temperature changes and was not significantly different from the control values. However, the $V_{\text{max}}$ values for preparations from both muscular-dystrophy and carrier subjects were significantly higher than those for controls at all temperatures examined. Arrhenius plots of Ca$^{2+}$-ATPase activity also revealed an elevated enzyme activity at all temperatures (15–37°C) in preparations from both muscular-dystrophy and carrier subjects. The activation energy in all preparations above 20°C was 74.9 kJ/mol (17.9 kcal/mol). Below 20°C the activation energy in normal membranes was 114 kJ/mol (27.2 kcal/mol), but was elevated in preparations from muscular-dystrophy and carrier subjects to 130 and 133 kJ/mol (31.05 and 31.8 kcal/mol) respectively.

The results demonstrate an augmented activity of Ca$^{2+}$-ATPase activity in erythrocyte membranes from muscular-dystrophy patients. It appears that it is the $V_{\text{max}}$ for the reaction that is increased, while the affinity of the enzyme for its substrate appears unchanged. The characteristics of the temperature response of the enzyme preparations from muscular-dystrophy patients suggest that changes in the lipid domain of the membrane may be involved in the altered properties of the Ca$^{2+}$-ATPase.

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The effect of temperature on Na$^{+}$-stimulated and basal Ca$^{2+}$ efflux from cardiac and skeletal-muscle mitochondria

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The release of Ca$^{2+}$ from mitochondria is one source of the ion contributing to the steady-state concentration of free Ca$^{2+}$ in the cytosol. As the efflux increases, so does the cytosolic concentration of Ca$^{2+}$, with a concomitant increase in energy dissipation on account of Ca$^{2+}$ cycling. Certain myopathic conditions, malignant hyperthermia and the porcine stress syndrome, which render individuals and animals susceptible to volatile anaesthetics, muscle relaxants and stress, have been proposed to be associated with elevated sarcoplasmic Ca$^{2+}$ concentration (Briott, 1979; Gronert & Heffron, 1979) and increased Ca$^{2+}$ efflux from muscle mitochondria (Cheah & Cheah, 1979). Muscle of susceptible pigs exhibits a decreased mechanical threshold in the presence of volatile anaesthetics and succinylcholine (Gallant et al., 1979) suggesting increased Na$^{+}$ influx into the muscle fibres. This influx of Na$^{+}$ may then set off an energy-dependent cycling of Ca$^{2+}$ by mitochondria, leading to the characteristic hyperthermia. For this reason we have studied the effect of temperature on Ruthenium Red-insensitive Ca$^{2+}$ efflux from cardiac and skeletal-muscle mitochondria in the presence and absence of NaCl.

Mitochondria were prepared from rat hearts as described previously (Harris et al., 1979) and from the hindlimb muscles by digestion of the finely minced muscle with trypsin (1 mg/g) for 15 min at 0–2°C in a medium containing 0.15M-KCl, 2.5 mM-EGTA and 20 mM-Tris/HCl (pH 7.4). The digest was centrifuged at 10,000 g for 10 min; the pellet was rinsed and homogenized in 10 vol. of the same medium. Muscle mitochondria were isolated by the same centrifugal procedure as described previously.

Hepes-[4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid] (pH 7.4). Active uptake was then inhibited with 0.8 mM-Ruthenium Red (Moore, 1971) and Ca$^{2+}$ efflux was measured at a suitable sensitivity on the recorder. Calibrations by the internal-standard method were made several times during each run. Final efflux values were corrected for changes of sensitivity and for depletion of the Ca$^{2+}$ load as efflux proceeded. Cuvette temperature was measured with a thermistor.

Results are presented as an Arrhenius plot. Continuous lines were obtained by the least-squares method. Points joined by broken lines were omitted because they show temperature inactivation of efflux. Ca$^{2+}$ efflux was stimulated by 8 mM-NaCl. Cardiac mitochondria: $\bigcirc$, basal efflux; $\blacksquare$, Na$^{+}$-stimulated efflux. Skeletal-muscle mitochondria: $\bigcirc$, basal efflux; $\bullet$, Na$^{+}$-stimulated efflux.

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The efflux of Ca\(^{2+}\) from both cardiac and skeletal-muscle mitochondria loaded with 25 nmol of Ca\(^{2+}\)/mg of protein into a salt-free medium is shown in the form of an Arrhenius plot in Fig. 1. Basal efflux of Ca\(^{2+}\) from cardiac and muscle mitochondria increases as the temperature is raised from 17 to 41°C; the respective activation energies are 53 and 87 kJ/mol. Between 17 and 34°C Na\(^{+}\)-stimulated efflux of Ca\(^{2+}\) appeared to be only slightly dependent on temperature, as indicated by the low activation of 12 and 11 kJ/mol. At 37 and 41°C, Na\(^{+}\)-stimulated efflux from muscle mitochondria decreased dramatically compared with the efflux from cardiac mitochondria. Thus cardiac mitochondria seem to be able to resist increased temperature better than skeletal-muscle mitochondria. In the latter case there is an inverse relationship between basal and Na\(^{+}\)-stimulated Ca\(^{2+}\) efflux at the higher temperatures. The ability of Na\(^{+}\) to stimulate Ca\(^{2+}\) efflux appears to be contingent upon there being a low basal Ca\(^{2+}\) efflux. Even at low temperatures when the basal efflux has been stimulated, for example by thyroid hormone, the Na\(^{+}\)-stimulated efflux is diminished.

Increased intracellular Na\(^{+}\) could therefore initiate aerobic mitochondrial Ca\(^{2+}\) cycling in malignant-hyperthermia-susceptible muscle. But as the temperature increased Ca\(^{2+}\) cycling would become independent of the concentration of Na\(^{+}\).

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Transport and metabolism of choline in synaptosomes: energy requirement

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The uptake of low concentrations of choline (below 2 μM) into synaptosomes (commonly named ‘high-affinity uptake’ of choline, \(K_m\approx1 \mu\text{M}\)) has been suggested to be a cholinergic marker and a possible rate-limiting step in acetylcholine synthesis (Kuhar & Murnin, 1978). These suggestions have been of great importance since only choline acetyltransferase (EC 2.3.1.6, the enzyme responsible for the synthesis of acetylcholine) has been considered, and used, as a cholinergic marker and since the mechanism(s) regulating acetylcholine turnover is still unknown (Tücek, 1978).

Several recent findings have, however, indicated that non-cholinergic nerve cells and even non-nervous cells have a high-affinity uptake mechanism for choline and that no direct, or kinetic, coupling seems to exist between this uptake mechanism and the synthesis of the neurotransmitter (Massarelli, Kessler & Al-Shaikhaly, 1979). This led us to formulate the hypothesis that the transport of choline might be regulated by the endogenous metabolism of the choline pool (Massarelli, 1978). To support this hypothesis it was necessary to study some essential characteristics of the carrier mechanism responsible for the transport of choline. The experiments that are reported here have been performed with the aim of studying the energy dependence of the transport of choline into synaptosomal preparations.

Crude synaptosomal fractions from rat brain were obtained using standard procedures. Portions were preincubated in Krebs–Ringer phosphate solution containing 10 mM glucose (at a concentration of 0.4 mg/ml), pH 7.4, for 5 min at different temperatures and incubated with 1 μM-[Me-\(^{3}^{14}\)C]choline (sp. radioactivity 60 Ci/mol) for 1 min. The incubation was stopped by the addition of 0.1 mM-hemicholinium-3 (final concentration) and cooling in ice. The suspension was carefully layered on 0.32 M sucrose, centrifuged (5000 g for 20 min) and the sucrose was discarded. The pellet was homogenized in 0.4 M HCl (radioactivity was measured in portions of the homogenate to analyse the uptake) and rapidly freeze-dried. Ethanol (50%, 40 μl), containing 10 mM unlabelled carriers, was added to the dry material and portions (10 μl) were spotted on t.l.c. plates. The development was performed by the method of Marchbanks & Israel (1971). The spots containing \(^{14}\text{C}\)choline were afterwards scraped and the radioactivity counted in an Intertechnique SL 30 liquid-scintillation spectrometer with 10 ml of Formula 963 (New England Nuclear). The results, obtained over a range between 8 and 40°C, show that, similar to the results for neuroblastoma cells (Massarelli et al., 1979), the energy of activation shows more than one value after incubation with 1 μM-choline (Table 1). At physiological temperatures the energy of activation of the uptake of choline (30.0 ± 10.8 kJ/mol) was close to the value observed for the transport of choline, which was measured when the free choline compartment was analysed (26.3 ± 1.1 kJ/mol). After a breaking point at 26°C for the uptake and 23°C for the transport the second energy of activation had a value of 128.8 ± 18.0 kJ/mol for the uptake and 88.7 kJ/mol for the transport. The higher energy of activation found for the uptake reflects the energy required for the synthesis of acetylcholine (176.1 kJ/mol).

When synaptosomes were preincubated with unlabelled

<table>
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<tr>
<th>Uptake</th>
<th>Phosphocholine</th>
<th>Betaine</th>
<th>Choline</th>
<th>Acetylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.0 ± 10.8</td>
<td>31.6</td>
<td>24.0</td>
<td>26.3</td>
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<tr>
<td>128.8 ± 18.0</td>
<td>72.5</td>
<td>69.5</td>
<td>88.7</td>
<td>176.1</td>
</tr>
<tr>
<td>40.0 ± 9.6</td>
<td>26</td>
<td>20</td>
<td>23</td>
<td>25</td>
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</table>

**Table 1. Energies of activation of the uptake, transport and metabolism of choline in synaptosomes**

\(E_a\) is the energy of activation. The values are the averages of three (uptake) or two separate experiments. \(E_a\) values at 40–25°C and at 25–8°C are for incubation in 1 μM-[Me-\(^{14}\)C]choline and that at 40–6°C is for incubation in 100 μM-[Me-\(^{14}\)C]choline. For uptake, the breaking-point temperature refers to results for 1 μM-[Me-\(^{14}\)C]choline only.

**References**


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