Short Communication

Single and Multiple Early Afterdepolarization Caused by Nickel in Rat Atrial Muscle

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Abstract. We examined the possible role of the Na-Ca exchange (NCX) in the arrhythmogenesis in rat atrial preparations applying microelectrode technique. In control Tyrode solution preparations isolated from the sinoatrial area contracted with frequency of 48 ± 4 min^{-1} (group I) or 84 ± 7 min^{-1} (group II). In preparation beating with low frequency partial inhibition of NCX by administration of Ni^{2+} (0.3 mmol/l) to the bath solution caused single early afterdepolarization (EAD) on the 15th min. During the following five minutes they were transformed into multiple EADs from 4 to 47 (action potentials) with general duration of 1–12 s. The effects were reversible. Ni^{2+} (0.3 mmol/l) in the preparations beating with higher rate (group II) did not cause multiple EADs, but after higher Ni^{2+} concentration (0.5 mmol/l) single EAD was observed more often. It was concluded that Ca^{2+} overload due to partial block of the NCX can contribute to the development of atrial tachyarrhythmias.

Key words: Cardiac electrophysiology — Early afterdepolarization — Ni^{2+} — Action potential — Rat

The exact mechanisms of cardiac arrhythmias are not fully understood and both re-entry and enhanced triggered activity (Carmeliet 1999; Nuss et al. 1999) are postulated to be involved, but less is known about the Ca^{2+} overload which induces triggered mechanism especially in the atria. Since intracellular Ca^{2+} concentration critically depends on both Ca^{2+} entry *via* L-type Ca current (Bean 1985) and extrusion from the cell *via* Na-Ca exchance (NCX) (Kimura et al. 1987), we examined the possible effect of moderate NCX inhibition by Ni^{2+} administration into concentration (0.1–1.0 mmol/l) which does not influence significantly L-type calcium current in rat atrial preparations.

We applied standard microelectrode techniques to study action potentials (AP) from young (1.5 month) white rats ($n_{\mathrm{heart}} = 19$). The rats were anesthetized

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in aether under bell-glass and then sacrifices. Heart was quickly removed and placed into control solution (mmol/l): NaCl 118; KCl 4.7; $CaCl_2$ 2.7; $NaHCO_3$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.5; HEPES 3; glucose 5. Spontaneously beating right atrial preparations including sinoatrial valve and segment of sinoatrial ring (2×2 mm) were placed into a tissue bath and superfused with Tyrode solution equilibrated with air and warmed to 28 ℃ (pH – 7.3). The bath was connected to the ground with a 2.5 mol/l KCl/Ag/AgCl junction. Preparations were impaled with glass microelectrode having a long fine shank and tip resistance of 25–40 MΩ. AP parameters cells of sinoatrial area were examined. Only the preparations with stable impalements were used for data analyses.

In the control Tyrode solution the preparations from sinoatrial area spontaneously contracted with the frequency of 48 ± 4 min^{-1} (group I, $n_{\text{strips}} = 8$) and 84 ± 7 min^{-1} (group II, $n_{\text{strips}} = 11$). The duration of action potentials at the level of 100 % repolarization of cells without slow diastolic depolarization was equal to 270 ± 30 ms $\pm$ S.D. (230–330 ms, $n_{\text{cells}} = 15$) and 190 ± 20 ms (160–240 ms,

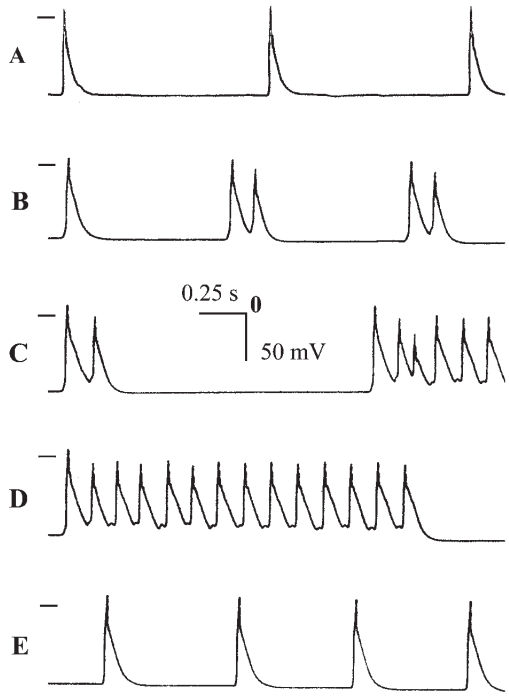

Figure 1. Effect of $[Ni^{2+}]_o$ in atrium type cells in sinoatrial area of rat heart. **A.** action potentials in control solution; **B.** effect of Ni^{2+} (0.3 mmol/l) at 14^{th} min exposition; **C.** 16^{th} min exposition; **D.** 19^{th} min exposition; **E.** recovery after washout of Ni^{2+} (10 min.) This recording was obtained from the same cell. Calibration: 50 mV; 0.25 s.

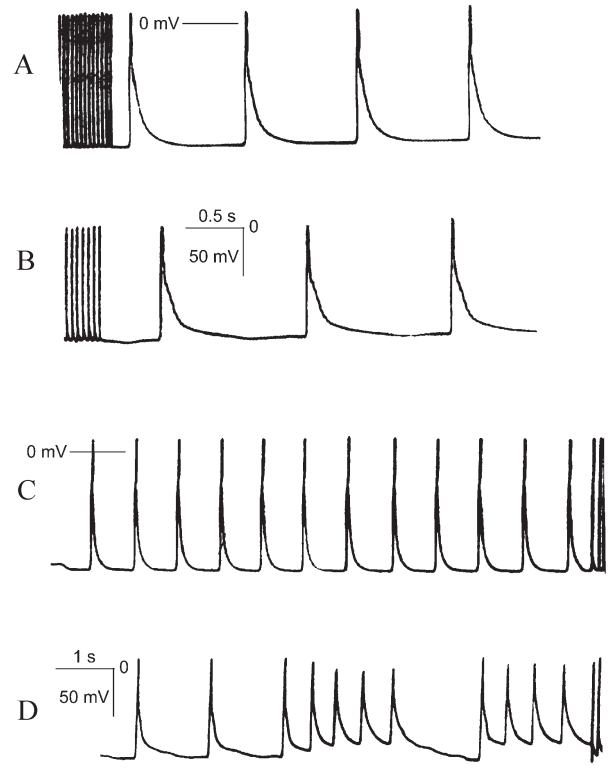

Figure 2. Recordings of membrane potential in normal spontaneously beating strips with different frequency. **A.** action potentials in control solution; **B.** effect Ni^{2+} (0.3 mmol/l) at 25^{th} min exposition; **C.** action potentials in control solution; **D.** effect Ni^{2+} (0.5 mmol/l) at 20^{th} min exposition.

$n_{\text{cells}} = 13$), respectively ($p < 0.01$). Administration of Ni^{2+} (0.3 mmol/l) to the bath caused "early" AP at 15±1 min in group I with low frequency (Fig. 1). During the following next five minutes they were transformed into multiple early afterdepolarization (EAD) from 4 to 47 EADs with the general duration from one to 12 s in a series. The effects were reversible. It should be noted that Ni^{2+} (0.3 mmol/l) with a higher beating rate did not (group II) induce multiple early AP, only occasional single early AP were observed when Ni^{2+} was increased up to 0.5 mmol/l in the tissue bath. Single early APs were observed more often, sometimes a series of multiple early APs from 3–7 APs were registered (Fig. 2).

The main finding of the present study is to show that partial block of NCX elicited EAD in rat atrial preparation. This observation can be best explained by assuming that the incomplete block of NCX (Hinata et al. 2002) would favor the increase of intracellular Ca^{2+} concentration but is not able to fully prevent the forward mode of NCX carrying sufficient amount of inward current resulting in

transient depolarization or EADs (Noble and Bett 1993; Viswanathan and Rudy 1999). Since in atrial cells which lack distinct plateau phase the Ca^{2+}-transient lasts longer than the AP, the extrusion of Ca^{2+} *via* the NCX occurs mostly during diastole i.e. under the conditions when actual membrane potential value is more negative than the expected reversal potential for NCX. Therefore if frequency is higher, the cells exhibit more Ca^{2+} release/Ca^{2+}-transients which would favor Ca^{2+} extrusion, and thereby reduce Ca^{2+}, overload. This may explain the observation that at higher rate low concentration of Ni^{2+}, i.e. mild inhibition of NCX did not evoke EADs. However, when concentration of Ni^{2+} was doubled, the more pronounced inhibition of NCX resulted in significant Ca^{2+} overload and consequently enhanced EAD activity. In our experiments we could not elevate Ni^{2+} further since we did not want to decrease the Ca^{2+} entry *via* the L-type Ca^{2+} channels. We can, however, speculate that more effective NCX block would decrease EAD formation completely abolishing the inward current generated by NCX itself. Unfortunately, we cannot test this latter hypothesis since we lack specific blocker of NCX.

Considering all the limitations of our study we can conclude that the actual activity of NCX may play an important role in the mechanism of atrial fluttering and fibrillation. Further studies with more specific drug are necessary, however, to evaluate the exact role of NCX in the arrhythmogenesis in cardiac preparations.

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