO_4^-$ 1.8; Cl^- 148.4; glucose 10. The solution was gassed with 95% O_2 — 5% CO_2 at 36—37 °C; pH 7.4. In experiments with 4-aminopyridine the solutions were buffered with 10 mmol/l Hepes and neutralised to pH 7.4 with 5 mmol/l NaOH. Double-distilled water was used to prepare the solution.

Compounds: 4-aminopyridine (Ralph N. Emanuel Ltd. Wembley, Middlesex); Tyramine, Alprenolol, CsCl (Serva). The compounds were added directly to the control solution without any compensation for osmolarity. Other reagents were of the highest grade from a standard commercial source.

Both the action potentials and the polarizing currents were recorded simultaneously on a dual-beam oscilloscope, and photographed.

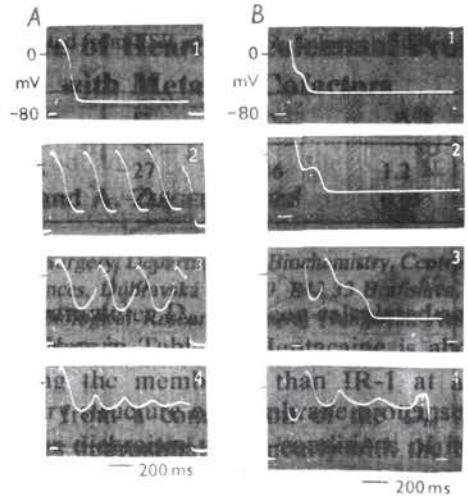

Fig. 1. Differences in current-induced electrical activity between guinea-pig (*A*) and rabbit papillary muscles (*B*). Depolarizing pulses of 1 s duration and of increasing intensity were introduced at 1 min intervals. Papillary muscles: 0.7 mm in diameter and 2.5 mm long

Results

Characterization of the double response in rabbit papillary muscles.

Fig. 1 illustrates the typical species-dependent pattern of electrical activity in depolarised guinea-pig and rabbit papillary muscles. Both preparations were of the same size, (as for both, the diameter and length). They were depolarised from the resting potential to various less negative membrane potential levels by a constant current of increasing intensity. Fig. 1 *A*, *C* illustrates automatic activity usually observed in the guinea pig myocardium in comparison with an atypical double response in rabbit papillary muscle (Fig. 1 *B*, 3). In the latter case, depolarisation to -60 mV induces the first, fast "spike-like" component lacking the plateau phase. At further depolarisation the second, slow component of the double AP appears. The threshold potential level for the slow phase was -50 mV ± 5 mV. Double APs were observed in all rabbit papillary muscles studied regardless of their size (0.5 mm—1.3 mm in diameter) ($n = 30$).

Effects of potassium channel blockers (Cs^+, 4-aminopyridine) and K^+ ions.

Cs^+. To examine the role of potassium current system in genesis of double AP, the effect of Cesium (Cs^+), a putative blocker of the "inward rectifying channel", was tested. No changes in the abnormal activity was found in the presence of Cs ions.

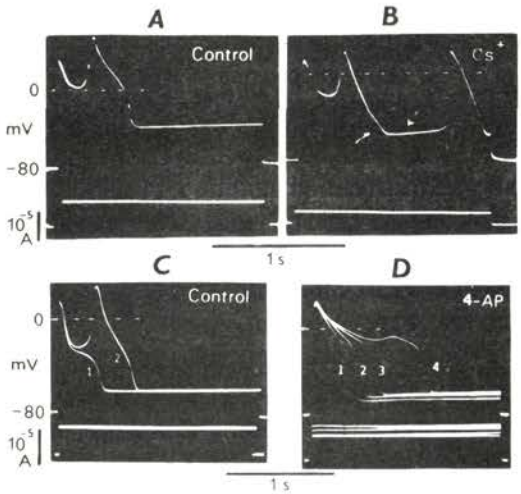

Fig. 2. Comparative effects of potassium channel blockers: on double action potential in rabbit papillary muscles CsCl (10 mmol/l, 3 min exposure) (*B*) and 4-aminopyridine (4-APy, 0.1 mmol/l, 30 min exposure) (*D*), superposition of action potentials (traces 1—4) under increasing current. Papillary muscle: 0.75 mm in diameter and 2.9 mm long (*C*, *D*) and 1 mm in diameter and 3 mm long (*A*, *B*).

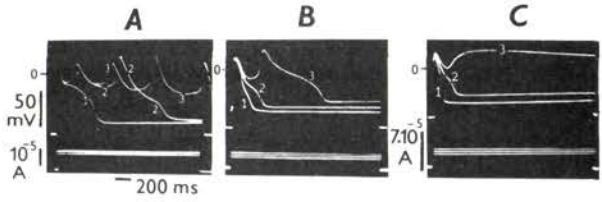

Fig. 3. Effect of changes in external K^+ concentration on double response (AP) in depolarized rabbit papillary muscle. *A*, *B*, *C* show subsequent records of electrical activity in Tyrode's solution containing: 2.4 and 10 mmol/l K^+ respectively; solution exchange is 20 min intervals. The top traces (1—3) represent superimposed membrane potentials, those at the bottom show the applied current. Papillary muscle: 0.9 mm in diameter, 3.0 mm long.

The addition of CsCl in concentrations ranging from 5—10 mmol/l for 20 min failed to abolish double activity in rabbit myocardium under a long depolarising current ($n = 5$). The effect of a 3 min exposure to 10 mmol/l CsCl is shown in Fig. 2*B*. Preservation of the double AP in the presence of Cs ions is obvious. In addition to depolarisation by about 10 mV, a prolongation of the final repolarisation phase and pacemaker potential (arrow) could be observed under direct current. All the effects observed are compatible with extracellular Cs^+ action on inward rectifying potassium channels (Isenberg 1976).

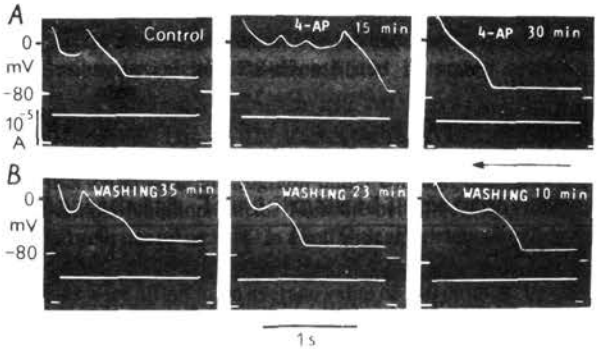

Fig. 4. Time-dependent transformation of a double action potential to a monophasic one in the presence of 0.2 mmol/l 4-APy (*A*) and reversion of its action (*B*). In each panel, the top trace represents the membrane potential, the bottom one shows the applied current. Papillary muscle: 0.8 mm in diameter and 2.9 mm long.

K^+ ions. The double AP, was highly sensitive to K^+ concentrations in solutions. The fast component was more pronounced at high K^+ (10 mmol/l, Fig. 3*C*) and diminished at low K^+ (2 mmol/l, Fig. 3*A*) as compared to the control (4 mmol/l, Fig. 3*B*). Moreover, under the current depolarisation in a low K^+ solution rabbit papillary muscles easily generated low voltage automacity instead of a double AP ($n = 7$). Fig. 3*A* shows a representative example of current-induced automacity induced in rabbit papillary muscle in a low K^+ solution.

4-aminopyridine (4-APy). Contrary to CsCl, 4-APy was found to abolish double AP in rabbit papillary muscle ($n = 12$). This effect was observed at relatively low concentrations ranging between 0.1 mmol/l to 0.2 mmol/l. It reached a maximum within 30 min of exposure and could be reversed. In the presence of 4-APy, rabbit papillary muscles generated augmented and prolonged monophasic AP_s upon a series of depolarizing currents of increasing intensity, as illustrated in Fig. 2*D* (traces 1—4).

To understand the action of 4-APy in more detail time-dependence of the effect was studied (Fig. 4). It is seen that transformation of a double AP to a monophasic one resulted from a gradual elimination of the spike-like component, namely its rapid repolarisation phase in the course of a 30 min exposure to 0.2 mmol/l 4-APy. On the other hand, this phase readily reappeared after the blocker had been removed from the solution (Fig. 4*B*). Low voltage oscillations of the membrane potential can be seen at the intermediate step of 4-APy action (15 min).

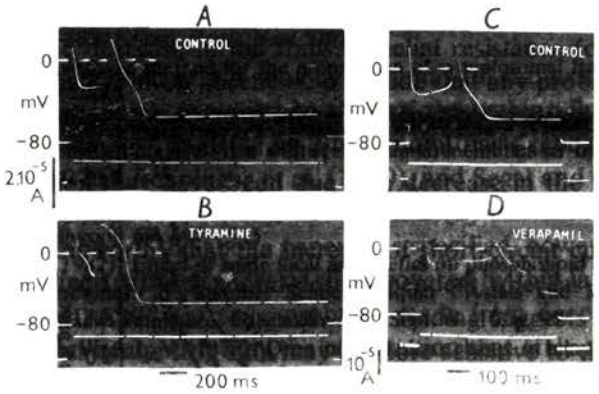

Fig. 5. Comparative effects of calcium channel modulators (tyramine — 0.1 mmol/l, 10 min exposure) (*B*) and verapamil — (2×10^{-6} mol/l, 40 min exposure) (*D*) on the second component or the double action potential in depolarised rabbit papillary muscle. Papillary muscle: 0.9 mm in diameter and 3.5 mm long.

Modulators of Ca^{2+} channels (tyramine, verapamil).

To exclude the possible role of neurotransmitter release, which is characteristic of pharmacological action of 4-APy on nerve and muscle cells (Thesleff 1980; Bowman and Savage 1981), atenolol, a β-blocker, was used. Pretreatment of rabbit papillary muscles with 1 μmol/l atenolol for 20 min ($n = 3$) did not significantly alter the electrophysiological effect of 4-APy on the double activity (results no shown).

Moreover, tyramine, (0.1 mmol/l; $n = 5$) a specific pontentiator of neurotransmitter release, could not mimic the electrophysiological action of 4-APy. It only caused a marked potentiation of the second component of the double AP (Fig. *5B*). Both the amplitude increase and the prolongation of the second component reached nearly 30% each. This contrasted with the action of Ca^{2+} channel blocker, verapamil (2×10^{-6} mol/l), which diminished the development of the slow phase of the double AP (Fig. *5D*). This result is representative of a total of five experiments in which the calcium antagonist had similar action.

Double AP rate-dependence.

The double patters in response to depolarising current was found to exhibit striking rate sensitivity and it could be induced by depolarising pulses applied at a low rate of 1 min (post-rest phenomenon Figs. 1—5). This special behaviour disappeared under higher rates of depolarising steps, or after a preliminary stimulation of the

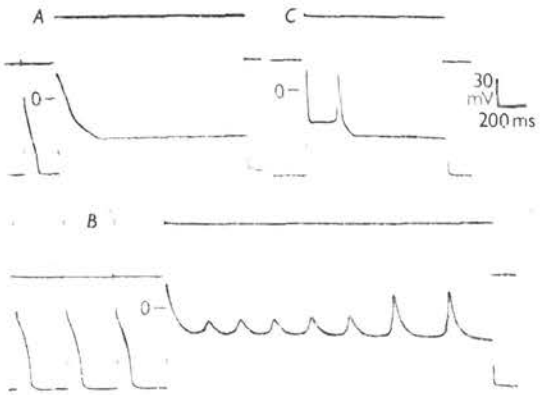

Fig. 6. Rate sensitivity of double AP in depolarized rabbit ventricular myocardium. Comparative effect of depolarising current (2 reobase) preceeded by short impulses (*A*, *B*) or a 1 min rest interval (*C*) in the same preparation. In each panel, the upper trace is the current, the lower trace is the membrane potential. A typical polygraph record. Papillary muscle: 1.0 mm in diameter and 3.2 mm long.

papillary muscle by short (3 ms) supratreshold impulses and a usual pattern appeared. This is illustrated in Fig. 6. It can be seen that the depolarising pulse closely following a short preliminary impulse elicited a normal monophasic AP (Fig. 6*A*) and not an anomalous double AP, which could in turn be produced by the same depolarisation following a 1 mm rest interval (Fig. 6*C*). The electrical response of the prestimulated papillary muscle was qualitatively similar to that obtained with 4-APy treated preparations. A prolonged monophasic AP deprived of the fast repolarising phase could be observed in the both cases. Low voltage automaticity could also be seen under these conditions (Fig. 4*A*, 15 min 4-APy action; Fig. 6*C*).

Discussion

The possible role of the transient outward current in the genesis of a double AP.

The new information that could be obtained by our pharmacological approach concerned the high sensitivity of a double AP to 4-APy: the latter has widely been used as a putative blocker of I_t in a number of cell systems (Kenyon and Gibbons 1979; Thompson 1982).

The following line of evidence suggests the specificity of the electrophysiological action of 4-APy on rabbit papillary muscles.

1. Double AP may be inhibited by 4-APy in concentrations which specifically

act on fast K channels leaving intact both the inward rectifying and the pacemaker currents in cardiac Purkinje fibres (Van Bogaert and Snyders 1982).

2. CsCl recognized as the inhibitor of inward rectifying potassium channels in specialized and working myocardium fibres (Isenberg 1976; Trautwein and Mc Donald 1978) had no effect on double AP.

3. The effect of 4-APy was maintained in the presence of the β-blocker atenolol, which was added to minimize the possible influence of endogenous catecholamine release. It therefore may be argued that the inhibitory action of 4-APy on double AP was not mediated by neurotransmitter release. In addition, tyramine, a stimulator of the neurotransmitter release could not mimic the electrophysiological action of the blocker. Thus, the high sensitivity of the double AP, in particular of its spike-like component, to 4-APy may be regarded as an indirect indication of I_t involvement in fast spike repolarisation. Striking similarities between the electrophysiological effect of preliminary stimulation and 4-APy on double AP should be emphasized. The finding of the rate dependence of the phenomenon favours the conclusion of the involvement of I_t in the genesis of double AP. As a matter of fact, this rapidly activating and inactivating current has a paradoxically long period of recovery ($\tau > 1$ s): a significant increase in I_t was shown after a rest period, in contrast to a marked depression observed during the stimulation of sheep Purkinje fibres (Boyett 1981). It may therefore be speculated that the rate sensitivity of the double AP could account for the rate dependence of I_t in rabbit ventricular and sheep Purkinje fibres, respectively. Our interpretation of the nature of double AP is further supported by the influence of various K^+ concentrations on the phenomenon. Thus, its rapid repolarisation phase was potentiated by high K (10 mmol/l) and diminished by low K (2 mmol/l). According to recent pharmacological data, K^+ seems to be a principal charge carrier in the transient outward current in cardiac Purkinje fibers (Isenberg 1978; Vereecke et al. 1980; Marban and Tsien 1982). On the other hand, the spike-component was eliminated by TTX, a selective blocker of fast sodium channels (Kukushkin and Gainullin 1978). The genesis of the first TTX- and 4-APy-dependent component of the double AP can therefore be adequately explained by consecutive activation of the fast inward and outward currents.

As for the origin of the second component, which is sensitive to both catecholamines and calcium antagonist, it may be due to a time-dependent inactivation of I_t with a parallel unmasking of the slow inward current, I_{si}, under long depolarisation of rabbit papillary muscle. In this respect it is interesting to mention that the principal problem of I_t in cardiac Purkinje fibres is its interference with and overlaping (masking) of I_{si}. Pharmacological dissection of I_t is followed by an increase in or unmasking of I_{si} (Marban and Tsien 1982). Such a mechanism could best account for the inhibitory action of 4-APy on double AP.

The functional role of such masking and demasking effects of I_t on the calcium

current seems more obvious in the nerve system where I_t gets involved into modulations of neurotransmitter release and in various forms of synaptic plasticity by the regulation of Ca^{2+} entry into nerve terminals during spike activity (Shapiro et al. 1980).

The possible mechanisms of double activities in cardiac tissue.

It is well known that double activity can be induced with various pathological agents (toxins, drugs, hypoxia or acidosis) in different cardiac preparations (Trautwein 1973; Weidmann 1973; Fabiato and Fabiato 1971; Honerjager 1982; Coraboeuf et al. 1980). The majority of the double APs described were induced by slowing down terminal repolarisation of the action potential (Cranefield and Wit 1979). Principally, two main mechanisms seem to underlie the alteration of the terminal repolarisation phase in cardiac AP: 1) abnormal inactivation of sodium channels, aconitin or batrachotoxin being a classical example of such effects (Weidmann 1973; Honerjager 1982); 2) inhibition of I_{k2} potassium channels. The latter mechanism favours double activity during acidosis in cardiac Purkinje fibres (Coraboeuf et al. 1980).

From the phenomenological point of view there is much similarity between double activity induced by the above factors and that found in rabbit papillary muscles during long depolarisation. However the basic ionic events responsible for these double APs seem to be rather different.

It is very likely that double AP in rabbit myocardium is related to the existence of I_t channels. This mechanism could contribute to the development of bigeminy in depolarised areas of myocardium at low beating rates due to slow repriming of I_t channels (Boyett 1981). Obviously, further voltage-clamp experiments are needed to verify our hypothesis concerning the nature of the double activity in depolarised rabbit ventricular preparations.

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