J Endocrinol Reprod 5(2) : 51 - 58 (2001)
JER 41

HYPOGLYCEMIC EFFECT OF SIALOADENECTOMY IN MICE

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(Received 8th May 2001)

SUMMARY

One and three months old mice of both the sexes were sialoadenectomised and maintained under normal conditions in the animal house upto the age of three and six months, respectively. During this period, their weight was recorded. Alloxan monohydrate was used to induce diabetes in some of them. Twenty four mice which were sialoadenectomised were subjected to fasting for 24 h and 36 h. Blood glucose, liver glycogen and alkaline phosphatase activity in liver, soleus muscle and kidney were determined in experimental and normal mice. The blood glucose level was considerably decreased in sialoadenectomised and sialoadenectomised diabetic mice compared to normal and diabetic cases. The decrease was much higher in the sialoadenectomised mice which were denied food for 24h and 36h. Glycogen content was decreased significantly in sialoadenectomised and sialoadenectomised diabetic mice. But in fasted sialoadenectomised mice, there was less decrease than fasted control. In sialoadenectomised and sialoadenectomised diabetic mice, alkaline phosphatase activity was decreased. The decrease was highly significant in liver and soleus muscle.

Key words: Glucagon- like substance; Hyperglycemia; Hypoglycemia; Submandibular gland

INTRODUCTION

The occurrence of glucagon or glucagon-like substance in the submandibular gland is well known since long (1-10). Earlier studies (8, 10) have shown the hyperglycemic effect of intravenously administered acid ethanol extract of submandibular gland. The hypoglycemic effect of salivary duct ligation in diabetic mice is also demonstrated (11). Three-fold increase in the glucagon level is shown over a 48h period in eviscerated and pancrectomised rats (2). In such rats according to them, the submandibular gland is the source of circulating glucagon. *De novo* synthesis and release of glucagon by submandibular gland has been shown by Perez Castillo and Blazaquez (12). Contrary to the above, there are reports mentioning the absence of hyperglycemic or glycogenolytic activity of the salivary glucagon (13, 14). Tahara *et. al.* (15, 16, 17) in their studies on perfused rat liver and chemical analysis of glucagon demonstrated no effect of the salivary gland glucagon on the carbohydrate metabolism.

Earlier reports pertaining to the role of salivary glucagon on the carbohydrate metabolism are mainly based on the studies of isolated glucagon. Tahara and his associates have demonstrated the degradation of submandibular glucagon molecule in the presence of

esteroprotease in the granular duct of submandibular gland. During isolation there is every possibility of denaturation of the glucagon molecule. Therefore, the present study was undertaken to evaluate the role of submandibular glucagon on the carbohydrate metabolism

MATERIALS AND METHODS

One and three months old mice of both sexes, weighing 25 ± 0.5 g and 30 ± 0.5 g respectively were sialoadenectomised under mild ether anaesthesia. They were maintained under the laboratory conditions until they reached the age of three and six months, respectively. Gold Mohur rat feed (Lipton India Ltd.) and water were supplied *ad libitum*. Their body weights were recorded every after five days. The experimental mice were grouped into three. The first group (F_1) served as control (normal). The second (F_2) served as sialoadenectomised group. The third group (F_3) included normal and sialoadenectomised mice, which received two injections of alloxan monohydrate (200 mg/ kg body wt.) on alternate day and used 48 hours after the 2nd injection, while the mice of F_1 and F_2 groups received only saline injections. The mice from all the groups were sacrificed by cervical dislocation and the liver, kidney and soleus muscle were dissected out. Blood was collected from the heart in heparinized tubes for the determination of glucose by Folin Wu method (18).

One gram of liver was used for the estimation of glycogen. Liver was rapidly minced, homogenized and transferred immediately to the centrifuge tube containing 30% KOH. The glycogen was estimated by the method of Hassid and Abraham (19). The tissue was digested by heating the tube in the boiling water bath, for 30 min. 0.8 ml of saturated sodium sulfate was added to precipitate glycogen with 1:2 volume of 95% ethanol. The tubes were heated again till the mixture begin to boil. Afterwards, the tubes were cooled and subjected to centrifugation at 3000 rpm. The supernatant was discarded. Traces of alcohol were removed by keeping the centrifuge tubes in an oven at 37^0C overnight. The glycogen powder was suspended in distilled water and hydrolysed in 6ml of 2N sulphuric acid. The hydrolysed glycogen was used for the determination of glucose by Nelson-Somogyi's method (20).

The liver, kidney and soleus muscle pieces were finely minced and homogenized in cold 0.05 M alkaline glycine buffer (pH 10.5). The homogenates were centrifuged at 11000 rpm for 10 min. at 4^0C and the supernatants were used for the determination of alkaline phosphatase activity. One unit of enzyme is defined as m mole *p-nitrophenol* formed at 37^0C per min/ mg protein (21).

The blood glucose and the liver glycogen were determined in the mice subjected to fasting For this purpose, the mice of six months age were selected from F_1, F_2 and F_3 groups.

RESULTS

A steady increase in the body weight of mice was seen from the age of one month to three months (Fig.1). The weight gain was not significant between the age of three months to six months (Fig. 2). The body weight was always higher in the sialoadenectomised mice than in the normal ones.

Fig. 1: Growth rate in Sialoadenectomised mice from age of 1 to 3 months

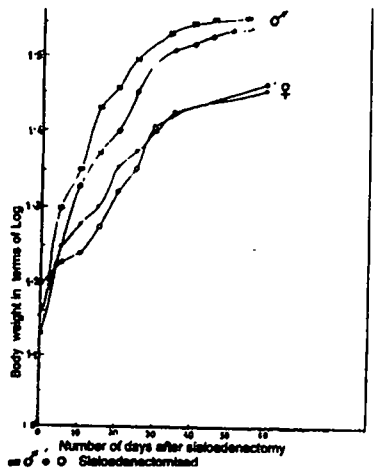
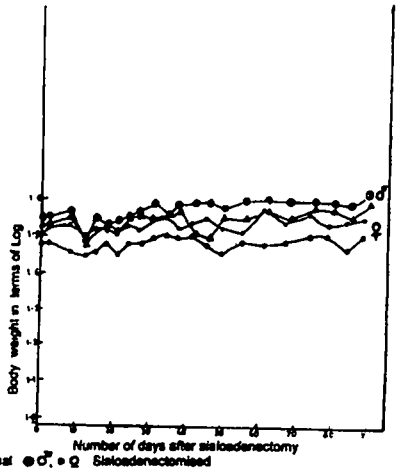


Fig. 2: Growth rate in Sialoadenectomised mice from age of 3 to 6 months



The blood glucose level was significantly decreased in both sialoadenectomised and sialoadenectomised diabetic mice than the respective controls (Table 1). Glucose values were still reduced in the sialoadenectomised mice which were fasted for 24 h and 36 h than in the normal mice which were fasted for the same period (Table 2).

The glycogen content was found to be decreased both in the sialoadenectomised and sialoadenectomised diabetic than in the normal and diabetic mice, respectively (Table 3). In the normal fasted mice, the decrease was more than in the sialoadenectomised fasted mice (Table 4).

Table 1 : Blood glucose level (mg/100ml) in sialoadenectomised and sialoadenectomised diabetic mice

Animal	Age in months	Normal	Sialoadenecto-mised	Diabetic	Sialoadenecto-mised+Diabetic
Male	3	60.83±1.541	46.66±2.057*	119.73±3.04	74.60±3.153*
Female	6	78.3±1.242	66.02±1.814*	115.55±0.786	102.22±1.698*
Male	3	57.77±2.553	37.5±1.424*	115.23±0.603	93.64±2.551*
Female	6	64.12±1.071	47.97±0.9534*	128.85±1.149	102.53±1.443*

Mean + S.D. of 6 animals (n=6) Sialoadenectomised compared with normal and Sialoadenectomised diabetic compared with diabetic. * $p < 0.001$ Highly significant

Table 2 : Effect of sialoadenectomy on blood glucose level (mg/100ml) after fasting in male and female mice

6 months old mice	Blood glucose	
	Normal	Sialoadenectomised
Male fasted for 24 hrs.	49.73±0.053	38.6±0.048*
Male fasted for 36 hrs.	43.90±0.048	28.6±0.039*
Female fasted for 24 hrs.	42.32±0.027	35.6±0.043*
Female fasted for 36 hrs.	36.93±0.042	24.34±0.0784*

Values are mean ± S.D. of 6 animals (n=6)
Fasted sialoadenectomised compared with fasted control
* p< 0.001 Highly significant

Table 3 : Glycogen content (mg/g wet tissue weight) in the liver of sialoadenectomised and sialoadenectomised diabetic mice

Animal	Age in months	Normal	Sialoadenectomised	Diabetic	Sialoadenecto-mised+Diabetic
Male	3	28.14±0.546	26.49±0.202*	22.27±1.252	15.37±0.634*
Female	6	38.53±2.24	66.02±1.46*	25.63±1.16	20.94±0.66*
Male	3	21.5±0.62	37.5±0.13*	16.613±0.512	13.22±1.032*
Female	6	34.46±1.16	24.46±0.61*	21.35±1.02	18.41±0.373*

Mean + S.D. of 6 animals (n=6) Sialoadenectomised compared with normal; Sialoadenectomised diabetic compared with diabetic.
* p< 0.001 highly significant

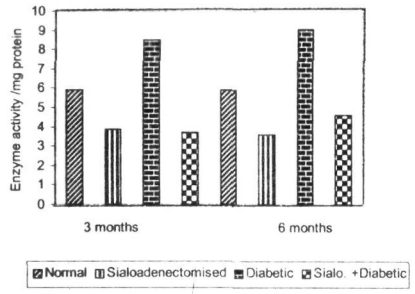
Table 4 : Effect of sialoadenectomy on liver glycogen (mg/g wet tissue weight) after fasting in male and female mice

6 months old mice	Liver glycogen	
	Normal	Sialoadenectomised
Male fasted for 24 hrs.	5.08±0.036	7.02±0.031*
Male fasted for 36 hrs.	1.06±0.023	3.55±0.02*
Female fasted for 24 hrs.	3.09±0.016	5.03±0.026*
Female fasted for 36 hrs.	0.9±0.023	2.04±0.031*

Values are mean ± S.D. of 6 animals (n=6)
Fasted sialoadenectomised compared with fasted control
* p< 0.001 highly significant

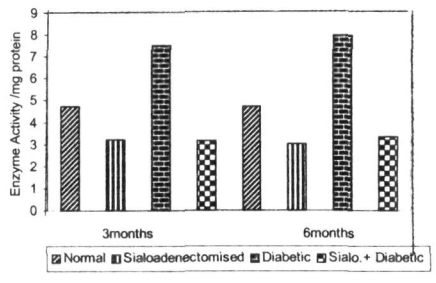
The alkaline phosphatase activity was increased in the liver (Fig. 3 and 4) and the soleus muscles (Fig. 7 and 8) of the diabetic mice compared to control mice but it was reduced in sialoadenectomised and sialoadenectomised diabetic mice. Significant changes were not observed in the kidney (Fig.5 and 6).

Fig. 3 Alkaline phosphatase activity/ mg protein in the liver of sialoadenectomised & sialoadenectomised diabetic male mice



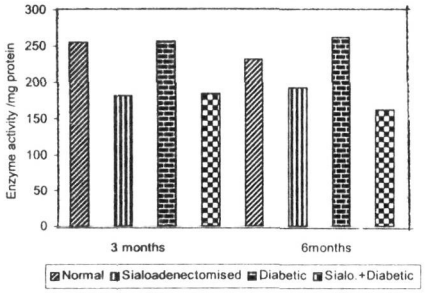
* P< 0.001 Each histogram denotes mean+ SD (n=6)
Normal Vs Sialoadenectomised
Diabetic Vs Sialoadenectomised diabetic

Fig. 4 Alkaline phosphatase activity/ mg protein in the liver of sialoadenectomised and sialoadenectomised diabetic female mice



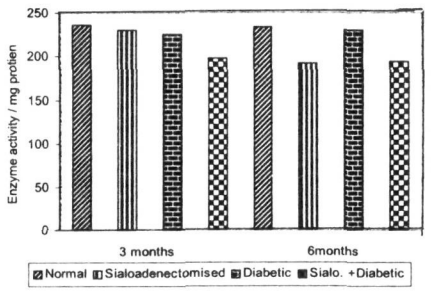
* P< 0.001 Each histrogram denotes mean + SD (n=6)
Normal Vs Sialoadectomised
Diabetic Vs Sialodenectomised diabetic

Fig. 5 Alkaline phosphatase activity/ mg protein in the kidney of sialoadenectomised & sialoadenectomised diabetic male mice



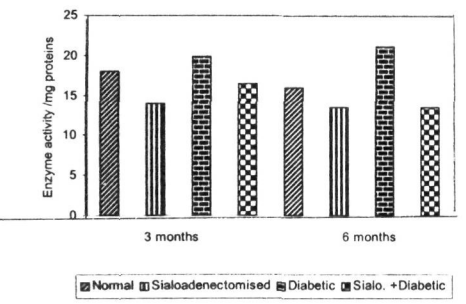
Each histogram denotes mean+ SD (n=6)
Normal Vs Sialoadenectomised
Diabetic Vs Sialoadenectomised diabetic.

Fig. 6 Alkaline phosphatase activity/ mg protein in the kidney of sialoadenectomised & sialoadenectomised diabetic female mice



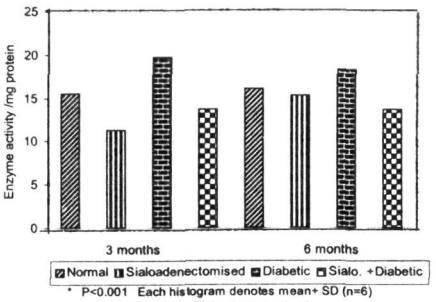
Each histogram denotes mean+ SD (n=6)
Normal Vs Sialoadenectomised
Diabetic Vs Sialoadenectomised diabetic.

Fig. 7 Alkaline phosphatase activity/ mg protein in the soleus muscle of sialoadenectomised & sialoadenectomised diabetic male mice



*P<0.001 Each histogram denotes mean+ SD (n=6)
Normal Vs Sialoadenectomised
Diabetic Vs Sialoadenectomised diabetic.

Fig. 8 Alkaline phosphatase activity/ mg protein in the soleus muscle of sialoadenectomised & sialoadenectomised diabetic female mice



* P<0.001 Each histogram denotes mean+ SD (n=6)
Normal Vs Sialoadenectomised
Diabetic Vs Sialoadenectomised diabetic.

DISCUSSION

Induction of hypoglycemia in sialoadenectomised and sialoadenectomised diabetic mice clearly indicates that the submandibular gland of mice secretes glucagon- like substance and/or may be regulating gluconeogenesis and glycogenolysis in the liver. Gluconeogenesis is under the control of glucagon and insulin (22). Glucagon exerts its effect on liver leading to hyperglycemia. Hyperglycemic action is associated with loss in body weight, decrease in liver glycogen (22) and increased alkaline phosphatase activity in various organs (23, 24). Except liver glycogen exactly opposite to hyperglycemia, results were obtained in sialoadenectomised condition. These studies suggest the removal of basal level of glucagon is necessary to decrease the gluconeogenic and glycogenolytic rate. The study of Jennings *et. al.* (25) showed that the induction of selective glucagon deficiency in dogs fasted overnight associated with decrease in gluconeogenesis and glycogenolysis. Though there was loss of blood glucose and liver glycogen during fasting, the loss of glycogen was reduced in sialoadenectomised fasted mice than fasted normal mice. It means, at least partly glycogenolysis must have been inhibited in sialoadenectomised mice due to unavailability of submandibular glucagon. Penhos *et. al.* (2) showed plasma immunoreactive insulin and immunoreactive glucagon after evisceration with and without functional pancreas. Perez-Castillo and Blazquez (12) showed *de-novo* synthesis and release of glucagon by submandibular gland. In earlier reports, the absence of salivary glucagon action may be due to isolated glucagon and during isolation there is a possibility of denaturation of glucagon molecules. Tahara *et. al.* demonstrated degradation of glucagon molecule (15,16,17) during isolation and therefore it must be inactive. Our reports demonstrated the action of submandibular glucagon *in vivo*.

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