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# Effect of Chemical Sympathectomy on Glycogen Storage in Rat Skeletal Muscle in Thermal Trauma

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## ABSTRACT

**The glycogen storage in rat skeletal muscle is reduced after a 20% third degree burn. The reason is probably a relative deficiency of insulin caused by insulin resistance at the tissue level. Posttraumatically increased sympatho-adrenal function has been suspected to cause this insulin resistance. In an earlier study, however, it has been shown that adrenal demedullation has no effect on the glycogen storage. In the present investigation an attempt was made to assess the importance of the increased peripheric sympathetic activity. Muscle glycogen, serum insulin and blood glucose were determined at the end of a glucose infusion after infliction of a burn both in 6-hydroxy-dopamine treated rats and rats with an intact peripheric sympathetic nervous system. It was found that a chemical sympathectomy did not improve the glycogen storage. The result indicates that the increased activity of the sympatho-adrenal system after a burn is not the main cause of the reduced skeletal muscle glycogen storage.**

## INTRODUCTION

After trauma and abdominal operations there is a marked reduction of the muscle glycogen depot (6). In previous studies of the glycogen storage in the skeletal muscle in rats subjected to a burn, it was found that the activity of glycogen synthetase is decreased (13). This might be the cause of the inhibited glycogen storage on glucose infusion after a burn. The reason for the reduced activity of the enzyme is not clear. A probable cause was thought to be the high adrenaline production from the adrenal medulla following trauma. However, adrenal demedullation did not improve the glycogen storage in the muscle (12). Another possible explanation is that the increased noradrenaline release from sympathetic nerves may cause this posttraumatic reduction of glycogen storage.

The aim of this investigation was therefore to study the importance of the increased peripheral sympathetic tone. The effect of chemical sympathectomy with 6-hydroxy-dopamine (6-OH-DA) on the glycogen storage during glucose infusion after burn injury was investigated in the rat.

## MATERIAL AND METHODS

48 male albino rats (Sprague-Dawley, Anticimex, Stockholm, Sweden) weighing about 250 g were used. The rats were fed on a standard commercial diet (Anticimex diet 210).

The animals were divided into the following groups: (I) non-burned, intact rats, (II) non-burned, chemical sympathectomized rats, (III) burned rats, and (IV) burned, chemical sympathectomized rats.

The glycogen storage in the skeletal muscle was studied in all groups during an infusion of 8 ml 50% glucose (4 g glucose). The animals were conscious on administration of the infusions which were given by means of an injection pump during 4 hours. The rate of infusion was 2 ml/h.

About 5 days before the start of the experiments a catheter was inserted in the superior vena cava. 48 hours before the infusions 50 mg/kg 6-OH-DA were given i.v. to the rats in groups II and IV. The 6-OH-DA was dissolved in saline and 0.2 g/ml ascorbic acid was added to prevent oxidation. The rats in the control groups received saline with the same amount of ascorbic acid.

All rats were deprived of food from 40 hours before the start of the infusion. Water was given ad libitum. Animals from the different groups were treated simultaneously.

In groups III and IV a standardized burn was produced under ether anaesthesia 20 hours before the infusion. A third degree burn covering about 20% of the body surface was produced essentially as described by Arturson (4).

Immediately after completion of the glucose infusion the rats were anaesthetized with ether. Biopsies were taken from the thigh muscle for glycogen assay. The muscle specimen was immediately frozen in liquid nitrogen and thereafter kept frozen until analysed. Blood samples were drawn with a syringe from the aorta for glucose determination. The rats were then killed and the position of the catheter was controlled. All animals, where it might be suspected that some part of the infusion had not been given intravenously, were excluded from the study.

Glycogen was isolated from KOH digest of muscle by ethanol precipitation and determined with an all-

Table I. *Glycogen storage (mg/g wet tissue) in skeletal muscle, glucose concentration in blood (mg/100 ml) and insulin concentration in serum (ng/ml) after an infusion of 4 g glucose i.v. Means and S.D. are given*

| Group | n | Glycogen | Glucose | Insulin |
|---|---|---|---|---|
| Intact control | 8 | 31.3±3.6 | 204± 40 | 2.5±0.8 |
| Burned control | 7 | 15.7±2.7 | 430±113 | 4.4±1.0 |
| 6-OH-DA treated | 11 | 27.6±4.1 | 222± 47 | 4.2±2.5 |
| 6-OH-DA treated, burned | 11 | 12.1±2.3 | 347±137 | 6.1±1.7 |

enzymatic method (for details see Adolfsson (1)). Rabbit liver glycogen (type III, Sigma, St. Louis, USA) was used as a standard. Glycogen concentration is expressed in mg/g wet tissue.

Blood glucose concentrations were determined by a commercial glucose oxidase method (Boehringer, Mannheim GMBH, Germany). Values are given in mg/100 ml.

Insulin was measured in duplicate samples of serum by radioimmunoassay, separating free and antibody bound insulin by ethanol precipitation (10). Crystaline mouse insulin, which reacts with the antibodies as rat insulin in this assay system (5) was used as a standard. $^{125}$I-insulin and insulinbinding reagent were obtained from the Radiochemical Center, Amersham, England. Values are given in ng/ml.

Mean values and standard deviations (SD) are given. Student's *t*-test was used to compare differences between mean values.

## RESULTS

Nine rats were excluded from the study because of problems with the catheter during the glucose infusion. Two rats died in the experiment. Both these rats belonged to the burned 6-OH-DA group. They died soon after infliction of the burn.

### *Muscle glycogen*

At the end of the glucose infusion the glycogen concentration in the muscle was 31.3 (S.D. 3.6) mg/g tissue in untreated intact rats and 15.7 (S.D. 2.7) mg/g tissue in untreated burned rats. This difference is highly significant ($p<0.001$). The corresponding values for the 6-OH-DA treated groups were 27.6 (S.D. 4.1) mg/g tissue in the non-burned rats and 12.1 (S.D. 2.3) mg/g in the burned group. This shows a considerable lower glycogen concentration in the muscle after glucose infusion in burned than in non-burned animals also after chemical sympathectomy ($p<0.001$).

The comparison between the glycogen concentrations found in the two non-burned rat groups shows no significant difference between untreated and 6-OH-DA treated rats. However, a small difference in glycogen concentration values was noted in the two burned rat groups. The glycogen concentration was somewhat lower in the burned 6-OH-DA treated rats than in the untreated burned rats ($p<0.01$).

### *Blood glucose concentration*

The glucose tolerance was lowered in the untreated burned rats compared to intact non-burned rats, giving higher glucose concentration after infusion of the same amount of glucose ($p<0.001$). A similar difference was noted between the non-burned and burned 6-OH-DA treated rats ($p<0.01$).

There was no significant difference between the blood glucose concentration in non-burned untreated and non-burned 6-OH-DA treated rats. The blood glucose concentrations were higher and more varied in both burned rat groups but there was no significant difference between the burned groups.

### *Insulin concentration*

The lowest serum insulin concentrations were found in the intact non-burned animals. In both the untreated burned rats and the 6-OH-DA treated burned rats we found significant higher insulin concentration ($p<0.01$). In burned rats 6-OH-DA treatment in itself gave higher insulin values in comparison with untreated rats ($p<0.05$).

## DISCUSSION

In a previous study it was found that 24 hours after infliction of a moderate burn the glycogen storage in the muscle during standardized glucose infusion was greatly reduced (14). In contrast there were no appreciable changes in the glycogen storage in the liver at this time. A decreased activity of glycogen synthetase might explain the reduced glycogen storage capacity (13). In the study reported in this paper the glycogen storage in the muscle after a burn was again significantly reduced. This reduction was accompanied by significant elevation of the serum insulin levels. In spite of this hyperinsulinism there was also an elevated plasma glucose level. Although it is known that the beta-cells are inhibited by increased secretion of catecholamines in the shock phase after a burn, the

insulin response to glucose loading, in agreement with our results often is higher than normal in later stages after injury (2). A relative deficiency of insulin caused by an insulin resistance at the tissue level seems to be the reason to the lowered glycogen storage as it can be normalized to some extent on administration of insulin (14). A probable cause of the insulin resistance might be an effect of increased sympatho-adrenal activity posttraumatically. *In vitro* experiments have shown inhibition of the glycogen synthesis in muscle tissue by adrenaline (18, 8). Catecholamines have been reported to inhibit glucose uptake by tissues in *in vivo* tracer studies (3). We have earlier (11) shown that both adrenaline and noradrenaline excretion are increased after the burn used in this experiment. However, adrenal demedullation did not improve the glycogen storage after a burn (12).

A high activity of the peripheral sympathetic nerves might be the reason to the insulin resistance. The present study shows, however, that the glycogen storage in a muscle 24 hours after the burn is considerably reduced also in chemical sympathectomized animals. The adrenals are supposed to be resistant to direct action of 6-OH-DA (15) so that some compensatory increase of catecholamine release from the adrenals in the chemical sympathectomized rats might be possible. Thus, in order to exclude any possible effect of catecholamines on the muscle glycogen storage after a burn, adrenal medullectomized, 6-OH-DA treated animals ought to have been included in our study. As in the control groups we found no absolute insulin deficiency but instead higher plasma insulin values and an impaired glucose tolerance after the burn. These results talk in favour of the importance of other peripheral insulin-antagonistic substances than catecholamines.

The increased insulin response observed in sympathectomized animals can be explained by the insulin-releasing effect of glucose not being inhibited by noradrenaline released from sympathetic nerves. It is well known that insulin secretion may be inhibited by stimulating alpha-adrenergic receptors of the pancreas islets (17, 16). Burr et al. (7) found high fasting insulin levels in Wistar rats treated with 50 mg/kg 6-OH-DA but in contrast to our results they found a markedly inhibited insulin release in response to glucose. As an explanation they proposed a chronic depletion of a critical pool of pancreatic insulin caused by an impairment of the beta-cell function secondary to adrenergic deficiency. One explanation to these differences in insulin response to glucose infusions after chemical sympathectomy may be that their studies were made 2–6 weeks after the administration of 6-OH-DA while ours were made already after 2 days. The impairment of beta-cell function may need several days to develop, although the direct effect of 6-OH-DA on the nerve endings in the insular tissue probably is much faster. For instance, only 5% of noradrenaline remains in the rat heart 2 hours after a single injection of 100 mg/kg of 6-OH-DA (9).

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