Effects of potassium depletion and insulin on resting and stimulated skeletal rat muscle

R. Dengler, W. W. Hofmann and R. Rüdel

From the Institute of Physiology, Technical University of Munich, GFR, and Department of Neurology, Stanford University School of Medicine, and Veterans Administration Medical Center, Palo Alto, California, USA

SUMMARY The electrophysiological and metabolic responses to insulin of skeletal muscles from control and potassium-depleted rats were compared. Membrane potentials, action potentials, contraction parameters, as well as oxygen uptake were measured in diaphragm strips or intact extremity muscles from the two groups, and similar measurements were made in vivo. The muscles were examined in solutions with normal potassium concentration [K]₀, reduced [K]₀, and in normal [K]₀ with ouabain, in each case before and after insulin, 400 mU/ml. In normal solution, the depleted muscle contractions were weaker and slower than control. The depleted muscles, already having low potassium conductance, are paralysed by the further reduction of potassium conductance after insulin. Hyperpolarising effects of insulin-induced Na/K pumping are offset in the depleted muscles with a high sodium conductance and low [K]₀. Respiration is about normal at rest in depleted muscles, despite increased [Na]ᵢ, suggesting that the sodium is sequestered. After insulin, reduction of [K]₀, or ouabain plus insulin, the depleted fibres take up more O₂ than controls. In the presence of ouabain, this respiratory stimulation is believed to represent response to Ca++ influx. The K-depleted rat does not seem to be an entirely satisfactory model of the human disease hypokalaemic periodic paralysis.

The skeletal muscles of potassium-depleted rats develop a peculiar sensitivity to insulin when the extracellular concentration of potassium ions is lowered experimentally (Offerijns et al., 1958; Otsuka and Ohtsuki, 1965; Gordon et al., 1970; Kao and Gordon, 1975). The muscles, depleted by some weeks of a potassium-deficient diet, become wholly or partially paralysed after exposure to the hormone in test media containing from one-tenth to half of normal potassium concentration, and even direct electrical stimulation is without effect. Recent studies (Kao and Gordon, 1975) have shown that the muscle inexcitability is the result of insulin-induced depolarisation which is enough to inactivate the rapid sodium-carrying mechanism responsible for the upsweep of the action potential. This finding has been attributed to the fact that insulin decreases potassium conductance to the point where the membrane potential is now more affected by other ions and moves toward the sodium equilibrium potential (Kao and Gordon, 1975). It has been found that the contractile mechanism is able to work normally, providing that the surface membrane can be made excitable again by restoring extracellular [K], or by applying hyperpolarising electrotonic currents (Kao and Gordon, 1975). Such physiological changes are quite different from the responses of normal rat skeletal muscles to the same experimental conditions, where consistent hyperpolarisation (Zierler, 1957, 1959) and an increase of membrane resistance, without weakness, follow exposure to insulin.

The weakness caused by insulin in the depleted muscles resembles that seen in certain human disorders, particularly rapidly treated coma of diabetes mellitus, and in the familial disorder known as hypokalaemic periodic paralysis (Otsuka and Ohtsuki, 1970; Marble et al., 1973). Because of the known, powerful actions of insulin on movements of glucose and potassium out of the serum and into the muscle interior, earlier investigators concluded...
Effects of potassium depletion and insulin on resting and stimulated skeletal rat muscle

that the paralysis arose from lowered external potassium concentration, [K+]o (Grob et al., 1957; Shy et al., 1961; Pearson, 1964). Later experiments suggested that such factors as sodium permeability changes (Rieker and Bolte, 1966; Hofmann and Smith, 1970) or defective ion pumping played a role (Niall and Pak-Poy, 1966) and that the drop in [K+]o was only a secondary effect (Hofmann and Smith, 1970).

In the case of potassium-depleted rats, the experimental results have also been subject to various interpretations. While one group concludes that the effect of exogenous insulin stems from an increase in sodium permeability (pNa) (Rieker and Bolte, 1966; Hofmann and Smith, 1970), the results of others suggest that the fundamental change is an insulin-induced reduction of potassium conductance (gK) (Kao and Gordon, 1975). It is now clear that lowered extra- or intracellular potassium concentrations, alone, are not enough to cause the paralysis, and so other actions of insulin have become of increasing interest. In addition to its well-known metabolic effects, the hormone can hyperpolarise a normal muscle membrane, raise membrane ohmic resistance (Rm), increase intrafiber potassium, and decrease intrafiber sodium (Zierler, 1960; Creese and Northover, 1961; Zierler et al., 1966; Creese, 1968; Clausen and Kohn, 1977). Such changes are considered by Zierler (1960) and Zierler et al. (1966) to represent molecular alterations in the membrane, as a result of which ions are redistributed in different concentrations. Other workers explain the same basic findings as the result of stimulation of the active transport system for potassium and sodium (Creese, 1968; Moore, 1973).

The present experiments extend the range of observations on the mechanisms of insulin effects on potassium-depleted muscles. We have examined the hormone's action on the resting oxygen consumption of the test muscles in solutions with normal and decreased [K+]o. Our electrophysiological results confirm those of previous workers and we find, in addition, that the metabolic requirements of the depleted muscles are higher than control after insulin. We also find that the depleted muscles show changes in their contraction properties. The results suggest that the potassium-depleted rat is not an entirely adequate model of human hypokalaemic periodic paralysis.

Material and methods

Male Wistar rats were used in all experiments. Control animals averaged about 200–400 g in weight, while, after three to five weeks on a potassium-deficient diet, depleted rats of the same age averaged 100–150 g. The special food was provided by Ralston Purina Co, St Louis, Missouri (USA), and was made up as follows: casein 21.3%; sucrose 15.0%; solka floc 3.0%; multivitamins mixture 2.0%; mineral w/o K 3.0%; DL-methionine 0.15%; choline 0.2%; corn oil 5.0%; lard 5.0%; dextrin 43.35%.

The experiments were designed to permit both electrophysiological and metabolic measurements in vitro and parallel observations in vivo.

For in vivo studies, control and test rats were anaesthetised with sodium pentobarbitone, 35 mg/kg. Tracheotomy was performed, and the right internal jugular vein was cannulated. In one leg the sciatic nerve was cut near the pelvis, suspended on a pair of silver electrodes, and stimulated with single supramaximal shocks. The Achilles tendon was severed and connected to an isometric force transducer. In the other leg the skin and fascia over gastrocnemius were arranged to form a small pool filled with warmed physiological saline, so that muscle fibres could be maintained in good condition for microelectrode recording. In these preparations isometric twitch forces and membrane potentials could be recorded before and after intravenous administration of glucose or insulin. In vitro three different solutions were used. The basic medium, called "external potassium normal," (EKN), was made up to contain in mmol/l: NaCl 107.7; KCl 3.48; CaCl2 1.53; MgSO4 0.69; NaHCO3 26.2; NaH2PO4 1.67; Na gluconate 9.64; glucose 5.55; sucrose 7.6 (Bretag, 1969). The second medium, called "external potassium low," (EKL), was the same as EKN, except that the K+ concentration was reduced to from 0–0.5 mmol/l. The third, called "normal potassium, ouabain," (NKO), contained 10–3 mol/l g-strophanthidin diluted in EKN. The solutions were oxygenated with a mixture of 5% CO2 and 95% O2. Isometric twitch forces, membrane potentials, and action potentials were recorded in strips of diaphragm arranged in a small bath at 31±1°C. The ribs were fixed and the tendon connected to an isometric force transducer. The strips were adjusted so as to give directly stimulated twitches of maximal amplitude. After 20–30 min to allow equilibrium in a given solution, about 20 fibres were impaled with KCl-filled microelectrodes. Membrane potentials in surface and next underlying fibres as well as twitch forces were recorded before and after addition of insulin, 400 mU/ml. This same sequence was followed in each of the test solutions. In preparations from a single control rat and two potassium-depleted rats action potentials were also recorded. The purpose of these experiments was to learn if voltage transients had been altered by either the dietary depletion or by the insulin used (Insulin,
Hoechst, bovine; Hoechst AG, Frankfurt/Main, FRG), which contained a small amount of Zn++ (0.012–0.042 mg/100 U). For focal stimulation in these studies, small bipolar tungsten electrodes were used with which a few fibres could be stimulated selectively, thus reducing movement artefacts. The rate of rise and fall of the action potential was displayed simultaneously by means of a differentiator.

While diaphragm strips were being studied, the extensor digitorum longus (EDL) and soleus (SOL) muscles from both hind legs of the same rat were prepared in the same test solutions for measurements of oxygen consumption. Each muscle was fixed at resting length or slightly less on a small, plastic holder/stirrer disc which could be inserted into a 2.0 ml bath in a glass vial. The vial was itself immersed in a water jacket, which maintained the temperature of the test solution at 31°C±0.1°C. Underneath the water jacket chamber was a magnetic spinner, which caused the muscle-bearing disc to rotate and stir the solution at a rate of about 60/min. The fluid agitation and muscle movement were quite adequate to ensure mixing and diffusion of the dissolved oxygen. To provide full equilibration of the medium with room air, an identical stirrer disc without a muscle was left in the open vial for 10–20 min before replacement with the test preparation. After the muscle-bearing disc had been introduced, the polarographic oxygen electrode was immediately inserted, thus sealing the chamber for measurement of the declining po2 in the medium. The apparatus contained other test chambers, one of which was always prepared with the test solution of interest plus insulin, 400 mU/ml, the same hormone concentration as used for the diaphragms. After 20–30 min, or after the oxygen concentration in the medium had fallen to about 50% of control, the muscle was then transferred to the insulin-containing solution, which was at exactly the same temperature and fully equilibrated with air by means of its own stirrer, as above. Thereafter, the muscles were gently blotted once on each side, trimmed of their tendons, and weighed. The slopes of 12–20 minute, steady portions of the oxygen extraction curves were used to determine muscle consumption after subtracting the previously determined uptake by the electrode itself. Necessary corrections were made for ambient temperature and barometric pressure to estimate the initial volume of oxygen in the test fluids. Measurements were made of resting VO₂ and relative changes after addition of insulin or change of potassium concentration. After testing and weighing, pairs of EDL and SOL muscles from 10 depleted and three control animals were fixed in formalin and prepared for routine histological examination. Before dissection of the diaphragm for the in vitro studies, blood was obtained by cardiac puncture and serum K⁺ of control and depleted animals was determined on a flame photometer.

Results

IN VITRO EXPERIMENTS

Comparison of normal and potassium-depleted muscles in EKN

Resting membrane potentials (RMP) as measured in fibres from normal and potassium depleted rats before and after insulin in EKN are presented in Table 1. The RMP of fibres from potassium depleted rats before insulin averaged about 6 mV less than control, a difference which is not statistically significant. In normal muscle after insulin application, hyperpolarisation was evident (P<0.02), while the potassium depleted rat muscles showed no change. The failure of RMP measurements in potassium depleted rats to achieve statistical significance may be explained as a result of variable fibre response to the K-depletion itself. In fact, a wide scatter of values was seen in all electrophysiological measurements in potassium depleted rats in vitro (compare SD values).

Since the study focused on paralytic mechanisms, action potentials were compared in EKL and EKL insulin solutions (Table 2). K-depleted rat fibres showed reduced amplitude and slowing of depolarisation. With insulin, these differences from control were increased. Repolarisation was not significantly changed in potassium-depleted rat muscle until after insulin, when it slowed. The response in normal fibres were not changed by insulin except for an increase in amplitude and faster rise velocity, both of which could be explained as a result of an enhanced function of the fast Na⁺-mechanism during hyperpolarisation. There was no evidence of a Zn++ effect on the time course of the action potential (Stanfield, 1975). Prolonged duration, caused by inhibition of the delayed K⁺ outward current did not develop in normal fibres with the concentration of Zn in

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of diaphragm membrane potentials in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals</td>
<td>n¹</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>5 EKN</td>
<td>82.8±2.8</td>
</tr>
<tr>
<td>5 EKL</td>
<td>92.7±2.9</td>
</tr>
<tr>
<td>2 NKO</td>
<td>55.5±3.8</td>
</tr>
<tr>
<td>Depleted</td>
<td></td>
</tr>
<tr>
<td>5 EKN</td>
<td>76.9±6.8</td>
</tr>
<tr>
<td>8 EKL</td>
<td>72.0±12.5</td>
</tr>
<tr>
<td>1 NKO</td>
<td>52.3±2.9</td>
</tr>
</tbody>
</table>

Values are means ± SD in mV; n¹=number of rats; n²=number of fibres tested; NT=not tested.
Effects of potassium depletion and insulin on resting and stimulated skeletal rat muscle

Table 2  Comparison of diaphragm fibre action potential parameters in vitro

<table>
<thead>
<tr>
<th>Animals</th>
<th>n1</th>
<th>Solution</th>
<th>Resting membrane potential</th>
<th>Amplitude</th>
<th>Duration</th>
<th>dV/dt Rise</th>
<th>dV/dt Fall</th>
<th>n²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
<td>EKL</td>
<td>96.3±46.0</td>
<td>114.3±5.1</td>
<td>0.93±0.05</td>
<td>720±37</td>
<td>268±20</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>EKL+insulin</td>
<td>95.2±4.0</td>
<td>108.8±9.2</td>
<td>0.96±0.04</td>
<td>702±48</td>
<td>248±22</td>
<td>10</td>
</tr>
<tr>
<td>Depleted</td>
<td>2</td>
<td>EKL</td>
<td>80.6±7.5</td>
<td>92.6±9.4</td>
<td>1.13±0.14</td>
<td>683±93</td>
<td>211±26</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>EKL+insulin</td>
<td>65.3±9.4</td>
<td>57.7±21.8</td>
<td>1.55±0.54</td>
<td>344±155</td>
<td>126±69</td>
<td>13</td>
</tr>
</tbody>
</table>

n¹ = number of rats; n² = number of fibres in which action potentials could be elicited. Tests in insulin made after 30–60 min of exposure. Membrane and action potentials in mV. Duration measured at half amplitude, in ms. Rise and fall velocities in V/s.

400mU/ml of the insulin used. The Figure illustrates typical results in diaphragm preparations tested in vitro in reduced [K⁺]o solution.

Mechanical responses of the diaphragm strips are shown in Table 3. The time courses of the twitches of the directly stimulated K-depleted rat preparations were clearly prolonged both in contraction and relaxation. It is of interest that the average twitch tension per gram of muscle was actually slightly higher in the depleted specimens. We have no explanation at present for the slight fall of twitch tension in both normal and K-depleted muscles after insulin. In neither the normal nor the prolonged K-depleted muscle twitches did insulin change during the time course.

Metabolic activity in normal and depleted leg muscles was assessed indirectly by comparing oxygen uptake rates under various conditions (Table 4). The values shown were all obtained from resting muscles at the same temperature and under the same ionic conditions used for the electrophysiological measurements in diaphragm. The oxygen consumption in soleus (SOL) and extensor digitorum longus (EDL) should thus indicate internal adjustment to experimental conditions but has nothing to do with contraction. The depleted muscles, weighing on the average about a quarter to one-third of controls and having a much more favourable volume/surface ratio, used no more oxygen per gram of tissue than normal muscles in EKN solution. This finding suggests that abnormal intracellular concentrations of K⁺ and Na⁺ (Offerjns et al., 1958) do not, themselves, cause greater fuel consumption in these muscles. The cells appear to be well adjusted, both

![Figure](http://jnnp.bmj.com/)

**Figure**  Representative action potentials from normal and potassium-depleted rats in reduced [K⁺]o and insulin. All tracings were obtained in vitro and in EKL solution. Records in B and D, 30 min after introduction of insulin into bath at 31.0±0.1°C. Direct stimulation, see text.
electro-physiologically and metabolically, at rest and in normal \([K^+]_0\).

Table 4 compares \(\dot{V}O_2\) of normal and K-depleted fibres in vitro and in EKN solution before and after insulin. The great majority of measurements were made from 20–30 min after exposure to the hormone, or at a time when insulin-receptor interactions would have produced their usual effects, both at the surface membrane (Mullins and Awad, 1965; Erlij and Grinstein, 1976) and on various intracellular energy transfer mechanisms (Buse et al., 1965; Wool, 1965). The respiratory stimulation observed thus represents the sum of all the metabolic activation, from which no single process can be isolated with this technique, but the parameter of interest in these experiments was the relative change produced by the hormone in each of the two muscle groups. It is clear that the depleted EDL muscles began to produce more oxygen after insulin than controls (see, however, Zierler et al., 1966), while K-depleted SOL tended to decrease its respiration. On the other hand, when \([K^+]_0\) was reduced (EKL), soleus \(\dot{V}O_2\) in KDR was more strongly stimulated than that of EDL. These changes may result from the major enzymatic and other differences between the two muscle types. While the insulin-receptor interaction, which is limited to the surface membrane (Levine, 1965; Schudt et al., 1976) produces little or no effect on the polarisation of K-deficient muscles, it is nevertheless able to activate intracellular energy exchanges. The metabolic response thus suggests that membrane receptor activation is still able to release intracellular "messenger" (Kissebah et al., 1975). When ouabain was added to the normal potassium solution (NKO), the EDL muscles of control animals respired as if there had been no change, while those of potassium-depleted rats required considerably more oxygen. Soleus seemed unaffected in both groups. After insulin in this medium the average response of EDL and SOL of both groups of animals was up by about a third, EDL lagging behind SOL in the K-depleted rats. It should be noted, however, that the fractional rise in \(\dot{V}O_2\) after insulin in K-depleted fibres was superimposed on an already elevated base level. Thus, the alkaloid, in concentration far above that required to block active \(Na^+\)/K+ transport, still permits a metabolic response to insulin. The one ion which may have increased its intracellular concentration in this medium is calcium (Glynn, 1964).

Comparison of normal and potassium-depleted muscle in EKL

In EKL the differences between control and depleted fibres became more pronounced. Normal muscles were hyperpolarised by about 10 mV (\(P<0.02\)), while the resting membrane potential of depleted muscles remained unchanged (Table 1). On this latter point, our findings disagree with Otsuka and Ohtsuki (1970) who recorded relative hyperpolarisation of depleted muscles in \(K^+\)-free solutions. The failure of K-depleted rat muscles to hyperpolarise here in EKL suggests that anomalous rectification is enough under these conditions to prevent reaching the expected new \(K^+\) equilibrium potential (Adrian and Freygang, 1962). Normal muscles remained hyperpolarised in this solution with insulin while the depleted muscles showed marked de-polarisation (Table 1).

When K-depleted fibres had been depolarised by insulin, the amplitude and maximal up-and-down-stroke velocity of the action potential decreased, prolonging the total time course. At this point, numerous K-depleted fibres proved to be electrically inexcitable. Table 2 compares the action potential parameters of the controls with the two K-depleted rat experiments. Action potential photographs of one experiment are illustrated in the Figure.

The results of twitch force measurements in EKL are also given in Table 3. As opposed to the response in EKN, depleted muscles became much weaker with insulin in EKL. Again, the wide individual variation, reduces the statistical significance of the pooled data, but the direction of change was consistent in each given preparation. As in EKN, the time to peak contraction and half relaxation of the depleted muscles was about twice that of controls. The difference was increased with insulin, especially the half decay time. The changes in the K-depleted

<table>
<thead>
<tr>
<th>Animals</th>
<th>Solution</th>
<th>Force Mean (mN±SD)</th>
<th>Mean %</th>
<th>Mean Force/mg</th>
<th>TTP (ms)</th>
<th>D/2 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>EKN</td>
<td>183 ± 47</td>
<td>100</td>
<td>2.63</td>
<td>25.3 ± 4.2</td>
<td>26.3 ± 11.9</td>
</tr>
<tr>
<td></td>
<td>EKN+insulin</td>
<td>155 ± 44</td>
<td>86</td>
<td>2.16</td>
<td>24.3 ± 5.1</td>
<td>29.0 ± 9.6</td>
</tr>
<tr>
<td></td>
<td>EKL</td>
<td>166 ± 71</td>
<td>100</td>
<td>1.54</td>
<td>24.0 ± 6.0</td>
<td>25.7 ± 11.0</td>
</tr>
<tr>
<td></td>
<td>EKL+insulin</td>
<td>138 ± 55</td>
<td>84</td>
<td>1.40</td>
<td>25.0 ± 3.0</td>
<td>31.0 ± 9.9</td>
</tr>
<tr>
<td>Depleted</td>
<td>EKN</td>
<td>153 ± 35</td>
<td>100</td>
<td>3.82</td>
<td>42.3 ± 6.9</td>
<td>59.0 ± 9.0</td>
</tr>
<tr>
<td></td>
<td>EKN+insulin</td>
<td>141 ± 37</td>
<td>90</td>
<td>3.09</td>
<td>42.5 ± 8.7</td>
<td>62.8 ± 14.2</td>
</tr>
<tr>
<td></td>
<td>EKL</td>
<td>97 ± 56</td>
<td>100</td>
<td>1.38</td>
<td>47.1 ± 6.4</td>
<td>55.9 ± 17.6</td>
</tr>
<tr>
<td></td>
<td>EKL+insulin</td>
<td>60 ± 36</td>
<td>65</td>
<td>0.92</td>
<td>50.9 ± 18.9</td>
<td>71.6 ± 36.2</td>
</tr>
</tbody>
</table>

Force in millinewtons (mN)±SD. TTP and D/2 in ms ± SD.
Effects of potassium depletion and insulin on resting and stimulated skeletal rat muscle

Table 4: Insulin effect on oxygen uptake by slow and fast muscles of normal and potassium-depleted rats

<table>
<thead>
<tr>
<th></th>
<th>Normal (mmol/l)</th>
<th>EDL (mmol/l)</th>
<th>SOL (mmol/l)</th>
<th>%Δ (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Potassium-depleted</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EKN†</td>
<td>148.8 ± 4.7</td>
<td>185.2 ± 2.2</td>
<td>200.1 ± 1.9</td>
<td>+34 ± 22</td>
</tr>
<tr>
<td>EKL†</td>
<td>148.3 ± 4.3</td>
<td>185.0 ± 2.3</td>
<td>200.0 ± 1.9</td>
<td>+34 ± 22</td>
</tr>
<tr>
<td>EK</td>
<td>148.0 ± 4.0</td>
<td>185.0 ± 2.0</td>
<td>200.0 ± 1.9</td>
<td>+34 ± 22</td>
</tr>
<tr>
<td>KCl</td>
<td>148.0 ± 4.0</td>
<td>200.0 ± 1.9</td>
<td>200.0 ± 1.9</td>
<td>+34 ± 22</td>
</tr>
<tr>
<td><strong>Control (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EKN‡</td>
<td>156.8 ± 2.2</td>
<td>185.2 ± 2.2</td>
<td>200.1 ± 1.9</td>
<td>+34 ± 22</td>
</tr>
<tr>
<td>EKL‡</td>
<td>156.8 ± 2.2</td>
<td>185.2 ± 2.2</td>
<td>200.1 ± 1.9</td>
<td>+34 ± 22</td>
</tr>
<tr>
<td>EK</td>
<td>156.8 ± 2.2</td>
<td>200.1 ± 1.9</td>
<td>200.1 ± 1.9</td>
<td>+34 ± 22</td>
</tr>
<tr>
<td>KCl</td>
<td>156.8 ± 2.2</td>
<td>200.1 ± 1.9</td>
<td>200.1 ± 1.9</td>
<td>+34 ± 22</td>
</tr>
</tbody>
</table>

Muscles, which are found before insulin, suggest that the dietary K⁺ depletion itself can also alter the contraction time course. Similar changes have been reported in cardiac muscle, where the abnormalities are thought to result from decreased Ca²⁺ binding by the sarcoplasmic reticulum (Harrison *et al.*, 1972; Sack *et al.*, 1974).

The oxygen uptake of the normal muscles rose by about 12% above that in EKN while, without any shift of the membrane potential, respiration was increased by nearly 50% in the others. With insulin in EKL, normal muscles raised their oxygen consumption by 10–11%, the depleted muscles by a further 25% or more (Table 4). It is of interest to contrast these metabolic responses of mammalian muscles to lowered [K⁺]₀ with those in elevated [K⁺]₀ where, despite depolarisation equal to or greater than that observed in KDR muscles after insulin, there is normally no increase in respiration (Hofmann, 1976). The insulin-induced depolarisation of the depleted muscles in EKL solution clearly calls forth a much more active metabolic response than is seen in normal muscles when the same or greater degree of depolarisation is produced by another mechanism. It thus seems unlikely that the change in resting membrane potential is, itself, the metabolic stimulant.

Both the reduction of resting potassium conductance in EKL solution and the depolarisation caused by insulin in this solution in K-depleted muscles could allow sodium and calcium ions to run down their gradients (Eckert and Lux, 1976). Enhanced oxygen requirements of the muscles may then reflect stimulation of either intracellular Na⁺- or Ca²⁺-dependent mechanisms (Sandow, 1965; Van der Kloo, 1967). As in normal [K⁺]₀ solution, this metabolic stimulation is greater in the potassium-depleted, sodium-loaded fibres.

**IN VIVO EXPERIMENTS**

In four control animals the resting membrane potential was 18.2 ± 1.7 mV (83 fibres). In two potassium-depleted rats, the value was 69.1 ± 2.9 mV (70 fibres). The serum concentration in normal animals averaged 5.3 ± 0.9 mmol/l and that in the K-depleted rats 2.3 ± 0.5 mmol/l. Resting membrane potentials in normal rats were unchanged after glucose (1.5–2.0 g/kg) but were raised after insulin by about 8 mV (133 fibres). This change corresponds with that reported above for in vitro preparations in EKN. As in vitro, the K-depleted fibres were depolarised before any treatment, and, after insulin, the resting membrane potential dropped to 61.8 ± 7.8 (71 fibres). The resting depolarisation in K-depleted fibres could be explained as a result of the observed hypokalaemia and effects of endogenous insulin. The
depolarising effect of glucose in these animals presumably represents a response to increased endogenous insulin. The injection of additional insulin gave no further depolarisation.

HISTOLOGICAL CHANGES
Normal and test muscles, including soleus, extensor digitorum longus, and diaphragm strips, were stained with haematoxylin and eosin and examined for evidence of gross structural abnormalities. The only consistent change noted in the potassium-depleted fibres was generalised atrophy. No sign of vacuolisation or swelling was seen, even in preparations which had been soaked for one or more hours in solutions containing low potassium with insulin.

Discussion
The basic electrophysiological abnormalities found by earlier workers in potassium-depleted muscles can be combined with the present data to give a more comprehensive picture of the total disturbance. To explain electrical inexcitability and depolarisation of these preparations in low \([K^+]_o\) and insulin, it is helpful to take as proven a few assumptions. First, anything causing a drop in potassium conductance \((g_K)\) produces an increase of the \(g_{Na}/g_K\) ratio, with movement of the membrane potential toward the sodium equilibrium potential \((E_{Na})\) (Adrian and Freygang, 1962; Adrian, 1969). This depolarisation, in turn, favours further movement of Na. Second, a loss of either external or internal potassium reduces \(g_K\) (Adrian, 1969). Third, insulin further reduces \(g_K\) (Kao and Gordon, 1975), while it stimulates ionic pumping (Creese, 1968; Moore, 1973). On the basis of such observations, the sensitivity of membrane potential in the depleted fibres to manipulation of \([K^+]_o\) or to insulin can be attributed in part to the already low potassium conductance. We confirm that the K-depleted fibres are slightly depolarised, even in normal \([K^+]_o\) (EKN) in vitro, and are further depolarised when in EKL solution. The in vitro findings are duplicated in the living potassium-depleted rat where the muscles were also slightly depolarised, presumably because of low \([K^+]_o\) and circulating endogenous insulin.

When insulin is added to normal fibres in EKN in vitro, they become hyperpolarised, while the depleted fibres do not. This difference could be explained in the following way: in both normal and depleted preparations insulin stimulates electrogenic ionic pumping (Gourley, 1961) but, because of the greater \(g_{Na}\) in the depleted muscles, the expected voltage change is neutralised. The effect of EKL in normal muscles is also to hyperpolarise, as predicted from the new K equilibrium potential. The same hyperpolarisation cannot develop in the K-depleted muscles, however, because of the lowered ratio \(K^+/K^+\). Any contribution to the resting membrane potential by the active transport mechanism (Frumento, 1965) will also be blocked in these preparations by the low \(K^+\). The depolarising action of insulin on K-depleted muscles in EKL as well as in vivo is explained as a result of a further reduction of \(g_K\) (Kao and Gordon, 1975). Under these conditions many fibres become inexcitable, leading to the observed decay in force. The question of interest is whether the present combined electrophysiological and metabolic studies support these inferences.

The respiratory studies were mainly carried out with limb muscles, but in a few experiments diaphragm strips were found to behave similarly and the results are taken to apply generally to skeletal muscles. What is of interest is that, despite a large internal load of sodium (Offerjns et al., 1958), the resting \(V_{O_2}\) of the potassium-depleted fibres was about the same as that of control muscle in normal \([K^+]_o\). In view of the much smaller size of the K-depleted preparations, their failure to take up more \(O_2\) per gram than control muscles at rest may actually indicate a relative reduction of \(V_{O_2}\). Since a rise of \([Na]^+\) is known to be one of the stimuli to muscle respiration (Keynes and Maisel, 1954), the Na accumulation in the K-depleted muscles, along with the maintenance of a nearly normal membrane potential in vitro suggests that the incorporated sodium exists partly in some bound form. Such compartmentalisation of intracellular sodium has been suggested in muscle by Zierler et al. (1960) and would not allow it to affect the membrane potential or resting \(V_{O_2}\). In any case, dietary K depletion, though it leads to complex shifts of intracellular ionic ratios, does not increase resting muscular fuel consumption, as long as the muscle is bathed in a medium with normal \([K^+]_o\).

Despite their normal respiration in EKN, the depleted EDL muscles responded to insulin, reduced \([K^+]_o\), and ouabain with a greater rise in oxygen consumption than controls. Soleus responses to these manipulations were generally in the same direction as EDL, but tended to be variable. The differences in \(V_{O_2}\) of EDL and SOL under the conditions of these experiments presumably reflect the differences of basic metabolic properties in fast and slow muscles.

The ouabain response is of some interest, as with lowered \([K^+]_o\) or a very high concentration of the glycoside, it would not be expected that the sodium/potassium exchange pump system could be more active (Sjödin, 1971). In fact, from the estimates that have been made of the fraction of resting muscle energy exchange required by the pump itself, it is
Effects of potassium depletion and insulin on resting and stimulated skeletal rat muscle

doubtful if even intense activation would account for the VO₂ increases noted here (Keynes and Maisel, 1954). Nevertheless, in both normal and K-depleted muscles, insulin respiratory stimulation continued after that caused by ouabain and was additive. The increased metabolic activity of the muscle cells in these tests is not likely to reflect simply active ion transport.

Since the [Ca++] of the cytoplasm is believed to regulate many metabolic activities in a resting muscle (Rasmussen and Goodman, 1977), the increased VO₂ after insulin or lowered [K+] in the depleting preparations suggests that the reduction of potassium conductance may have permitted Ca++ ions to run inward, down their electrochemical gradient. The fact that respiration is not increased in these muscles in EKN solution itself may indicate that the calcium which has accumulated during the depletion period has also been bound, perhaps at the inner surface of the plasma membrane. Under these conditions, insulin or depolarisation from any cause could act by releasing more Ca++ from a larger pool. Most of the elevated metabolism may be accounted for by an increased [Ca++] in the cytosol, with activation of any of several Ca++-dependent metabolic cascades (Kissebah et al., 1975; Schudt et al., 1976; Rasmussen and Goodman, 1977). Analysis of Ca++ exchange-ability in K-depleted, Na-loaded membranes after insulin will be of help in further clarifying this problem.

The somewhat slowed and weakened contractions of the potassium-depleted muscles in normal [K+] raise the question of defective excitation-contraction coupling. This, if Ca++ ions have been leaking inward, again suggests that sequestration of this ion may have occurred at some distance from the sarcoplasmic reticulum (Sandow, 1965).

A point of interest is the relationship between the effects of potassium-depletion tested here and the human muscle disease called hypokalaemic periodic paralysis. As previous workers have pointed out, there are numerous similarities, including increased intracellular [Na+] and decreased [K+] between paralytic attacks (Shy et al., 1961; Otsuka and Ohtsuki, 1965; Gordon et al., 1970; Hofmann and Smith, 1970), depolarisation plus inactivation of the fast Na mechanism during paralysis (Riecker and Bolte, 1966; Kao and Gordon, 1975) and sensitivity to both lowered [K+] and insulin (Offerjoins et al., 1958; Otsuka and Ohtsuki, 1965; Gordon et al., 1970; Kao and Gordon, 1975). There are, on the other hand, certain differences which require further investigation. In the potassium-depleted rat, the serum [K] is low continually, and there is no evidence that paralysis is preceded or accompanied by an uptake of potassium from the extracellular space. In hypokalaemic periodic paralysis the serum K+ remains normal between attacks and it is known that paralysis is associated in most cases with a remarkable shift of K ions into muscle, at which time serum [K] drops.

From the evidence at hand, it would appear that chronic potassium depletion so changes the electrophysiological properties of muscle membranes that other cations, such as Na+ and Ca++, move inward. The response is exaggerated by insulin, which leads to depolarisation inexcitability, and hypermetabolism. In hypokalaemic periodic paralysis, on the other hand, inexcitability is preceded by a large K+ uptake, and insulin seems to return electrical excitability towards normal (Hofmann and Smith, 1970).

There is no reason to expect that dietary depletion of K would change any particular aspect of the insulin-receptor interaction on a muscle membrane, though in K-depleted rat fibres the hormone may find more metabolic "coupler" (Ca++) available for release. In hypokalaemic periodic paralysis, however, the incomplete data currently available raise the question of an abnormality in the receptor itself, perhaps associated with excesses of insulin release. In this human disease, the large fluctuation of serum [K+] in response to glucose and insulin could be explained either as a result of increased glucose transport itself or energy-dependent K+ uptake in response to an induced Na+ influx. Measurements of membrane resistance, VO₂ and Ca++ fluxes before and after insulin in human muscle will be useful steps in answering these questions.

We dedicate this work with great respect and gratitude to Professor Albrecht Strupp, Director, Department of Neurology, Technical University, Munich, on his 60th birthday. We are deeply indebted to Mrs E. Koster for expert technical assistance. The work was supported by The Sander Stiftung, Technical University of Munich.

References


Effects of potassium depletion and insulin on resting and stimulated skeletal rat muscle.

R Dengler, W W Hofmann and R Rüdel

J Neurol Neurosurg Psychiatry 1979 42: 818-826
doi: 10.1136/jnnp.42.9.818

Updated information and services can be found at:
http://jnnp.bmj.com/content/42/9/818

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/