*J. Physiol.* (1975), **251**, *pp.* 711–735
*With 8 text-figures*
*Printed in Great Britain*



# THE EFFECT OF ZINC IONS ON THE GATING OF THE DELAYED POTASSIUM CONDUCTANCE OF FROG SARTORIUS MUSCLE

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(*Received 20 January 1975*)

SUMMARY

1. A voltage-clamp method was used to examine the effects of zinc ions on the delayed potassium currents and also the slowly activating potassium currents that are turned on by depolarizing the membrane of frog sartorius muscle fibres.

2. In a control solution, the delayed potassium conductance had a maximum value of $17{\cdot}8 \pm 2{\cdot}5$ mmho.cm$^{-2}$. The reversal potential for the currents was $-76{\cdot}9 \pm 2{\cdot}5$ mV. The membrane potential where $n_\infty$ had the value 0·5 was $-49$ mV.

3. The major effect of zinc ions was to slow the delayed potassium currents. The value of $\tau_n$ was increased approximately tenfold in 0·1 mM zinc. Zinc does not alter the effective valency of the gating particles of the potassium channel, but the conductance was shifted to more positive membrane potentials: in 0·1 mM zinc, the membrane potential where $n_\infty$ had the value 0·5 was $-32$ mV.

4. Zinc ions, at a concentration of 0·1 mM, also reduced the maximum potassium conductance by about 60% to $7{\cdot}3 \pm 0{\cdot}8$ mmho.cm$^{-2}$; they did not alter the reversal potential of the currents, which had a value in 0·1 mM zinc of $-74{\cdot}6 \pm 1{\cdot}5$ mV.

5. Zinc ions had little or no effect on the rate of inactivation of the potassium currents.

6. Zinc ions had little effect on the conductance attributable to the slowly activating potassium system. In 0·01 mM zinc this conductance had a value at 0 to $+10$ mV of $1{\cdot}25 \pm 0{\cdot}29$ mmho.cm$^{-2}$. Zinc did not alter the reversal potential of the slow potassium currents from the value of

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$-85{\cdot}3 \pm 1{\cdot}6$ mV in the absence of zinc, and had no effect on the time course of the turn-off of these currents at $-60$ mV.

7. The delayed potassium currents obtained in 0·002 and 0·01 mM zinc could not be fitted exactly with a simple fourth order equation, but were well fitted by a model proposing that zinc ions slow the opening and closing of the gating mechanism to one tenth the normal rate when they bind to the gating molecule. If the binding sites are not saturated, those gating molecules that do not bind zinc are assumed to be quite unaltered in their properties, though the potential dependence of their rate constants $\alpha_n$ and $\beta_n$ was assumed to be shifted to more positive levels. In one fibre in 0·01 mM zinc, the model fitted the currents best if 50% of the gating molecules bound zinc.

## INTRODUCTION

It is well known that zinc ions prolong the action potential of skeletal muscle (for review, see Sandow, 1965) as well as of nerve (Takahashi, Murai & Sasaki, 1960). The rate of onset of the action of zinc is quite slow, even in dissected single muscle fibres (Stanfield, 1973), but it seems likely that this effect is on the surface, as opposed to the T-system membrane, especially in view of the recent analysis of the muscle action potential by Adrian & Peachey (1973). Certainly, the effect of zinc may be made to reverse rapidly if zinc is chelated by some suitable agent such as EDTA (Sandow & Isaacson, 1966); and, although the effect is hard to explain, zinc acts quickly if the muscle fibres are detubulated with the glycerol technique of Howell & Jenden (1967; Stanfield, 1973). Kao & Stanfield (1970) showed that zinc depressed the delayed potassium conductance, which seems to lie mainly on the surface membrane (Adrian, Chandler & Hodgkin, 1970*a*), and suggested also that zinc and uranyl ions 'characteristically slowed the rate of development of the outward current'. This view has recently been taken up by Begenisich & Lynch (1974), who showed that zinc applied to the inside of the squid axon slowed the potassium conductance in that preparation and also that it reduced the sodium conductance.

With the discovery of charge movements within the membrane of excitable cells (Armstrong & Bezanilla 1973, 1974; Keynes & Rojas, 1974; Schneider & Chandler 1973) the pharmacological actions of zinc have acquired a new significance, since zinc, applied internally to the squid axon at the high concentration of the 10 mM (Armstrong & Bezanilla, 1974), blocks the movements of charge within the membrane that are considered to be the gating currents of the sodium channel. This paper describes an investigation in some detail of the effect of zinc ions on the two potassium conductances of skeletal muscle that are turned on by

depolarization (Adrian *et al.* 1970*a*, *b*; Stanfield, 1970). It did not prove possible to look at the effects on the sodium conductance, since the voltage clamp method used here controls the membrane potential well during the sodium conductance over only a small range of its potential dependence. But there is evidence from other sources (Sandow, Taylor & Preiser, 1965; Edman & Grieve, 1966; Stanfield, 1973) that zinc has no effect on the rate of rise or rise time of the action potential of skeletal muscle, and it is improbable that zinc has any effect, at least when applied externally, on the activation of the sodium conductance in this excitable cell.

## METHODS

*Voltage clamp.* The voltage clamp technique used to obtain the results of this paper has been described before (Adrian *et al.* 1970*a*; Stanfield, 1970), and requires only brief description here. Three micro-electrodes impaled a muscle fibre near its end.

Two electrodes, spaced at distances $l$ and $2l$ from the end of the fibre, were used for recording: these electrodes were filled with 3 M-KCl and had tip resistances between 5 and 20 MΩ (usually between 7 and 15 MΩ) and tip potentials less negative than $-5$ mV. The membrane potential was controlled by a feed-back amplifier at the electrode impaling at the distance $l$ from the fibre end. The potential difference across the membrane recorded at this point is termed $V_1$. The potential difference across the membrane recorded at distance $2l$ from the fibre end is $V_2$. The third micro-electrode, filled with 2 M potassium citrate, had a tip resistance less than 10 MΩ and was used to pass current. It impaled the fibre at a distance $2l + 50$ μm from the fibre end.

Adrian *et al.* (1970*a*) have shown that the membrane current flowing around the micro-electrode that records $V_1$ is given by the approximation

$$I_m = \frac{a(V_2 - V_1)}{3l^2 R_i} \text{ A.cm}^{-2}, \quad (1)$$

where $a$ is the fibre radius, $V_2$ and $V_1$ the membrane potentials at distances $2l$ and $l$ from the fibre end, and $R_i$ the specific internal resistance of the muscle fibre. Adrian *et al.* (1970*a*) have shown that the approximation of eqn. (1) is accurate to within 5% if $(V_2 - V_1)/V_1$ is less than 6. This condition was always kept in the present experiments, and $l$, the inter-electrode distance, was set at 125 μm in most cases.

The sartorius muscle of the frog (*Rana temporaria*) was used throughout the present experiments, which were performed mainly in the cold, at about 4° C. A few experiments were carried out at room temperature, about 20° C.

*Solutions.* The Ringer solution used had the following composition: 115 mM-NaCl, 2·5 mM-KCl, 1·8 mM-$CaCl_2$, and 5 mM Tris-HCl buffer (pH 7·2). Solutions were made hypertonic by the addition of sucrose to a concentration of 350 mM: this was done to prevent contractions, which dislodge the micro-electrodes. A few experiments were carried out in solutions containing the impermeant anion gluconate in place of chloride, to check that the currents measured were potassium currents.

Zinc was added as zinc acetate to the solution described. In order to block sodium currents, all solutions contained tetrodotoxin at a concentration of $10^{-6}$ g/ml.

## RESULTS

A summary of the main effects of zinc ions on the delayed potassium currents (i.e. on $I_{K_1}$ of Adrian *et al.* 1970*b*) is given in Table 1. Descriptions of the results in the control solution and then of the effects of zinc are given in the next two sections.

### *Experiments in hypertonic Tris-buffered Ringer*

Fig. 1 offers a description of the main properties of the delayed potassium conductance of skeletal muscle fibres. The results are very similar to those obtained previously by Adrian *et al.* (1970*a*) though the currents appear to turn on slightly slower, and at membrane potentials about 3 mV more negative. Kao & Stanfield (1970) found that the 'threshold for delayed rectification' was slightly more negative in tris-buffered Ringer ($-54{\cdot}8$ mV) than in phosphate-buffered Ringer ($-52{\cdot}0$ mV).

Fig. 1*A* shows tracings of two records of membrane current obtained during depolarizing pulses that take $V_1$ to $-5$ mV and $-14$ mV. The current turns on, after the capacitative transient, along an S-shape time course to a maximum level, and then begins slowly to inactivate. Fig. 1*B* plots out the current–voltage relation for the fibre, plotting the maximum ionic currents against voltage. The delayed currents begin to turn on noticeably at membrane potentials positive to $-50$ mV.

The rest of the analysis of Fig. 1 follows the model of Hodgkin & Huxley (1952). The details of the model need not be given here, except to say that $n_\infty$, which may be taken as describing the steady-state distribution of particles that gate the potassium channel, at a given membrane potential is defined as follows

$$n_\infty = \sqrt[4]{\left(\frac{g_{K\infty}}{\bar{g}_K}\right)} = \frac{\alpha_n}{\alpha_n + \beta_n}, \tag{2}$$

where $g_{K\infty}$ is the peak potassium conductance at that membrane potential, the slow inactivation being ignored in the analysis; $\bar{g}_K$ is the maximum potassium conductance, measured during large depolarizing pulses, and $\alpha_n$ and $\beta_n$ are rate constants, depending on membrane potential, but not on time, for the opening and closing of the gates.

In describing the turn-on of conductance, eqn. (11) of Hodgkin & Huxley (1952) may be modified to

$$g_K = g_{K\infty}\,[1 - \exp(-t/\tau_n)]^4, \tag{3}$$

where

$$\tau_n = 1/(\alpha_n + \beta_n),$$

since $n_\infty$ is virtually zero in skeletal muscle fibres at the holding potential of $-100$ mV.

In Fig. 1*C* are plotted potassium currents predicted by eqn. (3) when $\tau_n$ is 8·05 and 10·57 msec; these curves fit the records of Fig. 1*A* rather well, after subtraction of leak current and capacitative transients found by scaling the currents flowing during small depolarizing pulses. The

reciprocals of the time constants at various membrane potentials are plotted against $V_1$ in Fig. 1*E*. The line through the points is drawn by eye and is not a prediction from Hodgkin–Huxley equations for the rate constants.

Fig. 1*D* gives the relation between $g_K/\bar{g}_K$ and also $n_\infty$ as defined in eqn. (2) and the membrane potential $V_1$. The line through the points representing $n_\infty$ is a Boltzmann distribution whose formula is given on page 6 in eqn. (4), while the values of $V_{\frac{1}{2}n}$ and of $kT/z'e$ are given in the legend of Fig. 1.

The fibre of Fig. 1 had a maximum potassium conductance, $\bar{g}_K$, of 20·5 mmho.cm$^{-2}$, calculated on the assumptions that the fibre radius in the hypertonic solution was 40 $\mu$m, and that the specific internal resistance of the fibre was 370 $\Omega$ cm. The last value follows from the measurements of $R_i$ in single dissected muscle fibres in hypertonic solutions by

TABLE 1. The effects of zinc ions on the delayed potassium conductance of frog sartorius muscle

| Zinc concn. (mM) | Resting potential (mV) | $\bar{g}_K$ (mmho.cm$^{-2}$) | $V'_{K_1}$ (mV) | $V_{\frac{1}{2}n}$ (mV) | $\tau_n$ ($V_1 = 0$) (msec) |
|---|---|---|---|---|---|
| 0 | $-85{\cdot}1 \pm 1{\cdot}8$ (13)* | $17{\cdot}8 \pm 1{\cdot}7$ (13)* | $-76{\cdot}9 \pm 2{\cdot}5$ (13)* | −49 | $7{\cdot}1 \pm 0{\cdot}3$ (13)* |
| 0·002 | $-86{\cdot}8 \pm 3{\cdot}0$ (5) | $13{\cdot}2 \pm 1{\cdot}3$ (5) | $-76{\cdot}8 \pm 2{\cdot}4$ (5) | −47 | $9{\cdot}2 \pm 0{\cdot}8$ (5) |
| 0·01 | $-89{\cdot}0 \pm 1{\cdot}0$ (13) | $9{\cdot}4 \pm 0{\cdot}6$ (13) | $-74{\cdot}1 \pm 1{\cdot}2$ (13) | −44 | $73{\cdot}4 \pm 5{\cdot}0$ (13) |
| 0·1 | $-82{\cdot}2 \pm 2{\cdot}3$ (14) | $7{\cdot}3 \pm 0{\cdot}8$ (14) | $-74{\cdot}6 \pm 1{\cdot}5$ (14) | −32 | $93{\cdot}2 \pm 5{\cdot}8$ (14) |

$V_{\frac{1}{2}n}$ is the value of $V_1$, where $n_\infty = 0{\cdot}5$; the values of $\tau_n$ are those at a membrane potential ($V_1$) of zero.

* Mean ± S.E. of the mean, followed by the number of fibres in parentheses.

The values for $\bar{g}_K$ in 0·01 and 0·1 mM zinc are significantly different from that in the control solution ($P < 0{\cdot}001$ in each case), while that in 0·002 mM zinc is not ($0{\cdot}18 > P > 0{\cdot}13$). The values for $V'_{K_1}$ in the zinc solutions are not significantly different from that in the control solution ($P > 0{\cdot}33$ in each case).

Hodgkin & Nakajima (1972) and from their value for the $Q_{10}$ of $R_i$. The value for $\bar{g}_K$ also depended on the measurement, by means of two pulse experiments, of the reversal potential of the delayed potassium conductance ($V'_{K_1}$) which in the fibre of Fig. 1 was −62 mV. The mean values for $\bar{g}_K$ and $V'_{K_1}$, in fibres in the control hypertonic solution at 4° C, were $17{\cdot}8 \pm 1{\cdot}7$ mmho.cm$^{-2}$ (mean ± S.E. of the mean, thirteen fibres) and $-76{\cdot}9 \pm 2{\cdot}5$ mV (mean ± S.E. of the mean, thirteen fibres). These values are also given in Table 1. The mean resting potentials of these fibres was $-85{\cdot}1 \pm 1{\cdot}8$ mV.

Fig. 2 summarizes the results from the fibres immersed in the control hypertonic solution. In Fig. 2*A* are plotted the relations between both $g_K/\bar{g}_K$ and $n_\infty$ and membrane potential. The line through the points in

the case of the $n_\infty$ relation is a Boltzmann relation of the kind used by Keynes & Rojas (1974) to describe the distribution of the charges in the membrane of the squid axon whose movement is held to gate the sodium conductance (Armstrong & Bezanilla, 1973, 1974; Keynes & Rojas, 1974). The $n_\infty$ relation is given by the formula

$$n_\infty = \frac{\exp\{(z'e/kT)(V_1 - V_{\frac{1}{2}n})\}}{1 + \exp\{(z'e/kT)(V_1 - V_{\frac{1}{2}n})\}}, \quad (4)$$

where $V_{\frac{1}{2}n}$ is the value of $V_1$ when $n_\infty = 0{\cdot}5$. $z'$ is the effective valency of the gating particle, $e$ is the value of the protonic charge (equal and opposite

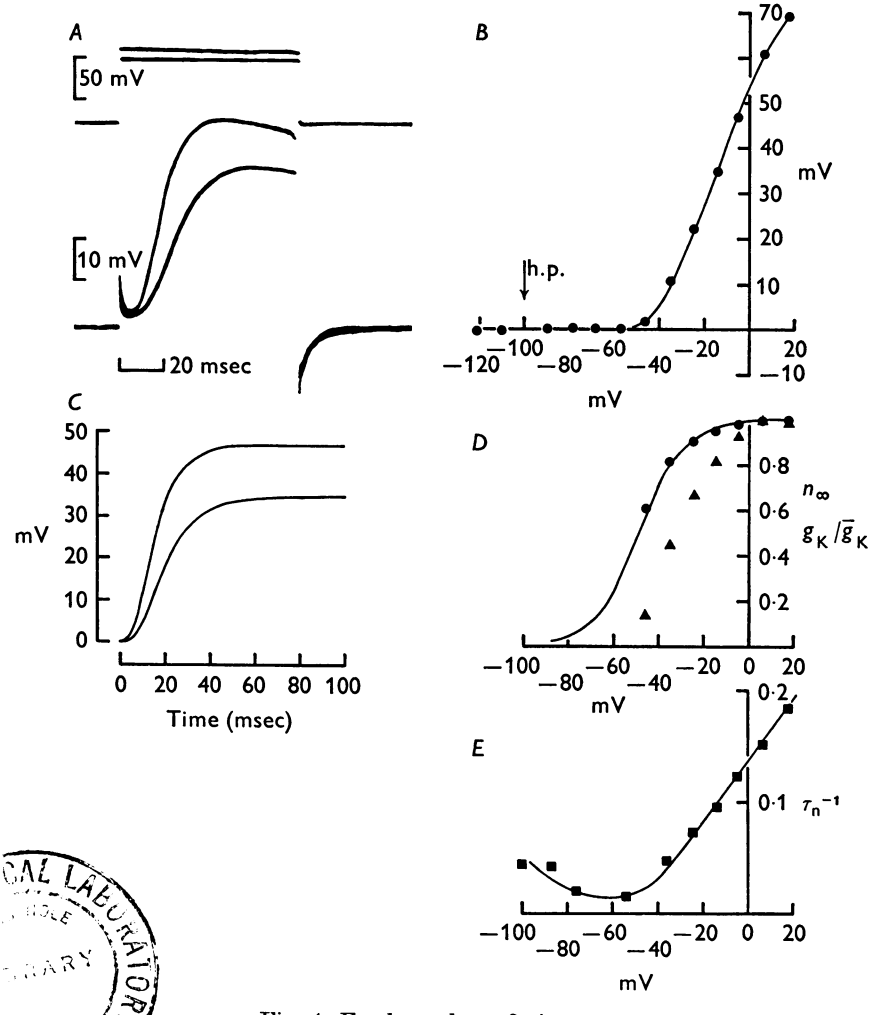




Fig. 1. For legend see facing page.

in sign to that of an electron), $k$ is Boltzmann's constant, and $T$ is the temperature in degrees absolute. In the relation of Fig. 2, $z'$ is 2·65 (= 23·9/9) and $V_{\frac{1}{2}n} = -49$ mV. The line through the points representing $g_K/\bar{g}_K$ is given by the fourth power of $n_\infty$ as defined by eqn. (4).

In Fig. 2*B* are plotted out the reciprocals of $\tau_n$, found for all thirteen fibres, against membrane potential. The points are distributed in the way described by Hodgkin & Huxley (1952) for nerve, and by Adrian *et al.* (1970*a*) for skeletal muscle.

The line through the points in Fig. 2*B* represents the sum of the rate constants $\alpha_n$ and $\beta_n$ given by the eqns.

$$\alpha_n = \frac{\bar{\alpha}_n (V - \bar{V}_n)}{1 - \exp\{-\frac{1}{7}(V - \bar{V}_n)\}}, \tag{5}$$

where $\bar{V}_n = -45$ mV and $\bar{\alpha}_n = 0{\cdot}0032$ msec$^{-1}$, and

$$\beta_n = \bar{\beta}_n \exp\{-\tfrac{1}{40}(V - \bar{V}_n)\}, \tag{6}$$

where $\bar{\beta}_n = 0{\cdot}0133$ msec$^{-1}$. These equations are those formulated by Adrian *et al.* (1970*a*) to describe the potential dependence of the rate constants for the opening and closing of the delayed potassium conductance. The $n_\infty$ relation in *A* may also be fitted reasonably well by $\alpha_n/(\alpha_n + \beta_n)$ using the values from these two equations, though the predicted relation was slightly less steep than that found. The $n_\infty$ relation was fitted by eqn. (4) because the fit could be made more exact, and because it gave a direct prediction of the effective valency of the postulated gating particle.

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Fig. 1. Properties of the delayed potassium conductance in a fibre immersed in the control hypertonic solution. Fibre resting potential, −90 mV; holding potential (h.p.), −100 mV; inter-electrode distance (*l*), 125 $\mu$m; T°, 4° C; tetrodotoxin, $10^{-6}$ g/ml.

*A*, tracings of records or membrane potential ($V_1$) and membrane current ($V_2 - V_1$). The depolarizations are to −5 and −14 mV.

*B*, current–voltage relation for the delayed potassium conductance. Ordinate: membrane current in terms of $V_2 - V_1$ (mV); abscissa: membrane potential, $V_1$ (mV). The arrow at −100 mV marks the holding potential.

*C*, curves representing the current in *A*, after subtraction of leak currents and capacitative transients, modelled according to eqn. (3) of text. The time constants, $\tau_n$, are 8·05 and 10·57 msec for the larger and smaller current respectively.

*D*, $n_\infty$ relation for this fibre. Ordinate: $n_\infty$ of $g_K/\bar{g}_K$; abscissa: membrane potential, $V_1$ (mV); ▲, $g_K/\bar{g}_K$; ●, $n_\infty$. The line through the points (●) is given by the formula of eqn. (4) of the text, where $V_{\frac{1}{2}n} = -49$ mV and $kT/z'e = 10$ mV. Fibre $\bar{g}_K = 20{\cdot}5$ mmho.cm$^{-2}$; reversal potential for delayed currents, $V'_{K_1} = -62$ mV; reversal potential for slow potassium currents, $V'_{K_2} = -82$ mV.

*E*, relation between $\tau_n^{-1}$ and membrane potential. Ordinate: $\tau_n^{-1}$ (msec$^{-1}$); abscissa: membrane potential, $V_1$ (mV). The line is drawn by eye.

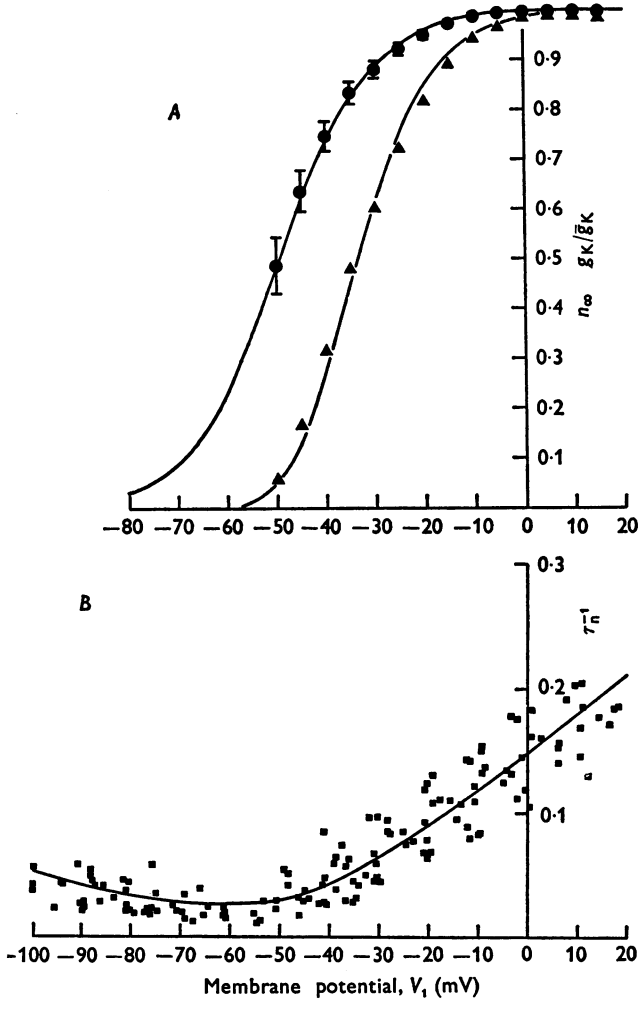


Fig. 2. *A*, $n_\infty$ relation for fibres in the control hypertonic solution. Ordinate: $n_\infty$ or $g_K/\bar{g}_K$; abscissa: membrane potential, $V_1$ (mV); ●, $n_\infty$; ▲, $g_K/\bar{g}_K$. Vertical bars represent ± S.E. of the mean. The line representing $n_\infty$ and joining the points (●) is given by equation (4) of the text, where $V_{\frac{1}{2}n} = -49$ mV and $kT/z'e = 9$ mV. The line representing $g_K/\bar{g}_K$ and joining the points (▲) is given by the fourth power of $n_\infty$ as given by eqn. (4).

*B*, relations between $\tau_n^{-1}$ and membrane potential. Ordinate: $\tau_n^{-1}$ (msec$^{-1}$); abscissa: membrane potential, $V_1$ (mV). The line through the points represents the sum of two rate constants $\alpha_n$ and $\beta_n$ given by eqns. (5) and (6) of text, where $\bar{V}_n = -45$ mV, $\bar{\alpha}_n = 0{\cdot}0032$ msec$^{-1}$ and $\bar{\beta}_n = 0{\cdot}0133$ msec$^{-1}$.

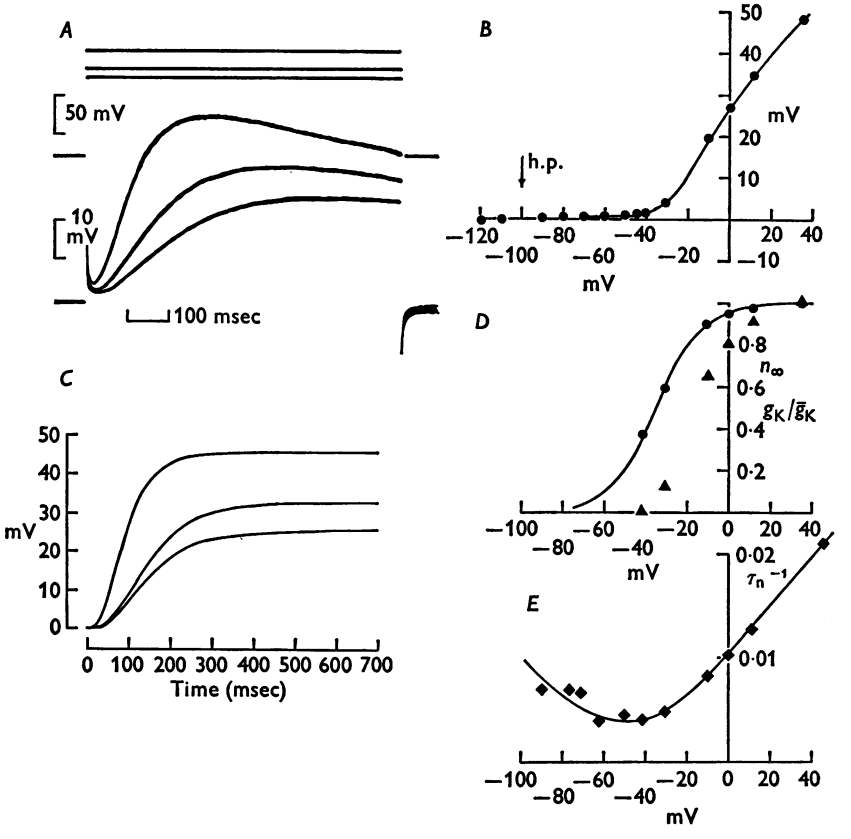


Fig. 3. Properties of the delayed potassium conductance in a fibre immersed in a hypertonic solution containing 0·1 mM zinc. Fibre resting potential, −99 mV; holding potential (h.p.), −100 mV; inter-electrode distance ($l$), 125 $\mu$m; T°, 4° C; tetrodotoxin, $10^{-6}$ g/ml.

*A*, tracings of records of membrane potential ($V_1$) and membrane current ($V_2 - V_1$). The depolarizations are to +36, +12 and 0 mV.

*B*, current–voltage relation for the delayed potassium conductance. Ordinate: membrane current in terms of $V_2 - V_1$ (mV); abscissa: membrane potential, $V_1$ (mV). The arrow at −100 mV marks the holding potential.

*C*, curves representing the currents in *A*, after subtraction of leak currents and capacitative transients, modelled according to eqn. (3) of the text. The time constants, $\tau_n$ are 47·3, 78·2 and 98·4 msec.

*D*, $n_\infty$ relation for this fibre. Ordinate: $n_\infty$ or $g_K/\bar{g}_K$; abscissa: membrane potential, $V_1$ (mV); ●, $n_\infty$; ▲, $g_K/\bar{g}_K$. The line joining the points (●) is given by eqn. (4) of the text, where $V_{\frac{1}{2}n} = 35$ mV and $kT/z'e = 11$ mV. Fibre $\bar{g}_K$: 9·0 mmho.cm$^{-2}$; $V'_{K_1}$, −85 mV.

*E*, relation between $\tau_n^{-1}$ and membrane potential. Ordinate: $\tau_n^{-1}$ (msec$^{-1}$); abscissa: membrane potential, $V_1$ (mV). The line is drawn by eye.

Fibres with small and slowly developing potassium currents that Adrian *et al.* (1970*a*, their Fig. 6) found occasionally were never seen in the hypertonic chloride solutions used here. Such fibres were occasionally found in the hypertonic solution containing gluconate as the impermeant anion. The reason for the occasionally deleterious effect of hypertonic solutions containing impermeant anions on the delayed rectifier is obscure.

Similar results were obtained at room temperature, where the maximum potassium conductance, $\bar{g}_K$, was $37{\cdot}4 \pm 7{\cdot}1$ mmho.cm$^{-2}$ (mean $\pm$ S.E. of mean, six fibres). Here the fibre diameter was assumed to be 40 $\mu$m, $R_1$ to be 220 $\Omega$ cm, and $V'_{K_1}$ to be $-75$ mV.

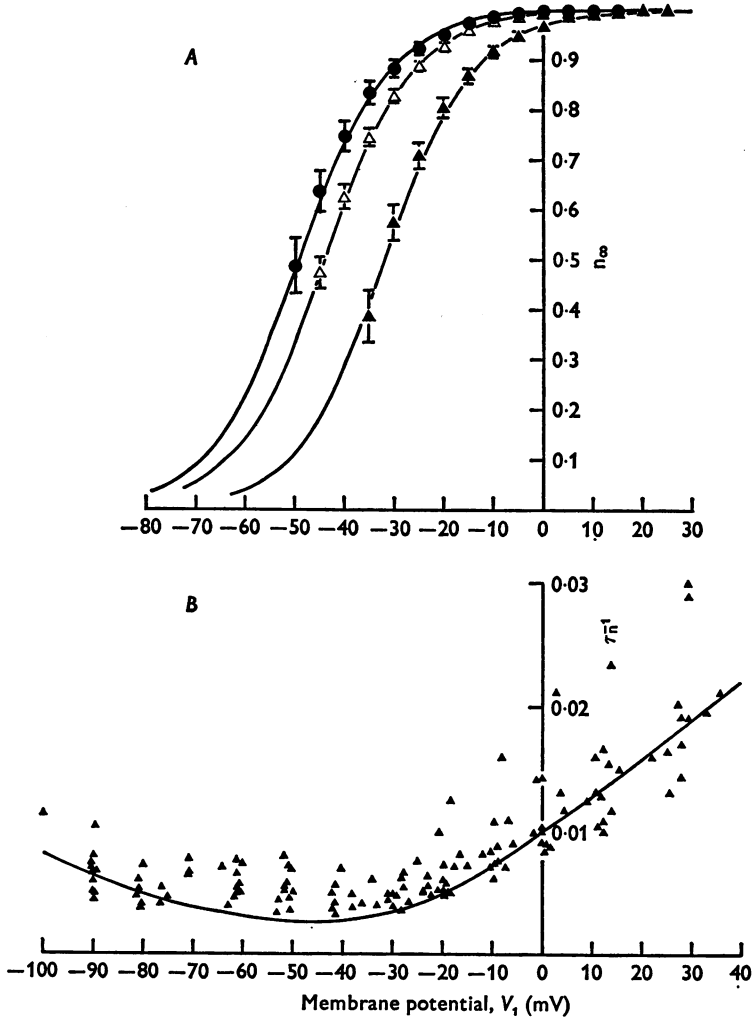


Fig. 4. For legend see facing page.

*Experiments in solutions containing zinc ions*

In summary, the effects of zinc are to reduce the maximum potassium conductance and radically to slow the rate of onset of the potassium currents. The relation between conductance and membrane potential is also shifted to more positive membrane potentials. As described in a later section, zinc ions probably do not have much effect on the slow potassium conductance described by Adrian *et al.* (1970*b*) and called by them $I_{K_2}$.

Fig. 3 summarizes the results from a muscle fibre immersed in the hypertonic Ringer solution containing zinc acetate at a concentration of 0·1 mM. The layout of the Figure is similar to that of Fig. 1. Fig. 3*A* shows tracings of current records obtained in response to depolarizing pulses to +36, +12 and 0 mV. The time scale is five times slower in Fig. 3*A* than in Fig. 1*A*, and it may be seen that the currents turn on more slowly. The current–voltage relation for the fibre is plotted out in Fig. 3*B*, plotting maximum ionic current against voltage.

The currents shown in Fig. 3*A* are S-shaped in the way eqn. (3) predicts and are therefore plotted in Fig. 3*C* according to this equation with time constants, $\tau_n$, of 47·3, 78·2 and 98·4 msec when the fibre was depolarized to +36, +12, and 0 mV respectively.

In Fig. 3*E*, the reciprocals of $\tau_n$ are plotted out against membrane potential. The scale on the ordinate is ten times greater than in Fig. 1*E*: the time constants are increased about tenfold in 0·1 mM zinc, at least at positive membrane potentials.

Fig. 3*D* shows the relations between both $g_K/\bar{g}_K$ and also $n_\infty$ and membrane potential for the fibre in 0·1 mM zinc. The value of $V_1$ where $n_\infty$ is 0·5 is −35 mV in this fibre, compared to −49 mV in the fibre of Fig. 1.

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Fig. 4. *A*, $n_\infty$ relations for fibres in control hypertonic solution, and solutions containing zinc at concentrations of 0·01 and 0·1 mM. Ordinate: $n_\infty$; abscissa: membrane potential, $V_1$ (mV); ●, control experiments; △, experiments in 0·01 mM zinc; ▲, experiments in 0·1 mM zinc; vertical bars: ± S.E. of mean. The line joining the points (●) is given by eqn. (4) of the text. $kT/z'e$ = 9 mV in each case, and $V_{\frac{1}{2}n}$ = −49 mV in the control solution, −44 mV in 0·01 mM zinc, and −32 mV in 0·1 mM zinc.

*B*, relations between $\tau_n^{-1}$ and membrane potential in fibres in a solution containing 0·1 mM zinc (▲). Ordinate: $\tau_n^{-1}$ (msec$^{-1}$); abscissa: membrane potential, $V_1$ (mV). The line through the points is given by the sum of two rate constants $\alpha_n$ and $\beta_n$, given by eqns. (5) and (6) of the text, where $\bar{V}_n$ = −28 mV, $\bar{\alpha}_n$ = 0·00032 msec$^{-1}$ and $\bar{\beta}_n$ = 0·00133 msec$^{-1}$. Thus 0·1 mM zinc reduces the rate constants for the opening and closing of the potassium conductance to 10% of their value in the control solution. The shift in potential dependence of $\alpha_n$ and $\beta_n$ has been taken to be the same as that for the $n_\infty$ relation.

The fibre of Fig. 3 had a $\bar{g}_K$ of 9·0 mmho.cm$^2$, with the reversal potential of the currents, $V'_{K_1}$, being $-80$ mV. The mean values for $\bar{g}_K$ and $V'_{K_1}$ in fourteen fibres in 0·1 mM zinc were $7·3 \pm 0·8$ mmho.cm$^2$ (mean $\pm$ S.E. of the mean) and $-74·6 \pm 1·5$ mV (mean $\pm$ S.E. of the mean) respectively. The value for $\bar{g}_K$ is significantly lower than that in the control solution ($P < 0·001$), while there is no significant difference between the values for $V'_{K_1}$ in the presence or absence of zinc ($P = 0·33$). Values for $\bar{g}_K$ and $V'_{K_1}$ in other concentrations of zinc (0·002 and 0·01 mM) are given in Table 1.

Fig. 4 summarizes the two other effects of zinc in addition to that on $\bar{g}_K$. Fig. 4*A* gives $n_\infty$ relations for fibres in the control solution and in 0·01 and 0·1 mM zinc. It may be seen that these relations are shifted significantly to more positive membrane potentials by zinc ions. The relations are fitted by the formula of eqn. (4), modified only to account for the shift: thus the value of $V_1$ where $n_\infty$ is 0·5 is $-49$ mV in the control solution, $-44$ mV in 0·01 mM zinc, and $-32$ mV in 0·1 mM zinc. In 0·002 mM zinc $n_\infty$ was 0·5 at $-47$ mV. These values are also given in Table 1. In none of the zinc concentrations was the apparent effective valency of the gating particle altered from its value of 2·65 in the control solution.

The shift in $n_\infty$ relation may appear to conflict with the finding of Kao & Stanfield (1970) who show zinc (at 0·05 mM) does not alter the 'threshold' for delayed rectification. But it does not necessarily do so if the slow potassium conductance also has a threshold, at about $-50$ mV, which is not altered by zinc.

Fig. 4*B* plots out values for the reciprocal of $\tau_n$ found in fourteen fibres in 0·1 mM zinc. The points representing experiments in zinc are consistently lower than in the control experiments. This appears to be the most significant effect of zinc in its slowing of the repolarization of the action potential.

The line in Fig. 4*B* is drawn according to eqns. (5) and (6), with $\bar{V}_n = -28$ mV, $\bar{\alpha}_n = 0·00032$ msec$^{-1}$ and $\bar{\beta}_n = 0·00133$ msec$^{-1}$. Thus 0·1 mM zinc is assumed to reduce the rate constants for the opening and closing of the potassium conductance to 10 % of their values in the control solution. The potential dependence is taken to have been shifted 17 mV to more positive values.

The records of current obtained in intermediate concentrations of zinc usually did not turn on exactly in the way that eqn. (3) would predict: they were not convincingly fourth order. Fig. 5 shows such records from two fibres, one in 0·002 and one in 0·01 mM zinc. In the lower concentration (Fig. 5*A*) the currents rose sharply at first and then more slowly towards a maximum. Fitting the sharper part of the current by eqn. (3) yielded values for $\tau_n$ little different from those in the control solution. In

0·01 mM zinc (Fig. 5*B*), the current records, often looked to be second or even first order; they also often had a discontinuity on the rising phase of the current, as if some of the potassium channels were turning on at their

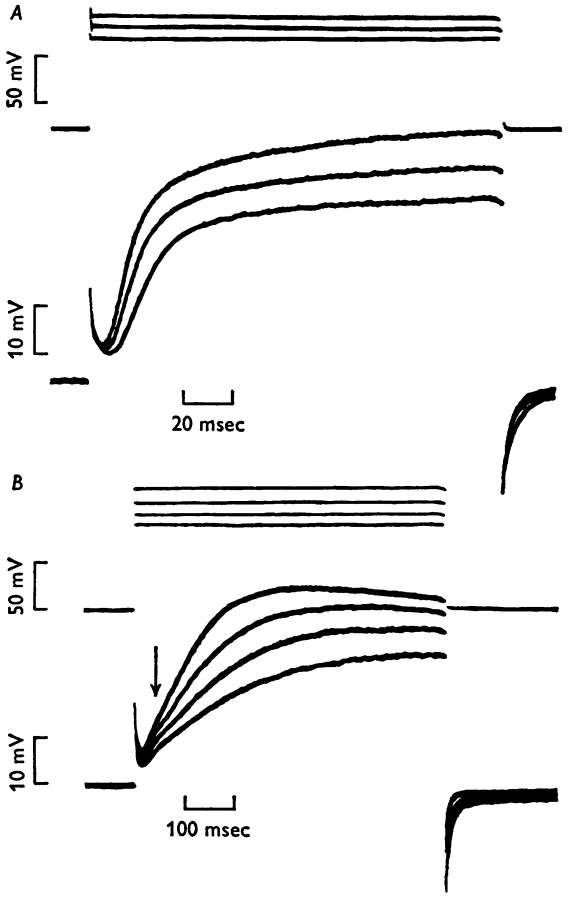


Fig. 5. Delayed potassium currents in muscle fibres in 0·002 and 0·01 mM zinc.

*A*, tracings of records of membrane potential, $V_1$ (above) and of membrane current in terms of $V_2 - V_1$ (below) from a fibre immersed in a solution containing 0·002 mM zinc. The depolarizations are to +20, +9 and −1 mV. Fibre resting potential: −85 mV; holding potential, −100 mV; inter-electrode distance (*l*), 125 $\mu$m; T°, 4° C; tetrodotoxin, $10^{-6}$ g/ml.

*B*, tracings of records of membrane potential, $V_1$ (above), and of membrane current in terms of $V_2 - V_1$ (below) from a fibre immersed in a solution containing 0·01 mM zinc. The depolarizations are to +27, +15, +2 and −8 mV. The vertical arrow shows the slight hump on the rising phase of the three smaller currents. Fibre resting potential, −93 mV; holding potential, −100 mV; inter-electrode distance (*l*), 125 $\mu$m; T°, 4° C; tetrodotoxin, $10^{-6}$ g/ml.

normal rate. Even in 0·1 mM zinc, the records were not always convincingly fourth order.

The records of Begenisich & Lynch (1974) show a similar result (their Fig. 4), though these authors do not comment on the effect. In skeletal muscle two factors may contribute to the apparent alteration in the onset of the potassium currents. One is that the turn-on of the slow potassium conductance, which is little altered by zinc, alters the apparent onset of the faster conductance when that is slowed. This factor clearly plays no part in the case of the squid axon since the slow conductance is not present there. As it turns out, results presented in a later section (p. 726, Fig. 7) suggest that there is little error caused by the presence of the slow conductance in skeletal muscle.

The second and more interesting possibility is that zinc ions bind to molecules that control the gating of the potassium conductance, as Begenisich & Lynch (1974) suggest: if one zinc ion binds to one gating molecule, the molecule might well exist either in an altered state, with rate constants for the opening and closing of the gate reduced, or in an unaltered state, with normal rate constants. The possibility that this happens will be dealt with further in the discussion section. However, the finding that the $n_\infty$ curve is unaltered in shape in 0·01 mM zinc suggests that zinc ions alter the potential dependence of the opening and closing of all gating particles.

A further possible source of error needed to be investigated. The three-electrode clamp does not achieve perfectly uniform control of membrane potential over the region of membrane – approximately over the end $3l/2$ in length of the fibre – where current is measured. Agents that reduce the conductance reduce the amount of decrement of potential towards the end of the fibre. Adrian *et al.* (1970*a*) provide clear evidence that this does not cause error in measurements of steady-state conductance, even when the membrane current–voltage relation is non-linear. However the amount of decrement of potential towards the end of the fibre might affect the apparent time-dependence of the currents and reducing the conductance might increase the measured value of $\tau_n$.

One method of testing this might be to control the potential at either $V_2$ or $V_1$ and see how the apparent $\tau_n$ is altered. A better way, which was adopted in the present experiments, is to inactivate the potassium currents by repeatedly depolarizing the fibre in the control solution with pulses of about 100 msec duration, and see whether $\tau_n$ is altered. In a fibre pulsed to +17 mV every sec for one min, the potassium conductance fell by 78 %. The apparent $\tau_n$ did increase, but by only 8·3 %. It follows that this problem is not a serious source of error either in these experiments or in those described in a previous paper (Stanfield, 1970).

In experiments at room temperature, $\bar{g}_K$ was $23{\cdot}9 \pm 2{\cdot}0$ mmho.cm$^{-2}$ (mean ± S.E. of mean, eight fibres) in 0·1 mM zinc, and $20{\cdot}6 \pm 3{\cdot}2$ mmho.cm$^{-2}$ (mean ± S.E. of mean, nine fibres) in 0·5 mM zinc. The fibre diameter was assumed to be 40 $\mu$m, $R_i$ to be 220 $\Omega$ cm, and $V'_K$ to be −75 mV. The $n_\infty$ curve is shifted about 8 mV towards more positive membrane potential

levels in 0·5 mM zinc. A similar reduction in rate constants for the turn-on of the currents is found at room temperature in the presence of zinc, though the analysis was not carried out in as much detail as for the experiments done in the cold.

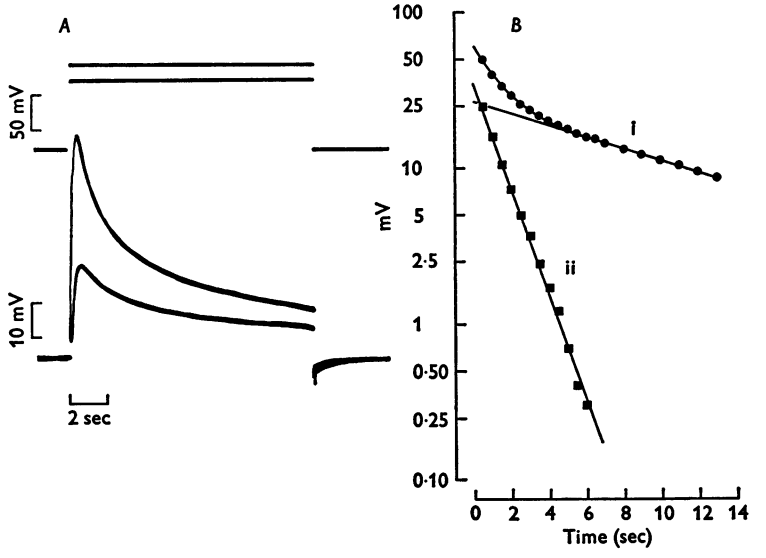


Fig. 6. Inactivation of delayed potassium currents.

*A*, tracings of records of membrane potential, $V_1$ (above) and of membrane current in terms of $V_2 - V_1$ (below) from a fibre immersed in a solution containing 0·01 mM zinc. The depolarizations are to +21 and −1 mV.

*B*, measurement of time constants for inactivation for the depolarization to +21 mV. Ordinate: $V_2 - V_1$ (mV) on a logarithmic scale; abscissa: time (msec) after the start of the depolarization; ●, membrane current, in terms of $V_2 - V_1$, after subtraction of leak current. The curved line through the points is the sum of two exponentials with time constants of 1·3 and 11·6 sec; (i) straight line gives slower exponential process; ■, membrane current, in terms of $V_2 - V_1$, after subtraction of the slowly inactivating current given by the straight line in (i). The straight lines were drawn by eye.

Fibre resting potential, −81 mV; holding potential, −100 mV; interelectrode distance (*l*), 125 μm; T°, 4° C; tetrodotoxin, $10^{-6}$ g/ml.

*Inactivation of potassium currents.* It is well known that the delayed potassium currents of skeletal muscle inactivate (Nakajima, Iwasaki & Obata, 1962; Adrian *et al.* 1970*a*; Stanfield, 1970). The effect of zinc ions on the inactivation of the potassium currents has been examined in 0·01 mM zinc. Detailed results come from only one fibre and the effect of depolarizing this fibre to +21 mV for 13 sec is shown in Fig. 6. The current inactivates, but at 4° C the inactivation has not run to completion

even after 13 sec. Adrian *et al.* (1970*a*) have shown that the fast currents ($I_{K_1}$) do inactivate almost completely, but felt (Adrian *et al.* 1970*b*) that the slow currents ($I_{K_2}$) did not. However, in fitting the currents of Fig. 6, it has been assumed that both conductances inactivate fully, and the current at the completion of inactivation has been predicted by scaling the currents obtained during small hyperpolarizing pulses. Fig. 6*B* shows the currents obtained, after subtracting the predicted current at the end of inactivation, plotted on a log scale against time.

As described before (Stanfield, 1970), the inactivating current is very well fitted by the sum of two exponentials which in this fibre, at this potential, have time constants of 1·3 and 11·6 sec. The longer time constant probably corresponds to the inactivation process for the slow component of the potassium current. The faster time constant will approximate that for the inactivation of the fast component of potassium current, though it may over-estimate it slightly, since at short times the rapid decline of current would contain both decreasing fast and increasing slow components. Nevertheless the fast component decays, in zinc, as quickly as the currents described by Adrian *et al.* (1970*a*) in fibres whose delayed rectifier was subjected to no pharmacological treatment. In their experiments, the time constants for inactivation were between 1·5 and 3 sec at +10 mV and 3° C. It therefore seems likely that, although zinc has substantial effects on the conductance and its activation (Table 1, Fig. 4*B*), there is very much less alteration in the rate of inactivation: probably zinc does not affect this process at all.

The faster time constant was 1·45 sec and 1·5 sec when the depolarizing pulse takes $V_1$ to −1 and −22 mV respectively. The slower time constant was 12·1 sec and 14·2 sec in each case.

In another fibre where $V_1$ was pulsed to +20 mV, the time constants were 1·3 sec and 8·2 sec respectively.

In assessing $\bar{g}_K$ (Table 1), no allowance was made for the inactivation of the potassium conductance. But even in 0·1 mM zinc, where the potassium currents take 200–300 msec to reach their peak, the error will be reasonably small – of the order of 10–15 %. Since the presence of the slow conductance may lead to an over-estimate of $\bar{g}_K$ in fibres whose delayed rectifier is slowed, it did not seem worth while to attempt to correct for the inactivation process, especially as it was not possible to measure the rate of inactivation in every fibre at every potential.

*Zinc ions and the slow potassium conductance*

The experiments to be described in this section were undertaken with the aim of assessing whether the presence of the slow potassium conductance might affect the measurements of the increase in $\tau_n$ in the presence of zinc ions. The results suggest that the presence of this conductance does not lead to any substantial error. Within the limits of the method used to

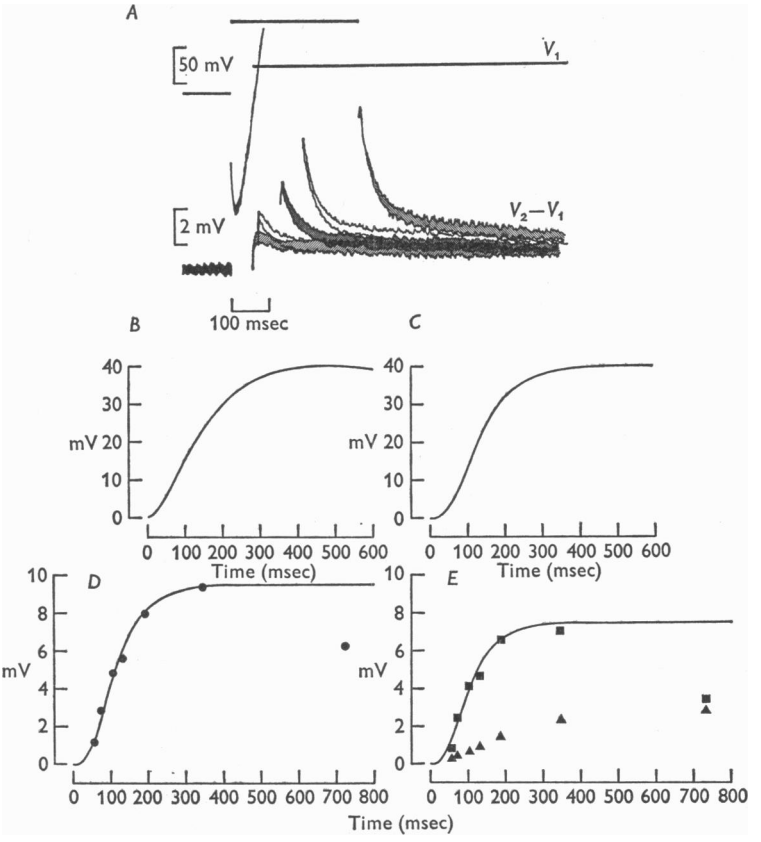


Fig. 7. Fast and slow currents in the presence of zinc ions (0·01 mM).

*A*, tracings of records of membrane potentials, $V_1$ (above) and membrane current in terms of $V_2-V_1$ (below). The larger depolarization, whose duration was varied between 56 and 722 msec during the course of the experiment, took the membrane potential, $V_1$, to +3 mV. The smaller depolarization, which followed the larger one immediately, took the membrane potential to −60 mV.

*B*, plot of the current (after subtraction of leak current and capacitative transient) flowing during the larger pulse in *A*. Ordinate: $V_2-V_1$ (mV); Abscissa: time (msec).

*C*, fit of current in *B* by eqn. (3) of the text. $\tau_n$, 70 msec; ordinate, $V_2-V_1$ (mV); abscissa, time (msec).

*D*, ●, ionic current flowing at start of second pulse; ordinate, $V_2-V_1$ (mV); abscissa, time (msec). The line is drawn according to eqn. (3) of the text, with $\tau_n$ = 59 msec.

*E*, ■, current flowing through the delayed potassium channel at the start of the second pulse; ▲, current flowing through the slow potassium channel at the start of the second pulse. Ordinate, $V_2-V_1$ (mV); abscissa, time (msec). The line is drawn according to eqn. (3) of the text, with $\tau_n$ = 54 msec.

Fibre resting potential, −97 mV; holding potential, −100 mV; inter-electrode distance (*l*), 125 μm; *T*°, 4° C; tetrodotoxin, $10^{-6}$ g/ml.

record the amount of slow conductance present in muscle fibres and its time course, zinc appears to be without effect on this slow potassium permeability mechanism.

Fig. 7 shows an experiment using two pulses to investigate the development of both fast and slow conductances during a large depolarizing pulse in a fibre immersed in a solution containing 0·1 mM zinc. The fibre is depolarized during the first pulse ($V_A$) to +3 mV for a varying period and is then repolarized during the second pulse ($V_B$) to −60 mV for several hundred msec before being repolarized to the holding potential of −100 mV. Two min rest was allowed between each experimental record to permit recovery of the conductance from inactivation. When the fibre is repolarized to −60 mV, an outward tail of current is recorded which shuts off approximately as the sum of two exponentials. One of these has a time constant of 24 msec ($\tau_n$ = 96 msec) and the other of about 335 msec. These two tails correspond to the shutting off of the two conductances. Fig. 7*A* shows the tracings of the current tails that flow during the second pulse. In Fig. 7*B* is plotted the current that flows during the first depolarizing pulse ($V_A$ alone), after subtraction of leak and capacitative transient. In Fig. 7*C* is a fit of this current by eqn. (3): as described before (p. 722) the fit is not particularly good in fibres in 0·01 mM zinc, for the modelled current has a longer delay and steeper rise than does the real current. The time constant, $\tau_n$, of the current drawn in Fig. 7*C* is 70 msec.

In Fig. 7*D* is plotted out the initial amplitude of the sum of the fast and slow tails as a function of the duration of $V_A$. The line through the points is drawn according to eqn. (3), with a time constant, $\tau_n$, of 58·8 msec. It is not clear why this should appear to be faster than the current of Fig. 7*B*.

In Fig. 7*E*, the fast and slow tails are separated and the fast conductance may be seen to turn on in 200–300 msec. The slow conductance turns on over 600–700 msec. Fitting the initial amplitudes of the fast tails by eqn. (3) yields a value for $\tau_n$ of 53·6 msec. This is slightly faster than the value obtained in Fig. 7*D*. However, it is not different enough to suggest that the presence of the slow conductance has led to any substantial overestimate in $\tau_n$ in the presence of zinc.

Assuming reversal potentials for the fast and slow conductances of −75 and −85 mV respectively, the fast and slow conductances are approximately 11·6 mmho.cm$^{-2}$ and 2·6 mmho.cm$^{-2}$. The latter figure suggests that the slow conductance is not reduced by zinc. Values for the slow conductance from this and five other fibres in 0·01 mM zinc have a mean of $1{\cdot}25 \pm 0{\cdot}29$ mmho.cm$^{-2}$.

Mean values for the time constant for the turn-off of the slow conductance at about −60 mV are 530 msec in the control solution, and

495 msec and 472 msec at a similar potential in 0·01 and 0·1 mM zinc respectively. Further, zinc appears to be without effect on the reversal potential of the slow permeability system. The values for this reversal potential, $V'_{K_2}$, are $-85{\cdot}3 \pm 1{\cdot}6$ mV (eleven fibres) in the control solution, and $-83{\cdot}7 \pm 1{\cdot}5$ mV (ten fibres) and $-82{\cdot}0 \pm 1{\cdot}9$ mV (ten fibres) in 0·01 and 0·1 mM zinc respectively. Neither of the values in the zinc solutions is significantly different from that in the control solution ($P > 0{\cdot}33$ and $= 0{\cdot}20$, respectively).

In one or two fibres, an attempt was made to study the onset of the slow potassium permeability by repolarizing the fibre to about $-140$ mV as well as to $-60$ mV as was done in the case of the fibre of Fig. 7. As Adrian *et al.* (1970*b*) point out, repolarizing to $-140$ mV is the better way of looking at the slow conductance since the fast conductance turns off very rapidly at such potentials, and does not interfere with the measurements. In such experiments, the slow permeability appeared to turn on more slowly when it was measured by repolarizing to $-140$ mV, and usually appeared to be still increasing after about 750 msec, while it appeared to begin declining after such times when it was measured by repolarizing to $-60$ mV.

One explanation for this effect is that the conductance lies at least partly within the T-system, as Adrian *et al.* (1970*b*) suggest, so that potassium accumulation there alters the driving force on potassium ions. Unfortunately the results are not complete enough to allow any useful guess as to the fraction of this conductance that is present in the T-system.

## DISCUSSION

The results of the present paper show that the major effect of zinc ions on the delayed potassium currents ($I_{K_1}$) of skeletal muscle is radically to slow their onset. In 0·1 mM zinc, the rate constants for the opening and closing of the gating mechanism of the channels fall to about one tenth their control values. There is also a reduction in the maximum conductance, and a shift in the potential dependence to more positive membrane potentials. Zinc ions appear not to alter the effective valency of particles considered to gate the potassium conductance, and they have no effect on the reversal potential of the currents. The rate of inactivation of the currents is probably also unaltered. At the concentrations of zinc used, there appeared to be little effect on the slow potassium currents ($I_{K_2}$), though this could be measured only indirectly.

Zinc ions alter the appearance of the turn-on of the delayed potassium currents ($I_{K_1}$) at concentrations of 0·002 and 0·01 mM zinc. Often the currents appeared second or even first order in their turn-on in 0·01 mM zinc, and the currents often turned on in a discontinuous way. This kind

of behaviour is perhaps likely in any non-saturating concentration of zinc, since at equilibrium between zinc ions and their binding site, presumably on a molecule that controls the gating of the channel, it is unlikely that all channels will be equally affected.

Two hypotheses may be considered. One is that a number of zinc ions bind to each gating molecule and the degree of slowing depends on the number of ions bound. In saturating concentrations of zinc, a slowing so profound might be expected, that there would be little turn-on of conductance. This hypothesis cannot be rejected on the basis of the experiments of this paper, because of the reduction in $\bar{g}_K$ seen in zinc. But two findings make it less attractive. First, since an effect on the onset of the currents is seen in 0·002 mM zinc, the range of concentrations used might be expected to cover most of the range of that effect, yet the slowing in 0·1 mM zinc is only to about 10% of the control value. Secondly, if a number of ions bind to the gating molecule, a change in the slope of the $n_\infty$ relation might be expected. Although the value (2·65) for the effective valency of the charged particle moving in the membrane depends on the correctness of the model used to fit the currents, the result that zinc does not alter its value is likely to be correct for other models.

The second hypothesis is simpler and is that one zinc ion binds to one gating molecule and, as a result, alters the rate constants for the opening and closing of the gate. Molecules that do not have a zinc ion bound are supposed to be unaltered, except that the potential dependence of the rate constants $\alpha_n$ and $\beta_n$ is supposed to be shifted to more positive values of membrane potential. This supposition is necessary because the $n_\infty$ relation in 0·01 mM zinc has the same shape as in 0·1 mM zinc (where the rate constants of nearly all the gates are supposed to be slowed) except that its potential dependence is shifted to a lesser extent. If the potential dependence of rate constants that were not slowed was not shifted, the $n_\infty$ relation would have a complex shape, and would represent the sum of an element of conductance that had an $n_\infty$ relation the same as that found in fibres in the control solution and an element with an $n_\infty$ relation the same as that in the presence of 0·1 mM zinc. But the hypothesis is broadly similar to Hille's that agents like tetrodotoxin and TEA block ionic channels only if a molecule of the agent is bound (Hille, 1967, 1968).

It will be supposed that zinc slows the rate constants $\alpha_n$ and $\beta_n$ to one tenth their control values. The altered time constant of eqn. (3) will be termed $\tau'_n$, and the fraction of altered gates in the open position will be termed $n'$.

Eqn. (2) may be rewritten

$$g_{K_0} = n^4 \bar{g}_{K_0}$$

to describe the time-dependent conductance ($g_{K_0}$) of that element of the

delayed potassium system that is made up of channels in an unaltered state. $\bar{g}_{K_0}$ is the maximum conductance of this group of channels.

If the idea of four independent gates is followed further, there may be taken to exist groups of potassium channels having either 0, 1, 2, 3 or 4 altered gating molecules per channel in non-saturating concentrations of zinc. The conductance of any group may be written

$$g_{K_x} = n^{(4-x)}n'^x\bar{g}_{K_x}, \tag{7}$$

where $x$ is 0, 1, 2, 3 or 4.

To describe the turn-on of the conductance in the form of eqn. (3), that eqn. must be modified to

$$g_{K_x} = g_{K\infty,\,x}[1-\exp(-t/\tau_n)]^{(4-x)}\,[1-\exp(-t/\tau'_n)]^x, \tag{8}$$

where $g_{K\infty,x}$ is the peak conductance at a given potential for that group of potassium channels having $x$ altered gates.

If the number of potassium channels in the membrane is large, as is likely from Hille's estimate of the upper limit of the conductance of a single channel (Hille, 1970), the number of channels having 0, 1, 2, 3 or 4 altered gating molecules may be predicted by simple probability theory. Thus if half the gating molecules are in an altered state, 6·25% of the channels will be unaltered, and 25, 37·5, 25 and 6·25% of channels will have one, two, three or four altered gating molecules respectively. In general the time-dependent conductance will be given by the equation

$$g_K = \bar{g}_K\,\{p_0n^4+p_1n^3n'+p_2n^2n'^2+p_3n\,n'^3+p_4n'^4\}, \tag{9}$$

where $p_0$, $p_1$, $p_2$ etc. give the probabilities of channels having zero, one, two etc. altered gates, $g_K$ and $\bar{g}_K$ are the time-dependent and maximum conductances for the muscle fibre, and $\bar{g}_Kp_0n^4 = g_{K_0}$; $\bar{g}_Kp_1n^3n' = g_{K_1}$; etc.

Fig. 8 shows the currents of the fibres of Fig. 5 fitted (after subtraction of leak current and capacitative transient) by eqns. (8) and (9). The values chosen for the time constants $\tau_n$ and $\tau'_n$, are given in the legend of Fig. 8: the values for $\tau_n$ are within the range of the control values. In computing the curves of Fig. 8, it has been assumed that 10% of the gating molecules are in the altered state in the fibre in 0·002 mM zinc, and 50% in the fibre 0·01 mM zinc. It may be seen that the model outlined above fits the currents rather well. If the relation between zinc concentration and the number of altered gating molecules is a rectangular hyperbola of the conventional kind, the value for $K_1$ for zinc binding to the gating molecule might be between 0·01 and 0·02 mM – similar to that for other related effects of zinc (Isaacson & Sandow, 1963; Mashima & Washio, 1964; Stanfield, 1973).

The hypothesis has the weakness that it does not account either for the reduction in conductance seen in zinc, or for the shift in its potential

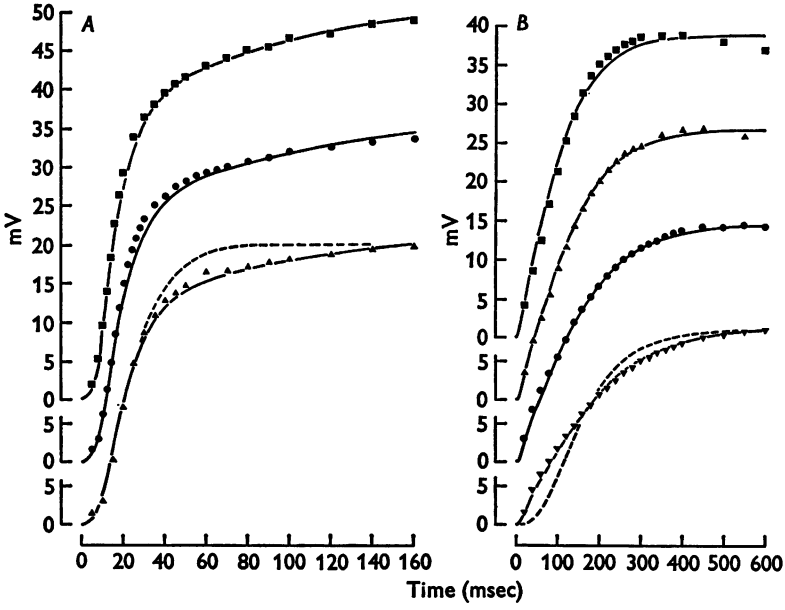


Fig. 8. Delayed potassium currents fitted by a model that assumes that zinc ions slow the rate constants for opening and closing of the gating mechanism of the potassium channels tenfold. It is also assumed that where gating molecules have not bound zinc ions, because the zinc concentration is not saturating, the gates open and close at unaltered rates, except that there is a shift in potential-dependence. The currents are those of Fig. 5.

*A*, currents from the fibre of Fig. 5*A* (0·002 mM zinc). The continuous lines were computed assuming that 10 % of the gating molecules are in an altered state, owing to binding of zinc, and thus, by simple probability theory, that 65·61, 29·16, 4·86, 0·36 and 0·01 % of channels have 0, 1, 2, 3 and 4 altered gates per channel. ■, current (after subtraction of leak current and capacitative transient) flowing when the fibre was depolarized to +20 mV; the continuous line is computed assuming an unaltered time constant, $\tau_n$ of 8·15 msec, and altered time constant, $\tau_n'$ of 81·5 msec. The model predicts that the current is 93 % complete at the end of the 160 msec pulse. ●, current flowing during a depolarization to +9 mV; $\tau_n$, 8·50 msec; $\tau_n'$: 85·0 msec. Current 93 % complete at end of pulse. ▲, current flowing during a depolarization to −1 mV; $\tau_n$, 10·0 msec; $\tau_n'$, 100·0 msec. Current 94 % complete at end of pulse. Dashed line: fourth order fit of current, with $\tau_n$ = 12·5 msec.

*B*, currents from the fibre of Fig. 5*B* (0·01 mM zinc). The continuous lines are computed assuming that 50 % of the gating molecules are in an altered state, owing to binding of zinc, and thus that 6·25, 25, 37·5, 25 and 6·25 % of channels have 0, 1, 2, 3 and 4 altered gates per channel. ■, current flowing during a depolarization to +27 mV; $\tau_n$, 7·26 msec; $\tau_n'$, 72·6 msec. ▲, current flowing during a depolarization to +15 mV; $\tau_n$, 8·85 msec; $\tau_n'$, 88·5 msec. ●, current flowing during a depolarization to +2 mV; $\tau_n$, 10·24 msec; $\tau_n'$, 102·4 msec. ▼, current flowing during a depolarization to −8 mV; $\tau_n$, 12·20 msec; $\tau_n'$, 122·0 msec. Dashed line: fourth order fit of current, with $\tau_n$ = 81·3 msec. Ordinates: $V_2 - V_1$ (mV); abscissa: time (msec).

dependence. The latter could be a simple surface charge effect of the kind first proposed for the effects of calcium on excitability (Frankenhaeuser & Hodgkin, 1957). However, Begenisich & Lynch (1974) found a shift in the same direction as that found here when zinc was applied inside the squid axon, not outside, and which cannot therefore be explained in a similar way. But they point out that their shift could have been caused by a small uncompensated series resistance: they did not measure reversal potentials for the delayed currents. As pointed out on page 730, it is assumed here that the potential dependence of the rate constants of all gates is shifted.

The hypothesis of Armstrong & Hille (1972) is that the activation gate of the potassium channel of the frog node of Ranvier is towards the inside of the membrane, and that there is presumed to be a selectivity filter or pore (Hille, 1973) outside of this. The potassium channel of skeletal muscle may be of similar design, though the pore cannot be identical in structure since the permeability ratio $P_{Na}/P_K$ is 0·03 for skeletal muscle (Adrian *et al.* 1970*a*) while it is less than 0·01 for frog node (Hille, 1973) and since TEA may well affect the kinetics of the delayed potassium conductance when applied from the outside (Stanfield, 1970), while it does not do so in the frog node of Ranvier (Hille, 1967). But, even if the pore diameter is much the same in muscle as in nerve (about 3 Å according to Hille, 1973) zinc ions, with an ionic radius of 0·74 Å (Robinson & Stokes, 1965), are at any rate small enough to pass through. Thus the effects on kinetics and conductance could be different ones – zinc binding at some external site to block conductance. The affinity for zinc at this external site would need to be lower than that on the gating molecule to account for the approximately 60 % reduction in $\bar{g}_K$ in 0·1 mM zinc. It may be noted that zinc appears not to alter the potassium conductance when applied inside the squid axon (Begenisich & Lynch, 1974).

This last finding meets a further possible objection to the model used to fit the currents of Figs. 5 and 8, that since zinc blocks the movements of the charges in the squid axon that are considered to gate the sodium conductance (Armstrong & Bezanilla, 1974) it might be supposed to do the same to the potassium channel. Begenisich & Lynch (1974) showed that zinc did very much reduce the sodium conductance – 10 mM zinc reduced it by more than 90 % – so it may be argued that the effect of zinc on the sodium channel is to bind the gating mechanism in the closed position (there being no increase in resting conductance). Such an explanation predicts a large effect on conductance, but no effect on the time course of the currents. In fact little effect was found: in 1 mM zinc the time to peak of $I_{Na}$ was 20 % slower on average, and 2 % faster in one fibre. It is suggested here that zinc has the effect of blocking the opening of the gating mechanism of the sodium conductance of the squid axon, but slows the gating mechanism of the potassium channel. It should be emphasized though that in skeletal muscle, zinc applied outside has little or no effect on the sodium conductance, as pointed out in the introduction.

In summary, the results of the present paper show that zinc helps distinguish between two potassium conductances that turn on when the muscle fibre membrane is depolarized. This effect of zinc may be added to the evidence from study of the physiological properties of the two systems (Adrian *et al.* 1970*a*, *b*) and from the different effects of TEA upon them (Stanfield, 1970).

The main effect of zinc on the delayed potassium conductance is to produce an approximately tenfold slowing of the rate constants for opening and closing of the gating mechanism of the conductance. This is probably achieved by a reaction between zinc ions and a molecule, probably a protein (Armstrong, Bezanilla & Rojas, 1973; Begenisich & Lynch, 1974), which controls the gating of the potassium channel.

The author is grateful to the Royal Society for a grant for equipment; to Mr R. H. Cook and Mr W. Smith for technical assistance; to Dr R. H. Adrian and Dr W. Almers for helpful discussion during the course of the experiments; and to Dr R. A. Chapman for his comments on the manuscript.

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