GLYCOLYTIC ENZYME PROFILES IN THE ARRECTOR PILI MUSCLE AND THE SUBCUTANEOUS MUSCLE

COMPARISON BETWEEN SMOOTH AND SKELETAL MUSCLE*

MICHAEL J. C. IM, M.S., PH.D. AND KENJI ADACHI, M.D., PH.D.

Although modern biochemical techniques have done much to elucidate various metabolic processes in epidermis, data on the metabolism of skin appendages are scanty and limited. In particular, muscle structures in skin have never been subjected to metabolic study. This paucity of information is largely due to the difficulty of obtaining enough material for standard biochemical analysis. Dissection of the arrector pili muscle, in particular, from unstained fresh skin is nearly impossible and the prolonged dissection procedure lowers the metabolic capacity of the tissue. In this study, the technical problem was solved by using an adaptation of Lowry's method (1, 2), which permits enzyme assay of minute samples (microgram quantities) that can be accurately dissected from frozen-dried sections of the original tissue without losing glycolytic enzyme activities.

The present short communication deals with profiles of enzyme activities related to energy metabolism in both the arrector pili and subcutaneous muscles. It is hoped that these results will substantiate, at least partially, the comparison between smooth and skeletal muscle metabolism.

MATERIALS AND METHODS

Fresh biopsy specimens were obtained without anesthesia from the middle back region of 1/2 year old rhesus monkeys, and the frozen-dried sections were prepared as described previously (1). From these sections accurate tissue sampling was easily done by free-hand dissection under the microscope. All arrector pili muscle samples were obtained from those attached to terminal anagen V or VI hair follicles (Figs. 1 & 2). Several dissected tissue samples were stained and examined for possible dermal contamination. The purity of these samples with respect to muscle structures is more than 95%. The dissected arrector pili and subcutaneous muscles, averaging 0.5 to 1.0 g, were weighed on a "fish pole balance" (1) and subsequently used for assay. The enzyme activities assayed in these muscle structures are phosphorylase (microadaptation of Ref. 3), hexokinase (4), phosphofructokinase (5), aldolase (6), glyceraldehyde-3-phosphate dehydrogenase (7), enolase (8), pyruvate kinase (9), lactate dehydrogenase (10), glucose-6-phosphate and 6-phosphogluconate dehydrogenases (11), isocitrate dehydrogenase (12), fumarase (12), and adenosine triphosphatase (the reagent mixture was the same as described in Ref. 13, but the product adenosine diphosphate was measured fluorometrically as described in Ref. 14).

RESULTS

Table I summarizes the levels of glycolytic enzyme activities in the arrector pili and subcutaneous muscles as compared with those in the epidermis. Little difference in enzyme activities, in general, was observed between arrector pili muscle and epidermis. On the other hand, there were marked differences between the enzyme activities of the arrector pili and those of subcutaneous muscle. The phosphorylase activity in subcutaneous muscle was about 25 times higher than in the arrector pili muscle, whereas in the subcutaneous muscle hexokinase activity was lower than in the arrector muscle. Glycolytic enzymes are generally 1.2 to 2.7 times more active in the subcutaneous muscle than in the arrector pili muscle. One of the exceptions was lactate dehydrogenase activities which were similar in the two muscle structures and the epidermis. No attempt was made to examine the possible alteration in the isozyme patterns.

Characteristic differences between smooth and skeletal muscles were also encountered in the levels of the two dehydrogenases participating in the pentose phosphate cycle. Both glucose-6-phosphate and 6-phosphogluconate dehydrogenase activities were 4–6 times higher in the arrector pili muscle than in the sub-
Fig. 1. Frozen-dried preparation of skin sample. The arrector muscle is easily identified (see arrow).

Fig. 2. The dissected arrectores pilorum muscles
cutaneous muscle. These activities were still higher in the epidermis.

Table II summarizes adenosine triphosphatase (ATPase) activities in the arrector pili and the subcutaneous muscles. The ATPase activities in both muscles are activated by Mg2+ and inhibited by the simultaneous addition of Na+ and K+. It must be stated that under our assay condition, both muscle tissue may contain an endogenous amount of these ions. Therefore the actual rates of inhibition or activation may be greater than the values presented in this Table.

**DISCUSSION**

Perusal of the available literature indicates that a comparative study of the energy metabolism between the smooth and skeletal muscle is scanty. In spite of abundant data on the skeletal muscle, metabolic studies on smooth muscle have been confined to the carbohydrate metabolism of the uterus (15, 16). For comparison of smooth and skeletal muscle metabolism, the muscle samples should be obtained from the same organ. Accordingly, monkey skin may be a suitable organ since it contains skeletal muscle (panniculus carnosus) immediately below the panniculus layer and smooth muscle (arrectores pilorum) in the corium.

Data on the enzyme activities in these two muscle structures provoke some speculation on the different metabolic capacities of smooth and skeletal muscles. One such difference is the markedly different levels of both glucose-6-phosphate and 6-phosphogluconate dehydrogenase.

**TABLE II**

<table>
<thead>
<tr>
<th>Activator or inhibitor</th>
<th>Arrector pili muscle</th>
<th>Subcutaneous muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg2+ (5 mM)</td>
<td>1.9 ± 0.48</td>
<td>2.8 ± 1.3</td>
</tr>
<tr>
<td>No Mg2+</td>
<td>.55 ± 0.06</td>
<td>1.6 ± 0.30</td>
</tr>
<tr>
<td>Mg2+ (5 mM)</td>
<td>.64 ± 0.02</td>
<td>1.9 ± 0.51</td>
</tr>
<tr>
<td>Na+ (80 mM) plus K+ (40 mM)</td>
<td>1.0</td>
<td>.51</td>
</tr>
</tbody>
</table>

* All values are expressed as moles of ATP converted to ADP per kg dry wt tissue per hour ± standard deviation.

**TABLE I**

<table>
<thead>
<tr>
<th>Enzymes**</th>
<th>Arrector pili muscle</th>
<th>Subcutaneous muscle</th>
<th>Epidermis†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylase</td>
<td>.03 ± .001</td>
<td>.73 ± .16</td>
<td>.03 ± .01</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>.42 ± .18</td>
<td>.17 ± .07</td>
<td>.52 ± .05</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>3.1 ± .44</td>
<td>7.8 ± .78</td>
<td>4.2 ± .48</td>
</tr>
<tr>
<td>Aldolase</td>
<td>1.1 ± .14</td>
<td>7.8 ± 2.3</td>
<td>.42 ± .17</td>
</tr>
<tr>
<td>G-3-PDH</td>
<td>9.2 ± 1.6</td>
<td>19.6 ± 3.8</td>
<td>7.0 ± .88</td>
</tr>
<tr>
<td>Enolase</td>
<td>9.2 ± .48</td>
<td>25.0 ± 6.5</td>
<td>13.2 ± 1.1</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>13.4 ± 3.0</td>
<td>22.2 ± 2.5</td>
<td>14.3 ± 1.7</td>
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<tr>
<td>LDH</td>
<td>15.6 ± 2.3</td>
<td>17.4 ± 2.3</td>
<td>14.5 ± 8.8</td>
</tr>
<tr>
<td>G-6-PDH</td>
<td>.26 ± .07</td>
<td>.04 ± .01</td>
<td>.61 ± .25</td>
</tr>
<tr>
<td>6-PGDH</td>
<td>.15 ± .03</td>
<td>.04 ± .01</td>
<td>.38 ± .18</td>
</tr>
<tr>
<td>ICDH</td>
<td>.61 ± .09</td>
<td>2.5 ± .54</td>
<td>.93 ± .23</td>
</tr>
<tr>
<td>Fumarase</td>
<td>2.8 ± .54</td>
<td>2.8 ± .57</td>
<td>3.1 ± .91</td>
</tr>
</tbody>
</table>

* Activities are expressed as moles/kg dry wt. tissue/hr ± standard deviation. Each figure represents an average enzyme activity from 3 separate animals. Quintuplicate tubes were run for each enzyme assay.

** The abbreviations used are G-3-PDH = glyceraldehyde-3-phosphate dehydrogenase; LDH = lactate dehydrogenase; G-6-PDH = glucose-6-phosphate dehydrogenase; 6-PGDH = 6-phosphogluconate dehydrogenase; ICDH = isocitrate dehydrogenase.

† Data on the enzyme activities in epidermis are taken from previous reports (see References 4-11; data on phosphorylase, isocitrate dehydrogenase and fumarase activities are unpublished ones) and are based on the activities in scalp epidermis.
genes in the two different muscle structures. In spite of certain limitations and assumptions in the use of C-1 and C-6 labeled glucose for the evaluation of the pentose cycle contribution (17, 18), the literature affirms that normal skeletal muscle (in a physiological condition) has an insignificant pentose cycle (19), whereas the smooth muscle (uterus) shows an appreciable one (15). Therefore, our data suggest the active participation of this alternative pathway of glucose catabolism in the arrector muscle and practically no participation of it in subcutaneous muscle.

No further speculation on the different levels of other carbohydrate enzymes will be discussed here since there are many alternatives. An over-interpretation of data can be erroneous, and the steady state measurements and considerations of allosteric relationships are required to yield the most probable speculation. Present methods are, unfortunately, not sensitive enough to measure the intermediate substrate levels. Further studies on the over-all metabolic pathways of these muscles are also necessary to draw a final conclusion although in order to carry them out a number of technical difficulties must be surmounted.

A common feature of the arrector pili and subcutaneous muscles found by us was that the ATPase of each muscle is an Mg**-activated enzyme. Also, the ATPase activity of each muscle is inhibited by the addition of both Na* and K* together, in striking contrast to the ATPase associated with active sodium transport in the sweat gland that is activated by Na* and K* together.* It has been reported that Mg** is required with ATP for the contraction of uterine muscle (20). The nature of the ATPase in both arrector pili and subcutaneous muscle is in accord with the common physiological property of the muscles, namely, function as a contractile mechanism.

### Summary

The activities of twelve enzymes participating in carbohydrate metabolism as well as adenosine triphosphatase activities were assayed in both arrector pili and subcutaneous muscles in young rhesus monkey skin. While the profiles of the enzyme activities in the arrector pili muscle differ considerably from those in the subcutaneous muscle, both muscles possess a common enzymatic feature for their physiological role—"contraction"—i.e. high adenosine triphosphatase activities which are activated by Mg** and inhibited by the simultaneous addition of Na* and K*.

### References


