Short communication

Current-dependent Gating of Single Cardiac Sodium Channels?

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Based on studies of differences in physicochemical properties of antagonists and agonists of ionic channels it has been proposed that the magnitude of the elementary currents might influence the gating properties of ionic channel molecules (Marinov and Saxon 1985; Marinov 1985, 1986). This idea was supported by measurements of Khodorov and coworkers (personal communication) who reported that in inwardly rectifying potassium channels increased unitary currents induced prolonged single channel openings. A similar finding has already been reported by Payet et al. (1985) working on the inward K^+ rectifier channel in myocytes of newborn rat hearts. However, for the delayed K^+ rectifier in skeletal muscle no similar correlation could be found (Standen et al. 1985). Herein we report data on single cardiac sodium channels suggesting a positive correlation between the magnitude of single channel currents and the mean duration of the open state when the unitary currents were changed only by variation of the concentration gradient for sodium.

Experiments were carried out on single ventricular cells of guinea pig hearts dissociated by a method described elsewhere (Kao et al. 1980). The solution in the experimental chamber for patch clamp studies contained (mmol/l): 140 K-aspartate; 10 EGTA; 1 $MgCl_2$; 5 Hepes; pH 7.4 adjusted with KOH. Single channel recordings were performed in this medium in cell attached patches only. The cells were zeroed close to 0 mV resting potential in the medium used. The patch clamp pipettes contained (mmol/l): 0.5 $CaCl_2$; 0.5 $MgCl_2$; 11 glucose; 5 Hepes; pH 7.4 adjusted with NaOH. NaCl was changed between 140 and 250 mmol/l, and osmotically balanced by sucrose. Experiments were performed at room temperature. The patch clamp device used was standard (Hamill et al. 1981) and has been described in detail elsewhere (Nilius et al. 1986, 1987).

Figure 1 shows single channel currents with two different sodium concentrations in the patch pipette. Short openings cluster at the very beginning of the

depolarizing step from −120 to −50 mV. From sufficiently long openings the magnitude of the single channel currents was measured in 140 mmol/l Na, being 1.7 ± 0.2 pA (61 traces) and 2.2 ± 0.1 pA in 250 mmol/l Na (52 traces). At both concentrations the time course of the averaged current (76 sweeps) shows the typical fast activating and inactivating Na currents. Obviously, an inspection of the ten consecutive sweeps reveals some strikingly prolonged openings together with an increase in the magnitude of the single channel currents in 250 mmol/l Na.

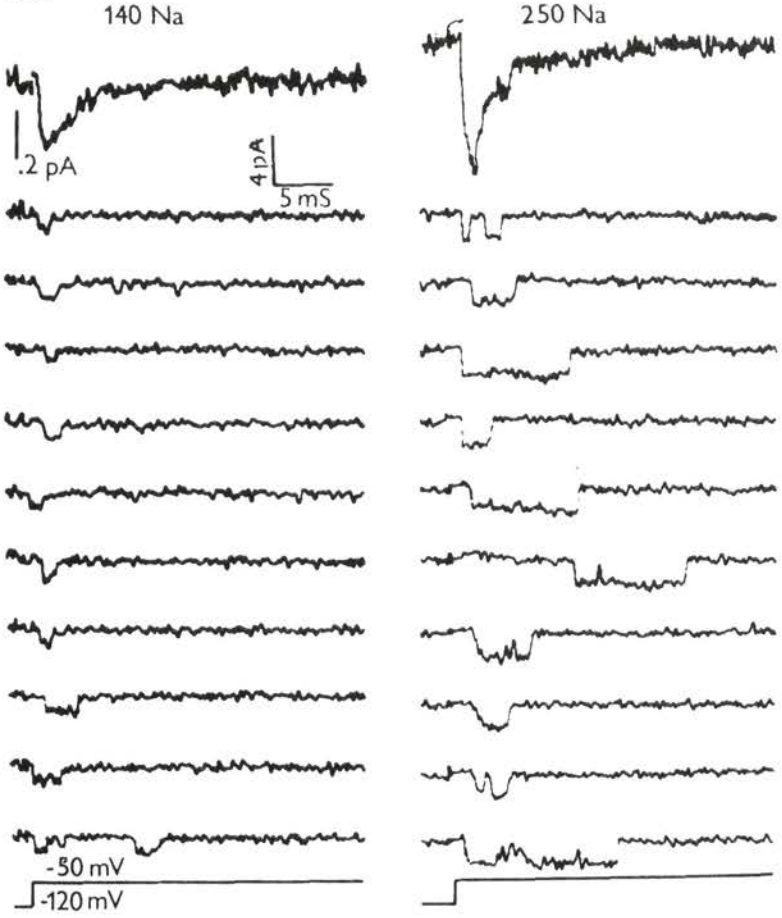

Fig. 1. Changes in sodium channel gating induced by varying extracellular Na concentration. *A*: Single channel currents obtained with 140 mmol/l NaCl in the patch pipette. The average current is from 76 sweeps (cell attached patch with supposingly 1 Na channel; sampling rate 10 kHz, 2 kHz filter, $i = 1.7 \pm 0.3$ pA, $\tau_0 = 0.7$ ms, 10 consecutive sweeps, cell 121286-01). *B*: Currents through Na channels at 250 mmol/l NaCl in the external solution. The average current is from 76 sweeps (cell attached 1-channel patch, same voltage protocol and conditions of recording as in *A*, $i = 2.2 \pm 0.1$ pA, $\tau_0 = 2.8$ ms, cell 121286-05).

Figure 2 shows an analysis of the mean open times as measured from the fits of the open time distributions. In patches shown in Figure 1*A*, *B* and with the same voltage protocol the mean open time increased from 0.7 ms (140 mmol/l Na) to 2.8 ms (250 mmol/l Na). From 3 patches (4 measurements) we obtained an average unitary current of 1.6 ± 0.2 pA and a mean open time τ_0 of 0.4 ± 0.3 ms in 140 mmol/l Na using voltage steps from -120 to -50 mV. With the same voltage protocol, the following data were obtained from 3 patches (6 measurements) in 250 mmol/l Na: $i_{Na} = 2.2 \pm 0.3$ pA, $\tau_0 = 2.1 \pm$ ± 0.3 ms. With 0.5 mmol/l Ca in the patch pipette the single channel conductance was close to 25 pS (see also Nilius et al. 1987). Therefore, the expected change in the unitary current of about 0.5 pA as calculated from the changed electro-chemical gradient for sodium, nicely matches the measured change in the unitary currents.

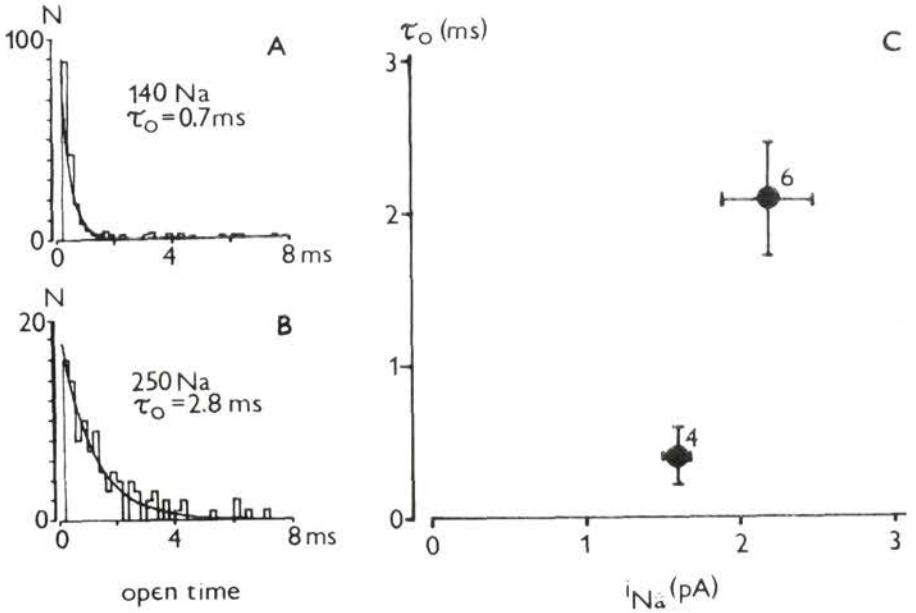

Fig. 2. Correlation between the magnitude of single Na channel currents and the mean open time. *A*, *B*: Open time distribution and fits using Marquardt-algorithm from the same patches as in Figure 1*A*, *B*. *C*: 6 measurements from 3 patches revealed a unitary current of 2.2 ± 0.3 pA and a mean open time of 2.1 ± 0.3 ms in 250 mmol/l Na; however, in 140 mmol/l Na (4 measurements from 3 patches) the values were: $i = 1.6 \pm 0.2$ pA and $\tau_0 = 0.4 \pm 0.3$ ms. The voltage protocol used for all the measurements was the same as shown in Figure 1.

These unexpected findings that a change in the magnitude of single cardiac Na currents at the same test and holding potentials might induce alterations in

the gating properties of ionic channels had been predicted by the concept of channels considering them proteinic moeities in which gating is coupled to electron transfer similarly as it is the case in photochemical reactions (Marinov 1985). We also argue that the presence of a monovalent cation in the pore inhibits channel closing as already suggested by Matteson and Swenson (1986).

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