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Forum Mini Review

Oxidative Enhancement of Insulin Receptor Signaling: Experimental Findings and Clinical Implications

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ABSTRACT

Signaling through the insulin receptor and several other receptor tyrosine kinases is subject to redox regulation. Prolongued exposure to hydrogen peroxide impairs the action of insulin, and may account to some extent for the decreased insulin responsiveness in hyperglycemic diabetic patients. However, insulin receptor kinase (IRK) autophosphorylation and/or kinase activity were found to be markedly enhanced by a more limited exposure to hydrogen peroxide or by an oxidative shift in the thiol/disulfide redox status. Oxidative enhancement of IRK function may be mediated by two different mechanisms with similar effects, *i.e.,* by direct oxidative activation of IRK activity or by oxidative inactivation of a protein tyrosine phosphatase, which otherwise down-regulates IRK-mediated signaling. As both mechanisms enhance IRK activity in the absence of insulin, there is a strong possibility that the background IRK activity in the postabsorptive period may be abnormally increased in certain oxidative conditions and thereby disturb the metabolism of glucose and other energy substrates. This remains to be tested. In line with the oxidative enhancement of IRK activity, clinical studies have shown that treatment with a thiol-containing antioxidant increases the postabsorptive glucose and/or insulin concentrations (*i.e.,* the HOMA-R index) at least under certain conditions. This effect may have therapeutic implications. *Antioxid. Redox Signal.* 7, 1071–1077.

INTRODUCTION

The diverse physiological functions of the insulin receptor (IR)

Insulin and its receptor are best known for their role in glucose homeostasis, intracellular glucose metabolism, lipid metabolism, and the synthesis of proteins at the transcriptional and translational level (62, 69). In line with these functions, abnormally low insulin receptor kinase (IRK) activity and/or impaired insulin responsiveness are common findings in obesity, non–insulin-dependent diabetes mellitus (NIDDM), and old age (15, 16, 19, 27, 28, 74). A muscle-specific IR knockout was shown to exhibit features of NIDDM (13), and dysregulation of insulin signaling in the central nervous system was found to be associated with neurodegenerative diseases, dysregulation of food intake, and diet-sensitive obesity (14). A series of more recent studies revealed, however, that signals from the IR or IR-analogous structures may also have negative functional implications. Studies in several distantly related species such as *Caenorhabditis elegans, Drosophila melanogaster,* and mice have shown that a decrease in IR signaling increases longevity and resistance to oxidative stress (12, 53, 63). Mice with fat-specific IR knockout also showed a relatively lower body fat mass in spite of normal food intake, suggesting that also in humans IR signaling may be an attractive target for therapeutic intervention in obesity (12). It is reasonable to assume that the activity of the IR may have to be delicately balanced in order to optimize the important physiological functions while minimizing the negative implications of IR signaling.

Redox regulation of signaling pathways

Free radicals and radical-derived reactive oxygen species (ROS) play a central role in numerous physiological and pathological processes by interacting with redox-responsive signaling pathways (for review, see 23). In many cases, sig-

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naling involves a redox-sensitive cysteine residue of a signaling protein. Certain redox-sensitive signaling pathways, therefore, respond not only to changes in ROS concentrations, but also to changes in the thiol/disulfide redox status (1, 5, 8, 29, 37, 50, 70). The IR signaling pathway is a particularly interesting case in point, because changes in insulin responsiveness were found in conditions with increased ROS concentrations or conditions with altered plasma thiol/disulfide redox status.

MODULATION OF IR SIGNALING BY ROS AND BY CHANGES IN THIOL/DISULFIDE REDOX STATUS

Insulin signaling requires autophosphorylation of the IR β-chain at Tyr^{1158}, Tyr^{1162}, and Tyr^{1163} (40, 65). Oxidants such as thiol-reactive agents, millimolar concentrations of hydrogen peroxide, or vanadate and pervanadate were found to exert insulin-like effects on intact cells in the absence of insulin (17, 18, 24, 35, 36, 44, 47, 54, 70, 71, 75, 80). Whenever tested, these agents were found to enhance tyrosine phosphorylation of the IR β-chain (24, 36, 75).

Various lines of evidence suggest that this enhancement is mediated by two different mechanisms that are not mutually exclusive (see Fig. 1), *i.e.,* by direct enhancement of the IR tyrosine kinase activity and by the oxidative inhibition of tyrosine phosphatase(s). Several signaling pathways involving tyrosine phosphorylation, including the IR signaling pathway are negatively regulated by protein tyrosine phosphatases (2, 3, 46, 51, 61, 82). These phosphatases are controlled by redox regulation through the reversible oxidation of the catalytic cysteine residue to sulfenic acid and sulfenylamide derivatives (7, 11, 21, 22, 52, 59, 68, 78). Whenever tested, this mechanism was found to enhance IR autophosphorylation and kinase activity in an insulin-dependent fashion. In addition, there is clear evidence for the enhancement of IR autophosphorylation and kinase activity by direct oxidative modification of the IRK domain, but the structural and functional details of this process are less well characterized. By exposing IR-transfected Chinese hamster ovary (CHO-HIR) cells to procedures that intracellularly induce mildly oxidative conditions, the number of IR β-chain sulfhydryl groups was shown to be decreased and IR tyrosine phosphorylation was concomitantly increased (70). More recent experiments with highly purified fragments of the IRK domain and several mutant proteins indicated that hydrogen peroxide at 30–100 μ*M* concentrations interacts directly with a redox-sensitive cysteine in the IRK domain and modulates its kinetic properties (T. Schmitt and Dröge, unpublished observations). The direct oxidative enhancement of IRK activity was also found to occur independently of insulin. The more detailed analysis revealed that oxidative derivatization of the IRK domain reverses the inhibition of kinase activity by ADP, *i.e.,* one of the products of the kinase reaction (T. Schmitt and W. Dröge, unpublished observations). As ADP is rapidly converted into ATP by cytoplasmic creatine kinase, and because creatine is utilized selectively by the brain and muscle tissues (73), the available evidence suggests (a) that the creatine kinase system may normally provide an important advantage for the insulin reactivity of muscle tissue over that of adipocytes, and (b) that this advantage is lost under more oxidative conditions that suppress the redox-sensitive ATP-dependent inhibition of IRK activity. The details of this process are still under investigation.

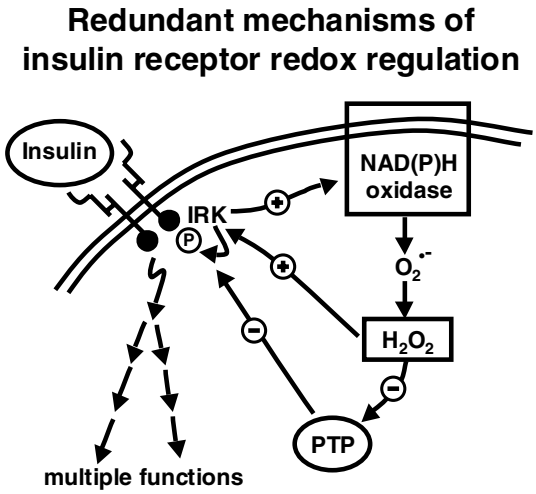

FIG. 1. Redundant mechanisms of IR redox regulation. Hydrogen peroxide (H_2O_2), which is produced in response to insulin signaling or other physiological or pathological processes, enhances IRK activity either by direct interaction with a redox-sensitive cysteine residue of the IRK domain, or by reversible inhibition of a protein tyrosine phosphatase (PTP), which normally down-regulates signaling pathways by dephosphorylating tyrosine-phosphorylated signaling proteins. $O_2^{\cdot-}$, superoxide anion.

Prolongued exposure of adipocytes or hepatocytes to 30–50 μ*M* hydrogen peroxide impairs insulin action (10, 30, 34, 45, 66, 67, 76, 77). Specifically, hydrogen peroxide was shown to impair insulin-induced IR autophosphorylation, stimulation of glucose transporter-4 (GLUT4) translocation, phosphatidylinositol 3-kinase (PI3-kinase) activation, and Akt activation (34, 66, 67, 76). In vascular smooth muscle cells, hydrogen peroxide was shown to decrease the autophosphorylation of the IR β-chain (30).

THE SOURCE OF ROS IN INSULIN SIGNALING: EVIDENCE FOR AN AUTOREGULATORY LOOP

Insulin stimulates rapid hydrogen peroxide production in a variety of cell types. In adipocytes, insulin was shown to induce NAD(P)H oxidase activity, which generates superoxide, *i.e.,* the precursor of hydrogen peroxide (49, 55, 56, 58, 60). In view of the positive and negative effects of hydrogen peroxide on insulin signaling, it is reasonable to assume that the endogenous induction of hydrogen peroxide production plays an autoregulatory role in insulin signaling. The positive autoregulatory role of ROS production has been studied in detail by Mahadev *et al.* (56), using the NAD(P)H oxidase inhibitor diphenyleneiodonium (DPI). DPI was shown to block

insulin-stimulated cellular hydrogen peroxide production, and to inhibit the insulin-induced activation of PI3-kinase, Akt kinase, glucose uptake, and GLUT4 translocation to the plasma membrane (for review, see Mehdi *et al.* in this volume).

PHYSIOLOGICAL IMPLICATIONS AND CLINICAL ASPECTS

Diabetes, obesity, and hyperglycemia

Diabetic hyperglycemia leads to glucose oxidation and oxidative degradation of glycated proteins, which result in ROS production (for review, see 57). As hyperglycemia was found to be ameliorated in diabetic patients by certain antioxidants, such as α-lipoic acid and *N*-acetylcysteine (NAC) (31, 57), it is suggested that oxidative inhibition of insulin signaling accounts, at least partly, for the decrease in insulin responsiveness in this condition.

Nondiabetic obese or hyperlipidemic subjects, in contrast, are not exposed to the extremely high ROS concentrations that are found in hyperglycemic subjects. However, they are exposed to moderately oxidative conditions as the plasma thiol level of these patients is, on average, abnormally low (39). A series of recent studies on nondiabetic subjects has shown that NAC treatment causes in these cases an increase in the postabsorptive glucose and/or insulin concentrations at least under certain conditions, indicating that in nondiabetic subjects oxidative enhancement of IRK activity prevails over oxidative inhibition (39). In two randomized double-blind trials involving a total of 140 nondiabetic subjects, NAC was found to increase the HOMA-R (homeostasis model assessment of insulin resistance) index (which is representative for the fasting insulin and glucose concentrations) in smokers and obese patients, but not in nonobese nonsmokers. This is compatible with the interpretation that the abnormally low plasma thiol level of these patients was indeed associated with an increased background IRK activity in the postabsorptive period, *i.e.,* in a state with low plasma insulin level. In obese patients, NAC also caused a moderate, but statistically significant, decrease in glucose tolerance (*i.e.,* after glucose loading) and a significant decrease in body fat. If NAC treatment was combined with creatine, a metabolite utilized mainly by brain and skeletal muscle tissues (6, 25, 38, 41, 72, 73, 81), the reduction in glucose tolerance as a *bona fide* indicator of muscular insulin reactivity was reversed, whereas body fat mass was still decreased (39). These observations suggest that creatine may selectively spare muscle tissues from the inhibitory effect of NAC on insulin reactivity. This conclusion is in line with the notion (see *Modulation of IR Signaling by ROS and by Changes in Thiol/Disulfide Redox Status*) that the creatine kinase system may normally provide an important advantage for the insulin reactivity of muscle tissue over that of adipocytes. The effect of creatine on NAC-treated obese patients also suggests that insulin reactivity is normally limited in these patients by low creatine availability. However, as the enhancing effect of creatine on insulin reactivity in the oral glucose tolerance test was seen only in NAC-treated and not in control patients, the effect of the muscular creatine kinase system on insulin reactivity may also have been compromised in these obese subjects by their relatively low thiol level. The fact that NAC treatment with or without creatine supplementation was found to decrease body fat mass suggested (a) that obesity may be, at least partly, the consequence of the abnormally low plasma thiol (mainly cysteine) levels in these patients, (b) that NAC treatment decreases the IRK activity of the adipose tissue, and (c) that the combined treatment of obese patients with NAC plus creatine may improve the balance between the insulin reactivity of skeletal muscle tissues and that of adipose tissues (39). This hypothetical mechanism clearly needs further investigations. But the observed effect is potentially of clinical interest irrespective of its mechanism. The hypothesis that obesity is at least partly related to the fat-specific IRK activity is in line with a recent study, showing that mice with fat-specific IR knockout accumulated less body fat mass in spite of normal food intake (12).

The age-related increase in the incidence of obesity is in line with the age-related decrease in the mean plasma cysteine concentration (33). Healthy young subjects have, on average, a higher plasma cysteine concentration than older subjects (33) or obese and hyperlipidemic subjects (39) and are able to ingest relatively large amounts of calories without gaining weight, *i.e.,* without storing all the calories they ingest.

Another mechanism that potentially accounts for the differential regulation of IRK activity in muscle and adipose tissues is schematically shown in Fig. 2. Glutathione (GSH) is the quantitatively most important intracellular thiol of low molecular weight and therefore an obvious inhibitor of the oxidative enhancement of IRK activity. In several cells and tissues, the intracellular GSH level was previously shown to depend decisively on the extracellular cysteine concentration (*i.e.,* in first approximation, the acid-soluble plasma thiol concentration). If the plasma thiol concentration is relatively high, intracellular GSH levels increase accordingly and

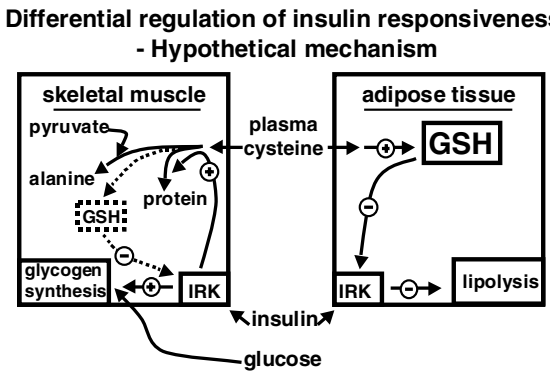

FIG. 2. Differential regulation of insulin responsiveness: hypothetical mechanism. The oxidative enhancement of IRK activity is down-regulated by GSH, *i.e.,* the quantitatively most important intracellular thiol compound of low molecular weight. Cysteine, *i.e.,* the quantitatively most important low-molecular-weight thiol in the plasma, is a limiting precursor for GSH biosynthesis. GSH biosynthesis in skeletal muscle tissue is limited by the fact that it has to compete for cysteine with protein synthesis and pyruvate-dependent transamination, which generates alanine.

down-regulate the oxidative enhancement of IRK activity. Skeletal muscle tissue, however, was found to have an exceptionally low intracellular GSH level, and the glycolytically active skeletal muscle type II fibers were found to have an even lower GSH concentration than type I fibers (9, 32, 42, 43, 48). This relatively low intracellular GSH level is tentatively explained by two processes that compete for cysteine, *i.e.,* protein synthesis and the pyruvate-dependent transamination of cysteine (see Fig. 2).

Longevity: positive consequences of a decrease in IRK activity

As a decrease in IRK activity is widely considered as merely disadvantageous, the observed decrease in insulin reactivity after NAC treatment may raise at face value a note of caution with regard to the use of NAC or other cysteine derivatives in hyperlipidemia and cardiovascular diseases as suggested previously (4, 20, 26, 64, 79). However, an abnormal insulin-independent increase in the IRK background activity may have negative consequences for the glucose, glucagon, and cortisol concentrations in the postabsorptive period. In line with this notion, a series of recent animal studies collectively showed that a decrease in IR signaling may increase longevity and resistance to oxidative stress (12, 53, 63), suggesting that a (moderate) decrease in IR signaling may have potentially positive effects. It may be important to note in this context that the various reports about positive consequences of IRK knockouts did not allow us to distinguish whether the apparent negative effects of IRK signaling were related to insulin-dependent IRK signaling or to the hormone-independent IRK background activity in the postabsorptive state.

FUTURE DIRECTIONS

As the oxidative enhancement of IRK activity was seen even in the absence of insulin, it will be important to study *in vivo* the IRK background activity at times of low insulin concentrations (*i.e.,* in the postabsorptive period) and the potentially negative consequences of an abnormally high IRK activity during this period. It may be reasonable to assume that IRK activity has to be delicately balanced in order to optimize the physiologically important positive functions of insulin signaling, while minimizing its negative implications.

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ABBREVIATIONS

DPI, diphenyleneiodonium; GLUT4, glucose transporter-4; GSH, glutathione; IR, insulin receptor; IRK, insulin receptor kinase; NAC, *N*-acetylcysteine; NIDDM, non–insulin-dependent diabetes mellitus; PI3-kinase, phosphatidylinositol 3-kinase; ROS, reactive oxygen species.

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