Physiol. Res. 51: 261-266, 2002

Effect of Hypothermia on Insulin - Receptor Interaction in Different Rat Tissues

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Received November 6, 2000
Accepted December 17, 2001

Summary

Experimental hypothermia caused extensive changes in the number of both classes of insulin receptors in different rat tissues. In the liver, the number of high affinity insulin receptors (HAIRs) decreased by 50 % (from 25.3 to 12.6 fmol/mg membrane protein), whereas number of low affinity insulin receptors (LAIRs) was almost unchanged in comparison to normothermic animals (5.63 and 4.39 pmol/mg, respectively). In the adipose tissue, number of both classes was reduced – HAIRs by 81 % (from 24.0 to 4.50 fmol/mg) and LAIRs by 92 % (from 16.0 to 1.29 pmol/mg). In the skeletal muscle, capacity of HAIRs was not changed (16.2 and 19.3 fmol/mg in normo- and hypothermic animals, respectively), whereas number of LAIRs increased by 150 % (from 6.65 to 16.6 pmol/mg). Hypothermic rats also showed lower amount (by 85 %) of LAIRs in the heart muscle (9.37 and 1.43 pmol/mg in control and experimental animals, respectively). Simultaneously, no significant changes were found in HAIRs (16.3 and 11.9 fmol/mg, respectively) and LAIRs (4.43 and 3.88 pmol/mg, respectively) in the brain. These differences in insulin receptors responses to hypothermia may reflect different physiological role of insulin in the regulation of target cell metabolism and/or the differences in tissue distribution of the insulin receptor isoforms.

Key words

Hypothermia • Insulin receptors • Liver • Adipose tissue • Skeletal muscle • Heart • Brain

Introduction

The first step in insulin action is its fast and reversible interaction with the specific protein receptor. The insulin receptor is located on the cell surface, within the fluid mosaic matrix of the plasma membrane (Curry and Curry 1970, Flint 1980, Benzing *et al.* 1983, Camps *et al.* 1992). Insulin binding initiates receptor internalization and subsequent sorting of the internalized receptor through recycling or degradative pathways (Olefsky 1990). Geiger *et al.* (1989) claimed that receptor - mediated internalization is a major mechanism of down - regulation of insulin receptors. Because of the intimate contact of the receptor with membrane lipids, alterations in the composition or the physical state of the lipids might affect the properties of the insulin receptor. Previous studies of the interaction of the insulin receptor and the plasma membrane indicated that temperature

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changes are responsible for the alterations in membrane fluidity (Benzing *et al.* 1983, Foreman and Tylor 1989, Geiger *et al.* 1989, Camps *et al.* 1992). The effect of the membrane lipid environment under hypothermic conditions on the properties of insulin receptors may play an important role in changes of the hormone - receptor interaction. Amatruda and Finch (1979) examined the binding of insulin to its receptors in the adipose tissue as a function of temperature. They reported that less insulin was bound at 37 °C than at 25 °C. High temperature increased membrane fluidity and this was associated with lower insulin binding by insulin receptors. Ginsberg *et al.* (1981) demonstrated that increased membrane fluidity of the Friend erythroleukemia cellsleads to an increase in the number of insulin receptors and a decrease in their affinity.

Another mechanism, which could be due to the altered interaction between insulin and its receptor, is hypoinsulinemia resulting from hypothermia (Hirvonen *et al.* 1978, Loubatieres-Mariani *et al.* 1980, Steffen 1988).

Our review of literature failed to find any current study on the insulin receptor properties in different rat tissues during hypothermia.

The aim of the study was to investigate the effect of hypothermia on (^{125}I) - insulin binding to plasma membranes isolated from rat skeletal muscle, adipose tissue, liver, heart and brain, and to determine whether the decrease in blood insulin concentration could affect the number and/or affinity of insulin receptor sites according to the down and up regulation theory.

Methods

Adult male Wistar rats weighing 200-220 g were used in the experiments. They were housed individually under standard room conditions with free access to a LSM laboratory standard diet and water, but the animals were deprived of food for 12 h before the experiments. All investigations were carried out taking into consideration the ethical aspects of the experimental procedure and were approved by the Ethical Committee of the Poznan University of Medical Sciences.

On the mornings of the experimental days, the animals were randomly divided into a normothermic (control) group and hypothermic group, each consisting of 12 rats. After induction of general anesthesia with thiobutabarbital i. p. (12-30mg/kg body weight) the control group was kept at room temperature, while the second group of rats was subjected to hypothermia as described previously (Torlinska *et al.* 1993). Hypothermia in anesthetized rats was induced by placing them in a special box filled with ice. The time of exposure for both groups was about 25-30 min. The mean rectal temperature was 36.6 ± 0.2 °C and 26.6 ± 0.2 °C for normothermic and hypothermic rats, respectively. Animals were sacrificed by cardiac puncture and the tissues were removed immediately after exsanguination and kept at -20 °C. Plasma insulin was measured by radioimmunoassay (Rat Insulin RIA kit, Linco Research Inc. USA). Protein concentration was estimated by the method of Lowry *et al.* (1951). Glucose concentration was determined by the glucose oxidase method (POCH Gliwice, Poland).

Isolation of crude membrane fraction

Plasma membranes from the tested rat tissues were prepared and purified according to the method of Havrankova *et al.* (1978). Briefly, tissues were homogenized in 20 ml of 0.001 M $NaHCO_3$ and centrifuged at 600 x *g* for 30 min. The resulting supernatant was centrifuged at 20 000 x *g* for 30 min and the pellet was washed twice with 0.001 M $NaHCO_3$. After protein determination, the final suspension was centrifuged again as above. The final pellet of membranes was resuspended in an incubation buffer (0.04 M Tris - HCl, pH 7.4) containing 0.2 g/l bovine serum albumin (BSA). All the procedures described above were carried out at 4 °C. All the chemical reagents were produced by Sigma Chemicals Co., USA.

Binding assay

Insulin binding activity was measured by incubating the membrane preparations (0.25 mg of protein) at 4 ^{0}C for 16 h with 80pg ^{125}I labelled porcine insulin (specific activity 8.0 GBq/mg, IBJ Swierk, Poland) in a final volume of 0.5ml 0.04 M Tris buffer (pH 7.4) containing 0.1 % BSA. Nonspecific binding was determined in the presence of 10 µmol/l unlabelled insulin. Bound and free fractions of insulin were separated by centrifugation at 20 000x*g* for 8 min and then the radioactivity of the pellets was determined using a gamma counter. For the competition binding assay, increasing amounts of unlabelled insulin were added to the reaction mixture to give a final concentration of 0-700 nmol/l. Data from competition binding studies were analyzed by the Scatchard method (Scatchard 1949) using the LIGAND Pc.v.3.1 computer program (Munson *and*

Rodbard 1980). Results were expressed as means ± S.E.M. and were interpreted statistically according to Student's t-test. The probability $p < 0.05$ was considered to be statistically significant.

Results

At the end of the cold exposure, plasma immunoreactive insulin concentration was significantly decreased in hypothermic rats (by about 50 %) as compared to the control group (Table 1). Despite the magnitude of hypoinsulinemia, plasma glucose concentration remained unchanged. Consequently, the IRI-to-glucose ratio in the hypothermic rats was markedly decreased. As is shown in Fig. 1, the number of high affinity binding sites in the liver, expressed as the binding capacity, differed in normothermic and hypothermic rats showing decrease by about 50 % in the group of hypothermic rats. The binding capacities of low affinity receptors in the liver of normothermic and hypothermic animals were similar.

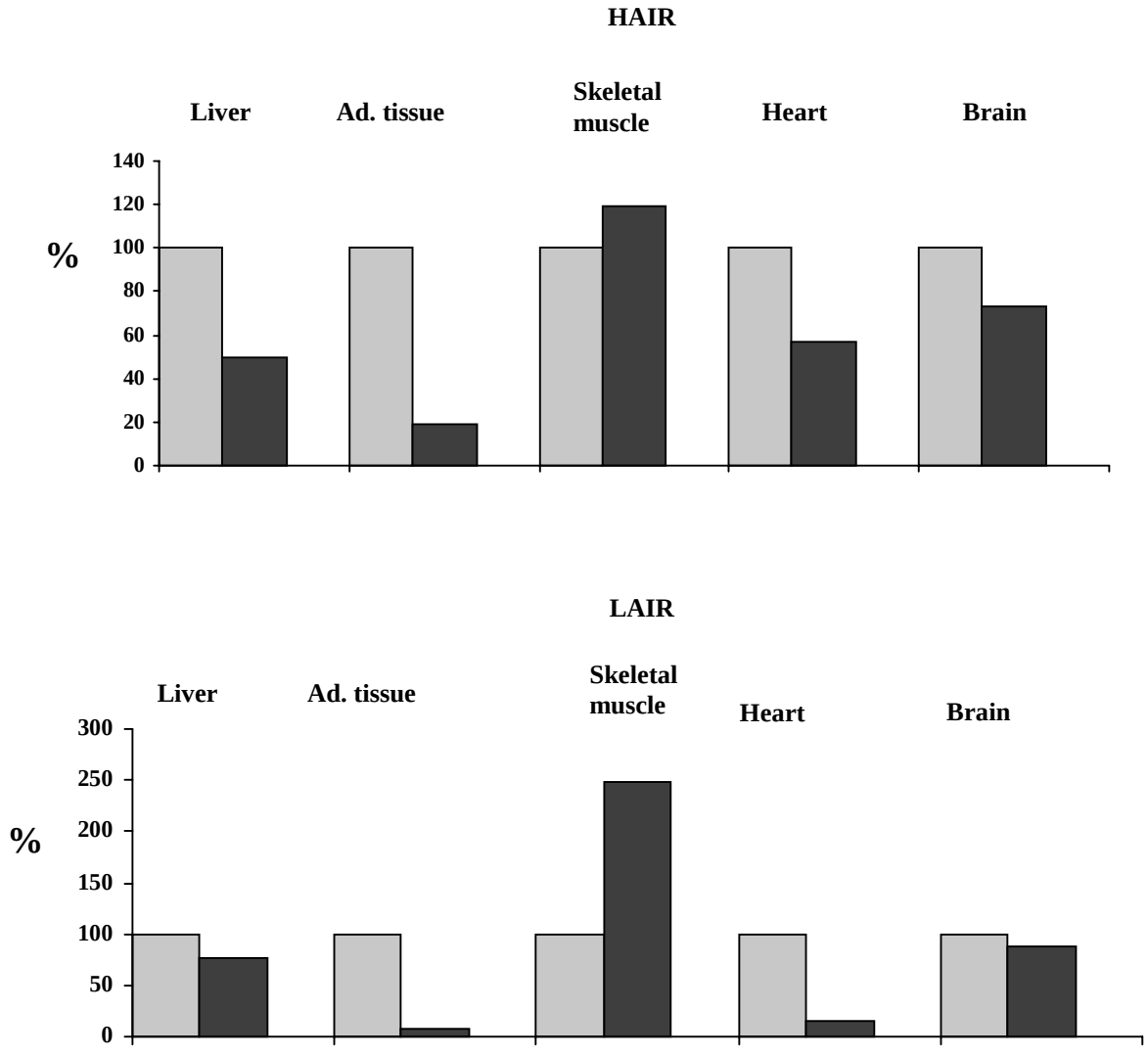

Fig. 1. *Binding capacity of high affinity (HAIR) and low affinity (LAIR) insulin receptors in the liver, adipose tissue, skeletal muscle, heart and brain of hypothermic (black columns) and normothermic (grey columns) rats. Binding capacity of HAIR and LAIR in normothermic (control) rats was taken as 100 %.*

The number of high affinity binding sites in the adipose tissue of hypothermic rats was decreased by approximately 81 % as compared with normothermic rats. The binding capacities of low affinity receptors in normothermic and hypothermic rats were also significantly different. We observed a decrease of insulin receptors by about 92 % in the hypothermic group.

Table 1. Serum glucose, insulin, and insulin/glucose ratio in normothermic and hypothermic rats.

	Normothermic (n=12)	**Hypothermic (n=12)**
Glucose (mmol/l)	8.99 ± 0.66	9.96 ± 2.06
Insulin (pmol/l)	134.90 ± 13.60	52.30 ± 5.12 *
Insulin/glucose (pmol/mmol)	15.00± 1.31	5.25± 0.80 *

** Significantly different from normothermic (control) rats (p<0.05)*

Table 2. Characteristics of insulin receptors in different tissues of normothermic and hypothermic rats.

	Normothermia				**Hypothermia**			
	HAIRs (fmol/mg)	**LAIRs (pmol/mg)**	**Kd_1 (nmol/l)**	**Kd_2 (nmol/l)**	**HAIRs (fmol/mg)**	**LAIRs (pmol/mg)**	**Kd_1 (nmol/l)**	**Kd_2 (nmol/l)**
Liver	25.3±4.7	5.63±0.88	0.23±0.03	300.0±60.0	12.6±3.0*	4.39±0.36	0.30±0.04	250.0±20.0
Adipose tissue	24.0±6.8	16.0±8.95	0.17±0.06	50.3±13.3	4.50±0.9*	1.29±0.40*	0.15±0.06	30.1±11.8
Skeletal muscle	16.2±3.1	6.65±1.3	0.82±0.21	282.0±29.0	19.3±3.8	16.6±1.76*	0.72±0.16	693.0±78.0
Heart	6.20±1.45	9.37±1.86	0.13±0.23	313.0±62.0	3.56±0.94	1.43±0.61*	0.58±0.12*	27.51±9.6
Brain	16.3±3.5	4.43±1.65	0.24±0.06	260.0±11.0	11.9±3.32	3.88±1.36	0.16±0.04	190.0±60.0

*Data are expressed as means ± S.D.; *Significantly different from normothermic rats (p<0.05); HAIRs - binding capacity of high affinity insulin receptors; LAIRs - binding capacity of low affinity insulin receptors; Kd_1, Kd_2 - dissociation constant for HAIRs and LAIRs, respectively.*

In skeletal muscle, the number of high affinity binding sites in normothermic and hypothermic rats did not differ significantly. On the other hand, the binding capacity of low affinity receptors was significantly increased by about 150 % in the hypothermic rats. A different situation was found in the case of hypothermic hearts where the number of insulin receptors was decreased as compared with normothermic hearts, but it was only significant in the class of low affinity insulin receptors (by about 85 %). By contrast, the number of both high and low affinity insulin receptors did not differ significantly in the brains of normothermic and hypothermic rats. The characteristic features of insulin receptors in the tested rat tissues (dissociation constant and binding capacity - B_{max}) are summarized in Table 2.

Discussion

Insulin receptors have been demonstrated in cells of a large variety of tissues from different animal species. It has been shown that binding of insulin receptors in different tissues is heterogeneous which may be due to a multiple class of receptors, to negatively cooperative interactions between receptors or combination of both (Gammeltoft 1984). The Scatchard analysis of binding data in our experiments has also shown nonlinear curves for both normothermic and hypothermic rat tissues which might indicate the existence of two receptor population with a high and low affinity to insulin.

Hypothermia accompanied by hypoinsulinemia and reduced glucose metabolism is regarded as a state of energy deficiency but the insulin receptor capacity or

affinity in multiple rat tissues has not yet been compared in hypothermic conditions. Our results indicate that there are basically three types of insulin receptor responses to hypoinsulinemia induced by hypothermia. The number of insulin receptors in the liver, adipose tissue, and heart is significantly reduced. These changes occur despite the significant decrease in blood insulin concentration, which seems to be in apparent conflict with the theory of down and up regulation (Geiger *et al.* 1989).

It appears that the decrease in specific insulin binding in hypothermia is due to a decrease in the number of insulin receptors in the liver plasma membranes, rather than to a change in the receptor affinity to insulin. The results obtained by Flint (1980) who worked on isolated rat hepatocytes have shown that the number of insulin receptors declines during lactation, whereas the serum insulin concentration is significantly reduced by 15th day of lactation. It seems probable that in situations such as lactation or hypothermia in which the need for energy substrates is apparently increased, the normal regulatory mechanism according to the down and up regulation theory does not work. On the other hand, the insulinopenia induced by hypothermia may be beneficial for the organism by favoring lipolysis in response to the need for energy substrates. The decrease in insulin concentration in hypothermic rats correlates well with the increase in serum free fatty acids (FFA) as we have shown previously (Torlinska *et al.* 1995).

The suggestion that an inverse relationship exists between the number of insulin receptors at target tissues and circulating insulin is also not consistent with our findings in hypothermic hearts. On the contrary, the number of insulin receptors in skeletal muscle is significantly increased under hypothermic conditions. These improvements of insulin binding in skeletal muscle binding sites of hypothermic rats probably result from an up regulation process induced by the low concentration of circulating insulin. Interestingly, the changes take place mainly in the low affinity receptor class. This phenomenon probably results from the lack of spare high affinity insulin receptors in the skeletal muscle as was shown by Camps *et al.* (1992). In our previous study we have shown that hypothermia decreases the rate of 2-deoxy-[2,6-^{3}H] glucose transport into skeletal muscle whereas muscle insulin sensitivity, measured either as lactate production or glycogen synthesis, is significantly increased. In our opinion, the increase in the number of insulin receptors plus increased sensitivity to insulin in the skeletal muscle of hypothermic rats may be a part of compensatory mechanisms stabilizing muscle glycogen stores despite hypoinsulinemia and a decreased rate of glucose transport (Torlińska *et al.* 1997).

Finally, brain insulin receptors do not respond to hypoinsulinemia under the conditions of our experiments, which could be attributed to the existence of blood-brain barrier. This is probably due to the different role played by insulin in the central nervous system, where it is thought to modulate sympathetic transmission and act as a tropic and growth-promoting factor.

In conclusion, the functional differences among insulin receptor responses to hypoinsulinemia may be accounted by for different physiological role of insulin in the regulation of the target cell metabolism (Joost 1995).

Another explanation of the differences observed could involve the distribution of receptor isoforms, which differs in various tissues. Cloning of the insulin receptor mRNA has revealed two forms of insulin receptors, differing by the absence (–12) or the presence (+12) of 12 amino acids near the COOH-terminus of the alpha-subunit. As a consequence, isoform A (+12) is predominant in the liver, while skeletal muscles express isoform B (-12). By contrast, heart and epididymal adipose tissue express both isoforms (Vidal *et al.* 1995).

In view of this, it is not surprising that the response of the tissues to hypoinsulinemia, which we observed in our study, was not identical.

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Reprint requests

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