Moderate physical training increases brain insulin concentrations in experimental diabetic rats

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Received 5 April 2007; revised 4 April 2008

Insulin is an important modulator of growth and metabolic function in the central nervous system. The aim of this study was to investigate the influence of swimming physical training (at 32°C ±1ºC, 1 hr/day, 5 days/week, with an overload equivalent to 5% of the body weight, for 4 weeks) on brain insulin concentrations in alloxan induced type 1 diabetic rats. Training attenuated hyperglycemia but had no effect on insulinemia in diabetic rats. Hematocrit and blood albumin values remained without changes. Brain insulin did not change in diabetic rats. However, physical training increased the concentration in both control and diabetic rats. It is concluded that in the present experimental conditions, diabetes had no influence on brain insulin, however moderate physical training increased the hormone in both control and diabetic animals.

Keywords: Brain, Diabetes, Insulin, Physical training, Rat

Insulin plays a complex role in fuel homeostasis regulation and in anabolic effects in peripheral tissues. The brain was considered an insulin-insensitive tissue, however, recent molecular studies indicated that insulin is present in several regions of the central nervous system (CNS) acting as a neuromodulator, inhibiting food intake and stimulating fat oxidation.

Diabetes is associated with an increased relative risk for developing dementia. Studies have pointed an elevated risk for Alzheimer disease (AD) in diabetics patients. Studies performed with human postmortem brain tissues associated molecular and pathological features of AD to reduced expression of the insulin and insulin-like growth factor (IGF) genes and receptors.

Brain insulin source is uncertain however, it was reported that human blood-brain barrier insulin receptor can act as a transport system. The hypothesis of insulin biosynthesis in brain was based on the disagreement between central and peripheral insulin profile and presence of preproinsulin-I mRNA expression in rat brain.

The effects of physical training on brain insulin concentrations are observed in studies that demonstrate increase in insulin receptors and insulin mRNA in hippocampus after physical and mental training. However, less is known about the role of swimming exercise on brain insulin concentrations in diabetic animals and humans. In this communication, the effects of moderate physical training on the brain insulin concentrations in experimentally induced diabetic rats are reported.

Materials and Methods

Male Wistar rats (175-200 g) from the Botucatu Central Animal Breeding Center São Paulo State University (UNESP), were used and maintained in Biodynamic Laboratory in Department of Physical Education, in São Paulo State University (UNESP) Rio Claro, at 25°C with 12:12 hr L:D cycle and fed with Purina rat food and water ad libitum. All experiments with animals were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe no123, Strasbourg, 1985).

Diabetes induction and experimental design — Diabetes was induced by an intravenous injection (32 mg/kg body weight) of alloxan (Sigma). After 5 days, blood samples were obtained with animals in the fed state to determine the plasma glucose concentration. Rats that were not diabetic (<200 mg/dl) or too severely diabetic (>600 mg/dl) were eliminated from the study. Rats injected with citrate buffer (vehicle for alloxan) were used as controls.
The rats were randomly distributed into following 4 groups of 8 animals each: sedentary control (SC), trained control (TC), sedentary diabetic (SD) and trained diabetic (DT). Training included daily swimming with a load of 5% of the body weight, 1 hr/day, 5 day/week, for 4 weeks.

Serum and tissue analyses — At the end of the experimental period, the rats were sacrificed 48 hr after their last exercise bout by decapitation. Trunk blood was collected in a glass tube, centrifuged for 5 min at 3000 rpm for glucose, insulin, albumin and hematocrit determinations. Blood samples for hematocrit were collected before sacrifice through a small cut at the top of the tail. Samples of the liver for glycogen and the whole brain for insulin content were collected. These evaluations aimed at verification of the efficiency of alloxan in inducing a diabetic picture in the animals in the conditions of the present study and also to evaluate the efficiency of the exercise training protocol.

Analytical methods — Serum glucose concentration was measured by a colorimetric method. Serum insulin concentration was determined by radioimmunoassay (RIA Kit Coat-A-Count, USA). Serum albumin and liver glycogen were estimated. The brain was weighted and homogenized in 5 ml of insulin extractor liquid, centrifuged (3000 rpm) and 2 ml of the supernatant were taken and neutralized. After another centrifugation, 100 µl of the supernatant were taken for insulin determination by radioimmunoassay.

Statistical analysis — All dependent variables were analyzed by one-way analysis of variance (ANOVA) and a significance level of $P<0.05$ was used for all comparisons. The Bonferroni test was used for post-hoc comparisons. All values are expressed as mean±SD.

Results

Both diabetic groups showed decrease in serum insulin concentrations and increased blood glucose concentrations (Table 1). Physical training reduced blood glucose in TD group. Hematocrit and serum albumin were similar among all groups (Table 1).

Diabetic animals demonstrated lowered liver glycogen stores (51%), but physical training was able to counteract this alteration in TD group (reduction of 15%) (Fig. 1).

Brain insulin was not altered in sedentary diabetic animals and training increased brain insulin both in diabetic and control rats ($P<0.05$; TC=62.3%; TD=70.9%) (Fig. 2).

Discussion

It is well recognized that insulin has a fundamental role in neural growth, differentiation and function. Furthermore, alterations in brain insulin concentrations have been associated with the development of cognitive disturbances in age-associated diseases, such as Alzheimer. In the present study, the effects of physical training on brain insulin concentrations in experimental diabetic animals were evaluated.

As expected, blood insulin was decreased and blood glucose was increased in both diabetic groups (SD vs TD). Physical training partially counteracted the blood glucose alterations in the diabetic group.

![Liver glycogen (mg%) after 4 weeks of training](image)

Fig. 1 — Liver glycogen (mg%) after 4 weeks of training. [The values are the mean ± SD of 8 rats/group. ANOVA $P<0.001$; DF (3,28); $F=11.9$; Bonferroni test $P<0.05$; a≠SC; b≠TC]

Table 1 — Blood glucose, insulin, albumin and hematocrit after four weeks of training in rats

<table>
<thead>
<tr>
<th>Groups Parameter</th>
<th>SC</th>
<th>TC</th>
<th>SD</th>
<th>TD</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>147.1±10.0</td>
<td>149.9±13.1</td>
<td>459.2±86.1$^{a,b}$</td>
<td>269.3±113.0$^{a,b,c}$</td>
<td>33.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>13.3 ± 8.0</td>
<td>14.1 ± 5.3</td>
<td>4.8 ± 1.2$^{ab}$</td>
<td>3.0 ± 0.9$^{ab}$</td>
<td>10.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Albumin (g%)</td>
<td>4.9 ± 0.4</td>
<td>4.6 ± 0.7</td>
<td>4.3 ± 0.6</td>
<td>4.3 ± 0.3</td>
<td>2.4</td>
<td>0.089</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>50.1 ± 2.1</td>
<td>52.0 ± 5.3</td>
<td>52.4 ± 2.7</td>
<td>52.1 ± 3.2</td>
<td>0.68</td>
<td>0.57</td>
</tr>
</tbody>
</table>

SC - sedentary control, TC - trained control, SD - sedentary diabetic, TD - trained diabetic. ANOVA; DF (3,28); $a$≠SC; $b$≠TC; $c$≠SD
(TD). Previous studies have also shown that chronic exercise decreases serum glucose in diabetic animals and humans \(^{20,21}\) since skeletal muscle responds to chronic exercise via a series of structural and functional adaptations. The mechanisms that improve glucose uptake through physical training include increased muscle blood flow, increased insulin binding to its receptor (IR) and increased IR turnover \(^{22,23}\). The increased responsiveness to insulin induced by swimming exercise in rat skeletal muscle may result partly from modulation of the insulin signaling pathway at different molecular levels \(^{24}\). In particular, the IRS/IP3-kinase pathway may be involved in the activation of glucose transport and glycogen synthesis in muscle, and an increase in this association in the muscle of trained animals and humans may have an important role in the responsiveness to insulin \(^{24,25}\).

No difference in hematocrit and serum albumin concentrations was observed among the groups in the present study. This suggests that the alterations observed in blood glucose probably were not influenced by dehydration \(^{26}\).

In the present study, diabetes decreased glycogen content in liver and the physical training restored it in diabetics. These results are in agreement with other studies, showing that endurance exercise induces chronic adaptations in liver, enabling diabetic rats to restore their hepatic glycogen stores \(^{21,27}\). Insulin deficiency leads to an increase in phosphorylase activity and to a decrease in glycogen synthase activity \(^{28}\). Pettersen \textit{et al}. \(^{29}\) showed that relatively high rates of net hepatic glycogen synthesis were observed in hyperglycemia and hyperinsulinemia due to combined stimulation of glycogen synthase and inhibition of glycogen phosphorylase flux \(^{30}\). In agreement, they showed that trained rats had a higher total hepatic glycogen synthetase activity and a lower phosphorylase activity, with increased glycogen content in liver.

The present study demonstrated that experimental diabetes induced by alloxan did not alter brain insulin concentrations. It is well established that insulin can cross blood-brain barrier and reach CNS \(^{18,31}\). On the other hand, there is no consensus among researchers about insulin biosynthesis in the brain \(^{14,18}\). In the present study, blood insulin was decreased in diabetic animals whereas brain insulin was unaltered in the sedentary diabetic group. The present data indicate a dissociation between blood and brain insulin concentrations, corroborating with the hypothesis that insulin synthesis may occur in CNS, as pointed in other studies \(^{4,6}\).

The type II diabetics were more susceptible to development of cognitive disturbances. Type II diabetes is caused by insulin resistance and possibly is associated with decreased brain insulin concentrations, decreased memory in elders and clinically detectable Alzheimer disease (AD) \(^{4,32}\). Nevertheless, many studies have investigated type II diabetes, whereas studies with type I diabetes are scarce. In the present study, though we have not investigated directly AD, we observed that alloxan diabetes induction did not alter brain insulin concentrations in type I diabetes rat model.

It is well recognized that physical and mental activities decrease the development of AD in elders. Nevertheless, exact mechanisms by which this occurs is not yet known \(^{33,34}\). In the present study, we found significant increase in brain insulin concentrations in trained groups (TC and TD). The increase in brain insulin concentrations in trained animals is an interesting finding. If this may be useful in AD prevention, needs further studies. Insulin does have a significant effect in global brain glucose metabolism and this effect occurs mainly in the cerebral cortex. Also, insulin modulate acetylcholine, norepinephrine and dopamine concentrations, important neurotransmitters for cognition process \(^{4}\). In summary, moderate physical training is able to increase brain insulin content both in normal and diabetic rats. Whether this increase in brain insulin induced by physical training is beneficial in AD prevention requires more studies.
References
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