The K^+ -sensitive Hyperpolarization of Rat Skeletal Muscle and Metabolic Inhibitors

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Abstract The electrogenic contribution of Na-pump to the resting potential was investigated in isolated “Na-rich” rat soleus muscles exposed to K-free medium for more than 4 hr at 37°C. When the resting potentials of fibres fell to around -75 mV in the K-free medium, the addition of K^+ to the bathing medium evoked a transient hyperpolarization. The K^+ -sensitive hyperpolarization was reproducible in repeated K^+ challenged at 45 or 60 min cycles. The K^+ -sensitive hyperpolarization was completely abolished by cooling, by adding ouabain, or by substituting Li^+ for Na^+ , and reduced by low and high pH. DNP and hypoxia had little effect on the K^+ -sensitive hyperpolarizations.

The resting potential of “Na-loaded” and “K-depleted” soleus muscles (“Na-rich” SOL) of hypokalemic rat is described as the sum of the ionic diffusion potential predicted by either the Nernst equation or the “constant-field” equation and of the metabolic potential induced by an electrogenic Na-pump over a wide range of external K^+ concentration (AKAIKE, 1975a, b, 1976, 1979). According to DOCKRY *et al.* (1966), “Na-rich” rat skeletal muscles, which had been immersed overnight in a cold K-free Krebs, failed to excrete Na^+ in Krebs with 10 mM K^+ . However, the “Na-rich” SOL muscles obtained by exposing the muscle to a K-free Krebs containing 140 mM Na^+ for more than 4 hr at 37°C were transiently hyperpolarized on adding 5 mM K^+ to the K-free medium (Akaike and Kuraoka, in preparation). It was suggested that the K^+ -sensitive hyperpolarization resulted from operating an electrogenic Na-pump. However, the supporting evidence is incomplete in that it rests entirely on resting potential measurements after adding K^+ .

Therefore, the aim of the present study was to test, in the presence of metabolic inhibitors, whether an electrogenic Na-pump contributes to a transient K^+ -sensitive hyperpolarization of “Na-rich” SOL exposed to K-free medium for a long time.

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METHODS

The experiments were carried out on soleus muscles dissected from male Wistar rats weighing 140 to 200 g. The isolated muscles were immersed in K-free medium for at least 4 hr, and then the muscles were prepared for membrane potential measurements. Full details of dissection and potential measurements have been described previously (AKAIKE, 1975a).

The normal Krebs solution contained (mM): 135 Na^+ , 5 K^+ , 2.5 Ca^{2+} , 1.2 Mg^{2+} , 110 Cl^- , 1.2 $\text{H}_2\text{PO}_4^{2-}$, 0.4 HPO_4^{2-} , 28 HCl_3^- , 1.2 SO_4^{2-} , 2.5 gluconate $^-$ and 11 glucose. K-free Krebs was prepared by replacing K^+ by an equivalent amount of Na^+ . A rapid change in the external solution was made by flushing through about 30 ml of a test solution within 1 min. Then, the muscle was superfused at a rate of 2 ml/min. All solutions were bubbled with 95% O_2 +5% CO_2 , but with 95% N_2 +5% CO_2 in the experiments on hypoxia. The pH of test solutions was adjusted to 7.3 to 7.4 by adding sufficient HCl or NaOH.

The numerical values are given as the means $\pm$ 1 S.D. of the mean.

RESULTS

Resting potentials in K-free medium

On removing $[\text{K}]_o$ the fibres hyperpolarized slowly as seen in Fig. 1A, in which the maximal hyperpolarization was induced between 30 and 120 min after the beginning of exposure. Afterwards, the fibres depolarized gradually during the successive exposure to K-free medium. After 5 hr, the fibres in K-free medium depolarized to the steady-state potential of about -75 mV.

On adding 5 mM K^+ to the bathing medium while the fibres had hyperpolarized, there was a rapid depolarization. In contrast, the addition of 5 mM K^+ to the preparation exposed to K-free medium for 5 hr elicited a transient hyperpolarization (Fig. 1A). To know the relationship between the K^+ -sensitive depolarization or hyperpolarization and the magnitude of resting potential of muscles exposed to K-free medium, therefore, brief applications of 5 mM K^+ were repeated for the preparations exposed to K-free medium at various times during 6 hr. The results are summarized in Fig. 1B, in which the K^+ -sensitive hyperpolarization appears to occur at the depolarized potentials of less than about -90 mV and saturate at potentials of less than about -75 mV. Therefore, the following experiments about K^+ -sensitive hyperpolarization were made on preparations which had depolarized around -75 mV in the K-free medium; K^+ -sensitive depolarization appeared at resting potentials greater than -90 mV.

Repeated K^+ challenge on "Na-rich" muscles

As shown in Fig. 2, K^+ -sensitive hyperpolarizations were repeatable in the "Na-rich" SOL muscles exposed to a K-free medium for more than 4 hr. The magnitude of K^+ -sensitive hyperpolarization was reduced when the 5 mM K^+

Fig. 1. A: depolarization following hyperpolarization evoked in rat soleus muscles exposed to K-free medium with 140 mM Na^+ , and K^+ -sensitive depolarization or hyperpolarization. Upper line on graph A shows the experimental maneuver. Each point represents an average of 4-6 fibres of a muscle. Ordinate: potential (mV). Abscissa: time (hr). B: relationship between resting potential in K-free medium and K^+ -sensitive hyperpolarization or depolarization. Each point represents an average value of 4-10 fibres from individual muscles. Ordinate: K^+ -sensitive potential (mV). Abscissa: potential in K-free medium (mV).

Fig. 2. Effects of repeated K^+ challenges on K^+ -sensitive hyperpolarization. Each point indicates the mean value of resting potential of 16–19 fibres of 4 preparations. Vertical bars are ± 1 S.D. of the mean. Ordinate: potential in mV. Abscissa: time in hours.

challenge was repeated in 15 min cycles. However, the repeated K^+ challenges in 45 or 60 min cycles could elicit a hyperpolarization as rapid and large as that observed in the first K^+ challenge. From the experiment described above, the repeated K^+ challenge procedure at 45 or 60 min intervals was performed on “Na-rich” SOL muscles in the following experiments.

Effects of temperature

The muscles in this series were exposed to K-free medium for 4 to 7 hr, at which time “Na-rich” SOL fibres gave resting potentials of about -70 to -75 mV. During the long exposure to K-free medium, the preparations were immersed repeatedly in normal Krebs containing 5 mM K^+ at various temperatures for 15 min.

The “Na-rich” SOL fibres bathed in normal Krebs containing 5 mM K^+ at 37°C hyperpolarized to -96.3 ± 3.3 mV (38 fibres in 6 experiments). When the cold fibres were challenged with normal Krebs, however, the K^+ -sensitive hyperpolarization was markedly reduced (Fig. 3). At temperatures between 0 and 4°C the cooled fibres showed no hyperpolarization on adding 5 mM K^+ . It is also evident in the upper diagram of Fig. 3 that cooling hyperpolarizes the resting potentials of fibres exposed to the K-free medium although the effects were slight. These effects of cooling were completely reversible. The lower diagram of Fig. 3 shows the relationship between temperature and maximal membrane potential in medium containing 5 mM K^+ at various temperatures. It is clear in this figure that K^+ -sensitive hyperpolarization is completely abolished on cooling to around 4°C .

Fig. 3. Effects of cooling on K^+ -sensitive hyperpolarization. Upper panel: typical records from a preparation. Each point represents the mean value of 6–10 fibres. Vertical bars are ± 1 S.D. of the mean. Ordinate: potential (mV). Abscissa: time (hr). Lower panel: relationship between temperature and resting potential. Each point is the mean of maximal hyperpolarizations of the resting potentials of 25–30 fibres in 5 preparations measured in a medium containing 5 mM K^+ at different temperatures. Ordinate: potential (mV). Abscissa: temperature ($^{\circ}C$).

Effect of pH

The preparations were immersed first in K-free medium at pH 6.0, 6.5, 7.0, 7.5 or 8.0 for 15 min. Then, the "Na-rich" SOL muscles were exposed to normal Krebs having a pH the same as that of the K-free medium for 15 min. The maximal hyperpolarization was evoked in the medium at pH 7.0, but the K^+ -sensitive hyperpolarization became significant at either high or low pH as compared with that at pH 7.0 (Fig. 4A). These pH effects were reversible. The effects of various pH on the K^+ -sensitive hyperpolarization are summarized in Fig. 4B.

Fig. 4. Effects of various pH on K^+ -sensitive hyperpolarization. A: typical pH responses of K^+ -sensitive hyperpolarization. Each point represents the mean of 4-6 fibres of a muscle. Vertical bar represents ± 1 S.D. of the mean. Ordinate: potential (mV). Abscissa: time (hr). B: relationship between various pH and K^+ -sensitive hyperpolarizations. Different symbols show different preparations. Each point is an average value of 4-8 fibres. Ordinate: K^+ -sensitive hyperpolarization (mV). Abscissa: pH.

Fig. 5. Effects of metabolic inhibitors on K^+ -sensitive hyperpolarization. A: effect of ouabain. B: effect of DNP. DNP could not prevent the K^+ -sensitive hyperpolarization. Each point is the mean of 10 to 16 fibres in 4 preparations. The vertical bar for each point is ± 1 S.D. of the mean. Ordinate: potential (mV). Abscissa: time (hr).

Effects of ouabain and DNP

Addition of 0.05 mM ouabain to the K-free Krebs had no effect on the potentials of 'Na-rich' SOL fibres depolarized to around -70 to -75 mV in K-free medium but completely abolished the K^+ -sensitive hyperpolarization as seen in Fig. 5A. The inhibitory action of ouabain on the K^+ -sensitive hyperpolarization was dose-dependent, in which ouabain had a threshold concentration of around 0.001 mM for the inhibition. Such ouabain actions were rapidly reversed by washing the muscles with normal Krebs without ouabain. On the other hand, 2,4-dinitrophenol (DNP) had a slight inhibitory action on the K^+ -sensitive hyperpolarization at a high dosage of 0.1 mM (Fig. 5B).

Effect of Li^+

Replacement of NaCl in the bathing K-free Krebs by LiCl was made on the "Na-rich" SOL which had been exposed to K-free medium for more than 5 hr. In Li, K-free medium for 45 min, the resting potentials of fibres changed little, as seen in Fig. 6. At this stage, however, the hyperpolarization in response to adding 5 mM K^+ was completely abolished. Successively, a return to the K-free medium

containing Na^+ for 60 min brought about a full recovery of the K^+ -sensitive hyperpolarization.

Fig. 6. Effect of Li^+ on K^+ -sensitive hyperpolarization. Each point is the mean of 17–20 fibres in 4 preparations. Vertical bars represent ± 1 S.D. of the mean. Ordinate: potential (mV). Abscissa: time (hr).

Fig. 7. Effect of hypoxia on K^+ -sensitive hyperpolarizations. The fibres immersed in K^+ -free medium depolarized faster in low P_{O_2} (A) than in high P_{O_2} (B). However, the K^+ -sensitive hyperpolarization was not affected by low P_{O_2} . Each point represents the average value of 12–18 fibres in 4 preparations. The records were obtained from the individual paired companion muscles. Vertical bar is ± 1 S.D. of the mean. Ordinate: potential (mV). Abscissa: time (hr).

Effect of hypoxia

Paired muscles from the same rat were immersed in K-free medium bubbled with 95% O₂+5% CO₂ or 95% N₂+5% CO₂. By the change of gas mixture from 95% O₂+5% CO₂ to 95% N₂+5% CO₂ the oxygen tension (P_{O_2}) in the bathing medium fell from 600 to 15 mmHg within 15 min. Figure 7A and B show the results, in which the fibres depolarized faster in low P_{O_2} than in high P_{O_2} . However, the K⁺-sensitive hyperpolarization was little affected by the reduction in P_{O_2} .

DISCUSSION

It is well known that the K⁺-sensitive hyperpolarization is due to a transitory activation of an electrogenic Na-pump in various cells such as skeletal muscle fibres (KERNAN, 1962; DOCKRY *et al.*, 1966; AKIYAMA and GRUNDFEST, 1971; AKAIKE, 1975a), heart muscle (PAGE and STORM, 1965; GLITSCH, 1972; HIRAOKA and HECHT, 1973; NOMA and IRISAWA, 1974), smooth muscle (TAYLOR *et al.*, 1970; CASTEELS *et al.*, 1971), snail neurons (KERKUT and THOMAS, 1965) and non-myelinated nerve fibres (RANG and RITCHIE, 1968) and that the hyperpolarization is completely abolished by K-free medium, cooling and ouabain. In the present experiments, the K⁺-sensitive hyperpolarization was also abolished by cold, ouabain and replacement of external Na⁺ by Li⁺, and reduced by low or high pH. The results suggest an involvement of an electrogenic Na-pump in the generation of the K⁺-sensitive hyperpolarization in "Na-rich" SOL fibres. It may also be concluded that there is no essential difference in the activation mechanism of an electrogenic Na-pump between the "Na-rich" SOL muscles obtained by long exposure to K-free medium at 37°C and those obtained from hypokalemic rats (AKAIKE, 1975a).

According to HOLMES (1962), the post-tetanic hyperpolarization (PTH) of C-fibres in rabbits, rats and cats was irreversibly increased at a low pH medium (pH 6.4). He suggested that the facilitated hyperpolarization could be explained in terms of a Na-pump as H⁺ reduced the Na-K coupling. However, the K⁺-sensitive hyperpolarization of "Na-rich" SOL fibres in the present experiments was maximum at pH 7.0 from pH 6.5 to 8.0. This result appears to be supported by previous enzymatic observations that ouabain-sensitive Na⁺-K⁺ ATPase in the red cell, nerve and muscle of cat is fully activated around pH 7.0 to 7.2 (BONTING *et al.*, 1961).

It is well known that DNP, as well as other metabolic inhibitors such as ouabain, NaN₃, NaCN and N₂ gas (low P_{O_2}), can abolish the Na⁺ efflux from squid axon (HODGKIN and KEYNES, 1955; CALDWELL *et al.*, 1960a, b), the PTH in non-medullated nerve fibres of rabbit (RITCHIE and STRAUB, 1957) or in stretch receptor neuron of crayfish (NAKAJIMA and TAKAHASHI, 1966), and the hyperpolarization on rewarming "Na-loaded" cat heart muscle (TAMAI and KAGIYAMA, 1968). In the present experiments, however, the K⁺-sensitive and ouabain-sensi-

tive hyperpolarization in "Na-rich" SOL fibres of rats was little affected by DNP and hypoxia. HASHIMOTO (1964) obtained a similar result in recovering "Na-loaded" toad sartorius muscles in Ringer containing K^+ . However, further studies to determine whether these differences are due to differences in the tissue should be conducted.

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