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Hyperinsulinemia induces a reversible impairment in insulin receptor function leading to diabetes in the sand rat model of non-insulin-dependent diabetes mellitus

(nutrition-induced diabetes/tyrosine kinase activity/insulin resistance)

HANNAH KANETY*, SMADAR MOSHE*, ELEAZAR SHAFRIR†, BRUNO LUNENFELD*, AND AVRAHAM KARASIK*

*Institute of Endocrinology, Chaim Sheba Medical Center, Tel Hashomer and the Tel-Aviv University Sackler School of Medicine, Tel-Aviv 52621, Israel; and †Department of Biochemistry, Hebrew University-Hadassah Medical School, and Hadassah University Hospital, Jerusalem 91010, Israel

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ABSTRACT **The insulin receptor was evaluated at different disease stages in the sand rat (*Psammomys obesus*), a model for nutrition-induced diabetes. Nondiabetic sand rats showed markedly low receptor number in liver compared with albino rats. Their receptor had an intact tyrosine kinase activity but a higher K_m for ATP in the phosphorylation reaction of exogenous substrates. The initial effects of overeating (i.e., development of hyperinsulinemia without hyperglycemia) were associated in the sand rat with a dramatic decrease in *in vitro* and *in vivo* insulin-induced receptor tyrosine kinase activity in both liver and muscle. In muscle, this coincided with a decrease in receptor number and an increase in basal tyrosine kinase activity. Similar changes were observed upon development of hyperinsulinemia with hyperglycemia. Upon recovery from the diabetic state by diet restriction, the impaired receptor kinase activation was corrected. Complete restoration occurred only in animals that fully recovered from the diabetic state and became normoinsulinemic. These observations indicate that loss and gain of receptor tyrosine kinase activity were dependent on insulin levels. Thus, overeating may lead to the development of hyperinsulinemia through ineffective extraction of excess insulin by the scarce liver receptors. Hyperinsulinemia, in turn, causes a reversible reduction in receptor kinase activity, leading to insulin resistance. This sequence of events may be relevant to diet-related changes in human non-insulin-dependent diabetes mellitus.**

The development of non-insulin-dependent diabetes mellitus (NIDDM) in the majority of patients occurs in parallel to weight gain (1). Overeating is initially followed by hyperinsulinemia and insulin resistance, which gradually develop into pronounced hyperglycemia. This diabetogenic process may be reversed by diet restriction and weight loss (2). Since both hyperinsulinemia and insulin resistance coincide with weight gain in humans, it is not feasible to determine which is the primary event mediating the effect of obesity. We have addressed this query in an animal model of NIDDM. The sand rat is a desert-adapted gerbil that in its natural habitat is nondiabetic (3–7). In captivity, on an ad libitum rodent diet, it develops obesity and hyperinsulinemia without or with hyperglycemia (6, 7). Through control of the animals' diet, it is possible to discern several stages of development of glucose intolerance, as well as reverse them, thus addressing the sequence of events that lead to induction of diabetes. We studied the insulin receptor (IR) function in these well-characterized animals and found alterations that may be relevant to human nutrition-induced diabetes.

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MATERIALS AND METHODS

Materials. Human recombinant insulin and ^{125}I-monoiodo-labeled (tyrosine-14 of the A chain) human insulin were gifts from Novo Biolabs (Bagsvaerd, Denmark). [γ-^{32}P]ATP was from Amersham. ATP, CTP, Hepes, Tween 20, poly(Glu^{80}Tyr^{20}), bovine serum albumin (insulin free), *N*-acetyl-D-glucosamine, leupeptin, aprotinin, soybean trypsin inhibitor, and phenylmethylsulfonyl fluoride were from Sigma. Wheat germ agglutinin (WGA) and Con A coupled to agarose were from Bio-Makor (Rehovot, Israel). Reagents for SDS/PAGE were from Bio-Rad. Anti-IR serum was raised in rabbits using a peptide corresponding to residues 1309–1324 of the carboxyl-terminal tail of the human IR β subunit according to Lerner *et al.* (8).

Animals. Sand rats (*Psammomys obesus*) were obtained from a colony established by J. H. Adler at the Animal Farm of the Hebrew University-Hadassah Medical School. The animals were fed either ad libitum standard rodent chow pellets (Amrod 935 produced by Ambar, Hadera, Israel) containing 30% (wt/wt) finely ground straw or the same food pelleted without straw. Details of the food composition, breeding, and maintenance have been reported (9, 10). Animals on both diets were checked periodically for their blood glucose and insulin levels in a nonfasting state at 9 a.m. Normoglycemia was defined as serum glucose <5.5 mM and normoinsulinemia as serum insulin <100 microunits/ml. Animals were divided into five groups by their diabetic and metabolic status. Group A were animals fed chow with ground straw and had consistent (at least four consecutive measurements) normoglycemia and normoinsulinemia. Animals that were fed the regular laboratory chow and became hyperinsulinemic but not hyperglycemic were assigned to group B; those that were fed the regular laboratory chow and developed both hyperinsulinemia and hyperglycemia were assigned to group C. Recovered (Rec) animals were originally hyperglycemic and hyperinsulinemic (group C) but became normoglycemic when placed on a high fiber, low calorie diet. This group was subdivided into animals whose serum insulin remained high and resembled group B (Rec B) and into fully recovered normoglycemic, normoinsulinemic animals resembling group A (Rec A). The characteristics (mean ± SEM) of animals in the different groups are given in Table 1. The albino rats of the Hebrew University strain, used as reference animals, were fed Amrod 935 diet without straw and were all normoglycemic and normoinsulinemic.

Manipulation of Animal Tissues. Animals were anesthetized i.m. with Nembutal (60 mg/kg). Insulin (100 μg) was injected in phosphate-buffered saline (PBS) via the portal vein. Control animals were injected with PBS alone. Livers

Abbreviations: IR, insulin receptor; IRTK, IR tyrosine kinase; WGA, wheat germ agglutinin; NIDDM, non-insulin-dependent diabetes mellitus.

Table 1. Characteristics of sand rats in the different insulin-resistance groups

Parameter	Group				
	A	B	C	Rec A	Rec B
n	14	6	17	6	10
Age, months	7.2 ± 0.7	4.0 ± 0.6	4.6 ± 0.7	5.5 ± 0.9	6.5 ± 1.3
Weight, g	156 ± 8	171 ± 12	180 ± 10	196 ± 14	207 ± 10
Serum glucose, mmol/liter	3.7 ± 0.3	3.4 ± 0.3	17.8 ± 1.4	3.4 ± 0.5	5.1 ± 0.4
Serum insulin, microunits/ml	33 ± 6	210 ± 59	272 ± 38	59 ± 11	300 ± 64

and hind leg muscles were removed 30 sec or 2 min, respectively, after the injection and immediately frozen in liquid nitrogen. Liver membranes were prepared by sequential centrifugation (11). For lectin purification, tissues were homogenized in a buffer containing 50 mM Hepes, 0.25 M sucrose, 2 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 50 mM NaF, 2 mM EGTA, 80 mM β-glycerophosphate, 1 mM phenylmethylsulfonyl fluoride, and aprotinin (10 μg/ml), leupeptin (10 μg/ml), and trypsin inhibitor (10 μg/ml) at pH 7.6. IR was purified from homogenized tissues on WGA-agarose as described (12). In several experiments, Con A was used instead of WGA, and elution was with 0.5 M α-methyl-D-mannoside. Protein content of the preparations was assayed by the Bradford method.

Insulin Binding. Insulin binding in membranes was measured in calcium-free Krebs–Ringer's phosphate buffer (pH 7.6) containing 1% bovine serum albumin using ^{125}I-labeled insulin (^{125}I-insulin) as tracer (0.03 nM) and membranes (100 μg/ml). After a 20-h incubation at 4°C, the membranes were spun down, the surface of the pellets was washed with chilled 0.25 M sucrose, and the pellets were counted in a γ spectrometer. Insulin binding in WGA eluates (10–50 μg) was performed as described (12, 13) using 0.09 nM ^{125}I-insulin. The nonspecific binding was represented by the radioactivity bound in the presence of 10 μM insulin (membranes) or 1 μM (WGA eluates). Values of the ratio of bound to free insulin at trace insulin concentration [$(B/F)_o$] were taken as a measure of the IRs present in each preparation. Binding data were compared using the Student *t* test.

Assay of IR Tyrosine Kinase Activity (IRTK). Assays were carried out essentially as described (12, 14) using poly(Glu^{80}Tyr^{20}) as an exogenous substrate and various concentrations of ATP. Tyrosine kinase activity was defined as the amount of enzyme required to incorporate 1 pmol of ^{32}P into poly(Glu^{80}Tyr^{20}) during 1 min.

Dephosphorylation Assay. After purification on WGA as described above, the albino rat IR was subjected to immunoprecipitation with a specific IR antibody coupled to protein A-Sepharose for 4 h at 4°C. The immunocomplexes were allowed to undergo insulin-induced autophosphorylation in 0.1% Triton X-100/50 mM Hepes, pH 7.5, in the presence of 5 μM [γ-^{32}P]ATP, 10 mM $MnCl_2$, and 100 nM insulin. Autophosphorylation was terminated by adding 10 mM EDTA and 1 mM ATP. Immunocomplexes were washed and resuspended in 25 mM Hepes (pH 7.5) containing 5 mM EDTA, 1 mM dithiothreitol, and protease inhibitors (15). Particulate and cytosolic fractions from sand rat liver, prepared as described (15), were added to the immunocomplexes to evaluate protein-tyrosine phosphatase activity. After incubation for 20 min at 30°C, the dephosphorylation reaction was terminated by adding Laemmli sample buffer and boiling for 5 min. Samples were analyzed by SDS/PAGE and autoradiography. The degree of dephosphorylation was evaluated by densitometry and expressed as density of the IR β-subunit band after incubation with buffer alone or with the appropriate hepatic fraction.

RESULTS

Insulin Binding in Liver and Muscle of Sand Rats in the Different Insulin-Resistance Groups. To establish the characteristics of the IR in the sand rat liver and muscle, we compared nondiabetic (group A) sand rats and albino rats. In sand rat liver membranes, insulin binding was 5-fold lower than in the albino rat (binding in albino rat liver membranes of 2.22 ± 0.15 vs. binding in sand rat liver membranes of 0.44 + 0.16 $[B/F]_o$ per mg of protein; $n = 7$ pairs, $P < 0.0001$). Insulin binding was even lower in WGA- or Con A-purified liver preparations from sand rats compared with albino rats (Fig. 1*A*). In contrast, muscle WGA-purified preparations demonstrated only a 2-fold lower binding in sand rats compared with albino rats. Scatchard analysis of the binding data in the membranes showed that low binding in the sand rat liver was primarily a result of a markedly low IR number compared with albino rat and a smaller difference in the affinity of the IR to insulin (Fig. 1*B*).

Interestingly, insulin binding was similarly low in liver of animals in the different groups of insulin resistance and did not correlate with the degree of hyperglycemia or hyperinsulinemia (Fig. 1*C*). In contrast, in muscle, hyperinsulinemia was negatively correlated with binding capacity. Binding was reduced by 60% in muscle from animals that developed hyperinsulinemia (groups B and C) compared with control sand rats (group A) and was still lower in animals that recovered from the hyperglycemic state but remained hyperinsulinemic (Rec B). Only upon full recovery—i.e., with the return to normoinsulinemia (Rec A)—did insulin binding revert close to the level of normoglycemic sand rats (Fig. 1*C*).

***In Vivo* and *in Vitro* Activation of IRTK in Liver and Muscle of Sand Rats in the Different Insulin-Resistance Groups.** Stimulation of IRTK is the first essential event for insulin cellular signaling after binding to its receptor (16, 17). To further define the properties of the sand rat IR, insulin-induced specific IRTK activity was measured in group A sand rats and in albino rats. Precautions involving use of multiple protease and phosphatase inhibitors were taken during purification to ensure preservation of intact IR. Insulin was added *in vitro* to hepatic preparations in the presence of 50 μM [γ-^{32}P]ATP, and the amount of phosphate incorporated into poly(Glu^{80}Tyr^{20}) substrate was measured. Insulin caused a marked increase in phosphate incorporation into the substrate when using albino rat WGA-purified preparations (Fig. 2, bars a and b) but only a nonsignificant increase in IRTK activity in the sand rat liver preparations (Fig. 2, bars d and e) under the same conditions. This lack of stimulation by insulin added *in vitro* was not due to the presence of a putative inhibitor of phosphorylation, since IRTK activity of WGA preparations from albino rat liver was not reduced by the addition of WGA eluates from sand rat liver (data not shown). In contrast, intraportal insulin injection *in vivo* caused a similarfold increase in liver IRTK activity in both albino and sand rats (Fig. 2, bars c and f). The finding that the receptor undergoes normal activation *in vivo* suggests that despite the very low insulin binding capacity in the nondiabetic sand rat, receptor function as a kinase was intact as long as the animal was not insulin resistant.

Large variations in the affinity of IRTK toward ATP were reported in different systems (17, 18). To elucidate whether low affinity for ATP is responsible for the inferior *in vitro* insulin activation of the sand rat hepatic IRTK, assays were

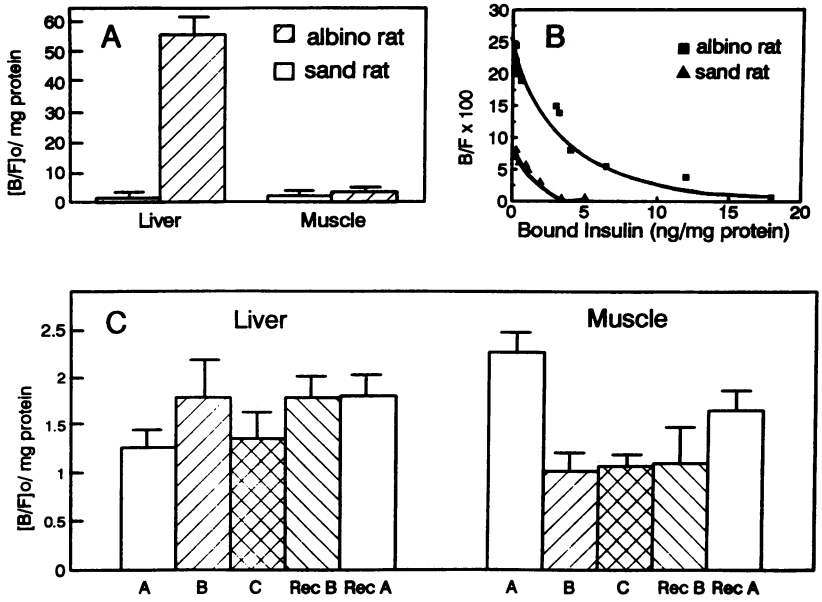

FIG. 1. Insulin binding in liver and muscle preparations. (*A*) Binding was determined as described in *Materials and Methods* in WGA-purified IR preparations from liver and muscle of group A sand rats and albino rats ($n = 7$ pairs). (*C*) Binding was also measured in liver and muscle WGA-purified IR preparations from sand rats of the different insulin-resistance groups (B and C) as well as animals that recovered from the diabetic state. Binding was expressed as the ratio of bound to free insulin at trace insulin concentration per mg of protein in the preparation examined. (*B*) Scatchard analysis of competition inhibition curve of insulin binding to hepatic membranes of group A sand rats and albino rats. Binding to membranes was performed as described in *Materials and Methods* except that increasing concentrations (0.1–10,000 nM) of unlabeled insulin were used for competition.

repeated at increasing ATP concentrations. Insulin *in vitro* activated the nondiabetic sand rat hepatic IRTK at higher ATP concentrations (Fig. 2, bars g and h). Lineweaver–Burk analysis revealed that the apparent K_m for ATP of the sand rat liver IR was ≈350 μM, which is much higher than that of the albino rat hepatic IR, which under the same conditions was 125 μM; both had a similar V_{max} (80 and 65 pmol per min per $[B/F]_o$, respectively). Thus, differences between *in vitro* and *in vivo* insulin-induced IRTK activity in sand rat liver are probably due to the requirement for high ATP concentration to achieve optimal phosphorylation.

In muscle, addition of insulin *in vitro* to sand rat WGA preparations led to a 2-fold increase in phosphorylation of exogenous substrate (Fig. 2, bars d′ and e′). This increase

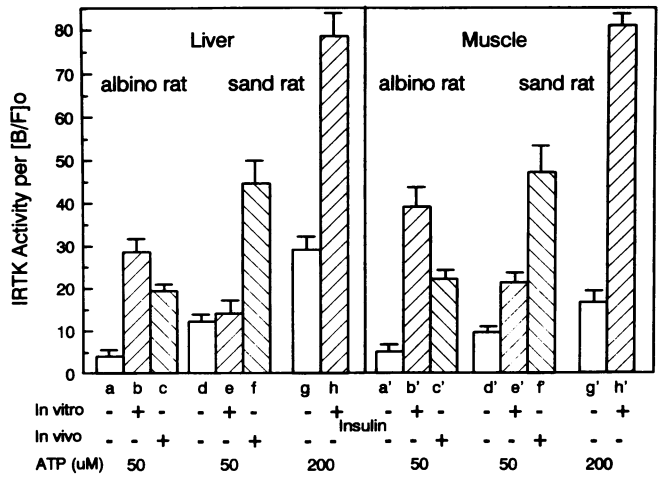

FIG. 2. Insulin-stimulated phosphorylation of poly($Glu^{80}Tyr^{20}$) by IR preparations from liver and muscle of the albino and sand rats. *In vitro* insulin-induced phosphorylation of poly($Glu^{80}Tyr^{20}$) was determined by incubating 50-μl aliquots of WGA eluates with 0.1 μM insulin. Phosphorylation was initiated by addition of the indicated concentration of [γ-^{32}P]ATP, 40 mM $Mg(CH_3COO)_2$, and poly($Glu^{80}Tyr^{20}$) at 2 mg/ml, as described in *Materials and Methods*. One unit of kinase activity was defined as the amount of enzyme required to incorporate 1 pmol of ^{32}P into poly($Glu^{80}Tyr^{20}$) during 1 min. *In vivo* insulin-induced IRTK activity was measured in the same way, except that insulin was injected intraportally to the animal prior to tissue removal and WGA purification rather than being added after tissue manipulation. In each experiment, IRTK activity was expressed per $(B/F)_o$ in the WGA preparation examined.

was significant but smaller in magnitude than in WGA-purified IR from control albino rats (Fig. 2, bars a′ and b′). *In vivo*, insulin-stimulated IRTK activity was similar foldwise in sand rat and albino rat (Fig. 2, bars a′ and c′, and bars d′ and f′). A comparable augmentation was observed when the *in vitro* assay was repeated with 200 μM ATP.

To define the effect of nutrition-induced changes on IR function, the *in vivo* activation of IRTK was evaluated in sand rats in the different stages of insulin resistance (Fig. 3). In contrast to intact IRTK activity in group A animals, virtually all insulin-induced hepatic IRTK activation was lost in animals from groups B and C. Upon recuperation from the diabetic state, there was also an improvement in receptor function. In the partly recovered animals, which reverted to normoglycemia but still had hyperinsulinemia (Rec B), a partial regain of IRTK was observed. Full restoration of *in vivo* insulin-stimulated IRTK occurred only upon return to normoglycemia and normoinsulinemia (Rec A) (Fig. 3 *Left*).

The most pronounced change in muscle, upon development of hyperinsulinemia, was a marked rise in the basal IRTK activity, which was observed in animals from both groups B and C. Almost no increase in IRTK activity was seen in the insulin-resistant animals after *in vivo* insulin administration. Maximal insulin-stimulated increase in IRTK activity was recovered only when animals returned to normal insulin levels (Rec A) (Fig. 3, *Right*).

To assess whether the impairment in IRTK activation upon development of insulin resistance is due to alteration in the receptor itself, and not to a cellular factor that prevents the activation of IRTK in the intact cell, the *in vitro* responsiveness to insulin was also evaluated. IR preparations purified from liver and muscle of noninjected sand rats were assayed with and without insulin. As shown in Fig. 4, insulin added *in vitro* in the presence of 100 μM ATP, to liver or muscle IR preparations from the nonresistant sand rat, caused a nearly 200% increase in IRTK activity. In contrast, only a minor (<50%) insulin-stimulated increase was observed in groups B and C. In muscle preparations from insulin-resistant animals, the increase in IRTK activity was half of that observed in the nonresistant sand rat. The *in vitro* results suggest that the impairment in IRTK activation in the insulin-resistant sand rats lies within the receptor itself.

Kinetic Parameters for the Phosphorylation Reaction of Exogenous Substrates in the Different Insulin-Resistance Groups. An inherent characteristic of the nondiabetic sand rat liver IR is a high K_m for ATP in the poly($Glu^{80}Tyr^{20}$)

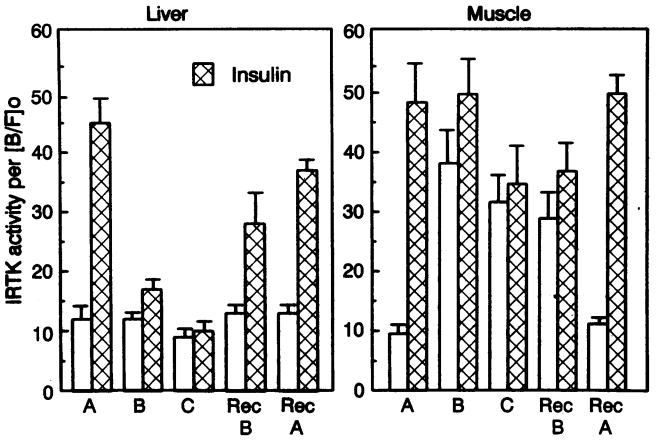

FIG. 3. *In vivo* insulin-stimulated hepatic and muscle IRTK activity in sand rats of the different insulin-resistance groups. *In vivo* insulin-stimulated IRTK activity was compared between hepatic (*Left*) and muscle (*Right*) WGA eluates from sand rats in the different insulin-resistance groups. PBS (open bars) and insulin (hatched bars) were injected intraportally prior to tissue removal and WGA purification. Phosphorylation of poly(Glu^{80}Tyr^{20}) was performed at 50 μM ATP, and data are expressed as described in Fig. 2. One unit of IRTK activity was defined as the amount of enzyme required to incorporate 1 pmol of ^{32}P into poly(Glu^{80}Tyr^{20}) during 1 min. In each experiment the phosphorylation was expressed per $(B/F)_o$ in the WGA preparation examined.

phosphorylation reaction. To assess whether a conformational change affecting the K_m for ATP forms the basis for the loss of IRTK activity upon development of diabetes, kinetic parameters for the phosphorylation reaction were evaluated in the different insulin-resistance groups (Table 2). Chronic hyperinsulinemia (groups B and C) was associated with a decrease in the K_m for ATP in the liver, whereas in muscle the affinity for ATP was similar in all groups. Acute *in vivo* insulin stimulation led to a 2-fold decrease in the K_m for ATP in liver of the group A sand rats but not in the hyperinsulinemic animals. In muscle the affinity for ATP was unaffected by acute insulin stimulation in either normoinsulinemic or hyperinsulinemic animals. The apparent V_{max} of poly(Glu^{80}Tyr^{20}) phosphorylation in the nondiabetic sand rat increased in both muscle and liver upon acute *in vivo* insulin administration. However, this increase was lost upon development of chronic hyperinsulinemia (groups B and C). Interestingly, in muscle the basal V_{max} was higher in the hyperinsulinemic than in the normoinsulinemic sand rats.

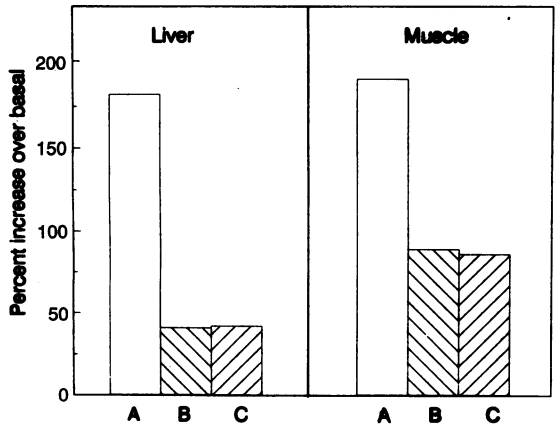

FIG. 4. *In vitro* activation of IRTK in sand rats of the different insulin-resistance groups. *In vitro* insulin-stimulated tyrosine kinase activity was compared between liver and muscle partially purified receptors from sand rats in groups A–C. WGA eluates from PBS-injected animals were incubated with 100 nM insulin for 50 min at 23°C, and phosphorylation reactions were performed at 100 μM ATP as described in Fig. 2. Results are expressed as percent increase over basal.

Protein-Tyrosine Phosphatase Activity in Sand Rats. To determine the possible involvement of specific protein-tyrosine phosphatases in impairing activation of IRTK in hyperinsulinemic sand rats, protein-tyrosine phosphatase activity toward the albino rat ^{32}P-labeled IR β subunit was measured in sand rat liver. A similar degree of dephosphorylation was found after incubating labeled rat IR with cytosolic preparations of group A or group C sand rats for 20 min at 30°C (61% ± 7% vs. 58% ± 9% decrease in IR phosphorylation). Particulate fractions of group A animals possessed higher protein-tyrosine phosphatase activity than group C sand rats (73% ± 6% vs. 59% ± 4%).

DISCUSSION

The sand rat is a unique model for evaluating the interplay between nutrition and genetics in the pathophysiology of NIDDM since development of diabetes in this rodent is not predetermined but rather dependent on caloric intake (3–7). Food restriction can revert the diabetic sand rat back to a normoglycemia and normoinsulinemia (7). We used this model to evaluate changes in the insulin transmembrane signaling through the sequence of events leading from obesity to diabetes.

The nondiabetic sand rat demonstrated a markedly low number of functionally intact IRs in liver. With the gradual development of hyperinsulinemia, liver and muscle from the sand rat lost insulin stimulation of IRTK, even before the appearance of hyperglycemia. Reversal by diet of the diabetic state to normoglycemia alone was not followed by the return of insulin-stimulated IRTK activity. Only when full recovery to normoinsulinemia occurred was the IRTK activity restored. These data point to hyperinsulinemia as a major noxious mechanism that leads to insulin resistance and glucose intolerance in the sand rat, by reducing the insulin-stimulated IRTK activity.

The phenomenon of attenuation of insulin-dependent responses secondary to chronic insulin exposure was described in several cells in culture (19–21). In NIH 3T3 cells expressing transfected human IRs, chronic insulin administration led initially to activation of the IRTK followed by a subsequent uncoupling of protein kinase activity (20). A similar result was observed in hyperinsulinemic transgenic mice (22). In rat

Table 2. Kinetic parameters of liver and muscle IRTK from uninjected or insulin-injected sand rats in the different insulin-resistance groups

	Liver		Muscle	
	K_m, μM	V_{max}, pmol per min per $(B/F)_o$	K_m, μM	V_{max}, pmol per min per $(B/F)_o$
Group A				
− insulin	350 ± 50	81 ± 15	64 ± 5	22 ± 3
+ insulin	153 ± 20	270 ± 30	88 ± 11	129 ± 6
Group B				
− insulin	190 ± 30	76 ± 10	74 ± 8	94 ± 6
+ insulin	171 ± 10	189 ± 11	80 ± 4	130 ± 10
Group C				
− insulin	244 ± 19	75 ± 17	74 ± 10	92 ± 8
+ insulin	238 ± 22	101 ± 5	73 ± 7	131 ± 4

Liver and muscle WGA-purified preparations were obtained from sand rats of groups A–C, injected with either insulin or PBS. IRTK activity [pmol per min per $(B/F)_o$] was assayed in the presence of poly(Glu^{80}Tyr^{20}) at 2 mg/ml and increasing concentrations of ATP (12.5–400 μM). Analysis of the results according to the Lineweaver–Burk equation yielded the K_m and V_{max} values presented here.

adipocytes exposed to insulin for a long time, a reduction in both K_m for ATP and insulin-stimulated V_{max} of the phosphorylation reaction was documented (19), similar to that observed in the sand rat liver receptor upon development of hyperinsulinemia. In HepG2 cells, chronic exposure to insulin increased the basal IRTK activity, like the effect of chronic hyperinsulinemia observed in the sand rat muscle (21).

The molecular basis that underlies the impaired activation of IRTK is presently unknown. Multisite phosphorylation of the IR β subunit, on serine and threonine residues, has been shown to be responsible for reduction of IRTK both in cells in culture and in the intact animal (23, 24). This serine/threonine phosphorylation could be induced by insulin through the activation of members of the recently described family of insulin-stimulated serine/threonine kinases (25). In Fao cells serine/threonine phosphorylation of the IR caused a severe impairment of V_{max} of IR tyrosine phosphorylation with a small decrease in the K_m for ATP (23). Our data, which show similar changes in these kinetic parameters with the development of hyperinsulinemia, are in agreement with this mechanism. The alternative explanation—that impaired IRTK activation is mediated by putative insulin-dependent phosphotyrosine phosphatases—is not supported by our data.

Though reduction of IRTK stimulation was observed in muscle and liver of group B and C sand rats, these tissues behaved differently. In muscle, loss of IRTK activation was accompanied by an increase in the basal tyrosine kinase activity of the WGA-purified preparation and a decrease in receptor number, while in liver no such changes were seen. The changes observed in muscle resemble those reported in HepG2 cells chronically exposed to insulin *in vitro* (21). The increase in basal activity in muscle may be due to a constitutively activated IR or be a manifestation of the insulin-like growth factor 1 receptor activation by hyperinsulinemia, because insulin-like growth factor 1 receptors exist in muscle.

We propose a model for the development of diabetes based on the low hepatic IR density in the nonobese nondiabetic group A sand rat and the acquired insulin-dependent reversible reduction in IRTK activity. As long as the animal is on a low caloric diet, insulin secretion is low and a balance between pancreatic production and hepatic extraction of insulin exists. At this stage, the animal is normoglycemic and normoinsulinemic. When the animal is exposed to an affluent diet, it responds with increased insulin secretion. In the sand rat, the threshold for nutrient-induced insulin secretion is lower than in other rodents (26). However, as insulin binding is scarce and insulin extraction by the liver low (27), hyperinsulinemia ensues in the periphery. In turn, the persistent hyperinsulinemia may cause both a functional impairment in the activation of IRTK and a further decrease in peripheral IR number, potentiating insulin resistance with a clinical picture of hyperinsulinemia and hyperglycemia.

Inference from this model can be made to diabetes induced by overnutrition and obesity in humans. The hepatic receptor was found to be low in number in NIDDM patients (28), and insulin extraction in liver of obese individuals has been shown to be decreased (29, 30). Liver, muscle, and adipose tissue IRTK activity is decreased in obese NIDDM patients (28, 31, 32). In adipose tissue the reduced IRTK activity could be reversed by dietary control that incites weight loss and normoinsulinemia (33). Thus, overeating, through increase in insulin secretion and low extraction, may create peripheral hyperinsulinemia leading to insulin resistance through changes in IR number and IRTK activity. Understanding of the cellular mechanisms that mediate the hyperinsulinemia-induced impairment of IRTK will allow interference with the deterioration in glucose tolerance in human NIDDM.

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