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Mechanosensitive potassium channels in locust muscle membrane

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Abstract Patch clamp recordings have been made from adult locust (*Schistocerca gregaria*) muscle membrane to study the mechanosensitivity of potassium channels (BK and IK) in cell-attached patches by transiently applying measured pressures to the contents of the patch pipettes. The aim of the investigations was to demonstrate a novel gating behaviour by pressure of the BK channel in contrast to the familiar behaviour of the IK channel. The open probability (p_o) of the IK channel increased rapidly in response to a pressure step and monotonically during a pressure ramp. This gating was readily repeatable and rapidly reversible. The relationship between $\ln[p_o/(1-p_o)]$ and transmembrane pressure was linear. In comparison, p_o for the BK channel was also increased by pressure, but its gating was delayed, cumulative, and hysteretic.

Key words Mechanosensitivity · Stretch sensitivity · Curvature sensitivity · Potassium channels · Locust muscle

Introduction

In 1950, Katz discovered that some cell membranes respond to stretch by an increase in membrane permeability, but it was much later that Edwards et al. (1981) and Corey and Hudspeth (1983) suggested that ion channels might be involved in this phenomenon. Soon afterwards, Brehm et al. (1984) and Guharay and Sachs (1984) described stretch-activated K^+ channels in patches of vertebrate skel-

etal muscle. More recently, stretch-inactivated channels have been described (Morris and Sigurdson 1989; Franco and Lansman 1990). Several reviews on stretch-sensitive channels have been published (e.g. Kullberg 1987; Morris 1990; Petrov and Usherwood 1994). Studies of stretch-activated channels in insects have been limited, hitherto, to *Drosophila melanogaster* larval muscle membrane (Zagotta et al. 1988; Gorczyńska and Wu 1991) and cultured myocytes (Zagotta et al. 1988). The aim of the present investigation is to show that two types of K^+ channel (BK and IK; Gorczyńska et al. 1996) found in locust skeletal muscle membrane are mechanosensitive, and that the BK channel displays a novel, hysteretic form of mechanosensitivity.

Materials and methods

We have studied the surface membrane of fibres of locust (*Schistocerca gregaria*) metathoracic extensor tibiae muscle (Bates et al. 1988; Gorczyńska et al. 1996) using cell-attached patches in order to maintain connection to the cytoskeleton. Patch pipettes were filled with either low- K^+ saline (180 mM NaCl, 10 mM KCl, 2 mM $CaCl_2$, 10 mM HEPES, pH 6.8) or high- K^+ saline (180 mM KCl, 10 mM NaCl, 2 mM $CaCl_2$, 10 mM HEPES, pH 6.8). A closed pressure system (comprising a peristaltic pump and an electronic pressure-to-voltage converter) was attached by plastic tubing and a two-way tap to the patch pipette holder. Pressure differentials (P) were identified as negative when the pressure was lower inside the pipette than outside. This system generated membrane tension T that was linearly proportional to the pressure differential P according to the Laplace law $P=T(2/R)$, providing that the patch radius of curvature R did not change with pressure (see Petrov and Usherwood 1994). Since the seal was usually destroyed by repetitive pressure in most patches before the resting membrane potential could be measured, pipette potential (V_{pip}), rather than membrane potential (E_m) has been used as the voltage reference. Four experimental protocols were em-

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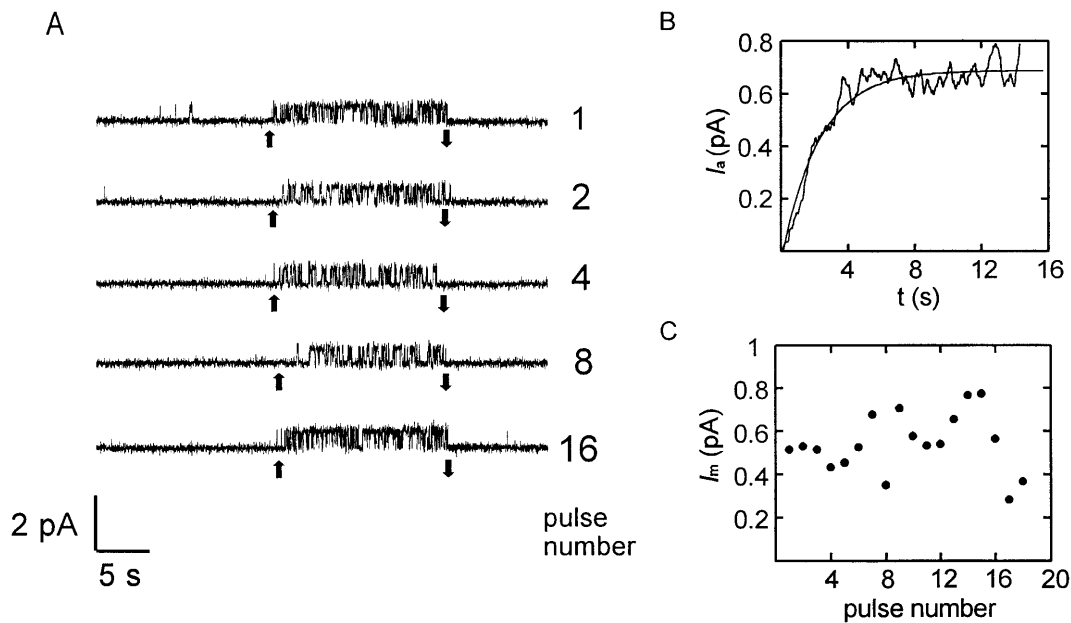


Fig. 1 **A** A series of -20 Torr pressure pulses of 15 s duration (indicated by arrows) applied to a cell-attached patch containing a single IK channel. The patch pipette contained low- K^+ saline. The patch was held at $V_{\text{pip}} = 40$ mV (protocol 2). Responses to the 1st, 2nd, 4th, 8th, and 16th pulses are shown. **B** Plot of average current (I_a) versus time. Current data were sampled into 1500 bins (10 ms each) and averaged over all 18 pressure pulses. **C** The mean current of each pulse (I_m) versus pulse number. The data in **B** have been fitted by a single first-order rate equation which gives a rate of 0.365 s^{-1} (time constant of 2.74 s)

ployed, i.e. (1) step changes in pressure at constant V_{pip} ; (2) constant V_{pip} during application of series of pressure pulses (the rise times and decay times of the pressure pulses were maximally 200 ms); (3) constant V_{pip} during application of a series of triangular pressure ramps; and (4) voltage ramps at various constant pressures.

Results and discussion

The BK channel is a Ca^{2+} -activated channel and has a maximum conductance of 170 pS; the IK channel is an inward rectifier with a maximum conductance of 35 pS (Gorczyńska et al. 1996) (conductances were measured using patch pipettes filled with high- K^+ locust saline; BK channel conductance depended steeply on K^+ concentration, while IK channel conductance probably saturated at low K^+ concentration). Both types of K^+ channel were observed routinely with pipettes containing high- K^+ saline, but openings of BK channels were either rare or absent with pipettes filled with low- K^+ saline (Gorczyńska et al. 1996). Neither the unitary conductances nor the single channel I/V characteristics of the two K^+ channels measured in either high- K^+ or low- K^+ saline were sensitive to changes on P (cf. also Guharay and Sachs 1984).

IK channel

The IK channel was studied in 121 patches. Most of these were made with pipettes containing low- K^+ saline such that openings of BK channels were rare or absent. p_0 was estimated from current amplitude histograms (Sokabe et al. 1991). With $V_{\text{pip}} = 40$ mV, p_0 varied between 0 and 0.5 at 0 Torr. p_0 increased reversibly, regardless of its value at 0 Torr, as P was stepped from 0 Torr to between -5 Torr and -40 Torr (protocol 1; data not shown). The relationship between $\ln[p_0/(1-p_0)]$ and P was linear, viz. linear fits to plots of $\ln[p_0/(1-p_0)]$ versus P for six patches yielded a mean slope of 0.13 ± 0.03 Torr $^{-1}$ (\pm SEM). In 90 of the patches (19 of them containing a single IK channel) there was a monotonic, rapidly reversible increase in p_0 when P was stepped from 0 Torr to either negative or positive values in the range of -50 to 50 Torr (protocol 2). This is exemplified in Fig. 1, which shows that effect on p_0 of a series of pressure pulses (-20 Torr) each of 15 s duration with a constant interval of 30 s between the pulses. The time span for the determination of p_0 was the duration of the pressure pulse, i.e. 15 s. At $P = 0$ Torr, $p_0 = 0.004$ ($V_{\text{pip}} = 40$ mV). p_0 increased reversibly with each pressure pulse. Data for 18 successive pressure pulses (Fig. 1B) show that the averaged current (I_a) per unit time in a pulse increased rapidly during the pulse, reaching a plateau after about 4 s. In Fig. 1B the rising phase of I_a is fitted with a first-order rate equation with a time constant of 2.7 s. It is clear from Fig. 1C that, despite the scatter of the data, the mean current per pulse ($I_m = I_{\text{unit}} p_0$, where I_{unit} is the unitary channel current, 2.5 pA in this case) does not change during a series of negative pressure pulses, which demonstrates the reversibility of the effect. We did not study the influence, if any, of V_{pip} on the relationship between pressure steps and p_0 . Figure 2A exemplifies the effect of pressure ramps (protocol 3) on a single IK channel. Reversibility from -50 Torr is also clear from Fig. 2A

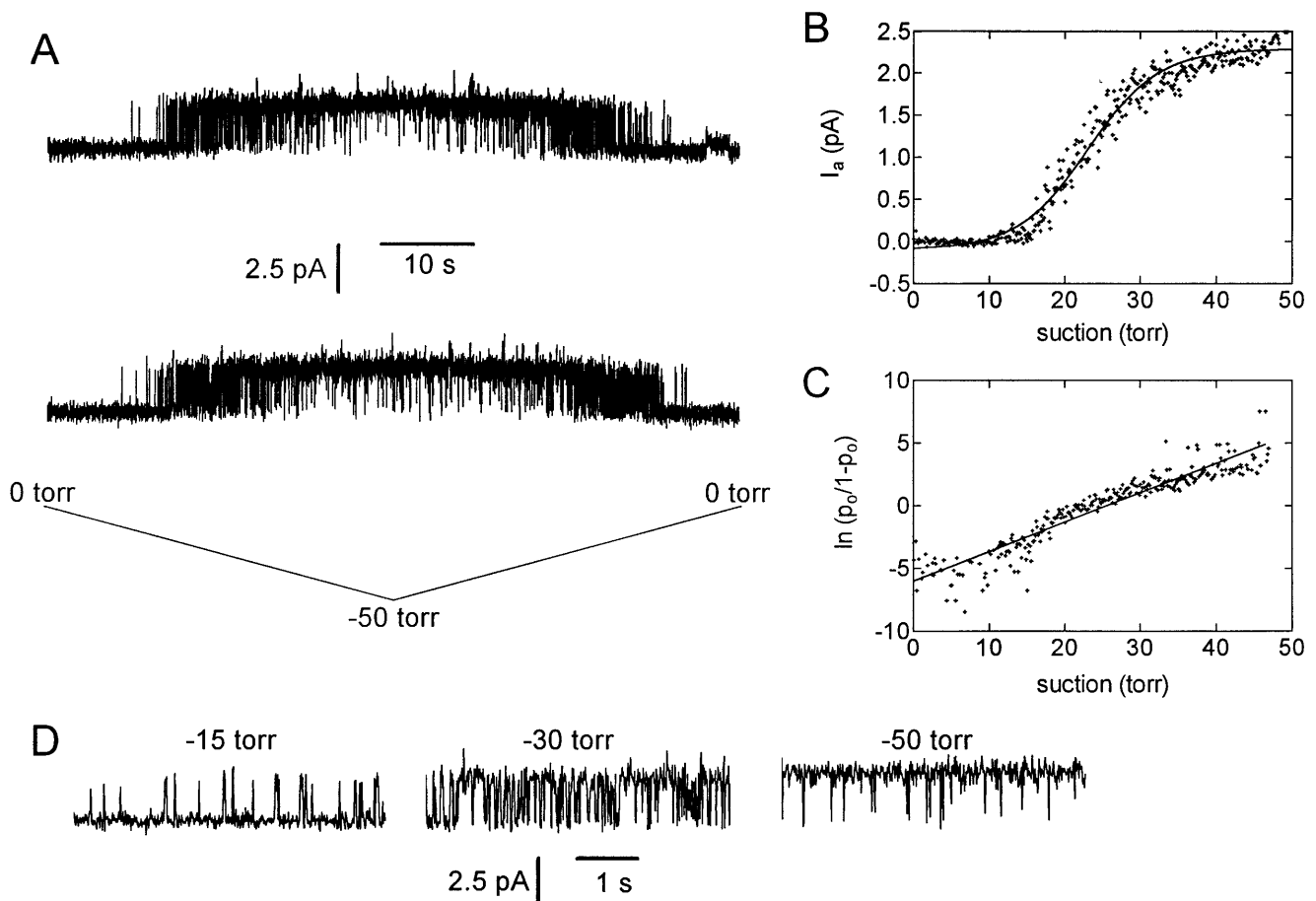


Fig. 2A–D Effect of pressure ramps on a cell-attached patch containing a single IK channel. The pipette contained low- K^+ saline and the patch was held at $V_{\text{pip}}=70$ mV (protocol 3). **A** Data from two pressure ramps of “0 Torr to –50 Torr to 0 Torr”. The total duration of a ramp was 75 s, i.e. the rate of change of pressure was 1.33 Torr s^{-1} . There were no rest periods between the ramps. **B** Average patch current expressed as a function of pressure. Current data were sampled into 380 pressure bins (0.13 Torr each) and averaged over seven rising pressure ramps (“0 Torr to –50 Torr”). The solid curve represents a fit by a Boltzmann function, with the $P_{50\%}=-23$ Torr. **C** A plot of $\ln[p_0/(1-p_0)]$ versus P . p_0 was calculated by dividing the averaged current (Fig. 2B) by the unitary channel current, $I_{\text{unit}}=2.3$ pA. The solid line represents a linear regression fit to the data (slope= $0.235 \pm 0.005 \text{ Torr}^{-1}$, correlation coefficient=0.94). **D** Higher time resolution traces from the rising phase of the second ramp in **A** at pressures of approximately –15, –30, and –50 Torr

(pressure ramp). The relationship between average current (I_a) versus pressure for seven pressure ramps of 0 to –50 Torr could be fitted by a Boltzmann distribution (Fig. 2B), giving a pressure for half-maximum activation ($P_{50\%}$) of –23 Torr. In Fig. 2C a plot of $\ln[p_0/(1-p_0)]$ versus P is fitted by a straight line to give a slope of 0.23 Torr^{-1} . In three other patches, values of 0.12, 0.11, and 0.07 Torr^{-1} were obtained, giving a mean value of $0.13 \pm 0.059 \text{ Torr}^{-1}$ ($n=4$). These results are in good agreement with those obtained using protocol 1. Insufficient data were obtained at positive pressures to construct a relationship like that in Fig. 2C, because the patch seal was quickly lost. The IK chan-

nel conductance did not change with P (Fig. 2D). Another interesting effect of pressure was on the shape of the macroscopic I/V relationship between average current, I_a , and V_{pip} of a single IK channel (protocol 4; data not shown). It was changed by altering P in a qualitative way, for example at 0 Torr this relationship showed an outward rectification (cf. Gorczynska et al. 1996), whereas at –40 Torr the relationship was almost linear.

BK channel

BK channels were studied in a total of 48 cell-attached patches made using pipettes containing high- K^+ saline. Suction clearly influenced BK channel activity in 32 of these patches. An increase in p_0 was observed when the pressure was stepped from 0 Torr to a negative value, but this change was delayed (Fig. 3, pulse 1), cumulative (Fig. 3B), and hysteretic (Figs. 3 and 4); the increase of BK activity reversed only very slowly when P was returned to 0 Torr. It was not possible to develop a strategy that provided patches exhibiting only BK channels, although one patch of this type was obtained by chance (see Fig. 4). In one set of experiments ($n=5$ patches), a series of pulses of negative pressure (~ 15 s duration separated by ~ 30 s rest periods) was applied (protocol 2). All five patches responded similarly to changes in P . Figure 3 illustrates data

Fig. 3A–C Application of 17 pressure pulses each of -20 Torr to a cell-attached patch held at $V_{\text{pip}}=40$ mV and with high- K^+ saline in the pipette. Each pressure pulse lasted 15 s and was followed by a 30 s rest period at 0 Torr (protocol 2). **A** Responses to the 1st, 2nd, 4th, and 16th pulses are shown. IK channels were activated immediately following application of pressure, whereas there was a gradual build-up in BK channel activation. **B** The mean current (I_m) for each pressure pulse is plotted against pulse number. **C** I_m during each rest period is plotted against preceding pulse number

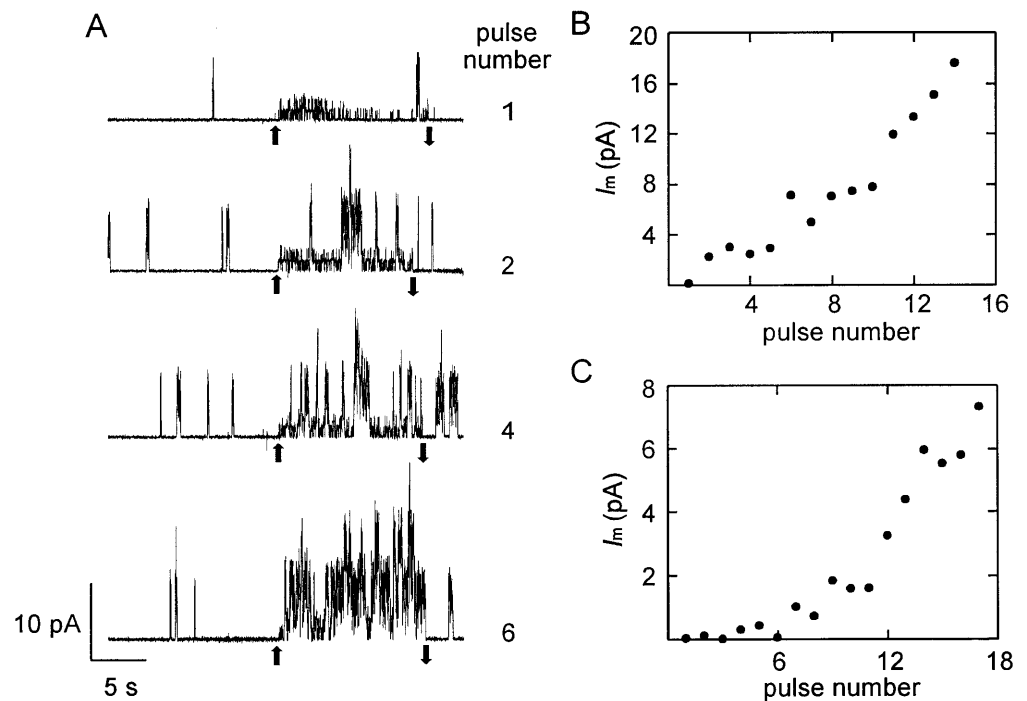
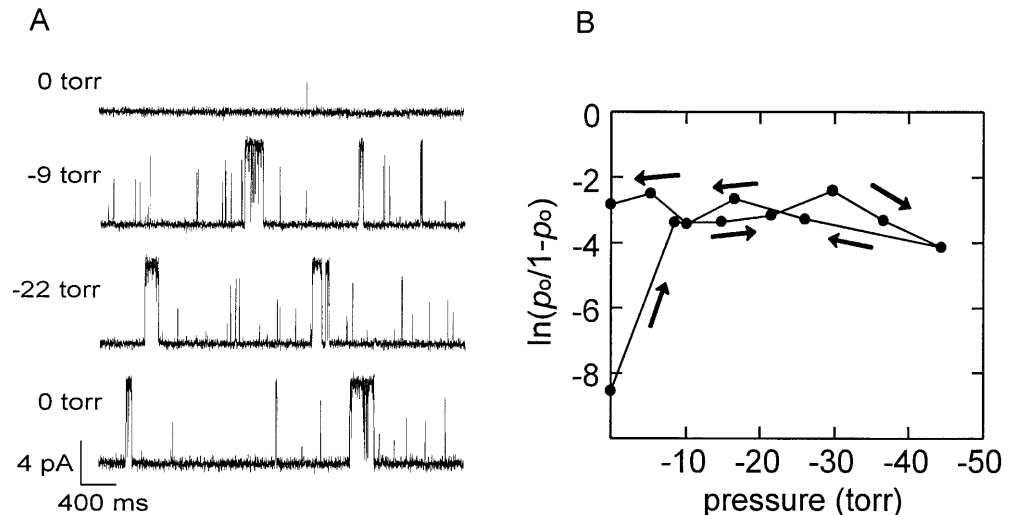


Fig. 4A, B Dependence of p_0 of a BK channel on static pressure (P). Data from a cell-attached patch with high- K^+ saline in the pipette and the patch held at $V_{\text{pip}}=25$ mV (protocol 1). **A** Examples of current traces at pressures of 0, -9 , -22 , and 0 Torr. **B** $\ln[p_0/(1-p_0)]$ versus P following increases and subsequent decreases of static pressure in the range 0 Torr to -45 Torr. p_0 was calculated from current amplitude histograms. Each static pressure was held for 2 min



from a patch that contained at least three BK channels. BK channel openings were rare before the pressure pulses were applied. There was little increase in activity during the first pulse of the series, just a burst of channel openings that occurred at the end of the pulse. Two BK channels opened during the second pulse, and a third one opened during subsequent pulses. The relationship between I_m per pulse and pulse number was nonlinear (Fig. 3B). BK channel hysteresis tendency (to stay open between the pressure pulses) also increased as the pulse series progressed (Fig. 3C).

In another set of experiments ($n=6$ patches), a series of up to 30 continuous triangular pressure ramps (0 Torr to -50 Torr to 0 Torr; 1.43 Torr s^{-1}) was applied (protocol 3). Very few, or no, BK channel openings were observed at

0 Torr for at least a 3 min period before and during the first few ramps. However, many more BK channel openings were observed during later ramps in the series. By averaging the BK channel currents generated during the later ramps, a relationship between I_a and P was obtained. This saturated above -20 Torr (data not shown).

The relationship between p_0 for the BK channel and constant pressure was studied for 21 patches (protocol 1). The data in Fig. 4 were obtained from a patch containing only a single BK channel and no IK channels such that it was possible to estimate p_0 despite the rapid open-close kinetics of the BK channel (recordings were made at a cut-off 10 kHz). Figure 4B demonstrates a steep activation of the BK channel between P values of -5 to -10 Torr, but

higher values of $|P|$ had no further effect. p_0 was still elevated 3 min after P was brought back to 0 Torr (Fig. 4B). Qualitatively similar data were obtained from other patches containing more than one channel, even though it was not possible to estimate p_0 .

Three models for stretch activation of ion channels have been proposed: a linear model (Corey and Hudspeth 1983; Martinac et al. 1987; Howard et al. 1988), a quadratic model (Guharay and Sachs 1984; Sachs 1986, 1988), and a model that combines these two (Sachs and Lecar 1991). The quadratic model implies that the submembraneous cytoskeleton plays an important role by collecting stresses from a large area of the muscle membrane and by concentrating them in the stretch-sensitive channel proteins that are anchored in the membrane. The relationship $\ln[p_0/(1-p_0)]$ versus P for the locust muscle IK channel is linear (Fig. 2C), thus implying a linear model (see also Petrov and Usherwood 1994).

In contrast, the relationship between $\ln[p_0/(1-p_0)]$ and P for the locust muscle BK channel is neither linear nor quadratic (see Fig. 4B) but is hysteretic. Residual stretching by progressive adhesion of the membrane patch to the patch pipette is not responsible for this, because when IK channels were present in the same patch as BK channels they always inactivated when P was returned to 0 Torr after application of a pressure differential, whereas the BK channels did not (see Fig. 3A). Perhaps the BK channel protein reacts directly to local patch curvatures (cf. Sokabe and Sachs 1990), as predicted by Petrov and Usherwood (1994) and Petrov (1998). This prediction required a conical shape of the channel protein; such a shape is nicely visualized by a very recent X-ray study of the KcsA K^+ channel structure (Doyle et al. 1998). Alternatively, or concomitantly, stretch activation may be mediated by fatty acids from mechanically activated phospholipases (Ordway et al. 1995).

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