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THE INFLUENCE OF INSULIN ON THE ACTIVITY OF ANTIOXIDANT ENZYMES IN THE RAT INTERSCAPULAR BROWN ADIPOSE TISSUE AND HYPOTHALAMUS

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The specific activity of some antioxidant enzymes: copper-zinc superoxide dismutase (CuZn SOD), manganese superoxide dismutase (Mn SOD) and catalase in the rat interscapular brown adipose tissue (IBAT) and hypothalamus were examined under the influence of chronic insulin treatment. Daily injection of both low (04 IU/kg b. m., i. p.) and high (4.0 IU/kg b. m., i. p.) doses of insulin over a 7 day period, induced a dose -dependent decrease in the blood glucose concentration. However, despite severe hypoglycemia, insulin stimulated significantly the activity of both superoxide dismutase (SOD) forms in the IBAT but had no effect on catalase activity. Moreover, insulin administration, irrespective of the dose applied, did not cause any change in the activity of any of the hypothalamic enzymes studied. These results suggest that the response of the main enzymes of antioxidant defence to chronic insulin treatment is tissue dependent. They also indicate that insulin probably regulates the activity of both SOD forms in the IBAT only, but is ineffective in the hypothalamus.

Key words: antioxidant enzymes, insulin, IBAT, hypothalamus.

INTRODUCTION

Interscapular brown adipose tissue (IBAT) is a highly specialized tissue, morpho-functionally adapted for heat production (thermogenesis).BAT thermogenesis is controlled by the sympathetic nervous system (SNS) (Girardier and Seydoux, 1986) with the integration of signals occurring in the hypothalamus. Moreover, many hormones have been shown to have a considerable influence on BAT metabolic activity. Some of them (catecholamines, insulin and melatonin) stimulate BAT activity and they are termed "pro-brown fat" hormones. Those which inhibit BAT activity, such as thyroid hormones and corticosteroids, are termed "anti-brown fat" hormones (Galpin et al. 1983). Despite the fact that insulin is considered as an anabolic hormone, it has been recently proposed that it can act as a catabolic hormone i. e. as a signal for

thermogenesis (Rothwell and Stock, 1988). Namely, it was shown that the peripheral injection of a relatively low dose of insulin may stimulate thermogenesis (Rothwell and Stock, 1988) and increase both the proton conductance pathway and the amount of uncoupling protein (UCP) in the IBAT (Seydoux et al. 1984). Conversely, in streptozotocin-diabetic rats, the capacity for thermogenesis is decreased and also the amount of the IBAT UCP (Seydoux et al. 1984; Géloën and Trayhurn, 1990). However, at present the precise mode of insulin action on BAT is not known. There is evidence that insulin stimulates BAT thermogenesis indirectly via central activation of the SNS (Landsberg and Young, 1984). It seems that the site of insulin action is within the ventromedial hypothalamus which contains insulin sensitive neurons in terms of glucose uptake and firing rate (Oomura, 1976) and which is involved in the regulation of body mass and BAT thermogenesis (Perkins et al. 1981). On the other hand, since insulin receptors are present in BAT (Géloën and Trayhurn, 1990) and having in mind the fact that insulin stimulates glucose transport (Czech et al. 1974) and lipogenesis (Mc Cormack, 1982) in this tissue, a direct effect of insulin on the brown adipocytes cannot be excluded. Bearing in mind that insulin can stimulate the sympathetic outflow and consequently BAT thermogenesis, there exists the possibility of increased generation of superoxide anion radicals (02) and consequently an enhanced production of hydrogen peroxide (H_2O_2) (Mc Cord Fridovich, 1969). It should be also noted that, in addition to other effects insulin increases H_2O_2 production in the brown adipocytes via stimulation of NADH and NADPH oxidase. Thus, H_2O_2 has been detected in brown adipocytes where it acts as a transmembrane messenger in insulin action (Halliwell and Gutteridge, 1989). Since superoxide anion radicals are very toxic, aerobic organisms possess antioxidant enzymes which maintain their intracellular concentration at a low level preventing membrane destruction. The main enzyme, which converts superoxide radicals into H_2O_2 plus O_2, is superoxide dismutase. Rat IBAT contains the two main forms of SOD: CuZn SOD and Mn SOD, localized in the cytosol and in the mitochondrial matrix respectively (Petrović et al. 1989).

Bearing in mind the evidence mentioned above, we consider that changes in the activity of some enzymes of the antioxidant system could be important indicators of metabolic activity in the IBAT under the influence of insulin. Therefore, we have investigated the effects of chronic administration of low and high doses of insulin on the activity of both SOD forms and catalase in the rat IBAT and hypothalamus.

MATERIALS AND METHODS

Male Wistar rats, weighing 218-256 g, at tha beginning of the experiment, were used. The animals were previously acclimatised to 22 ± 1°C, maintained under intermittent 12 h periods of light and dark and given water ad lib. The

rats were divided into three groups. The first group of rats (control) was treated with saline. The second group of animals was injected intraperitoneally with inculin (ICN Galenika, Beograd) in a dose of 0.4 IU/kg body mass over 7 days. The rats of the third group received a high dose of insulin i. e. 4.0 mg/kg b.m for 7 days. On day 7 of the experiment the animals of all the three groups were decapitated and their IBAT and hypothalamus removed, weighed and prepared for the measurement of both SOD forms and catalase activity. Namely, both IBAT and hypothalamus were minced and homogenized at 0-4^{0}C using 0.25 M sucrose, 0.05 M Tris and 0.1 nM EDTA adjusted to pH 7.34, with HCl. The homogenates were sonicated (at 50 W for 30 s in a Bronson model B-12 sonicator) to release Mn SOD. The homogenates were then centrifuged at 6,000 x g for 15 min. The supernatant was centrifuged at 85,000 x g for 90 min and used for the determination of CuZn and Mn SOD activities. SOD activity in the cytosol (CuZn SOD) and mitochondria (Mn SOD) was determined by the epinephrine method of Misra and Fridovich (1972) and expressed as units of SOD/mg of proteins. Total protein content was measured by the method of Lowry (1951). IBAT mitochondria were prepared by the method of Slinde et al. (1975) and mitochondrial protein content was estimated by a dye-reagent method (Bio-Rad) using bovine serum albumin standards. All results are presented as means ± SEM. The statistical significance of differences between the groups was evaluated by Student's t-test.

RESULTS

The results presented in Table 1 show that chronic insulin treatment of rats induced a dose-dependent decrease in blood glucose concentration ($P < 0.005$). At the same time, wet IBAT mass was significantly enhanced by each of the insulin doses applied ($P < 0.025$). In addition, insulin, irrespective of the applied dose, did not significantly alter the total protein content but markedly enhanced the mitochondrial protein content ($P < 0.005$).

Table 1. Effects of low and high doses of insulin on blood glucose and IBAT mass, total protein and mitochondrial protein content

Treatment	Control	Insulin	
	(Saline)	Low dose (o.4 IU)	High dose (4.0 IU)
Blood glucose (mM/1)	6.59 ± 0.18	4.50 ± 0.54	2.8 ± 0.26
IBAT mass (mg)	165 ± 13.22	191 ± 9.27	209 ± 14.5
IBAT protein content (mg/ml homog.)	4.72 ± 0.22	4.22 ± 0.22	4.16 ± 0.20
IBAT mitochondrial protein (mg/ml homog.)	0.21 ± 0.02	0.37 ± 0.05	0.27 ± 0.02

Mean values S. E. of mean (n=6)

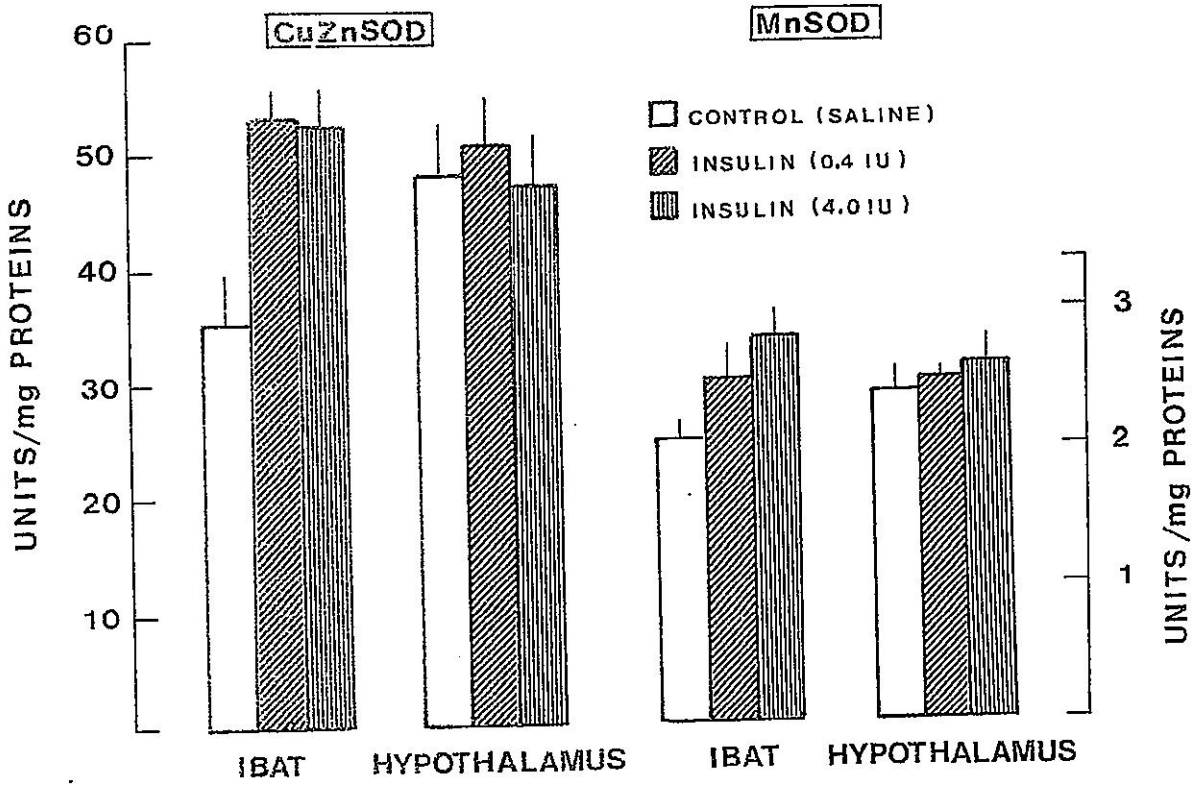

Figure 1. Effects of low (0.4 IU/kg b. m., i. p.)and high (4.0 IU/kg b. m., i. p.) doses of insulin on CuZn SOD and on Mn SOD activity in the interscapular brown adipose tissue and hypothalmus of rats. Each bar is the mean (SE) of 6 animals.

It is evident from Figure 1 that daily peripheral injection of either low or high insulin doses, for seven days, significantly increased the specific activity of CuZn SOD in the IBAT ($P < 0.025$). The values for control and insulin treated rats were 35.46 ± 6.02 and 54.1 ± 3.23 (low dose) and 53.34 ± 3.85 U/mg proteins (high dose) respectively. Similarly, a marked enhancement in Mn SOD activity in the rat IBAT was obtained also with the low and high doses of insulin (Figure 1) ($P<0.025$). The respective values for the control and insulin treated animals were 2.03 ± 0.0 and 2.5 ± 0.25 (low dose) or 2.8 ± 0.20 U/mg proteins (high dose). At the same time, the specific activity of both SOD forms in the hypothalamus (Figure 1) remained unchanged with respect to corresponding controls: 48.88 ± 4.5 (control), insulin treated 50.41 ± 6.6 (low dose) and 46.65 ± 4.7 U/mg protein (high dose) for CuZn SOD and 2.4 ± 0.18 (control), insulin treated 2.5 ± 0.06 (low dose) and 2.6 ± 0.32 U/mg protein (high dose) for Mn SOD.

In contrast to the increased specific activity of both SOD forms in the IBAT, catalase activity was not affected by either of the doses of insulin (Figure 2). Similarly, chronic treatment with insulin did not significantly change catalase activity in the hupothalamus.

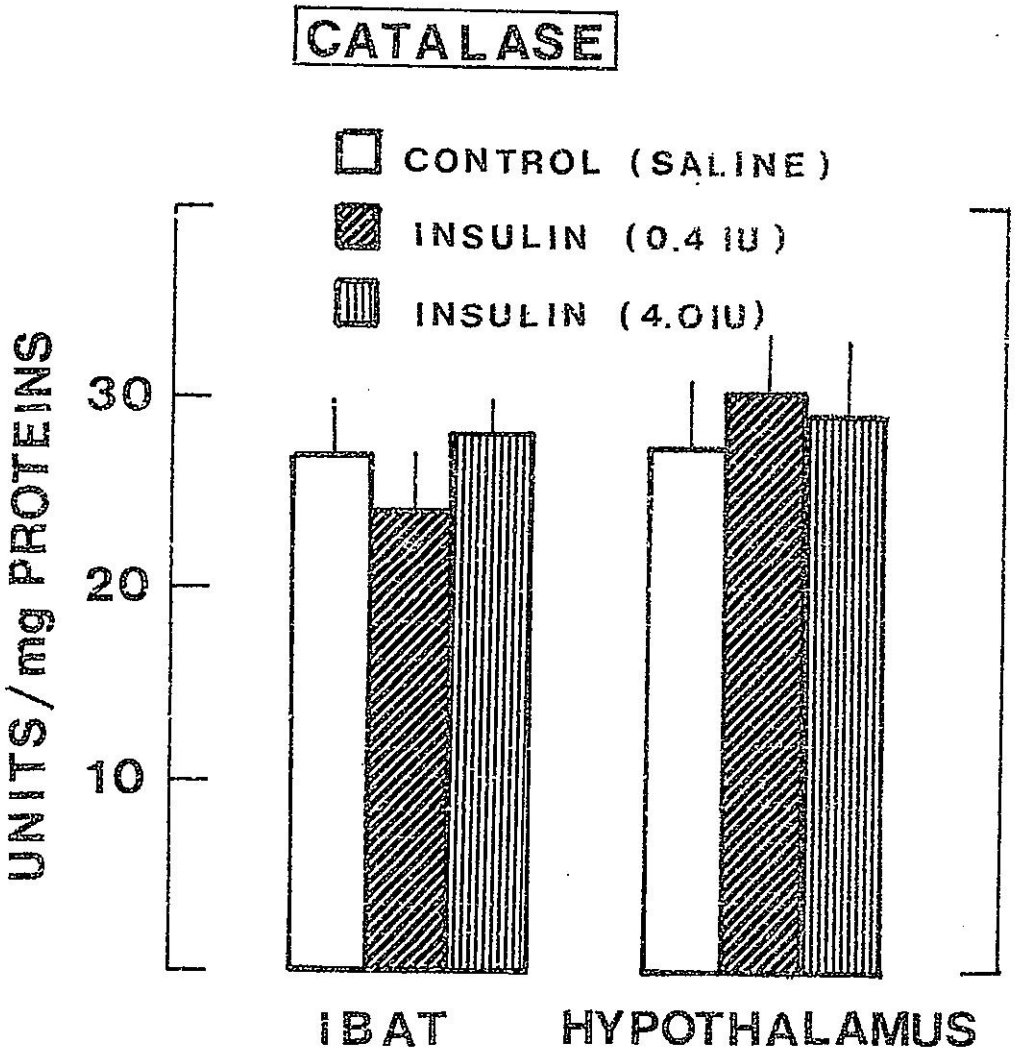

Figure 2. Effects of low (0.4 IU/kg b. m., i. p.) and high (4.0 IU/kg b.m., i.p.) doses of insulin on catalase activity in the interescapular brown adipose tissue and hypothalamus of rats. Each bar is the mean (SE) of 6 animals.

DISCUSSION

Our present results clearly show that chronic insulin treatment caused the well known dose-dependent hypoglycemia (Table 1). At the same time, insulin markedly increase both the IBAT wer mass and mitochondrial protein content but did not change the total protein content in this tissue. This insulin-induced increase in the BAT mass, wich is not accompanied by a change in the total IBAT protein content, may result from the possible very intense insulin-elicited lipogenesis (Granneman and Campbell, 1984). However, insulin produced a significant enhancement in the IBAT mitochondrial protein content. This increase might be the consequence of an insulin-induced increase in SNS activity (Landsberg et al. 1984) and enhanced noradrenaline secretion. This neurohormone, has a trophic effect leading to an increase in the mitochondrial protein content in the IBAT. Despite the fact that severe hypoglycemia occurs,

long-term insulin treatment, with either of the doses, resulted in an increased specific activity of both IBAT SOD forms. However, insulin did not change the IBAT catalese activity. To explain the obtained results we must take into account the following evidence: if insulin is one "pro-brown fat" hormone – which initiates thermogenin gene expression (Jacobsson et al. 1986) leading to an elevation of thermogenin synthesis (Seydoux et al. 1984), inducing a rise in the resting oxygen consumption (Andrews et al. 1985) and finally stimulating the total thermogenic activity of the IBAT - it is possible to suppose that, under these conditions, the generation of superoxide anion (02) radicals is increased and consequently the activity of the main enzymes of antioxidant defence (such as both SOD forms). These enzymes dismutate superoxide anion radicals into H_2O_2 plus 02. However, the exact mechanism of insulin stimulation of IBAT SOD activities, in vivo, is still obscure. It appears that insulin can initiate SOD gene expression and consequently SOD protein synthesis either directly, by acting on the brown adipocytes themselves, or indirectly by acting via higher centers and sympathetic stimulation. On the other hand, the failure of insulin to change the activity of the IBAT catalase, the enzyme which metabolizes H_2O_2 originating from the dismutation of superoxide anion radicals, might be interpreted as an adaptive response of the IBAT to maintain the optimal concentration of H_2O_2, which has been shown to act as a transmembrane second messenger for insulin action in adipocytes (Halliwell and Gutteridge, 1989). At the same time, the failure of insulin to alter the activity of all the hypothalamic enzymes studied might be the consequence of the low activity of the catecholamine degrading enzyme monoamine oxidase (MAO) which was obtained under the same experimental conditions (our unpublished results). Therefore, since insulin decreases hypothalamic MAO activity, the production of H_2O_2, which is generated in the processes of noradrenaline deamination (Cohen, 1986), is not possible.

In conclusion, these data suggest that the response of the main enzymes of antioxidant defence to insulin treatment is tissue specific. They also indicate that insulin plays a significant role only in the regulation of the activity of both IBAT SOD forms but is ineffective in the hypothalamus. However, the exact mechanism of insulin stimulation of the IBAT SOD acitivities remains to be established.

A c k n o w l e d g e m e n t

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UTICAJ INSULINA NA AKTIVNOST ENZIMA ANTIOKSIDATIVNE ZAŠTITE U INTERSKAPULARNOM MRKOM MASNOM TKIVU I HIPOTALAMUSU PACOVA

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SADRŽAJ

Izučavan je uticaj hroničnog tretiranja pacova insulinom na promene specifične aktivnosti enzima antioksidativne zaštite: bakar-cink superoksid dis-

mutaze (CuZn SOD), mangan superoksid dismutaze (Mn SOD) i katalaze u interskapularnom mrkom masnom tkivu (IBAT) i hipotalamusu. Dugotrajno tretiranje pacova (u roku od 7 dana) kako sa niskom (0.4 IU/kg, t. m., i. p.) tako i sa visokom (4. 0 IU/kg, t. m., i. p.) dozom insulina izaziva dozno zavisno smanjenje koncentracije glukoze u krvi. Međutim, uprkos izraženoj hipoglikemiji, insulin značajno stimuliše specifičnu aktivnost obe forme superoksid dismutaze (SOD) u IBAT-u ali ne menja aktivnost enzima katalaze u ovom tkivu. Istovremeno, insulin, nezavisno od primenjene doze, ne menja aktivnost ni jednog od izučavanih enzima u hipotalamusu. Ovi rezultati sugerišu da glavni enzimi antioksidativne zaštite u različitim tkivima različito odgovaraju na hronično tretiranje insulinom. Oni takođe ukazuju na mogućnost da insulin reguliše samo aktivnost obe forme SOD u IBAT-u ali da je bez efekta na aktivnost ovih enzima u hipotalamusu.