Potassium inhibition of sodium-activated potassium (K_{Na}) channels in guinea-pig ventricular myocytes

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1. Na^+-activated potassium channels (K_{Na} channels) were studied in inside-out patches from guinea-pig ventricular myocytes at potentials between −100 and +80 mV. External K^+ (K_o^+) was set to 140 mM. For inwardly directed currents with 105 mM internal K^+ (K_i^+), the unitary current–voltage relationship was fitted by the constant field equation with a potassium permeability coefficient, P_K, of $3{\cdot}72 \times 10^{-13}$ cm^3 s^{-1}. The slope conductance (−100 to −10 mV) was 194 ± 4·5 pS (mean ± S.D., $n = 4$) with 105 mM K_i^+ (35 mM Na_i^+) but it decreased to 181 ± 5·6 pS ($n = 5$) in 70 mM K_i^+ (70 mM Na_i^+).

2. K_{Na} channels were activated by internal Na^+ in a concentration-dependent fashion. With 4 mM K_i^+, maximal activation was recorded with 100 mM Na_i^+ (open probability, P_o, about 0·78); half-maximal activation required about 35 mM Na_i^+. When K_i^+ was increased to 70 mM, half-maximal activation shifted to about 70 mM Na_i^+.

3. With Na_i^+ set to 105 mM, channel activity was markedly inhibited when K_i^+ was increased from 35 to 105 mM. Channel openings were abolished with 210 mM K_i^+.

4. The inhibitory effect of internal K^+ was also observed at more physiological conditions of osmolarity, ionic strength and chloride concentration. With 35 mM Na_i^+ and 4 mM K_i^+, P_o was 0·48 ± 0·10 ($n = 6$); when K_i^+ was increased to 35 mM, P_o was reduced to 0·04 ± 0·05 ($n = 7$, $P < 0{\cdot}001$).

5. The relationship between P_o and Na_i^+ concentration at different levels of K_i^+ is well described by a modified Michaelis-Menten equation for competitive inhibition; the Hill coefficients were 4 for the P_o–Na_i^+ relationship and 1·2 for the P_o–K_i^+ relationship. It is suggested that Na^+ and K^+ compete for a superficial site on the channel's permeation pathway.

6. K_{Na} channels would be most likely to be activated *in vivo* when an increase in Na_i^+ is accompanied by a decrease of K_i^+.

Increases in potassium conductance activated by high levels of intracellular sodium (Na_i^+) have been reported in many different preparations. These include guinea-pig ventricular myocytes (Kameyama *et al.* 1984) as well as neurones from crayfish (Hartung, 1985), quail trigeminal ganglia (Bader *et al.* 1985), chick brainstem (Dryer *et al.* 1989), cat neocortex (Schwindt *et al.* 1989), rat olfactory bulb (Egan *et al.* 1992*b*), rat motoneurones (Safronov & Vogel, 1996), rat dorsal root ganglia (Bischoff *et al.* 1998) and frog spinal chord (Dale, 1993). In addition, sodium-gated potassium channels (K_{Na} channels) are present in *Xenopus* oocytes (Egan *et al.* 1992*a*), insect neurosecretory cells (Grolleau & Lapied, 1994) and frog taste cells (Miyamoto *et al.* 1996). In some preparations there is a sufficiently high density of voltage-gated sodium channels that the Na^+ accumulated during trains of action potentials may contribute to K_{Na} channel activation. The correlation between the numbers of voltage- and sodium-gated channels in individual membrane patches in *Xenopus* myelinated axons (Koh *et al.* 1994) supports this idea. In neurones there is enough K_{Na} channel activation at physiological levels of Na_i^+ to suggest that the sodium-activated potassium current may contribute to the resting membrane conductance (Haimann *et al.* 1990; Bischoff *et al.* 1998).

In cardiac preparations K_{Na} channels appear to require 20 mM Na_i^+ for activation, the response showing no saturation below 100 mM Na_i^+ (Kameyama *et al.* 1984). Guinea-pig myocytes have an intracellular sodium activity of about 7 mM (Rodrigo & Chapman, 1990), which may increase by over 30% during increased heart rate (Cohen *et al.* 1982). Nevertheless the relative insensitivity of the channels to Na_i^+ suggests that activation occurs (if at all) only during pathological conditions.

The K_{Na} channel binding site requires the co-operative action of at least two Na^+ to bring about activation (Kameyama *et al.* 1984; Haimann *et al.* 1990) but otherwise little is known about the characteristics of the site of action.

We therefore undertook a study of the effect of Na^+ in the presence of different concentrations of intracellular potassium (K_i^+). We report here that K^+ at the internal membrane surface competitively inhibits activation of K_{Na} channels in inside-out patches from guinea-pig ventricular myocytes. This may have some physiological significance, as an increase in Na_i^+ *in vivo* is likely to coincide with a decrease in K_i^+.

METHODS

Preparation

Adult guinea-pigs were killed by cervical dislocation. The heart was removed and perfused retrogradely via the aorta using a Langendorff apparatus. Single ventricular myocytes were prepared by enzymatic dissociation as described previously (Mitra & Morad, 1985; Rodrigo & Chapman, 1990) except that 40 mM taurine was included in the Tyrode solution used for the final perfusion. Myocytes were kept at room temperature (18–23 °C) and used within 12 h of isolation.

Recording conditions and analysis

Currents through K_{Na} channels were recorded using the inside-out configuration of the patch clamp technique (Hamill *et al.* 1981). Pipettes were made from borosilicate glass capillaries (Clark Electromedical Ltd) and had tip resistances of 3–10 MΩ when filled with pipette solution. We used a modified technique to excise inside-out patches; after seal formation, instead of removing a patch of membrane from the myocyte by withdrawing the pipette, the cell was dislodged and washed away by rapid superfusion of standard internal solution through the perfusion system (see Niu & Meech, 1998). One advantage of this approach was that although the cell was maintained in normal bathing medium, after patch isolation the inside surface of the plasma membrane was immediately exposed to a solution with an appropriate internal composition. By preparing detached patches in this way, K_{Na} channel activity was stable for periods of up to 30 min. Membrane potentials were corrected for the calculated loss of junction potential between the patch pipette and bathing solution upon seal formation (Barry & Lynch, 1991).

K_{Na} channel currents were recorded using an Axopatch-1D patch clamp amplifier and digitised via a TL-1 interface. For multi-channel analysis, currents were filtered at 0·5–1 kHz and digitised at 2–10 kHz. For single channel analysis the currents were filtered at 2 kHz and sampled at 20 kHz. All experiments were conducted at room temperature (18–23 °C). Channel open probability (P_o) was calculated as:

$$P_o = \left(\sum_{j=1}^{N} t_j j\right) \Big/ TN, \quad (1)$$

where N is the number of channels in the membrane patch and t_j is the time spent at each current level ($j = 1, 2, \ldots N$). The total duration of the recording, T, was at least 15 s. The 50% open/closed level was used to mark the beginning and end of each transition. Subconductance states, which were observed close to the full open and closed levels, were both rare and brief (i.e. less than 1% of open time); they were ignored in this analysis.

Solutions

Myocytes were maintained in normal saline (mM): NaCl, 140; KCl, 5·4; $MgCl_2$, 1; $CaCl_2$, 2; and Hepes, 5, at pH 7·25. Seals were obtained with patch pipettes that contained (mM): KCl, 140; $CaCl_2$, 1; and Hepes, 5, at pH 7·25 (adjusted with KOH). Once detached, the internal surface of the membrane patch was perfused with a standard internal solution composed of (mM): KCl + NaCl, 140; EGTA, 5; ATP, 2–5; and Hepes, 10, at pH 7·25 (adjusted with KOH). Test solutions are specified in the text; in some cases they were hyperosmotic to the standard solution; in other cases the osmolarity was made the same as the standard solution using either glucose or *N*-methyl-D-glucamine-HCl (NMDG-HCl). All chemicals were obtained from Sigma Chemical Co. Ltd.

RESULTS

During single channel recordings of K_{Na} channel activity from guinea-pig ventricular myocytes, we observed two other classes of potassium channel: the ATP-sensitive potassium channel (K_{ATP}) and the inwardly rectifying potassium channel (K_{IR}). K_{ATP} channel activity was inhibited by including ATP (at least 2 mM) in the perfusion solution (see Niu & Meech, 1998) but interference from K_{IR} was almost inevitable. However, K_{IR} channels had a smaller unitary conductance than K_{Na} channels, and their kinetics were significantly different. This meant that their activity could be excluded easily and had little impact on the analysis of K_{Na} channel properties. Furthermore, K_{IR} channels were generally less stable than K_{Na} channels and appeared to undergo rundown within 2–5 min after excision of the inside-out patch.

K_{Na} channels were present in about 10% of inside-out patches, and in most cases one to two channels were observed. Consistent with previously published work, the channels often had multiple subconductance states and their activity was significantly affected by changes in Na^+ concentration at the internal membrane surface. Figure 1*A* shows sections of a continuous recording from an inside-out patch containing two K_{Na} channels. Channel currents were recorded at different holding potentials in the presence of 140 mM K_o^+; the internal concentrations were 70 mM K^+ and 70 mM Na^+ (left column), or 105 mM K^+ and 35 mM Na^+ (right column). K_{Na} channel activity was markedly reduced when the patch was perfused with the 105 mM K_i^+, 35 mM Na_i^+ solution. The unitary current–voltage relationship (Fig. 1*B*) showed a significant inward rectification, which became even more marked as Na_i^+ was increased from 35 to 70 mM. The lines drawn through the data were calculated from the constant field equation (Goldman, 1943; Hodgkin & Katz, 1949) and are equivalent to a conductance of 199 pS in 140 mM external and internal K^+. The potassium permeability coefficient, P_K, was taken as $3{\cdot}72 \times 10^{-13}$ cm^3 s^{-1} and the P_{Na}/P_K ratio was set at 0·02 (see Kameyama *et al.* 1984; Wang *et al.* 1991). The mean slope conductance of the fully opened channel was $194 \pm 4{\cdot}5$ pS ($n = 4$) when measured with 140 mM K_o^+ and 105 mM K_i^+ (35 mM Na_i^+) for inward currents in the range −100 to −10 mV. The slope conductance of the inward current decreased slightly to $181 \pm 5{\cdot}6$ pS ($n = 5$) in 70 mM K_i^+ (70 mM Na_i^+). The values shown are means ± S.D. throughout.

Inhibitory effect of intracellular K^+

With Na_i^+ set to 105 mM there was a high level of K_{Na} channel activity in inside-out membrane patches exposed to

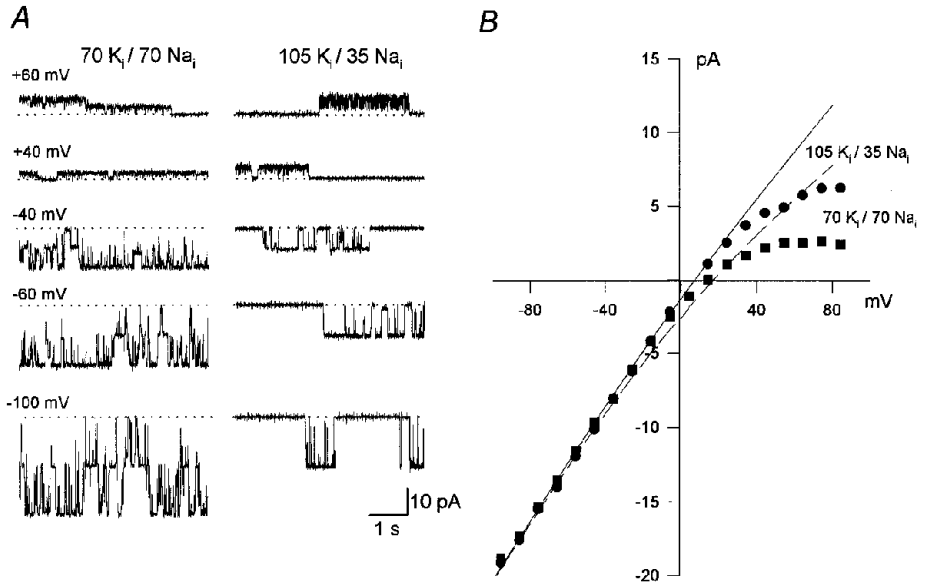

Figure 1. Effect of membrane voltage on K_{Na} channel currents

A, current traces from an inside-out patch containing two active K_{Na} channels recorded at potentials between +60 and −100 mV in the presence of 140 mM K_o^+. Internal solutions were 70 mM K_i^+, 70 mM Na_i^+ (left) and 105 mM K_i^+, 35 mM Na_i^+ (right). The dotted lines indicate the current level recorded with both channels closed. *B*, the unitary current–voltage relationship in 70 mM K_i^+, 70 mM Na_i^+ (■) and 105 mM K_i^+, 35 mM Na_i^+ (●). The lines drawn through the data were calculated from the constant field equation (Goldman, 1943; Hodgkin & Katz, 1949). The permeability coefficient, P_K, was taken as $3{\cdot}72 \times 10^{-13}$ cm^3 s^{-1} and the P_{Na}/P_K ratio was 0·02 (see Kameyama *et al.* 1984; Wang *et al.* 1991). Deviation from the expected relationship at membrane potentials more positive than +30 mV is due to channel block by Na_i^+ (Wang *et al.* 1991).

35 mM K_i^+ (Fig. 2, top trace) but the open probability was markedly reduced upon switching to a solution with 105 mM K_i^+. Channel activity was abolished completely in the presence of 210 mM K_i^+ but it returned to control levels once the bathing solution was returned to 35 mM K_i^+ (Fig. 2, bottom trace). Similar, fully reversible, effects were observed in three additional patches. Because Na_i^+ was set to 105 mM, the different test solutions were hyperosmotic. Thus the reduction in open probability could be the result of the greater osmolarity of the internal solutions, or their greater ionic strength and higher chloride concentration. Figure 3 shows that none of these factors are involved because in 35 mM K_i^+ the high open probability was retained even when 350 mM glucose (top trace), 175 mM lithium chloride ($n = 2$; middle trace), or 175 mM NMDG-HCl ($n = 4$; bottom trace) was added to the test solution. Thus,

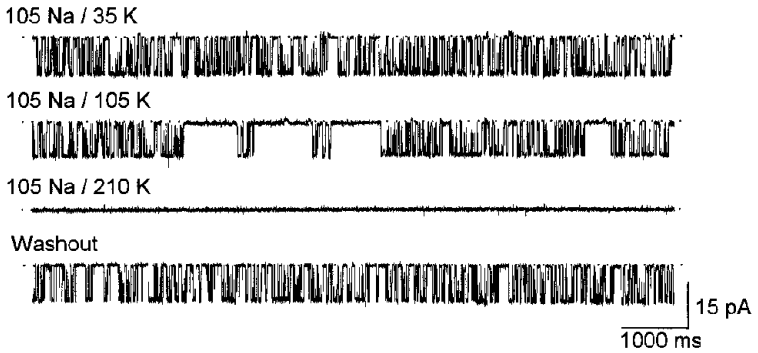

Figure 2. Effect of K_i^+ on K_{Na} channel activity

Single K_{Na} channel inward currents recorded from an inside-out membrane patch with 105 mM Na_i^+. The high open probability seen with 35 mM K_i^+ (top trace) was markedly reduced upon increasing K_i^+ to 105 mM and the channel was fully inhibited with K_i^+ at 210 mM (middle traces). The channel activity recovered to the control level after K_i^+ was returned to 35 mM (bottom trace). Note that because Na_i^+ was set to 105 mM the test solutions were hyperosmotic. The membrane potential was held at −60 mV.

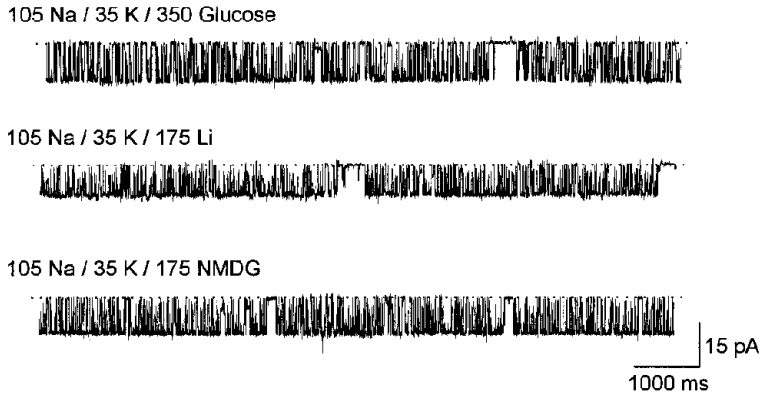

Figure 3. Effect of osmotic strength, internal Li^+ and NMDG-HCl on K_{Na} channel activity
Single K_{Na} channel inward currents recorded from an inside-out membrane patch with 105 mM Na_i^+ and 35 mM K_i^+. Top trace, high open probability with hyperosmotic solution (containing 350 mM glucose) at the internal surface. Middle trace, open probability was unaffected by the addition of 175 mM LiCl in place of glucose. Bottom trace, open probability was unaffected by the addition of 175 mM NMDG-HCl in place of glucose. The membrane potential was held at −60 mV.

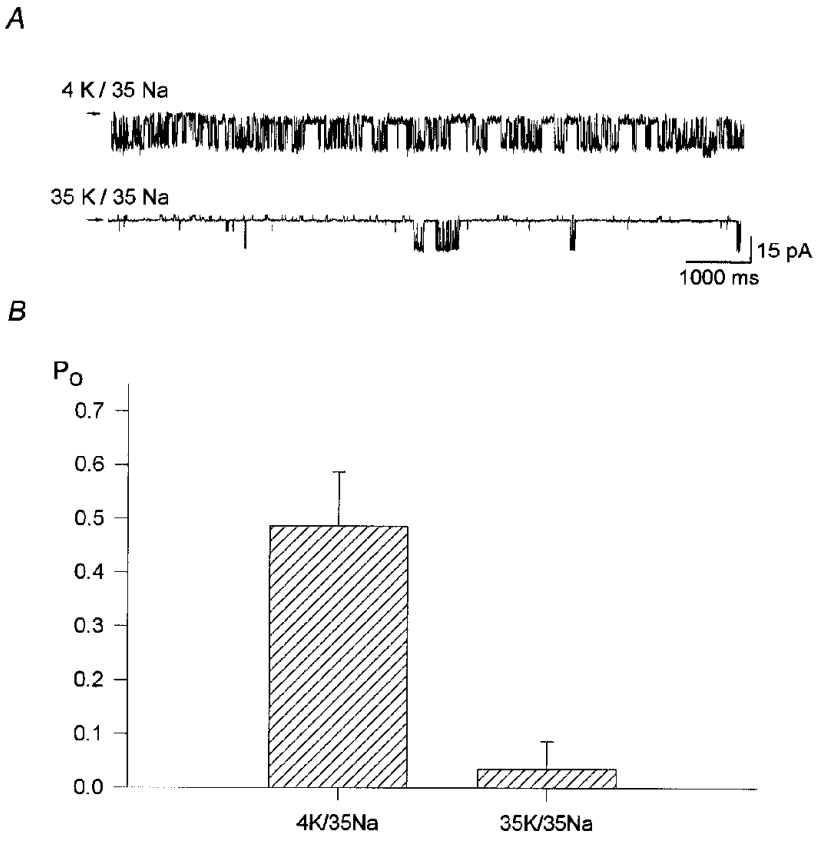

Figure 4. The effect of K_i^+ on K_{Na} channel activity at physiological osmolarity
A, examples of inward currents recorded from single K_{Na} channels in inside-out membrane patches with 35 mM Na_i^+. The high channel activity recorded with 4 mM K_i^+ (upper trace) was in contrast to the low number of openings seen with 35 mM K_i^+ (lower trace; different patch). Changes in osmolarity were corrected with NMDG-HCl and the membrane potential was held at −60 mV. *B*, bar graph to show that the open probability (P_o) was reduced from 0·48 ± 0·10 ($n = 6$) to 0·04 ± 0·05 ($n = 7$, $P < 0·001$) upon raising the internal potassium concentration from 4 to 35 mM in the presence of 35 mM Na_i^+. Values are means ± S.D.; P value is from Student's unpaired t test.

increased concentrations of K_i^+ appear to inhibit K_{Na} channel activity in a fully reversible fashion.

Internal K^+ was also inhibitory at lower concentrations and at a more physiological osmolarity (maintained using NMDG-HCl). Figure 4 shows K_{Na} channel activity with Na_i^+ set to 35 mM. Upon raising K_i^+ from 4 mM (upper trace) to 35 mM (lower trace) there was a clear reduction in the number of channel openings. The bar chart in Fig. 4 shows the open probability in the two solutions; the open probability was $0{\cdot}48 \pm 0{\cdot}10$ ($n = 6$) with 4 mM K_i^+ but became significantly smaller ($0{\cdot}04 \pm 0{\cdot}05$; $n = 7$; $P < 0{\cdot}001$, unpaired t test) upon raising K_i^+ to 35 mM.

From the data presented thus far, it is possible that internal Na^+ relieved the K_{Na} channel from inhibition by internal K^+. To determine whether internal Na^+ was essential for K_{Na} channel activation, we exposed the channel to a K^+–Na^+-free internal solution containing 105 mM NMDG-HCl (see Fig. 5). Although there was an almost immediate reduction in open probability upon switching to the K^+–Na^+-free solution, there was a delay of 12 s before channel opening was completely abolished. Upon returning to 105 mM Na_i^+, the increase in open probability followed an exponential time course with a time constant of about 1·2 s. The same result was observed for the two other patches tested.

While internal Na^+ was obviously necessary for K_{Na} activation, it is clear that there was also an interaction with internal K^+. To examine the inhibitory effect of K^+ on the sodium dependency in more detail, we first set K_i^+ at 4 mM and measured the channel open probability in different concentrations of Na_i^+ (see Fig. 6*A*, filled circles). For Na_i^+ concentrations below 140 mM, NaCl was replaced by equimolar concentrations of NMDG-HCl. When we compared the effect of Na_i^+ in the presence of 70 mM K_i^+ (Fig. 6*A*, open circles) the maximum open probability appeared to be as great but the data were displaced towards the right, i.e. the half-maximal open probability was observed at higher values of Na_i^+. In Fig. 6*B* the effect of different concentrations of K_i^+ on channel open probability is compared in the presence of 105 mM (open circles) and 35 mM (filled circles) Na_i^+. In Fig. 6 lines through the data points were drawn according to the following modified Michaelis-Menten equation, which can be derived assuming that there is a competitive interaction between Na_i^+ and K_i^+:

$$P_o = \frac{P_{o,max}}{1 + (1 + ([K^+]_i^g / k_b))(k_a / [Na^+]_i^h)}, \quad (2)$$

where k_a and k_b are dissociation constants, $[Na^+]_i$ and $[K^+]_i$ are the sodium and potassium ion concentrations at the intracellular surface of the membrane, and g and h represent Hill coefficients. The data were fitted by eye with

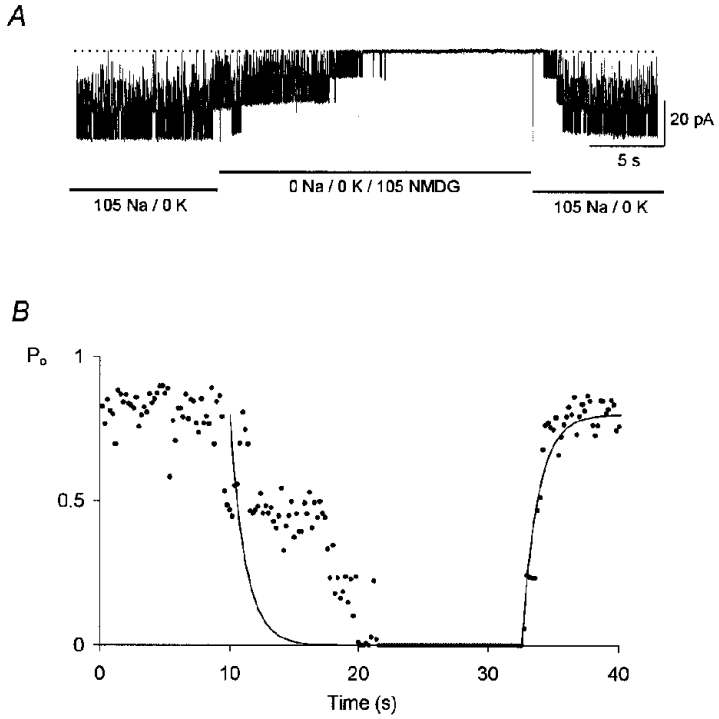

Figure 5. Effect of Na^+–K^+-free internal solution on K_{Na} channel activity

A, K_{Na} channel inward currents recorded from an inside-out membrane patch with 105 mM Na_i^+, 0 mM K_i^+. Three levels of unitary current are evident. Upon switching abruptly to a Na^+–K^+-free solution containing 105 mM NMDG-HCl, channel activity declined over a period of 12 s and was finally abolished. Activity quickly recovered when the patch was returned to the control solution. The membrane potential was held at −60 mV. *B*, change in P_o with time. The fitted line has a time constant of 1·2 s.

the k_a:k_b ratio set to $1{\cdot}75 \times 10^5$; the Hill coefficients g and h were 1·2 and 4·0; the maximum open probability ($P_{o,max}$) was 0·78.

Single channel kinetics

Patches with a single active K_{Na} channel were studied at −60 mV with 105 mM Na_i^+. Figure 7 shows the distributions of open and closed times in 4 mM K_i^+ (left) and 105 mM K_i^+ (right). Although obtained under slightly different conditions, the data confirm the findings of Mistry *et al.* (1997) and demonstrate the presence of two open and at least four closed states. The time constants obtained in the two studies are in broad agreement. In Fig. 7 the increase in K_i^+ from 4 to 105 mM had little effect on the open time constants or on the two shorter closed time constants but the longer closed time constants increased by factors of about 4 and 3. This matches the finding by Mistry *et al.* (1997) who showed that the time constants of these same closed states increased as the Na_i^+ concentration was decreased (with NaCl being replaced by Tris-HCl).

DISCUSSION

K_{Na} channel 'rundown'

K_{Na} channels recorded from detached inside-out membrane patches are particularly susceptible to a gradual loss of activity (or 'rundown') that is not seen in cell-attached patches (Egan *et al.* 1992*b*; Dryer, 1993). Egan *et al.* (1992*b*) suggest that the loss of some diffusible intracellular substance might reduce the sensitivity of the channel to sodium. Using techniques of myocyte preparation similar to those used here, Rodrigo (1993) found that in newly excised patches P_o was about 0·3 with 150 mM Na_i^+ and that it declined to 0·1 after 30 min. In cell-attached patches, activity remained constant for up to 140 min with P_o about 0·7 (Na_i^+ concentration was calculated to be 150 mM). In our hands high open probabilities could be obtained within seconds of patch isolation; most channels showed little rundown of activity in recordings lasting up to 30 min. The technique of patch isolation used (see Methods) has already proved effective in reducing K_{ATP} channel rundown (Niu & Meech, 1998) and it seems to be equally valuable here. In other respects the K_{Na} channels appeared to have properties identical to those reported previously. For example, for inward currents the unitary current–membrane potential relationship was well fitted by the Goldman-Hodgkin-Katz equation with P_K equal to $3{\cdot}72 \times 10^{-13}$ cm^3 s^{-1} (see Fig. 1 and Kameyama *et al.* 1984; Wang *et al.* 1991). A similar, though slightly lower value for P_K ($3{\cdot}17 \times 10^{-13}$ cm^3 s)$^{-1}$ has been reported for K_{Na} channels in quail trigeminal neurones (Haimann *et al.* 1990).

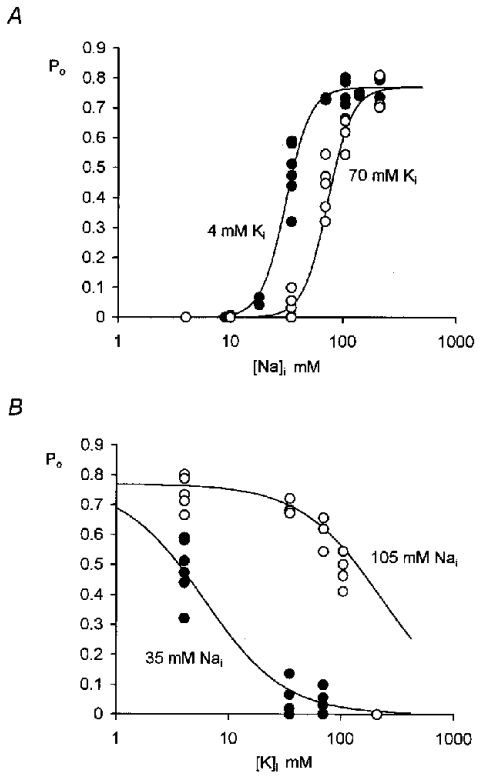

Figure 6. Effect of Na_i^+ and K_i^+ on K_{Na} channel open probability

Data are from 12 inside-out membrane patches. The membrane potential was held at −60 mV. *A*, K_{Na} channel open probability in different concentrations of Na_i^+ with K_i^+ kept constant at 4 mM (●) and 70 mM (○). For Na_i^+ concentrations below 140 mM, NaCl was replaced by equimolar concentrations of NMDG-HCl. *B*, the effect of different concentrations of K_i^+ on channel open probability in the presence of 105 mM (○) and 35 mM (●) Na_i^+. In each case the line through the data points was drawn according to the equation:

$$P_o = \frac{P_{o,max}}{1 + (1 + ([K^+]_i{}^g/k_b))(k_a/[Na^+]_i{}^h)},$$

where k_a and k_b are dissociation constants, $[Na^+]_i$ and $[K^+]_i$ are the sodium and potassium ion concentrations at the intracellular surface of the membrane, and g and h represent Hill coefficients. The data were fitted by eye with the k_a:k_b ratio set to $1{\cdot}75 \times 10^5$; the Hill coefficients g and h were 1·2 and 4·0; the maximum open probability ($P_{o,max}$) was 0·78.

Inhibition of K_{Na} channel activity by internal K^+

The main conclusion to be drawn from this work is that internal potassium ions have an inhibitory effect on the activity of K_{Na} channels in guinea-pig ventricular myocytes. Increasing K_i^+ from 4 to 70 mM increased the concentration of Na_i^+ needed for half-activation from about 35 mM to nearer 70 mM (see relationship between P_o and Na_i^+ in Fig. 6*A*). The latter value is close to that obtained in the same preparation by Kameyama *et al.* (1984) with K_i^+ equal to 40 mM or more. In quail trigeminal neurones, half-activation occurred in the range 25–30 mM Na_i^+ in the presence of either 75 mM (Haimann *et al.* 1990) or 50 mM K_i^+ (Haimann *et al.* 1992). With physiological concentrations of K_i^+ a lower sensitivity to Na_i^+ was reported so that here too Na^+ and K^+ may interact competitively (Haimann *et al.* 1992).

In a model to account for the channel kinetics in guinea-pig ventricular myocytes, Mistry *et al.* (1997) suggested that the presence of four closed states (see also Fig. 7) was consistent with the binding of 2 or 3 sodium ions before K_{Na} channels entered one of two open states. All the available data are consistent with the duration of the two longest closed states being dependent on the Na_i^+:K_i^+ ratio. We considered the possibility that the role of Na^+ was simply to displace K^+ from an inhibitory site. If so, 0 mM K_i^+ might permit activation even in the absence of Na_i^+. As Fig. 5 shows, the channel remained active for up to 12 s in Na^+–K^+-free solution, the change in P_o with time suggesting that the decline in activation had both fast and slow phases. Perhaps Na^+ is firmly bound at the activation site so that there is no requirement for Na_i^+ when the channel makes transitions between its shorter open and

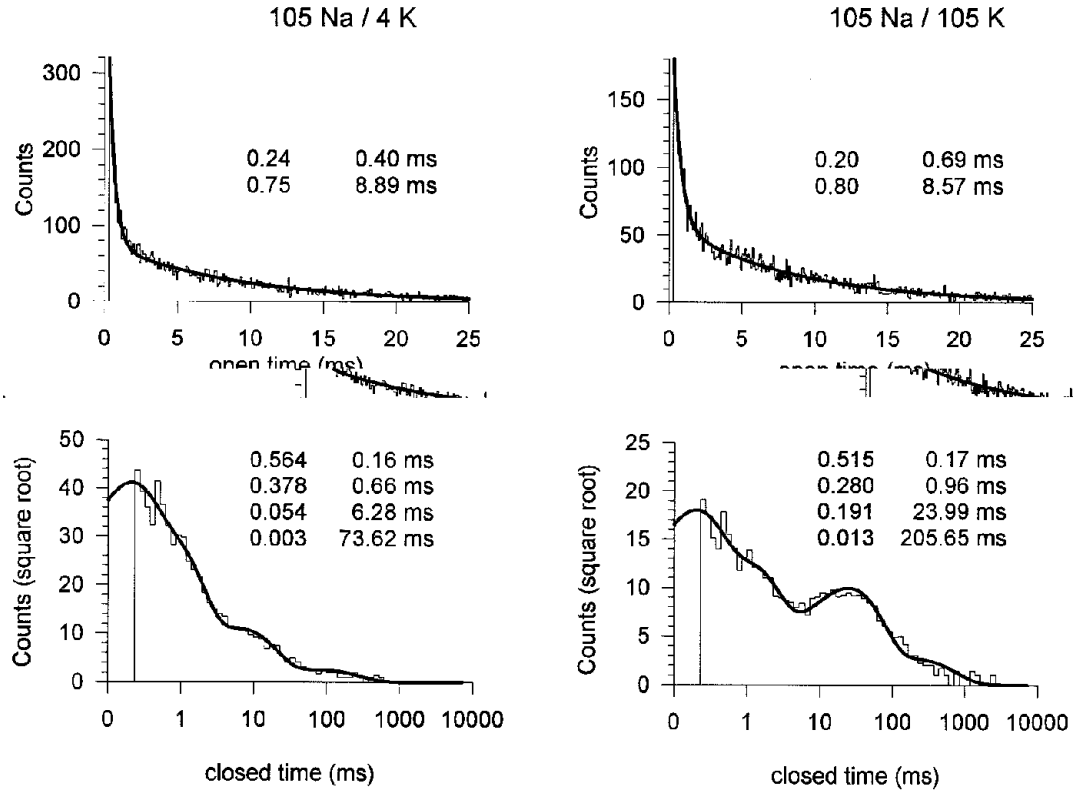

Figure 7. The effect of K_i^+ on open and closed times

Open and closed dwell time histograms for a single K_{Na} channel in a patch held at −60 mV and exposed to 4 mM (left) and 105 mM (right) K_i^+; Na_i^+ was kept constant at 105 mM. Open time distributions (upper graphs) are fitted by a curve that is the sum of two exponential terms. Closed time distributions (lower graphs) have logarithmic time axes and square root ordinates and were fitted to the equation:

$$f(t) = \sum_{j=1}^{m} (\alpha_j/\tau_j)\exp(-t/\tau_j),$$

where t is the measured dwell time, m is the number of components, α_j is the area of component j and τ_j is its time constant. Open time constants (and relative areas): 105 mM Na_i^+, 4 mM K_i^+: 0·4 ms (0·24), 8·9 ms (0·75); 105 mM Na_i^+, 105 mM K_i^+: 0·69 ms (0·2), 8·6 ms (0·8). Closed time constants (and relative areas): 105 mM Na_i^+, 4 mM K_i^+: 0·16 ms (0·56), 0·66 ms (0·38), 6·28 ms (0·05), 73·62 ms (0·003); 105 mM Na_i^+, 105 mM K_i^+: 0·17 ms (0·52), 0·96 ms (0·28), 24 ms (0·19), 206 ms (0·01).

closed states. However, reopening after prolonged closure is evident even after some seconds in Na^+-free solution so the possibility remains that incoming K^+ had access to an inhibitory site.

Hill coefficient

One explanation for the steep relationship between P_o and Na_i^+ concentration is that the binding of more than one sodium ion is necessary to open the channel. For Fig. 6*A*, K_i^+ was kept constant at either 4 or 70 mM, the ionic strength and osmolarity of the different solutions being maintained by replacing NaCl with equimolar NMDG-HCl. The P_o data were fitted by a modified Michaelis-Menten equation for competitive inhibition (eqn (2)). The Hill coefficients required to provide a satisfactory fit suggest that, while only 1–2 potassium ions are necessary for inhibition, at least 4 sodium ions are needed for activation of the channel. A similar high value, 4·8, has been reported for the effect of internal Na^+ on K_{Na} channels in the spinal neurones of the frog embryo (Dale, 1993), but values in the region of 2–3 are more generally reported (see Haimann *et al.* 1990; Rodrigo & Chapman, 1990). The Hill coefficient of 2·8 reported by Kameyama *et al.* (1984) was obtained in experiments where NaCl was replaced by equimolar KCl and it is likely that the inhibitory effect of increased K_i^+ concentration contributed to this low value. A similar explanation can account for the results of Veldkamp *et al.* (1994), who reported P_o values of 0·73 in 135 mM Na_i^+ and 0·62 in 100 mM Na_i^+.

Mechanism of K_{Na} channel activation

Permeant ions determine channel selectivity. In many K^+ channels the presence of potassium ions at binding sites within the pore seems to be essential for maintenance of selectivity. Under K^+-free conditions such channels permit a substantial Na^+ current (Zhu & Ikeda, 1993; Callahan & Korn, 1994; Korn & Ikeda, 1995; Starkus *et al.* 1997). K^+ channels that undergo C-type inactivation also appear to pass through an intermediate state with an increased Na^+ permeability before entering the non-conducting inactivated state (Kiss *et al.* 1999). Changing a single amino acid residue in the S6 segment of the channel decreases the ability of K^+ to block the Na^+ current (Ogielska & Aldrich, 1998). In the present study on K_{Na} channels the 13 pS decrease in the mean inward slope conductance when K_i^+ was reduced from 105 to 70 mM (see Results) might also indicate some form of K^+-modulated permeation.

Intracellular ions and gating. In small conductance calcium-activated potassium channels (K_{Ca}) an increase in P_o follows Ca^{2+} binding to a calmodulin-like receptor site (Xia *et al.* 1998). Functional studies show that activation by different divalent ions depends on their crystalline radius, as expected for a calcium-binding protein (Meech, 1980; Meech & Thomas, 1980). The receptor site responsible for sodium activation of the K_{Na} channel has been more difficult to pin down. Activation of the K_{Na} channel could follow the binding of Na^+ to a receptor site on or within the channel itself, or binding might occur some distance away and produce a more widespread change in the channel environment. K_{Na} channels show up to 12 subconductance states (Sanguinetti, 1990; Mistry *et al.* 1996) and Sanguinetti (1990) has suggested that the channel is actually a complex of 12 identical pores each of which gates independently of an over-riding main gate. One could imagine the cell membrane as a matrix of fixed charges with which Na^+ interacts to cause the conformation change necessary to open the 12-pore complex. The binding site itself need not be selective; the specificity of activation might lie in the conformation change that the cation brings about.

To envision a more discrete receptor we might compare its selectivity to that of other Na^+-binding sites. For example, H^+ and Li^+ can substitute for both Na^+ and K^+ during Na^+–K^+ active transport (Dunham & Hoffman, 1978) whereas the mammalian K_{Na} channel is insensitive to Li^+ and blocked by H^+ (Veldkamp *et al.* 1994). The enzyme dialkylglycine decarboxylase, which is activated by K^+ and inhibited by Na^+ (Aaslestad *et al.* 1968), contains an example of a more selective receptor. An increase in Na_i^+ from 75 to 130 mM (while reducing K_i^+ from 15 to 0 mM) substitutes Na^+ for K^+ at a site located near the reaction centre of the enzyme (site 1; Toney *et al.* 1993). The protein is too rigid to accept Na^+ without an additional water molecule that appears to cause a slight expansion of the structure around the metal ion. This in turn reorientates two amino acid residues within the reaction centre and produces the switch from the active to inactive form of the enzyme (Hohenester *et al.* 1994).

The K_{Na} channel site for which K^+ and Na^+ compete appears to bind 1–2 potassium or 4 sodium ions (see Fig. 6). The crystal radius of K^+ is 1·33 Å while that of Na^+ is 0·95 Å (Pauling, 1927, 1960), so K^+ has a volume about 2·7 times the volume of Na^+. Although it would be possible to pack 4 sodium ions in the same space as 1·5 potassium ions, electrostatic repulsion makes such close packing improbable and the receptor site must be large enough to accept the shell of water molecules that shield the individual ion charges.

Site of Na^+–K^+ competition. In a model to account for the crystal structure of the K^+ channel from *Streptomyces lividans* (Doyle *et al.* 1998), there is a 10 Å diameter water-filled cavity mid-way across the membrane which is large enough to accept all 4 sodium ions together with their associated water molecules. The cavity is accessible to cations from the cell cytoplasm, their position being determined in part by the electrostatic field set up by the negative charges associated with the COOH-terminals of the four α-helices of the selectivity filter (Roux & MacKinnon, 1999). Small movements of these pore helices may bring about subconductance states by altering the throughput of K^+ (Perozo *et al.* 1999). The presence of 4 or more sodium ions in this space might be all that is required to initiate activation. The induced strain on the pore helices might promote an initial entry of external K^+ into the narrow

external pore, the temporary presence of a single potassium ion at a binding site within the pore being sufficient to hold it open transiently once the Na^+ is flushed away by the entering K^+.

Estimates of the size of the internal opening (the internal pore) of voltage-gated K^+ channels, based on the access of large tetra-*N*-alkylammonium ions (French & Shoukimas, 1981), suggest a diameter of at least 12 Å; in inwardly rectifying K^+ channels the opening may be even larger (Lu *et al.* 1999). Assuming that the conductivity of the channel remains constant all the way across the membrane, most of the potential field will be associated with the high resistance external pore. Nevertheless up to 25% of the field is across the internal pore and the outward movement of 4 sodium ions through it should be markedly affected by membrane voltage. A difficulty with placing the Na^+ activation site within the central cavity is that P_o for K_{Na} channels has little or no voltage dependence (between −40 and +20 mV, Kameyama *et al.* 1984; between −80 and −20 mV, Wang *et al.* 1981; between −70 and −30 mV, Mistry *et al.* 1997). Hence the simplest explanation for the requirement for 4 activating sodium ions is that each ion binds to a separate channel subunit at some superficial site and that displacement of one sodium ion by a single potassium ion is sufficient to inhibit the process. The binding site appears to have a high affinity for K^+, Na^+ binding possibly being accompanied by water, to bring about the conformation change (c.f. dialkylglycine decarboxylase, above).

Physiological significance

The physiological significance of the K_{Na} channel in cardiac myocytes is unknown. The channels are inactive in normal saline because the cellular sodium concentration is less than 10 mM. However, they are significantly activated in intact guinea-pig ventricular myocytes when the Na_i^+ concentration is increased by superfusion with Ca^{2+}–Mg^{2+}-free solution (Rodrigo & Chapman, 1990). In addition, Luk & Carmeliet (1990) found that K_{Na} channel open probability increased when the Na^+–K^+ pump was blocked by ouabain. Under some experimental conditions, however, it is internal K^+ that changes not internal Na^+. In stimulated canine ventricular muscle, K_i^+ decreased to about 80 mM during hypoxia in acidic, glucose-free solution whereas Na_i^+ was not significantly altered (Nakaya *et al.* 1985). Overall the evidence indicates that K_{Na} channels would be activated during pathological conditions, such as prolonged ischaemia, Ca^{2+} paradox and digitalis toxicity when the increase of internal Na^+ is accompanied by a decrease of internal K^+.

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