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INHIBITION OF NA^+, K^+-ATPase OF INTACT MOUSE SOLEUS MUSCLE BY Mg^{++}

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SUMMARY: The effect of 10 mM $MgCl_2$ on the inhibition of respiration by ouabain was investigated with intact mouse soleus muscle preparations. Although ouabain caused a 19.7% inhibition of respiration of soleus muscle incubated in 1 mM $MgCl_2$ buffer, the response of respiration to ouabain was abolished upon incubation in buffer containing 10 mM $MgCl_2$. Initial respiration rates were significantly decreased in soleus muscle exposed to 10 mM, as contrasted to 1 mM, $MgCl_2$.

INTRODUCTION

Studies of active Na^+ transport conducted with intact muscle preparations have yielded conflicting results concerning the importance of Na^+, K^+-ATPase (Na^+, K^+-dependent adenosine triphosphatase EC 3.6.1.3.) as a component of cellular energy expenditure. Microcalorimetric studies of the ouabain-sensitive heat production of intact rat(1) and mouse soleus muscles(2) have led to the conclusion that active Na^+ transport accounts for no more than 6-8% of resting heat production. However, measurements of the ouabain-sensitive component of O_2 consumption of intact mouse soleus muscle have shown 14-22% of O_2 uptake to be due to the activity of Na^+, K^+-ATPase (3). In view of the key role proposed for Na^+, K^+-ATPase in thyroid thermogenesis (8), cold-induced thermogenesis (9), and as a primary mechanism in the development of obesity (10), it is imperative to resolve the current uncertainty concerning the physiological importance of the Na^+, K^+-ATPase in resting energy expenditure.

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The microcalorimetric determinations of the ouabain-sensitive heat production of mouse soleus muscle were conducted in a buffer containing 10 mM $MgCl_2$ to supress a secondary rise in heat production which occured following infusion with ouabain(1,2). However, previous investigators have found Na^+, K^+ -ATPase activity to be inhibited by high concentrations of Mg^{++} (4,6). Thus, it has been suggested (7) that inhibition by Mg^{++} of the Na^+ pump before challenge with ouabain may have contributed to the lack of inhibition of heat production by ouabain reported by Chinet et al.(1). The possibility that Mg^{++} will negate inhibition of respiration by ouabain was investigated in this study.

METHODS

Adult female mice, in the weight range of 20-25 g, were stunned by a blow to the head and bled from the neck. The intact soleus muscles were removed with care to minimize tissue damage following the procedure of Kohn and Clausen (5).

Measurement of ouabain-sensitive respiration of intact soleus muscle in control and experimental buffers.

The modified Krebs-Ringer HEPES buffer used as the incubation medium contained (mM): NaCl, 116.8; KCl, 5.9; $NaHCO_3$, 5.0; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $CaCl_2$, 1.0; HEPES (N-(2-hydroxymethylethyl piperazine-N'-2 ethanesulfonic acid), 10.0; $MgCl_2$, 1.0 (control) or 10.0 (experimental); glucose, 5.0; pH 7.3-7.4. Both soleus muscles were utilized from each mouse. Ouabain-sensitive respiration was measured in both the control and experimental buffers for each animal. To achieve thorough oxygenation, muscle preparations were individually equilibrated and incubated in 600 ml beakers containing 25 ml of control or experimental buffer in a shaking water bath at 37°C. Muscle preparations were equilibrated in either control or experimental buffer for 10-20 min and then transferred to the O_2 electrode chamber. O_2 consumption was measured with a YSI O_2 electrode for 10-15 min. The O_2 content of the buffer did not fall below 85% of the initial air-saturated level during the period of measurement. The muscle preparations were then incubated in control or experimental buffer containing 10^{-3} M ouabain for 45 min and O_2 consumption again measured. Muscle preparations from two mice were incubated in control or experimental buffers for 45 min without ouabain and found to maintain initial respiration rates throughout the incubation period.

Statistical Analysis

Respiration rates were compared between treatment groups by the unpaired Student's t-test.

TABLE 1. EFFECT OF $MgCl_2$ CONCENTRATION ON INHIBITION OF RESPIRATION OF INTACT MOUSE SOLEUS MUSCLE BY OUABAIN

$MgCl_2$ Concentration	n	Respiration[a] Initial	+Ouabain	Change(%)	
1.0 mM	12	4.65 ± 0.39	3.75 ± 0.29	-19.7	P<.001
10.0 mM	12	3.17 ± 0.21	3.37 ± 0.25	+6.1	N.S.

[a] $\mu l\ O_2$/ mg tissue dry wt/ hr (Mean ± SEM)

RESULTS AND DISCUSSION

The initial respiration rate of soleus muscle in the control buffer (4.65 ± 0.39 $\mu l\ O_2$/mg dry wt/hr)(Table 1), was higher than that measured previously for intact mouse soleus muscle (3.7 ± 0.10 $\mu l\ O_2$/mg dry wt/hr)(3), in a modified Krebs-Ringer bicarbonate buffer. The ouabain-sensitive component of the respiration of soleus muscle incubated in the control buffer was 19.7%, which is similar to the 14-22% inhibition of respiration reported previously for intact mouse soleus muscle (3). In contrast, using the experimental buffer, addition of ouabain resulted in a slight increase (6%) in the rate of O_2 consumption (Table 1). This finding is consistent with that obtained by Bond and Hudgins (4), in which progressive inhibition of red blood cell Na^+, K^+-ATPase was produced with increasing concentrations of Mg^{++} greater than 3.0 mM in a buffer with a high content of Na^+ and a low content of K^+. Thus, inhibition of respiration by ouabain is abolished in muscle tissue exposed to buffer containing 10 mM $MgCl_2$.

The respiration rate of soleus muscle in the experimental buffer was significantly lower ($p<.001$) than the initial respiration rate of soleus muscle in the control buffer. The

inhibitory effect of Mg^{++} on respiration was significantly greater ($p<.001$) than the inhibitory effect of ouabain on respiration. It is likely that high Mg^{++} is disruptive to other cell processes involved in energy transformations as well as to Na^+, K^+-ATPase although the mechanism of its inhibitory effect is not known.

We conclude that at least part of the lack of response to ouabain observed in microcalorimetric studies of active Na^+ transport in intact muscle preparations (1,2), was due to prior inhibition of the Na^+, K^+-ATPase by the high concentration of Mg^{++} included in the buffer rather than to an unimportant role of active Na^+ transport in the energy expenditure of physiologically intact muscle preparations.

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