The effects of manganese and barium on the cardiac pacemaker current, $i_f$, in rabbit sino-atrial node myocytes

D. DiFrancesco *, F. Porciatti and J. S. Cohen *

Dipartimento di Fisiologia e Biochimica Generali, Elettrofisiologia, via Celoria 26, I-20133 Milano (Italy), and *Department of Physiology and Biophysics, Health Sciences Center, SUNY at Stony Brook, Stony Brook (New York 11794–8651, USA)

Received 12 July 1990; accepted 2 October 1990

Summary. The isolation of ionic fluxes contributing to electric currents through cell membranes often requires block of other undesired components which can be achieved, among others, by divalent cations. Mn$^{2+}$ and Ba$^{2+}$ are often used, for example, to block Ca and K currents. Here we have investigated the effects of these two cations on the properties of the hyperpolarization-activated pacemaker current $i_f$, in rabbit sino-atrial node myocytes, as obtained by voltage clamp analysis. We find that 2 mM Mn$^{2+}$ shifts the $i_f$ activation curve by $3.2 \pm 0.3$ mV towards more positive values. However, when 1 mM Ba$^{2+}$ is also added, the positive shift is more than halved ($1.3 \pm 0.2$ mV). We find, too, that in the absence of blocking cations the ACh-induced $i_f$ inhibition is slightly higher than in their presence. These results indicate that the alteration of $i_f$ kinetic properties by Ba$^{2+}$ plus Mn$^{2+}$-containing solutions is minimal.

Key words. Pacemaker current; SA node; channel blockers.

Although the presence of $i_r$, a Na/K selective, inward current activated by hyperpolarization has been recognized in the mammalian SA node for more than a decade, its role in generating and modulating the normal diastolic depolarization remains controversial. A central part of this controversy concerns the voltage range of the pacemaker current $i_f$ and its modification by neurotransmitters and divalent cations.

Often, divalent cations such as Ba$^{2+}$ and Mn$^{2+}$ are used to dissect the current $i_f$ from interfering components (see for example DiFrancesco et al. 1). Acting through surface-charge screening or binding, divalent cations may alter the position of the activation curve (see for example DiFrancesco and McNaughton 2, for the pacemaker current in Purkinje fibers).

Recently DiFrancesco et al. 3 have reported that low (nM) concentrations of acetylcholine shift $i_f$ in a negative direction on the voltage axis and this effect of acetylcholine (ACh) is a major contributor to ACh's negative chronotropic effect. In their work, the dose-response relation for $i_f$ inhibition by ACh was measured in solutions containing Ba$^{2+}$ and Mn$^{2+}$. After that report appeared Brown et al. 5 suggested that the inclusion of Mn$^{2+}$ in the bathing Tyrode can shift the activation curve for $i_f$ as much as 10 mV in the positive direction on the voltage axis. This could affect the estimation of the relevance of $i_f$ to the generation and control of the diastolic depolarization. In the present report we have examined the effects of Mn$^{2+}$, and Mn$^{2+}$ plus Ba$^{2+}$ on $i_f$ with the aim of exploring the extent of the modification induced by these cations on the current activation range. Also we examined if Ba$^{2+}$ or Mn$^{2+}$ alter the dose response relation of ACh on $i_f$. We present data on the effects of ACh on the activation range of $i_f$ in the absence of these blocking cations, at ACh concentrations which only minimally affect the ACh-activated K-current $i_{K, ACh}$ (up to 30 nM). Our results indicate that the presence of Ca-current blockers does not affect significantly the analysis of the properties of $i_f$, and in particular does not alter the dose-response curve of the $i_f$ dependence on ACh.

Methods

The experiments were performed on acutely isolated SA node myocytes from the rabbit. The isolation procedure and electronic set up for whole cell voltage clamp have been described previously 1. The cells were aliquotted into petri dishes and directly placed on the temperature controlled microscope stage for study. The Tyrode solution contained in mM 140 NaCl, 5.4 KCl, 1.8 CaCl$_2$, 1.0 MgCl$_2$, 10 NaHCO$_3$, 10 d-glucose and 5.0 Hepes NaOH (pH = 7.4). We added BaCl$_2$ (1 mM), MnCl$_2$ (2 mM) and acetylcholine chloride (3–30 nM) as indicated. The dialyzing solution in the pipette contained (in mM): 10 NaCl, 130 K aspartate, 2.0 Mg-adenosine triphosphate (ATP), 0.1 guanosine triphosphate (GTP), 1.0 EGTA, and 10 mM Hepes-KOH (pH = 7.2). External solutions were superfused from a pipette placed over the myocyte under study. Exchange took less than 1 s. The temperature was maintained at 35–36°C. No corrections for liquid function potentials were applied in keeping with the previous study by DiFrancesco et al. 3. Voltage shifts of the $i_f$ activation curve under the action of shifting agents were measured as described in DiFrancesco et al. 3. The holding potential was set to $-35$ mV in the control (Tyrode) solution which is above the top of the activation curve, and $i_f$ was activated by a hyperpolarization to the mid-activation range applied every 2 seconds. In the presence of the shifting agent, the holding potential was adjusted manually to a new value by turning the holding potential knob (sensitivity = 0.1 mV), until the $i_f$ time-course in the test solution overlapped that in the control solution. The displacement from $-35$ mV of the new holding potential repre-
sented the measured shift of the $i_f$ activation curve. Given the high steepness of the $i_f$ activation curve, this method allowed resolution of fractions of a mV.

**Results**

The effects of $\text{Mn}^{2+}$ and $\text{Ba}^{2+}$ on the voltage dependence of $i_f$. We examined the actions of 2 mM $\text{Mn}^{2+}$ in the absence or presence of 1 mM $\text{Ba}^{2+}$ on the voltage-dependent activation of the current $i_f$. Our first protocol consisted of holding the myocyte at $-35$ mV and hyperpolarizing the cell to a voltage within the $i_f$ activation range. The holding potential of $-35$ mV was chosen because it is normally a few mV positive to the top of the $i_f$ activation curve. A sample set of results is illustrated in figure 1 A.

In this experiment the test potential was $-85$ mV, and the letters a–d indicate the order of solution application. Tyrode containing 2 mM $\text{Mn}^{2+}$ increases the amplitude of $i_f$, but this increase was strongly reduced when 1 mM $\text{Ba}^{2+}$ was also added. The effects of these solution changes were reversible.

A 3-pulse protocol was then used to see if the alterations in $i_f$ amplitude were due to a shift in the voltage dependence of $i_f$ activation (fig. 1 B). The first hyperpolarizing pulse was delivered to the middle of the $i_f$ activation curve and the second hyperpolarizing pulse was delivered to the bottom of the $i_f$ activation curve. A third depolarizing pulse rapidly deactivated $i_f$ before the cycle was repeated. A positive shift in $i_f$ activation was indicated by a larger time-dependent current in response to the first hyperpolarizing step and a smaller time-dependent current in response to the second hyperpolarizing step. Figure 1 B illustrates that for $\text{Mn}^{2+} (2 \text{ mM}) + \text{Ba}^{2+} (1 \text{ mM})$ (*), and $\text{Mn}^{2+} (2 \text{ mM}) (x)$ a shift in $i_f$ activation is observed. These results show that the action of the Ca-current blockers on $i_f$ is essentially that of shifting the current activation range to more positive voltages.

We next examined quantitatively the action of the blocking cations on $i_f$ by using the protocol shown in Figure 1 C. Here the test solution was superfused during a train of voltage steps of constant amplitude and the holding potential was then moved positive until the $i_f$ current was determined. Further addition of $\text{Ba}^{2+}$ partially reversed this effect. Cell 3-4. C Measurement of shifts of $i_f$ activation curve caused by either $\text{Mn}^{2+}$ plus $\text{Ba}^{2+}$ (upper) or $\text{Mn}^{2+}$ alone (lower). Hyperpolarizing steps of fixed amplitude (40 mV) were applied from a holding potential of $-35$ mV in the control solution, and the holding potential was then moved positive, during superfusion with the test solution, until the $i_f$ traces overlapped as shown. The shifts were $-1.5$ mV for the $\text{Mn}^{2+}$ plus $\text{Ba}^{2+}$ solution, and $-4.0$ mV for the $\text{Mn}^{2+}$ solution. Cell 1f-6.