Role of the Slow Inward Current during the Repolarisation Process in Frog Atrial Trabeculae

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Abstract. Voltage clamp experiments were performed to analyse the influence of the slow inward current (i_{si}) on the repolarisation process in frog atrium. $MnCl_2$ was used as i_{si} blocker. The action potential was prolonged by depolarising pulses applied during the plateau. This was an expected result considering that, at positive potentials the time constant for i_{si} inactivation increases. This effect was abolished by Mn ions which in turn block i_{si}. The results suggest that inactivation of i_{si} is of primary importance in determining the repolarisation rate. The action of Mn was not selective, since it reduced the background K current. This effect seems to be related to the decrease in Ca influx.

Key words: Frog atrial trabeculae — Slow inward current — Repolarisation — Manganese — Background K current

Introduction

The relative importance of outward currents in determining the repolarisation phase in cardiac fibers, varies among species (for a review see Carmeliet and Vereecke 1979). In the frog atrium, the identification of a delayed time-dependent outward current (i_X) has been important in explaining the repolarisation (Ojeda and Rougier 1974; Brown et al. 1976). On the other hand, Horackova and Vassort (1976) concluded that the normal repolarisation in the frog atrium is most dependent on the inactivation of the slow inward current (i_{si}). In a previous paper (Alvarez et al. 1983), we suggested that this could be the case for atrial trabeculae from *Rana catesbeiana*. At present, no definite conclusion exists about the mechanisms underlying the repolarisation process in the frog atrium.

The aim of the present study was to investigate the influence of i_{si} on the repolarisation phase of frog atrial trabeculae. $MnCl_2$ was used as an inhibitor of i_{si} (Rougier et al. 1969), and its effects on other ionic currents were also analysed since it has been demonstrated that Mn affects the outward current in other tissues from mammalian hearts (Ochi 1970; Kass and Tsien 1975).

Materials and Methods

Experiments were performed at room temperature (21°—23 °C) on isolated trabeculae (100—150 μm in diameter and 3—4 mm long) of frog atrium (*R. catesbeiana*). Preparations were placed in a double sucrose-gap perfusion system with a test node of 400 μm*. The voltage-clamp circuit included a MEZ—7101** differential amplifier, for measuring the membrane potential, whose output was fed into a CEZ—1100** voltage-clamp amplifier. Voltage steps were delivered by a SEN—7103** electronic stimulator coupled to a S—8300** step pulse generator. A standard Ringer solution of the following composition was used (in mmol/l): NaCl 111; KCl 5.4; $NaHCO_3$ 1.8; $CaCl_2$ 2. Isotonic KCl solution (121 mmol/l) and isotonic deionized sucrose (242 mmol/l) were used; pH of Ringer solution was adjusted between 7.4 and 7.5. Manganese chloride (Merck) was added to the Ringer solution at a concentration of 3 mmol/l. Experiments in which trans-gap action potentials exceded 80 mV, were selected for analysis. A paired "t" test was used for statistical analysis accepting $p < 0.05$ as the limit of significance.

Results

When a depolarising current pulse of 10 ms was imposed during the plateau phase (Fig. 1), the duration of the action potential, measured 20 mV positive to the resting potential, was increased by 25 ± 7.6 ms ($n = 6$, $p < 0.05$). This suggests that i_{si} inactivation might control the repolarisation phase (Reuter and Scholz 1977; Payet et al. 1981). In agreement with this finding is the fact that the time constant for inactivation (τ_f) of i_{si}, calculated by fitting the decay of the inward current up to 250 ms as one exponential, ranged (4 preparations) between 30—35 ms at -20 mV; 20—25 ms at 0 mV; and 45—55 ms at $+30$ mV (see also Alvarez et al. 1983). The slow inward current was separated from the fast i_{Na} by the double pulse technique (Fig. 1). The membrane was depolarized to -30 mV from a holding potential of -70 mV during 400 ms to inactivate i_{Na}. Subsequently, 400 ms steps of variable amplitudes were applied. i_{si} was measured as the difference between peak inward current and the stationary current level. This was possible since following such a short pulse there is little or no delayed rectification. The current-voltage relationship for i_{si} had a maximum between -10 and 0 mV and an apparent reversal potential of $+45$ to $+55$ mV (see also Alvarez et al. 1983).

In the presence of $MnCl_2$ (3 mmol/l) i_{si} was inhibited almost completely over the whole potential range studied (5 min $n = 4$). The duration of the action potential was significantly reduced by 61.6 ± 10.3 ms ($p < 0.05$). Under this condition, the depolarising pulse (with the same coupling interval) had no effect on the duration of the action potential. $MnCl_2$ also reduced the fast Na current by 27.3 ± 5.2 % ($p < 0.05$; Fig. 1). However, no exact quantitative description of this change could be made, since during the flow of i_{Na}, a significant lack of voltage control occurred (Alvarez et al. 1983).

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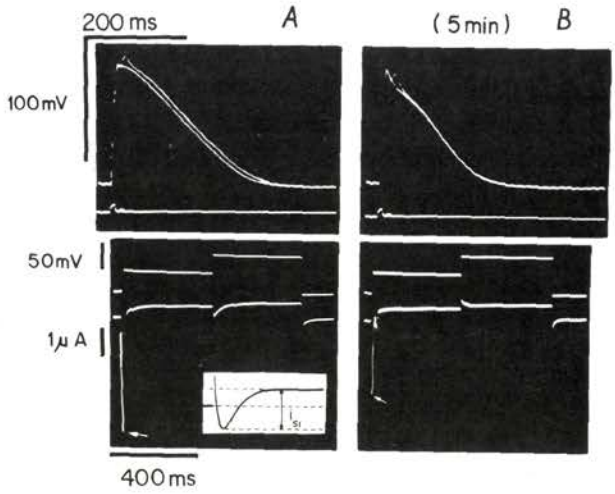

Fig. 1. Effect of a depolarising current pulse on action potential duration (*top*) and measurement of i_{si} (*bottom*), *A*: under control conditions: *B*: under the influence of $MnCl_2$. Stimulation rate: 12/min. The holding potential was set at −70 mV. The first pulse depolarised the membrane to −30 mV and the second one varied (up to +40 mV). Sodium spikes were slightly retouched. Arrows indicate maximum i_{Na}.

The outward current was measured by imposing pulses of 2 s duration from a holding potential of −70 mV (Fig. 2). In order to obtain an estimate of the outward background current (i_{K_1}), it was assumed that the delayed parts of the recordings could be extrapolated towards the onset of the pulse (see de Hemptinne 1971). The time-dependent outward current (i_X) was calculated by substracting i_{K_1} from the outward current value at the end of the clamp.

The current-voltage relationships for i_{K_1} and i_X (see Fig. 2) were similar to those reported by other authors (Rougier et al. 1969; de Hemptinne 1971).

Figure 2 shows the effects of $MnCl_2$ on these outward currents. In 4 experiments i_{K_1} was always reduced by 15—20% in the potential range from −30 to +40 mV. Between −70 and −40 mV this current remained unaffected. The delayed current, i_X, was not affected by $MnCl_2$ (5—10 min) over the potential range examined (−40 to +40 mV).

Discussion

Our results suggest that the repolarisation phase of the action potential in frog atrial trabeculae (*R. catesbeiana*) depends mainly on the inactivation of i_{si} against a background K current rather than on the activation of the time-dependent outward current (see Horackova and Vassort 1976). In our experiments, the action

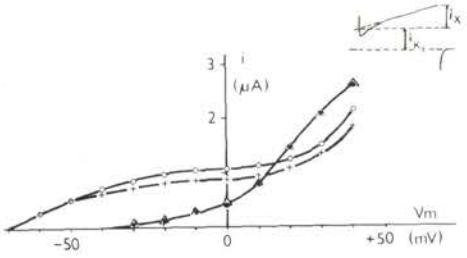

Fig. 2. Effects of $MnCl_2$ on outward currents. Pulse steps of 2 s duration were applied from −70 mV at 7 s intervals. The inset shows the procedure to determine i_{K_1} and i_X. (△) control i_X; (○) control i_{K_1}; (●) i_X under $MnCl_2$; (+) i_{K_1} under $MnCl_2$.

potential was lenghtened by a depolarising pulse applied during the plateau phase (cf. Reuter and Scholz 1977 and Payet et al. 1981). An increase in τ_f by the depolarising pulse, should prolong the action potential since the fraction of i_{X_1} activated during a single action potential is small (τ_{X_1} is greater than the duration of the action potential; 800 ms at 0 mV and 500 ms at +10 mV, Alvarez et al. 1983; see also de Hemptinne 1971). Thus, the rate-limiting mechanism during the repolarisation phase is inactivation of i_{si}, which is a relatively fast process (Horackova and Vassort 1976; de Hemptinne 1978; Alvarez et al. 1983).

In accordance with the above the depolarising pulse during the plateau phase did not increase the duration of the action potential in the presence of $MnCl_2$, since i_{si} was abolished. Under this condition, repolarisation is mainly controlled by the background K current.

It is evident that Mn action is not selective (see also Ochi 1970; Kass and Tsien 1975). It does not affect i_X while it reduces i_{K_1}. This reduction could well be explained in view of the direct relationship that exists between the g_{K_1} level and $(Ca^{++})_i$ (Bassingthwaighte et al. 1976; Isenberg 1977). A decrease in Ca influx through the slow channel due to the action of Mn, would then imply a reduction in i_{K_1} (cf. Delgado et al. 1979).

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