INHIBITORY ACTION OF GLUCAGON ON SARCOPLAMIC PROTEINS OF SKELETAL MUSCLES

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Abstract: Submandibular gland secretes a number of growth factors which are essential for cellular proliferation and differentiation. Glucagon like material is one of the growth factors secreted from submandibular gland. To explore the effect of this extrapancreatic glucagon on protein synthesis in skeletal muscles we have sialoadenectomised the male albino mice at the age of 20 days and allowed to develop in absence of submandibular gland. These animals were sacrificed on 40, 60, 80 and 100 days from birth. The gastrocnemius, soleus and rectus abdominis muscles were dissected out and subjected to protein estimation for evaluating the status of protein content in absence of glucagon. The results are consistent with literature available and showed the increased protein content in absence of glucagon. This confirms the inhibitory action of glucagon on protein synthesis of skeletal muscles.

Key words: Glucagon, Sarcoplamic protein Skeletal muscles

INTRODUCTION

Most of the studies on salivary glands are directed towards the secretion and distribution of proteins, enzymes and their hormonal regulation. But after the discovery of growth factors in the submandibular gland and their regulation by various hormones, the attention has been shifted to study the effect of submandibular gland secretion on the development and function of other organs of the body. Growth factors are proteins that bind to receptors on the cell surface, with the primary result of activating cellular proliferation and/or differentiation. Many growth factors like EGF, NGF, TGF, etc are quite versatile, stimulating cellular division in different cell types, while others are specific to a particular cell type. Among all growth factors secreted by submandibular gland, the glucagon is important and investigated in current work.

Silverman and Dunbar [1] first described IRG in extracts of rat submandibular gland, and showed that such extracts increased blood glucose level in intact, but not in eviscerated rats. They suggested that the submandibular gland participates in the entero-insulin axis by secreting glucagon, which in turn stimulates insulin secretion. The presence of IRG in the submandibular gland of several species including the mouse, rat, rabbit, dog, guinea pig, man has been confirmed by Lawrence and her co-workers [2-4] as well as several other scientists [5,6]. In contrast to submandibular gland only significant amounts of IRG are found in sublingual and parotid glands [3]. In general it has been suggested that IRG in the gland may explain the presence of glucagon in the blood of rats which were pancreatectomised and eviscerated but had functional liver [7,8].

The difference in IRG concentration between the arterial and venous blood suggests that the submandibular gland releases glucagon into the circulation [9,10]. IRG was released from the slices of the gland in vitro upon stimulation with glucose [2,4]. Somatostatin as well as high concentration of glucose stimulate the release of glucagon from salivary glands.
In current work in order to investigate the effect of glucagon from submandibular gland we have estimated the protein content of the skeletal muscle in absence of submandibular gland.

**MATERIALS AND METHODS**

Male albino mice were used for the present investigation. The animals were bred and reared in department animal house with proper care of light and temperature. The animals were provided with food and water *ad libitum*. These animals were sialoadenectomised on 20th day from their birth. After operation they were grouped according to the required age groups for the study as groups of 40 days, 60 days, 80 days and 100 days from birth. Then the animals were sacrificed on their respective age and the gastrocnemius, soleus and rectus abdominis muscles were dissected out. These muscles were subjected to further investigation.

**Technique used:** To study the sarcoplasmic fraction of muscle protein mass we used the water soluble extract of the muscles. The concentration of extract was 0.1 mg/ml of distilled water. Tissues were crushed at the bottom of the mortar for instantaneous freezing and gradual thawing with cold distilled water. The perfectly uniform homogenates were centrifuged at 10 °C at 5000 rpm for 10 minutes. The supernatant was used for estimation of proteins. The assay was carried out according to the Lowry’s method of protein estimation [11].

**RESULTS**

The results showed that the protein content of the gastrocnemius muscle in 40 days old sialoadenectomised mice increased five fold than the control mice, while in 60 days old mice the increase was three fold. Further, there was depletion in the increase of protein content. In 80 days old sialoadenectomised mice the increase was 1.3 fold but in 100 days old mice the increase in treated mice was not as much as the previous groups.

The protein content in the soleus muscle of 40 days old sialoadenectomised mice increased by about three fold as compared to control mice, while in 60 days old sialoadenectomised mice the increase was two fold, and in 80 days and 100 days old mice the increase was significant but not as much as the previous groups.

The protein content in the rectus abdominis muscle of 40 days old sialoadenectomised mice increased by about three fold as compared to control mice. In 60 days old sialoadenectomised mice the increase was two fold and in 80 days old mice increase was 1.5 fold but in 100 days old sialoadenectomised mice the increase was significant but not as much as the previous groups.

Here, we can see that the sarcoplasmic proteins have increased after sialoadenectomy. This can be the effect of glucagons like material secreted by submandibular gland.

**DISCUSSION**

To our knowledge the source of glucagons is pancreas but the extrapancreatic origin of the glucagons like material has been reported in recent days [1]. Rat salivary gland has significant amounts of polypeptides with the same physicochemical, immunological and biological properties as pancreatic glucagons [1]. The result obtained in current work is the outcome of the absence of glucagon like material due to sialoadenectomy.

Skeletal muscles are the major site of ATP production and hence important source of energy generation in vertebrates. ATPs are liberated as byproducts during glycolysis. But, under nutrient deprivation, the supply of carbohydrates spares the utilization of body nitrogen reserves which are primarily muscle proteins. The peptide products are degraded by cellular proteases so that carbon skeleton can enter the metabolic mainstream as precursors for gluconeogenesis or as citric acid cycle intermediates. This is achieved by the glucagon. Glucagon activates adenyl cyclase which is responsible for the formation of cyclic adenosine monophosphate. This AMP further activates protein kinase regulatory proteins, which in turn activates phosphorylase b kinase and convert it into phosphorylase a. The ultimate result of this reaction is the degradation of glycogen and energy production.

The effect of glucagon on muscle is direct rather than secondary to some other changes [12]. It has been shown that the addition of glucagons to perfused rat hemicorpus preparation resulted in lowering of rates of protein synthesis in plantaris and gastrocnemius muscle [13]. Further, in certain catabolic states, such as starvation, diabetes marked...
Fig. 1: Effect of sialoadenectomy on protein content of gastrocnemius muscle

Fig. 2: Effect of sialoadenectomy on protein content of soleus muscle

Fig. 3: Effect of sialoadenectomy on protein content of rectus abdominis muscle
Table 1: Effect of sialoadenectomy on protein content of skeletal muscles (mg protein/gm of muscle tissue). The number in parenthesis denotes the number of animals used

<table>
<thead>
<tr>
<th>Age of the animal</th>
<th>Gastrocnemius</th>
<th></th>
<th></th>
<th>Soleus</th>
<th></th>
<th></th>
<th>Rectus</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td>Sialoadenecotised</td>
<td></td>
<td>P value</td>
<td>Control</td>
<td></td>
<td>Sialoadenecotised</td>
</tr>
<tr>
<td>40 days (5)</td>
<td>95.6 ± 4.837</td>
<td></td>
<td>458.96 ± 2.933</td>
<td></td>
<td>P &lt; 0.0001</td>
<td>171.2 ± 1.304</td>
<td></td>
<td>457.8 ± 3.532</td>
<td></td>
</tr>
<tr>
<td>60 days (5)</td>
<td>203.0 ± 2.915</td>
<td></td>
<td>597.8 ± 5.263</td>
<td></td>
<td>P &lt; 0.0001</td>
<td>250.4 ± 2.074</td>
<td></td>
<td>414.2 ± 4.037</td>
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</tr>
<tr>
<td>80 days (5)</td>
<td>401.4 ± 2.048</td>
<td></td>
<td>548.2 ± 3.114</td>
<td></td>
<td>P &lt; 0.0001</td>
<td>459.3 ± 2.907</td>
<td></td>
<td>550.6 ± 2.702</td>
<td></td>
</tr>
<tr>
<td>100 days (5)</td>
<td>506.4 ± 6.107</td>
<td></td>
<td>605.0 ± 4.359</td>
<td></td>
<td>P &lt; 0.0001</td>
<td>508.0 ± 7.583</td>
<td></td>
<td>646.6 ± 3.975</td>
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</tr>
</tbody>
</table>

up-regulation of circulating glucagons is achieved [14,16]. In these conditions up-regulation of circulating glucagons resulted in decreased protein synthesis and increased proteolysis [17,18].

All above data indicates that after sialoadenectomy the absence of glucagon like material results in enhancement of protein accretion and enlightens the inhibitory ability of glucagons on protein synthesis. Therefore, the role of extrapancreatic glucagon on metabolism of muscle can be the subject of further study, which is in progress in our laboratory.

REFERENCES