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SIALOADENECTOMY EFFECT ON GASTROCNEMIUS MUSCLE OF MALE MICE

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ABSTRACT
Submandibular gland secretes a number of growth factors like epidermal growth factor (EGF), nerve growth factor (NGF), mesodermal growth factor (MGF), immuno reactive glucagon (IRG) etc, which are essential for cellular proliferation and differentiation. We investigated the role of submandibular gland secreted immuno reactive glucagon on blood glucose level of the mice, glycogen content and protein content in developing gastrocnemius muscles in mice. Twenty days old male mice were sialoadenectomised and these animals were maintained under normal conditions in the departmental animal house upto the age of 40, 60, 80 and 100 days from birth. Thereafter operated males were sacrificed after 40^{th}, 60^{th}, 80^{th} and 100^{th} days and used for estimation of blood glucose level; the gastrocnemius muscle was dissected out and subjected to glycogen and protein estimation. In sialoadenectomised mice the blood glucose level decreased whereas the glycogen content and protein content from the gastrocnemius muscle was increased in all the four groups as compared to control. These results reveal that submandibular gland secreted glucagon affect the metabolic regulation in the gastrocnemius muscle during development.

KEY WORDS: blood glucose, glycogen, gastrocnemius, protein, Sialoadenectomy,

INTRODUCTION

Submandibular gland synthesizes and accumulates a number of biologically active peptides that are released into both saliva and the bloodstream. The effects of these growth factors on various organs of the body have been investigated by many scientists. Atterdi in 1967 tried to determine possible mode of action of submandibular gland extract on embryonic muscle tissue and showed that mesodermal growth factors from submandibular gland is responsible for proper differentiation of muscle tissue. The existence of glucagon in salivary glands has been accepted and this salivary gland glucagon has been reported to be a hyperglycemic factor with a molecular weight much larger than pancreatic glucagon (Bhathena *et al.,* 1977; Dunbar *et al.,* 1977; Hojvat *et al.,* 1977; and Lawrence *et al.,* 1977). Silverman and Dunbar (1974) first described glucagon in extracts of rat submandibular gland and showed that such extracts increase blood glucose level in intact, but not in eviscerated rats. They suggested that the submandibular gland participates in the entro-insulin axis by secreting glucagon, which then stimulates insulin secretion. Preedy and Garlick in 1985 showed that administration of pharmacological dose of glucagon decreases protein synthesis in skeletal muscles and loss of muscle protein occurred as a result of decreased protein synthesis and increased proteolysis.

Therefore the present investigation was carried out to assess the role of submandibular gland glucagon on blood glucose level, glycogen content and protein content in gastrocnemius muscle during development.

MATERIALS AND METHODS

Male Swiss albino mice (*Mus musculus*) were bred and reared in the plastic cages in AC animal house (CPCSEA/233) under 12:12hr L: D cycle. The animals were provided with pelleted food from 'Pranav Amrut food' Sangli and water *ad libitum.* The sixteen animals were sialoadenectomised on 20^{th} day from their birth. After operation they were grouped according to the required age groups for the study as 40 days, 60 days, 80 days and 100 days from birth. Then the animals were sacrificed on their respective age and the gastrocnemius muscle was dissected out and used for following estimations.

- Estimation of blood glucose was carried out by using Folin and Wu, (1920) method using glucose as standard.
- Estimation of glycogen was carried out by using Pfleiderer, (1957) method by hydrolyzing glycogen to glucose.
- Estimation of protein was carried out by using Lowry's method (1951).
- Statistical analysis: – Results were interpreted with the help of ANOVA followed by Tukey's post hoc test.

RESULTS

There was increase in blood glucose level from juvenile to adult in both the control and sialoadenectomised mice. Whereas blood glucose level in sialoadenectomised male mice was decreased in all groups i.e. 40 days, 60 days, 80 days and 100 days as compared to control male mice and the decrease was significant. (Table 1).The glycogen content and protein content from gastrocnemius muscle in sialoadenectomised mice was increased as compared to control mice from 40 days to 100 days mice and the increase was significant (Table 2 and 3).

Table 1. Effect of Sialoadenectomy on blood glucose level of male mice (mg of glucose/ 100 ml of blood)

Groups	Control male	Sialoadenectomised male	Statistical significance
40 days(4)	68.47 ± 0.0084	64.62 ± 0.0089	P < 0.0001
60 days(4)	70.85 ± 0.0076	65.30 ± 0.0084	P < 0.0001
80 days(4)	75.79 ± 0.0084	66.66 ± 0.0089	P < 0.0001
100 days(4)	80.11 ± 0.0114	71.11 ± 0.008	P < 0.0001

Table 2. Effect of Sialoadenectomy on glycogen content of Gastrocnemius muscle of male mice (mg of glucose/ 100 mg of wet tissue)

Groups	Control male Gastrocnemius	Sialoadenectomised male Gastrocnemius	Statistical significance
40 days(4)	60.66 ± 0.0120	64.64 ± 0.0128	P < 0.0001
60 days(4)	62.64 ± 0.0112	67.36 ± 0.0114	P < 0.0001
80 days(4)	64.64 ± 0.012	68.08 ± 0.015	P < 0.0001
100 days(4)	67.36 ± 0.0165	71.91 ± 0.0114	P < 0.0001

Table 3. Effect of sialoadenectomy on protein content of Gastrocnemius muscle of male mice (mg protein / gm of the muscle tissue).

Groups	Control male	Sialoadenectomised male	Statistical significance
40 days (4)	17.6 ± 0.324	24.3 ± 0.3317	P < 0.01
60 days (4)	18.9 ± 0.4099	27.0 ± 0.5701	P < 0.01
80 days (4)	54.0 ± 0.3082	94.05 ± 0.2702	P < 0.01
100 days(4)	94.15 ± 0.7403	162.1 ± 0.8972	P < 0.01

DISCUSSION

In the present investigation in the sialoadenectomised mice the blood glucose level was decreased while glycogen content and protein content from the gastrocnemius muscle was increased as compared to control. Decrease in blood glucose level in the sialoadenectomised mice is due to absence of submandibular gland secreted glucagon.Increase in the protein content in the gastrocnemius muscle of sialoadenectomised mice is also due loss of glucagon like material secreted by submandibular gland. Preedy and Garlick in 1985 showed that the free and protein bound tyrosine in muscle of the perfused rat hemicorpus indicated an inhibitory action of glucagon. Preedy and Garlick in 1985 has done the direct measurements of the effect of this hormone on the muscle protein synthesis *in vivo* and has confirmed that effects of glucagon observed in isolated tissues *in vitro* can be reproduced in the whole animal. They indicated that the effect of glucagon on muscle may be direct rather than secondary to some other change. It has been shown that the addition of glucagon to perfused rat hemicorpus preparations resulted in lowering of rates of protein synthesis in plantaris and gastrocnemius muscle (Preedy *et al.,* 1980).

Effect of pancreatic glucagon on carbohydrate metabolism is to increase breakdown of liver glycogen to glucose and hence hyperglycemia, but it does not cause the breakdown of muscle glycogen and it has no effect on muscle phosphorylase. Next to liver, skeletal muscles are rich source of glycogen. In our observations we have found that in sialoadenectomised male mice glycogen content in all groups was increased as compared to normal. In all groups the glycogen content was low in juvenile mice gastrocnemius muscle and increased in adult. This indicates that there is no effect of pancreatic glucagon on muscle glycogen and perhaps salivary glucagon may not be having effect on liver glycogen. This indicates the possibility of two different species of glucagon. Pancreatic glucagon may not be having receptors on muscle cells and salivary glucagon may not have receptors on liver.

Induction of hypoglycemia in sialoadenectomised male mice in all groups indicates that submandibular gland of mice secretes glucagon like substance. Thus due to sialoadenectomy the glucagon which is secreted by submandibular gland is decreased that leads to increase protein content in gastrocnemius muscle and affect the metabolic regulation in the gastrocnemius muscle during development.

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