J. Physiol. (1985), **363**, *pp.* 119–134
With 10 *text-figures*
Printed in Great Britain

MECHANOTRANSDUCER ION CHANNELS IN CHICK SKELETAL MUSCLE: THE EFFECTS OF EXTRACELLULAR pH

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(*Received 12 October 1984*)

SUMMARY

1. The membrane of tissue-cultured chick pectoral muscle contains an ionic channel which is activated by membrane tension.

2. With 150 mM-external K^+ and 150 mM-internal Na^+, the channel has a conductance of 70 pS and a reversal potential of +30 mV. With 150 mM-external Na^+ and 150 mM-internal K^+ (normal gradient) the channel has a conductance of 35 pS and a reversal potential of −30 mV. The ratio of K^+ permeability to Na^+ permeability, $P_K:P_{Na}$, is 4 based upon reversal potentials and is 2 based upon conductance.

3. Kinetic analysis of single-channel records indicates that there are one open (O) and three closed (C) states. When analysed according to a linear sequential model: C_1–C_2–C_3–O_4, only the rate constant that governs the C_1–C_2 transition ($k_{1,2}$) is found to be affected by stretch or voltage.

4. The effects of stretch and voltage on $k_{1,2}$ can be summarized as $k_{1,2} = k^0_{1,2} \exp(\alpha V + \theta P^2)$, where $K^0_{1,2}$ is the voltage and stretch-independent part of the rate constant, α is the voltage sensitivity, V is the transmembrane potential, θ is the stretch sensitivity and P is the applied suction.

5. Increasing extracellular pH from 7·4 to 10·0 increases both α and θ in a manner suggesting titration of site(s) with a pK of 9·1. A single lysine of N-terminal amino acid may be be responsible for modulating both the voltage and pressure responses.

6. Extracellular pH does not affect $k^0_{1,2}$, the voltage- and stretch-independent part of $k_{1,2}$, suggesting that pH in the range 7·4–10 does not alter the local surface charge.

7. The conductance and reversal potential of the s.a. channel are unaffected by pH, suggesting that the titrated site(s) is not close to the mouth of the channel.

INTRODUCTION

In the membrane of cultured embryonic chick skeletal muscle there is an ion channel whose probability of opening increases with membrane tension (Guharay & Sachs, 1984). Stretch-activated (s.a.) channels have also been observed in tissue cultures of *Xenopus* muscle cells (Brehm, Kullberg & Moody-Corbett, 1984) and frog red blood cells (Hamill, 1983). The physiological function of these channels in the muscle cells is not yet known although they might be related to volume regulation as suggested for red blood cells. The channel kinetics are also similar to those found

in the auditory hair cells (Corey & Hudspeth, 1983), suggesting that the chick muscle s.a. channels may be considered to be prototypes of the transducers in free nerve endings or specialized mechanoreceptive organs such as the Pacinian corpuscle (cf. Edwards, 1983).

The s.a. channels are apparently linked in series with a cytochalasin-resistant component of the cytoskeleton and shunted by a cytochalasin-sensitive component of the cytoskeleton (Guharay & Sachs, 1984). Curve fitting open and closed time distributions suggests that there are three closed states and one open state. In a linear sequential model of these states, only one of the six rate constants is sensitive to membrane stretch, and this rate constant depends exponentially upon the square of the membrane tension.

In preliminary studies we noticed that increased extracellular pH caused the s.a. channel to become more sensitive to stretch. Extracellular pH is known to have effects on other types of channel. Acid pH depresses the peak Na^+ conductance, slows inactivation and shifts all voltage-dependent parameters of Na^+ channels to more positive voltage (Hille, 1968; Carbone, Fioravanti, Prestipino & Wanke, 1978; Yatani, Brown & Akaike, 1984). Increased $[H^+]$ also suppresses the Na^+ conductance of the outer segment of isolated retinal rods of frog (Mueller & Pugh, 1983). The alteration of the extracellular pH alters the relaxation parameters of miniature and end-plate potentials (Peper, Bradley & Dreyer, 1982) as well as the amplitude and the time-course of agonist-activated currents at a variety of nicotinic receptors (Scuka, 1977; Landau, Gavish, Nachshen & Lotan, 1981; Goldberg & Lass, 1983). The mechanoreceptive property of the lateral line organ of mudpuppy is also suppressed by acidic pH (Sand, 1975). It is generally agreed that the effects of extracellular pH are mediated by titration of surface charge and by proton block (Woodhull, 1973).

We have studied the effects of extracellular pH (range of 7·4–10·0) on the s.a. channel. This range of pH was determined by the limits of experimental reliability. In the course of the study, we found that the channel conductance is doubled when the extracellular Na^+ (150 mM) is totally replaced with K^+. The conductance and reversal potential of the s.a. channel are independent of pH. An increase of extracellular pH results in an increase in both the voltage and stretch sensitivities of the channel in a manner suggesting titration of a site or sites with pKs of about 9·0. In the model presented by Guharay & Sachs (1984) only one rate constant was sensitive to stretch. This same rate constant is the only one affected by voltage and pH. The change in extracellular pH seems to have a direct effect on the channel kinetics rather than through an effect on surface charge.

METHODS

Pectoral muscles were removed from 11-day-old embryos using sterile techniques, dissociated in divalent-ion-free saline containing collagenase (Sigma Type IA; 1 mg ml^{-1}) and plated in gelatin-coated 60 mm plastic tissue-culture dishes at a density of approximately 10^6 cells $dish^{-1}$. The cells were grown in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated horse serum, 2% (v/v) embryo extract, penicillin and streptomycin. The cells were grown in 95% relative humidity at 37 °C in a 5% CO_2–air atmosphere. After 2–3 days in culture, the cells were treated for 24–36 h with medium containing 10^{-5} M-cytosine arabinoside to reduce fibroblast growth.

Electrical recordings were made in HEPES-buffered saline. Our normal saline contained (mM):

NaCl, 150; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; and HEPES, 10. The pH of the experimental saline was varied from 7·4 to 10·0 by addition of NaOH. The experiments were done at room temperature (20–25 °C).

The membrane potential of intact cells was measured by breaking a cell-attached patch and adjusting the amplifier offset potential until there was no current flow. The average resting potential was *ca.* −60 mV. In cells where the recording did not last long enough to obtain a direct measurement of the membrane potential, we assumed a value of −60 mV. This was checked by comparing the amplitude of channel currents with the value expected from controlled measurements of channel conductance.

Patch pipettes were made from borosilicate-glass capillaries (Drummond), fire polished and coated with Sylgard (Dow–Corning, Midland, MI, U.S.A.) using standard techniques (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Unless otherwise stated all the experiments were done with cell-attached patches. After the formation of a gigaseal, membrane tension was changed by applying suction to the patch pipette using a micrometer-driven syringe; suction was monitored with a Hg manometer. The band width of the amplifier was greater than 10 kHz but the data were usually analysed with a 2 kHz band width imposed by an eight-pole Bessel filter.

Data were recorded on analog tape, digitized and transferred to nine-track digital tape for analysis. The digitized data were analysed using an automated pattern-recognition program (Sachs, Neil & Barkakati, 1982). For kinetic analysis, the program provided histograms of the time between transitions for single channels; i.e. times where more than one channel was open were ignored. Open and closed time histograms were fitted with one and three exponential distributions, respectively, using non-linear regression. To avoid biases related to finite band width, each bin in the histogram was compared to the integral of the probability density function evaluated over each bin. To avoid biases due to variance of the number of counts in a bin, the squared residuals were weighted inversely with the number of counts in that bin (Bevington, 1969). To avoid biasing due to the asymmetric shape of the Poisson distribution with small mean rates (empty bins, etc.), the histogram bin widths were adjusted so that at least five counts were in each bin (Sachs & Auerbach, 1983).

Since all records contained the activity of more than one s.a. channel, we could not interpret the exponential fit of the histograms to represent the activity of a single channel. For a patch containing N channels, the longest closed time constant is N times shorter than that from single-channel records. In initial analyses we took the longest closed time constant from a multi-exponential fit and multiplied it by the estimated number of s.a. channels in the patch (N). To estimate N for each patch, we studied the highest activity series and used either the maximum likelihood estimate of the number of channels (Patlak & Horn, 1982; Sachs, Neil & Barkakati, 1982) or, if that did not have a distinct peak, the maximum observed level. The usual number of channels was two or three.

Since the kinetics of an N-channel membrane are, in general, different from that of a one-channel membrane, it is not clear that a simple exponential fit will yield the correct time constants and relative proportions even though it appears to fit the data well. Jackson (1985) has derived the kinetics of an N-channel membrane in terms of the kinetics of a one-channel membrane. The analysis is in terms of transitions between compositions in which all channels are closed or one channel is open, i.e. transitions between higher levels are ignored.

The tail distribution for a single channel (the probability of an interval being longer than t) is given by

$$G_1(t) = \Sigma f_i \exp(-t/\tau_i),$$

where t is time, f_i is the fractional amplitude of component i and τ_i is the time constant of component i. Jackson showed that the tail distribution for N channels is given by,

$$G_N(t) = Q_1^{(N-1)}\, G_1(t), \tag{1}$$

where Q_1 is the single-channel first latency distribution and is given by,

$$Q_1(t) = \Sigma f_i\tau_i \exp(-t/\tau_i)/\Sigma f_i\tau_i. \tag{2}$$

We fitted the function $G_N(t)$ (eqn. (1)) to the observed closed time distribution to derive the single-channel kinetics. In practice, N cannot be evaluated in the fit since the form of the data is insensitive to N. For fitting histograms it is useful to note that the number of counts in each bin is proportional to $G_1(t)-G_1(t+t_B)$ where t_B is the bin width. Fitting s.a. channel kinetics with

eqn. (1) supported our values obtained from simple exponentials where the longest time constant was scaled by N. This result, however, is not general and depends upon both N and the values of the time constants.

The time constants and their respective fractional amplitudes were used to derive rate constants from a linear sequential model (Guharay & Sachs, 1984) using a numerical solution (ZXPOW from IMSL, Houston, TX, U.S.A.) of eqns. (3.65)–(3.67) and (3.70) of Colquhoun & Hawkes (1981).

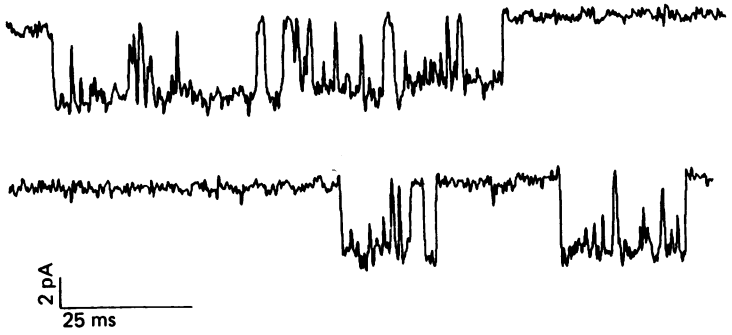

Fig. 1. Stretch-activated single-channel currents recorded from an excised inside-out patch of embryonic chick skeletal muscle. Current leaving the pipette is shown downward. The pipette contained normal saline (150 mM-Na^+, 5 mM-K^+, pH 7·4) and the bath contained saline with 150 mM-K^+ and 5 mM-Na^+ (22 °C). The pipette potential was +130 mV with respect to the bath and the data were low-pass filtered at 2 kHz.

RESULTS

A typical example of stretch-activated channel currents is shown in Fig. 1. As reported earlier (Guharay & Sachs, 1984), with 150 mM-K^+ on the extracellular face of the membrane and 150 mM-Na^+ on the intracellular face of an inside-out patch, the channel conductance is *ca.* 70 pS and the reversal potential is *ca.* +30 mV (K^+ face negative relative to Na^+ face). With the solutions reversed, typical of physiological concentration gradients, the channel conductance is reduced to *ca.* 35 pS and the reversal potential is *ca.* −30 mV (K^+ face negative; Fig. 2). We analysed the permeabilities in terms of the Goldman reversal potential equation and found that the channel appears somewhat more permeable to K^+ than to Na^+ ($P_K:P_{Na} = 4$).

In the hyperpolarizing quadrant, the channel conductance is 70 pS in symmetrical 150 mM-K^+ and 35 pS in symmetrical 150 mM-Na^+. Based upon the conductance difference, $P_K:P_{Na} = 2$.

When the pH of the extracellular solution was changed from 7·4 to 10·0, both the reversal potential and the conductance remained unchanged (Table 1). This implies that the rate-limiting step of permeation is determined by chemical groups which have pKs outside the range of pH 7·4–10·0. We were unable to perform the experiment in the acid range due to frequent 'seal breakdown'.

In all the experiments discussed hereafter, the extracellular solution contained 150 mM-Na^+, pH 7·4–10·0, and the patches were cell-attached.

Voltage dependence of the s.a. channel

We reported previously that the kinetics of the s.a. channel were nearly independent of the transmembrane potential (Guharay & Sachs, 1984). In the current work we

have found a distinct voltage dependence which increases with pH. We re-examined the earlier data to find the cause of the discrepancy and found that the reported lack of voltage dependency was a statistical fluke. Although the mean value of the time constants showed an insignificant trend with voltage, when we again fitted the data using squared residuals weighted inversely with the variance of the time constants, the resulting confidence limits on the voltage dependence were wide enough to

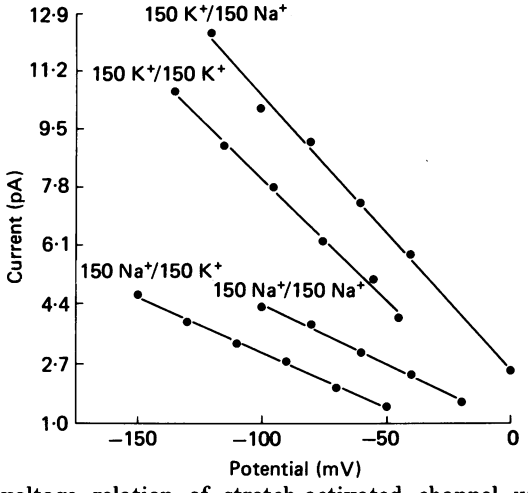

Fig. 2. Current–voltage relation of stretch-activated channel under different ionic conditions. Dominant ion species in mM noted as 'outside/inside' on the graph. With 150 mM-K^+ and 5 mM-Na^+ bathing the extracellular (pipette) face, the conductance was 70 pS but with 150 mM-Na^+ and 5 mM-K^+ bathing the extracellular face, the conductance was 35 pS. The reversal potential of the channel with 150 mM-K^+, 5 mM-Na^+ on the extracellular face and 150 mM-Na^+, 5 mM-K^+ on the cytoplasmic face was +30 mV (cytoplasmic face positive). With the reversed (normal) gradients the reversal potential was −30 mV (cytoplasmic side negative).

TABLE 1. Effect of extracellular pH on the conductance and the reversal potential of stretch-activated channels*

pH (*n*)	Conductance† (pS)	Reversal potential (mV)
7·4 (5)	36·3 ± 3·6	−30 ± 4
8·2 (4)	33·1 ± 4·1	−32 ± 6
9·0 (4)	35·8 ± 2·3	−31 ± 3
10·0 (5)	36·9 ± 4·1	−32 ± 2

Values are given as mean ± S.E. of mean.

* Channel currents were recorded from excised patches with 150 mM-Na^+, 5 mM-K^+ on the extracellular face and 150 mM-K^+, 5 mM-Na^+ on the cytoplasmic face, temperature 20–22 °C.

† From the slopes of current–voltage curves.

encompass the much more precise values reported here. In earlier work we concentrated on the pressure dependence and used relatively short records to evaluate the voltage dependence. Statistical scatter in the resulting time constants obscured the trend reported here.

As shown in Fig. 3, the probability of the channel being open (P_{open}) changes with membrane potential. Between −60 and −160 mV, P_{open} decreased in an exponential

manner with hyperpolarization. We could not study the voltage dependence in the depolarizing direction due to frequent openings of the Ca^{2+}-activated K^+ channel. Fig. 3 further shows that the voltage dependence of P_{open} is influenced by extracellular pH. The voltage sensitivity of the s.a. channel increases as the extracellular pH is raised. For example, at pH 7·4, an e-fold change in P_{open} requires 45 mV, whereas at pH 10·0 a similar change in P_{open} requires only 25 mV.

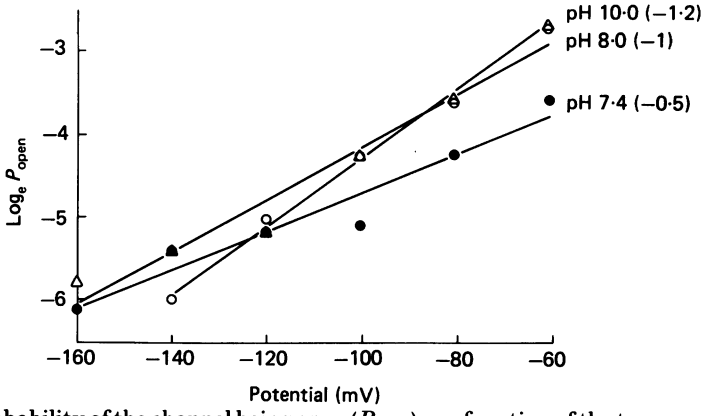

Fig. 3. The probability of the channel being open (P_{open}) as a function of the transmembrane potential at different values of extracellular pH. The probability increases exponentially with depolarization and the voltage sensitivity, α, of the channel increases with increasing pH. An e-fold change in P_{open} requires 45, 32 and 25 mV change in the membrane potential at pHs 7·4, 8·0 and 10·0, respectively. The numbers in parentheses represent the amount of suction in cmHg used for activating the channels for each experiment.

Stretch sensitivity: effects of pH

P_{open} increased exponentially with the square of the differential pressure across the patch (Fig. 4). The squared dependence on pressure is expected from a simple model of activation in which the energy of activation arises from linear distortion of the channel (Guharay & Sachs, 1984). The sensitivity to stretch is also influenced by the extracellular pH. As shown in Fig. 4 at pH 7·4, 3·12 cm^2Hg produced an e-fold change in the probability of the channel being open, whereas at pH 9·2 only 0·83 cm^2Hg were required for an e-fold change.

Effects of pH, stretch and voltage on s.a. channel kinetics

When the P_{open} is small and there is a single rate-limiting step for opening, the effects of voltage and pressure on the rate-limiting step are mirrored in the P_{open}. Analysis of the channel kinetics, however, reveals more detailed information. The distribution of open and closed times reveals multiple kinetic states: three closed states and one open state (Fig. 5*A* and *B*). We previously analysed the kinetics in terms of a four-state sequential model,

$$C_1 \underset{k_{2,1}}{\overset{k_{1,2}}{\rightleftharpoons}} C_2 \underset{k_{3,2}}{\overset{k_{2,3}}{\rightleftharpoons}} C_3 \underset{k_{4,3}}{\overset{k_{3,4}}{\rightleftharpoons}} O_4,$$

where the Cs represent closed states and O the open state (dubbed O_4 to simplify the numbering of the states). From our previous analysis (Guharay & Sachs, 1984), $k_{1,2}$

is the smallest and the only stretch-dependent rate. The closed time distribution (Fig. 5*B*) has three exponential components, and only the slowest is affected by stretch and transmembrane potential.

As shown in Fig. 6*A* and *B*, the effect of stretch and transmembrane potential was confined to only one of the time constants, τ_3, characterizing the longest closed interval distribution. As expected from the results on P_{open}, τ_3 increased exponentially with hyperpolarization (Fig. 6*A*) and decreased exponentially with the square of

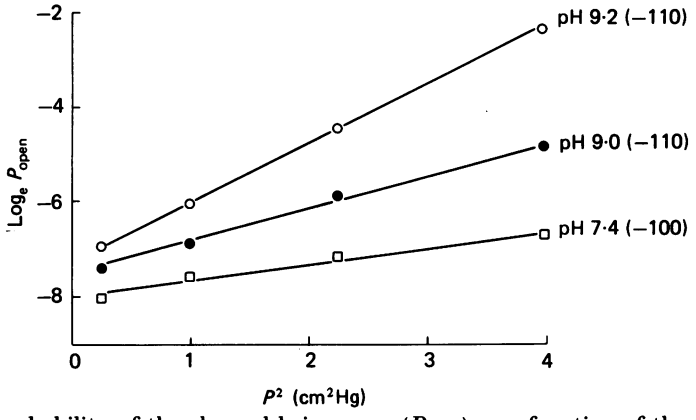

Fig. 4. The probability of the channel being open (P_{open}) as a function of the square of the applied suction (P^2) at different values of pH. The probability increases exponentially with P^2 and the pressure sensitivity, θ, increases with increasing pH. An e-fold change in the P_{open} requires 3·12, 1·49 and 0·83 cm^{2}Hg suction at pH 7·4, 9·0 and 9·2, respectively. The numbers in parentheses represent the transmembrane potential for each of the experiments.

applied pressure (Fig. 6*B*). It is also evident that the voltage and pressure sensitivity of τ_3 increased with increasing pH. For example at pH 7·4, either a 50 mV shift in membrane potential or 7·14 cm^{2}Hg of applied pressure caused an e-fold change in τ_3, whereas at pH 10·0 only a 17·8 mV shift in membrane potential or 1·06 cm^{2}Hg of applied pressure caused the same effect.

The open time, τ_1 and τ_2, time constants of the short and intermediate closed intervals were not affected by transmembrane potential (Fig. 7*A*) or stretch (Fig. 7*B*). This was the result at all extracellular pH values (7·4–10·0).

As expected from the results of our previous study, the only rate constant significantly affected by stretch is $k_{1,2}$ (Fig. 8*A*). We can further demonstrate that the effect of transmembrane potential on the s.a. channel is also confined to only one rate constant, $k_{1,2}$ (Fig. 8*B*). In line with the results on P_{open} and τ_3, $k_{1,2}$ increases exponentially with the square of the applied pressure and decreases exponentially with hyperpolarization of the membrane. An increase of extracellular pH increases the sensitivity of $k_{1,2}$ to voltage (Fig. 9*A*) and pressure (Fig. 9*B*). At pH 7·4, a 90 mV shift in membrane potential or 1·44 cm^{2}Hg of applied pressure is required to cause a e-fold change in $k_{1,2}$, whereas at pH 10·0 a 35 mV shift or 1·07 cm^{2}Hg of applied pressure can cause a similar change.

To examine the relation of pH and the voltage and pressure sensitivity of the s.a. channel, we plotted the normalized sensitivities against the extracellular pH

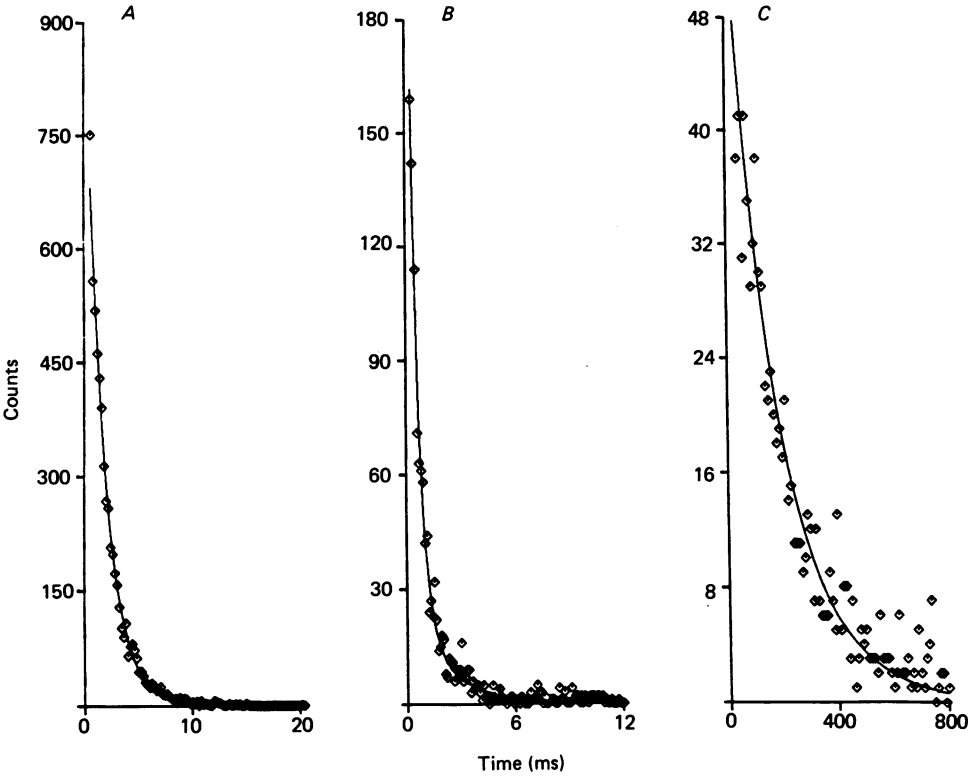

Fig. 5. The distribution of open time (*A*) and closed times (*B* and *C*) of the stretch-activated channel. *C* is a continuation, with a much longer time base, of the distribution shown in *B*. The theoretical curves (continuous lines) are calculated by a non-linear regression of the data to a single (*A* and *C*) or a sum of two exponentials (*B*). The data in panel *C* were fitted from 30 ms onward. The distribution of open times has a time constant of $1{\cdot}63 \pm 0{\cdot}038$ ms (90% confidence limit). The distribution of closed times has three time constants: $0{\cdot}40 \pm 0{\cdot}06$, $1{\cdot}91 \pm 0{\cdot}92$ and $176{\cdot}9 \pm 10{\cdot}29$ ms (transmembrane potential -120 mV; suction $-1{\cdot}5$ cmHg, temperature 22 °C, band width 2 kHz).

(Fig. 10*A* and *B*). The voltage and pressure sensitivities were normalized in the following manner,

$$\text{normalized sensitivity} = \frac{\text{sensitivity} - \text{minimum sensitivity}}{\text{maximum sensitivity} - \text{minimum sensitivity}}.$$

As shown in Fig. 10*A*, the relation between the voltage sensitivity and pH can be reasonably described by a titration curve where a single site of pK $9{\cdot}0 \pm 0{\cdot}2$ is being deprotonated with increasing pH. The relation between the pressure sensitivity and pH (Fig. 10*B*) also resembles a titration curve of a single site with a pK of $9{\cdot}3 \pm 0{\cdot}2$. It appears that both the voltage and pressure sensitivities of the s.a. channel are influenced by the same or similar chemical groups. We cannot yet determine how many groups per channel are titrated.

In order to model the effects of pH on $k_{1,2}$, we made a simple absolute reaction rate model for $k_{1,2}$,

$$k_{1,2} = k^0_{1,2}\, e^{(\alpha V + \theta P^2)}, \qquad (3)$$

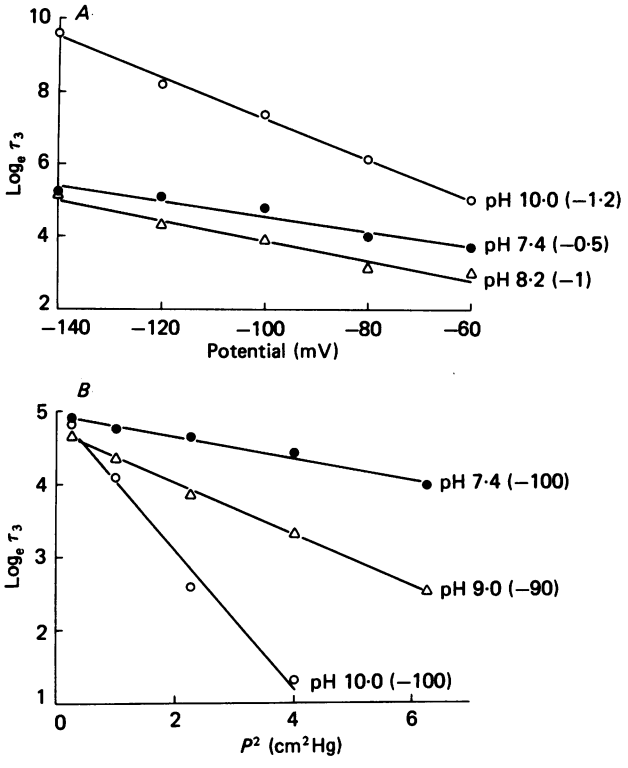

Fig. 6. The longest closed time constant, τ_3, as function of transmembrane potential (A) and square of applied suction (P^2) (B) at different pH. τ_3 increases exponentially with hyperpolarization and with P^2. Increasing extracellular pH increases the rate of change of τ_3 with voltage and pressure. The numbers in parentheses represent (in A) the amount of suction and (in B) the transmembrane potential. (Temperature 22 °C.)

where $k^0_{1,2}$ is the rate constant for transition at zero transmembrane potential (V) and applied pressure (P), α is the voltage sensitivity and θ is the pressure sensitivity. θ is a function of the patch diameter, d, the area elasticity of the channel, K_A, its equivalent area in the plane of the membrane, A, the efficiency factor, f, which reflects the fraction of available energy transferred to gating of the channel and kT is Boltzmann's constant times the temperature in °K (Guharay & Sachs, 1984),

$$\theta = f d^2 A/(32 K_A kT). \quad (4)$$

Using eqn. (3) we can extrapolate the data for zero applied pressure and voltage and solve for $k^0_{1,2}$. Table 2 shows $k^0_{1,2}$ at different values of pH along with the corresponding values of α and θ. It is evident that $k^0_{1,2}$ is not sensitive to the extracellular pH.

DISCUSSION

Ion permeation

For inward currents, the conductance of the channel is lower by a factor of two when Na^+ is substituted for K^+ on the extracellular side. There is no drop in inward

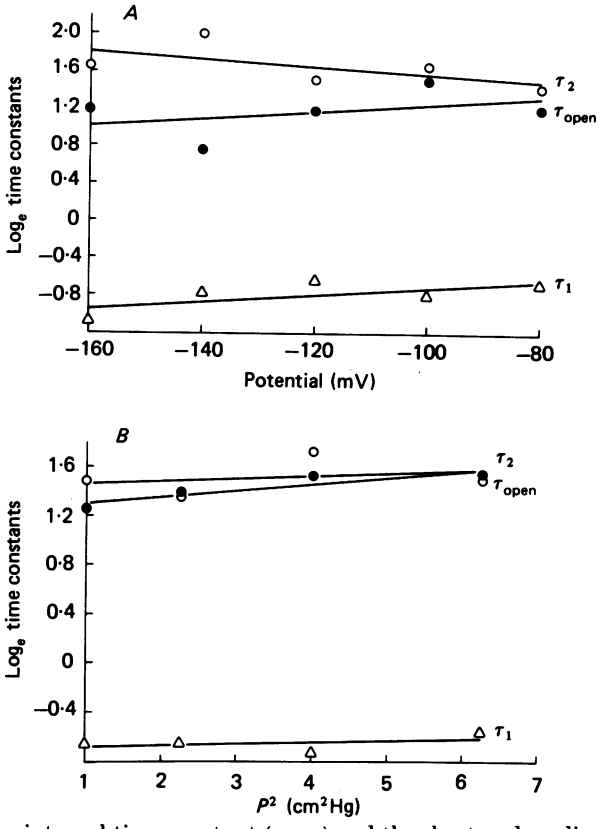

Fig. 7. The open interval time constant (τ_{open}) and the short and medium closed interval time constants (τ_1 and τ_2) as function of transmembrane potential (*A*) and the square of applied suction (P^2) (*B*), pH 10·0. None of these time constants are significantly affected by voltage or pressure. The observation is valid for all values of pH studied.

current when Na^+ is substituted for K^+ on the intracellular side. Preliminary results indicate that the conductance for Cs^+ is approximately the same as that for Na^+.

Voltage dependence of s.a. channel

The s.a. channel kinetics are voltage sensitive, the probability of the channel being open decreasing exponentially with hyperpolarization. The present results show clearly that the transmembrane potential does affect the kinetics and that it acts through the rate constant $k_{1,2}$ which decreases exponentially with hyperpolarization. This observation correlates with the results obtained in crayfish stretch receptor mechanotransducer, where the slope of the current–voltage curve for the stretch-induced current approaches zero at membrane potentials more negative than −100 mV (Brown, Ottoson & Rydqvist, 1978).

Effect of pH on α, θ and $k^0_{1,2}$

$k_{1,2}$ also increases exponentially with the square of the differential pressure. The effects of voltage and stretch can be summarized by assuming a Eyring rate model for $k_{1,2}$ (eqn. (3) in Results section).

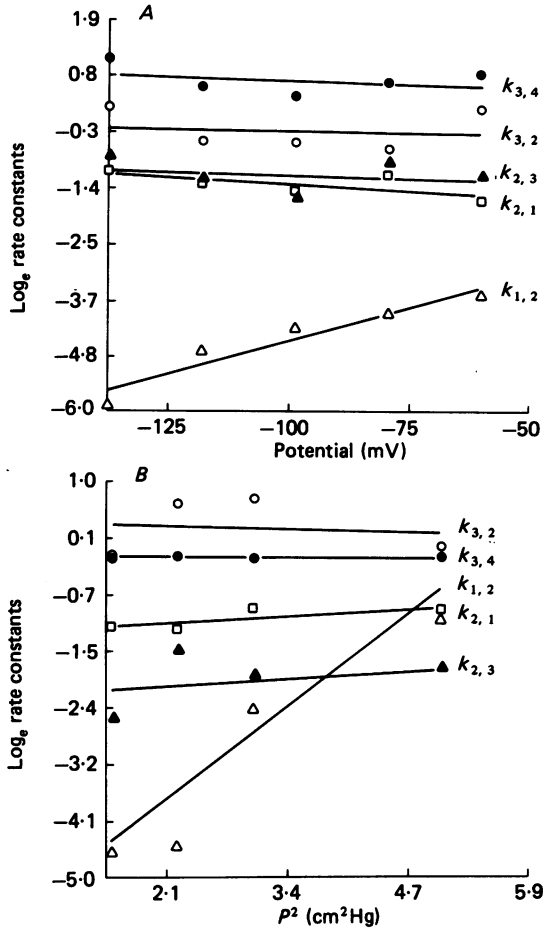

Fig. 8. The rate constants for the four-state sequential model of channel kinetics as function of transmembrane potential (*A*) and the square of the applied suction (P^2) (*B*) at pH 10·0. Only one rate constant, $k_{1,2}$ (determining the transition between states C_1 and C_2) is significantly altered by voltage or suction. $k_{1,2}$ increases exponentially with depolarization and P^2. At pH 10·0, a 35 mV depolarization or an increase of 1·07 cm^{2}Hg suction increases $k_{1,2}$ e-fold. (Temperature 22 °C, suction used in *A* was −1·2 cmHg and the transmembrane potential for *B* was −100 mV.)

Increasing extracellular pH from 7·4 to 10·0 results in an increase of the voltage sensitivity, α, in a manner suggsting titration of one or more independent sites with a pK of 9·01 ± 0·22. The effect of pH on the pressure sensitivity, θ, reflects titration of similar site(s) with a pK of 9·29 ± 0·23. Assuming that we are titrating a protein, this suggests involvement of the side-chain amide group of lysine or the α-amino group (N terminal) of any amino acid. There is no significant difference between the estimated pKs of α and θ (about 9·1), and it appears that similar and possibly identical chemical groups are involved in the voltage and stretch sensitivity of s.a. channel. Since both stretch and voltage affect the same rate constant and both sensitivities have the same pK, a single residue may be involved.

We found that the extracellular pH has no significant effect on the voltage- and

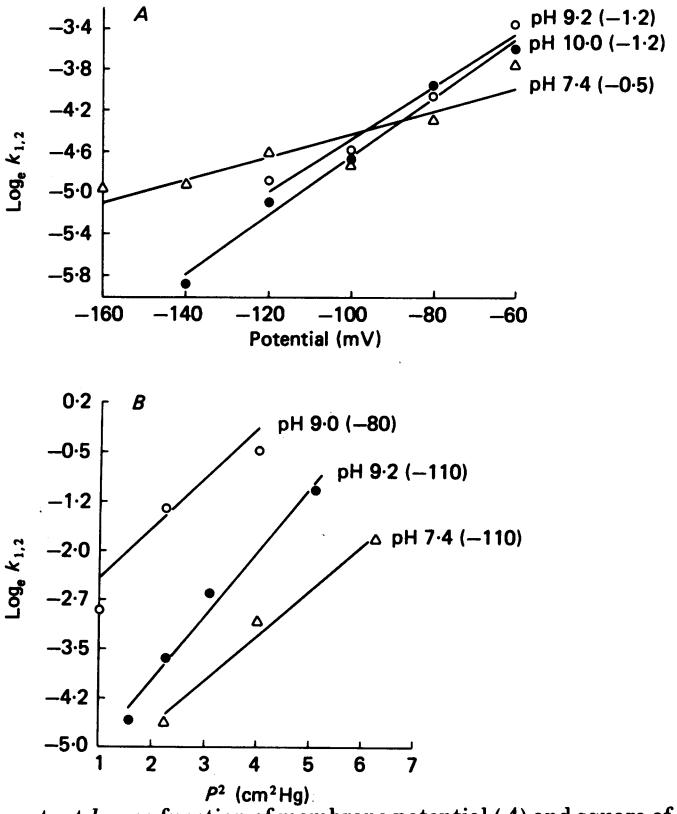

Fig. 9. The rate constant $k_{1,2}$ as function of membrane potential (*A*) and square of applied suction (P^2) (*B*) at different values of pH. Increase of extracellular pH increases the voltage and pressure sensitivities of $k_{1,2}$. For an e-fold change in $k_{1,2}$ at pHs 7·4, 9·2 and 10·0, the necessary changes in the membrane potential are 90, 40 and 35 mV, respectively. For a similar change in $k_{1,2}$, 1·44, 1·29 and 1·07 cm^{2}Hg suction is neccessary at pHs 7·4, 9·0 and 10·0, respectively.

pressure-independent rate, $k^0_{1,2}$ (Table 2). If the alteration of the extracellular pH caused a significant change in surface charge, the voltage sensitivity of the channel would have caused a change in $k^0_{1,2}$ with the pH.

The voltage sensitivity increased from 45 mV e^{-1} at pH 7·4 to 20 mV e^{-1} at pH 10. This change in voltage sensitivity corresponds to a change of the effective dipole moment of the channel during the $k_{1,2}$ transition. In the most simplistic interpretation, the titration to pH 10 results in the loss of a single positive charge from a site near the outside of the membrane and this site traverses approximately half of the membrane field during the $k_{1,2}$ transition. Alternatively, the deprotonation of the external site may result in a conformational change of the channel to a state where a larger change in dipole moment is associated with the $k_{1,2}$ transition. If the latter interpretation were true, we might expect that large conformational changes would also affect the voltage- and pressure-independent barrier causing changes in $k^0_{1,2}$, in contrast to our results.

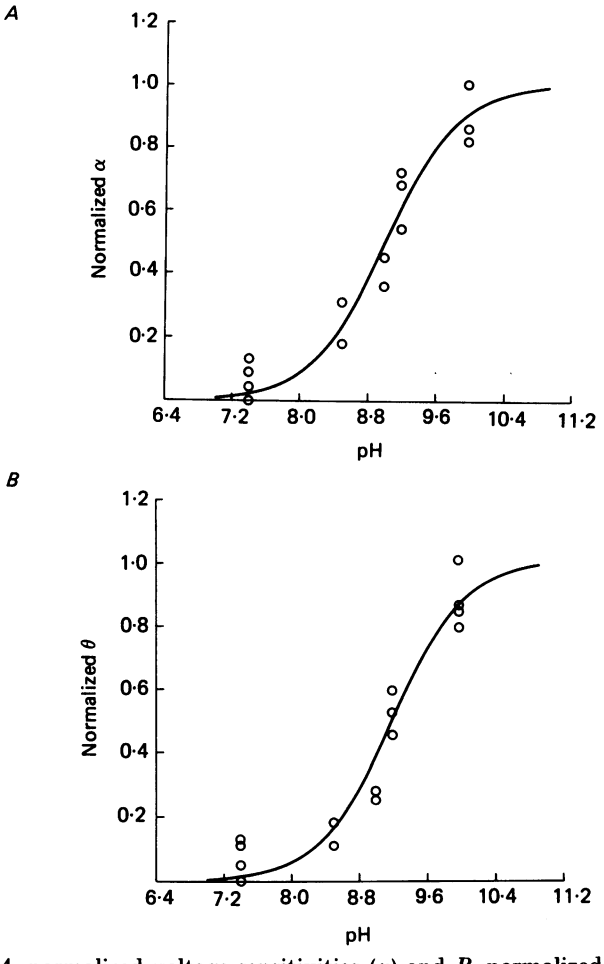

Fig. 10. *A*, normalized voltage sensitivities (α) and *B*, normalized pressure sensitivities (θ) as a function of the extracellular pH. The relations between α and θ, and pH can be reasonably described as titration curves where single sites are being deprotonated with p*K*s of $9{\cdot}0 \pm 0{\cdot}2$ and $9{\cdot}1 \pm 0{\cdot}2$, respectively.

In the range from pH 7·4 to 10·0, the effect of the extracellular pH on the s.a. channel kinetics can be summarized by the following equations:

$$k_{1,2} = k^0_{1,2} \exp(\alpha(\mathrm{pH})\ V + \theta(\mathrm{pH})\ P^2),$$

$$\alpha(\mathrm{pH}) = 0{\cdot}010 + 0{\cdot}018/(1 + 10^{(9{\cdot}1 - \mathrm{pH})}),$$

$$\theta(\mathrm{pH}) = 0{\cdot}66 + 0{\cdot}47/(1 + 10^{(9{\cdot}1 - \mathrm{pH})}).$$

The effect of pH on other ion channels is different from the effect we have shown for the s.a. channel. For the Na^+ channel, the literature suggests that pH does not alter the voltage sensitivity but causes a shift in the voltage-dependent parameters along the voltage axis (Hille, 1968; Carbone *et al.* 1978; Yatani *et al.* 1984). The effect can be adequately explained by the effect on the surface charge. However, in *Myxicola*

axons, an increase of pH significantly speeds up the activation of Na^+ channels, an effect which is beyond that to be expected from surface charge alteration (Schauf & Davis, 1976).

It is generally agreed that the decay time constant of the end-plate current in frog is shortened when the extracellular pH is increased. It has been suggested that the voltage dependence of the end-plate current is altered by pH (Scuka, 1977). It has also been suggested that intrinsic voltage dependence of the decay time constant is

TABLE 2. Effect of extracellular pH on voltage sensitivity (α), stretch sensitivity (θ) and $k^0_{1,2}$, the voltage- and stretch-independent part of the rate constant $k_{1,2}$

pH (n)	α (mV^{-1})	θ ($cm^{-2}Hg$)	$k^0_{1,2}$* (ms^{-1})
7·4 (5)	0·010 ± 0·001	0·66 ± 0·036	0·031 ± 0·002
9·0 (3)	0·018 ± 0·001	0·78 ± 0·014	0·028 ± 0·008
9·2 (3)	0·023 ± 0·002	0·92 ± 0·035	0·038 ± 0·005
10·0 (4)	0·028 ± 0·002	1·13 ± 0·051	0·033 ± 0·003

Values are given as mean ± S.E. of mean.
* Calculated according to eqn. (3) (Results section).

not affected by pH but the effect is mediated by alteration of surface charge (Mallart & Molgo, 1978; Peper *et al.* 1982). Results from fluctuation analysis in tissue-cultured chick muscle further show that the apparent open time of acetylcholine channels is shortened at alkaline pH (Landau, Gavish, Nachshen & Lotan, 1981; Goldberg & Lass, 1983). The apparent open time actually represents bursting activity which involves multiple processes (Auerbach & Sachs, 1984) so that an unambiguous interpretation of the data is not possible. We found that the open time of the s.a. channel was not affected by extracellular pH within the experimental range (pH 7·4–10·0).

Effect of pH on channel conductance and ion permeation

The fact that pH did not affect the reversal potential of the s.a. channel is in keeping with results on Na^+ channels (Hille, 1968) and acetylcholine channels (Goldberg & Lass, 1983).

The channel conductance of the s.a. channel is independent of pH. In contrast, the conductance of the acetylcholine channel, estimated from fluctuation analysis, increases with increasing pH in chick myoballs (Goldberg & Lass, 1983). The peak Na^+ current increases with high pH (Hille, 1968; Carbone *et al.* 1978), although it is not clear whether the increased peak is due to an increase in the channel conductance or the number of open channels. The fact that there was no change in s.a. channel conductance with titration of the channel suggests that the site(s) which was titrated is not close to the mouth of the channel. An increase in negative surface charge near the mouth of the channel could increase the local cation concentration and increase conductance of the channels as suggested for the Ca^{2+}-activated K^+ channel (Moczydlowski, Alvarez, Vergara & Latorre, 1985).

In conclusion, there exists in cultured chick skeletal muscle a channel whose gating is affected by stretch as well as voltage. The stretch and voltage both appear to be acting independently on a single conformational change of the channel. The voltage

and stretch sensitivities are both titrated at the same pK. Alteration of extracellular pH affects s.a. channel kinetics by titrating these sites and not by altering the surface charge. The channel conductance and the ion-selectivity of the channel are independent of pH, indicating that the sites are far from the mouth of the channel.

We would like to thank Richard McGarrigle for preparing the tissue cultures. We would also like to thank A. Auerbach, J. Hidalgo and J. Neil for their helpful criticism. This research was supported by grant no. NS 13194 from NINCD.

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