DECREASED BINDING OF INSULIN TO ITS RECEPTORS IN RATS WITH HORMONE INDUCED INSULIN RESISTANCE

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SUMMARY Insulin and glucagon receptor binding was studied in purified liver membranes from rats made insulin resistant by implantation of an MtT pituitary tumor which secretes growth hormone, prolactin, and ACTH. Insulin binding to its receptors was decreased and correlated with the degree of insulin resistance. In contrast, binding of glucagon to its receptors was unchanged.

In man the major clinical forms of insulin resistance are those due to obesity, glucocorticoid excess and hypersecretion of growth hormone (1). In all three there exists glucose intolerance, hyperinsulinemia, and diminished responsiveness to endogenous and exogenous insulin (1). Although the clinical manifestations of these forms of insulin resistance are well characterized (1-3), the cellular mechanisms underlying these conditions are only beginning to be understood.

The obese hyperglycemic mouse is an inherited syndrome characterized by obesity, glucose intolerance, hyperinsulinemia and resistance to exogenous insulin (4,5). We and others have found that the severe insulin resistance in these animals is associated with a marked decrease in insulin receptor concentrations in liver, fat, and thymic lymphocytes (4-7). However decreased insulin receptors are not a feature unique to genetic obesity. Insulin receptor defects are also found in liver membranes of mice made obese by gold thioglucose (8) and in lymphocytes and fat cells of obese humans (9,10). We therefore investigated whether hormonally induced insulin resistance was also associated with decreased insulin receptor concentrations.

TABLE I

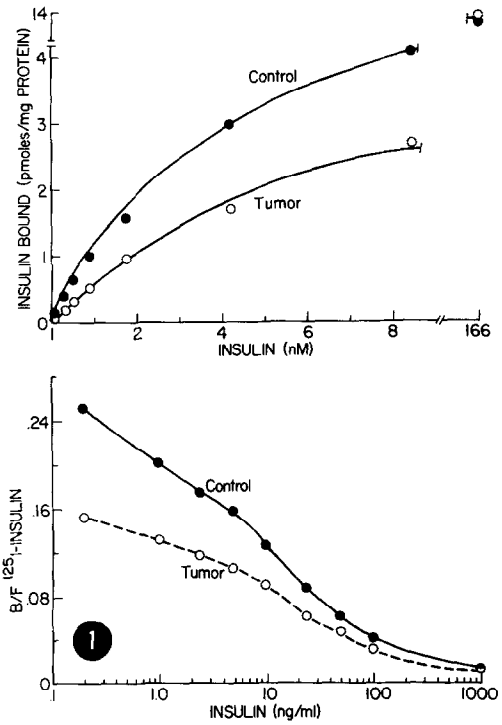
	Blood Glucose mg/100 ml	Plasma Insulin μU/ml	Plasma Corticosterone μg/100 ml	Plasma RGH ng/ml
Control	129 ± 21 (12)	33 ± 15 (11)	4.6 ± 4 (3)	112 ± 5 (4)
Tumor	106 ± 6 (4)	247 ± 38 (4)	53 ± 23 (5)	2381 ± 171 (6)

These assays were performed using standard techniques (15-18) and are the mean ± S.D. Figures in parentheses are the number of observations made. Animals were fed ad libitum and bloods obtained between 8:00 a.m. and 10:00 a.m.

In the rat, growth hormone and glucocorticords cause insulin resistance and under appropriate conditions also induce glucose intolerance (11-13). Rats implanted with MtT pituitary tumors, secreting high levels of growth hormone, ACTH, and prolactin are an animal model of this type of insulin resistance (14). In the present study we find that the binding of insulin to its receptors is decreased in purified liver membranes prepared from these rats. We propose that this decrease in insulin receptor binding is an important factor in the insulin resistance caused by these hormones.

METHODS AND RESULTS

MtT (F_4) tumors of pituitary origin were implanted subcutaneously in 2-4 month old female Fischer rats (14). When the tumors had reached 5 to 10 grams in weight, the animals underwent a rapid growth spurt. At that time, they had 10-fold elevations of circulating growth hormone and corticosterone which resulted in insulin resistance as manifested by normoglycemia in the presence of a seven-fold elevation in plasma insulin levels (Table I). These animals, along with age and sex matched controls, were then sacrificed and highly purified liver plasma membranes prepared (19). To measure insulin receptor binding 130-180 μg/ml of membrane protein were incubated with 30 pM ^{125}I-insulin (20) plus unlabeled insulin in 150 μl of Ringer's buffer (21) at 20°C. Maximum binding was reached after 6 hours and bound and free hormone were then separated by centrifugation (21).



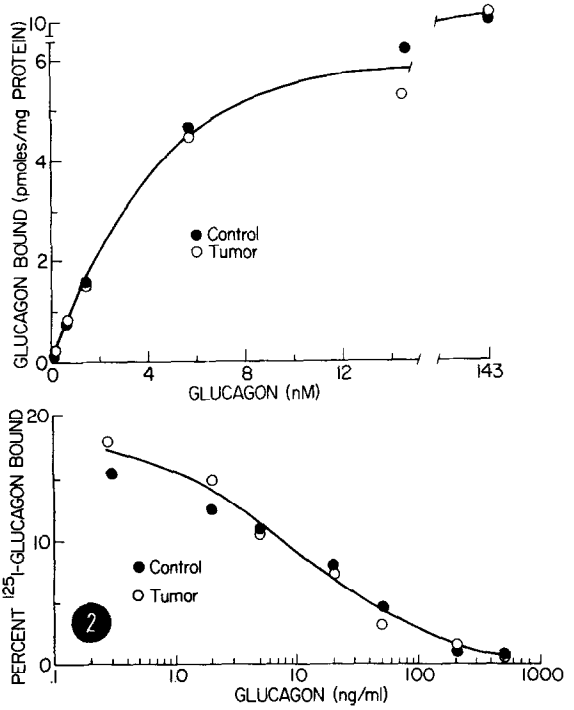


Figure 1. Insulin receptor binding in purified liver membranes from control and tumor rats. TOP Insulin bound as a function of the total insulin concentration. Each point is the mean of 3 separate experiments with one membrane preparation performed in triplicate. The standard deviation of all points was less than 10%. BOTTOM Bound/free ^{125}I-insulin per 100 µg protein as a function of the total insulin concentration.

Figure 2. Glucagon receptor binding in purified liver membranes from control and tumor rats. Membranes were incubated at protein concentrations of 50 to 90 µg/ml with 100 pM ^{125}I-glucagon (25) plus various concentrations of unlabeled glucagon in 200 µl Ringer's buffer at 20°C. After 1 hour bound and free hormone was separated by centrifugation (24,25). Each point is the mean of 2 separate experiments performed in triplicate. TOP Glucagon bound as a function of the total glucagon concentration. BOTTOM Percent ^{125}I-glucagon bound per 100 µg protein as a function of the total glucagon concentration.

When incubated under identical conditions, liver membranes from tumor animals demonstrated less insulin binding than controls (Fig. 1). At insulin concentrations of 0.03 nM to 9 nM, a range which includes the levels seen in portal blood (22), there was approximately a 40% decrease in insulin binding. This marked decrease in insulin binding was reproducible in eight separate experiments using four separate membrane preparations. Only at high concentrations of

insulin did the binding by tumor animal membranes approach normal. Using both low temperatures and low liver membrane concentrations insulin degradation (23) was minimized and did not differ in preparations from normal and tumor bearing animals. In contrast to insulin binding, glucagon binding to its receptor was unchanged over the entire dose-response range (Fig. 2).

Previous studies indicate that the decreased insulin binding in these membranes was not due to the occupation of binding sites by endogenous insulin. Injections of insulin to normal mice, which raised plasma insulin to levels higher than those seen in the obese hyperglycemic animals, did not reduce insulin receptor binding in both liver membranes (4,5) and isolated thymic lymphocytes (26). Further, addition of insulin *in vitro* to blood from humans did not alter insulin receptor binding in isolated lymphocytes (9).

DISCUSSION

The studies of Lefkowitz, et al. with radioiodinated ACTH (27) and Goodfriend and Lin with radioiodinated angiotensin (28) demonstrated that the binding of polypeptide hormones to their receptors could be studied directly in target tissues. This approach has been extended to study the characteristics of insulin receptors in several tissues (21,29,30) and to measure insulin receptor concentrations in conditions of altered sensitivity to the hormone. In thymocytes from hypophysectomized rats or liver membranes from fasted obese mice, an increased concentration of insulin receptors (7,8) was associated with increased sensitivity to insulin. Conversely, in obesity, decreased insulin resistance was associated with decreased insulin receptor concentrations (4-10). These studies indicated that changes in insulin receptor concentrations were a major factor in the altered cellular responses to the hormone. In the present study we have now demonstrated that hormonally induced insulin resistance is also associated with major alterations in insulin binding to its receptors.

In the obese hyperglycemic mouse, we and others demonstrated a decrease in the number of insulin receptors in liver (4,5), fat (6) and thymocytes (7).

In contrast neither glucagon, growth hormone or isoproterenol binding in the liver plasma membranes of these mice were significantly changed, nor was there any change in adenylate cyclase or 5'-nucleotidase activity (4,5). Analysis (31,32) revealed that the concentrations of both the high affinity-low capacity and low affinity-high capacity receptors were decreased.

We now find a similar decrease in insulin binding to receptors in liver plasma membranes from rats made insulin resistant by high levels of growth hormone and glucocorticoids. However in contrast to obese mice there was no significant change in the total number of binding sites. Rather, analysis of the data (31,32) revealed that there was a selective loss in binding to only the high affinity-low capacity site. Again no alteration of glucagon receptor binding was observed. When compared to controls, 5-fold increases in insulin concentrations over the physiologic range were needed to fill the same number of receptor sites in membranes from tumor animals. This correlated well with the 7-fold increase in plasma insulin levels in the tumor bearing animals.

In these animals we have not defined the individual roles of growth hormone or glucocorticoids. However, preliminary studies in this laboratory indicate that a 4 day course of ACTH or dexamethasone to normal rats can decrease insulin binding to liver membranes whereas adrenalectomy can restore insulin binding to normal in liver membranes from tumor animals (33). This would indicate a major role for glucocorticoids in the regulation of plasma membrane insulin receptors. Studies are now in progress to further clarify the effects of corticosteroids and growth hormones in liver and other insulin sensitive tissues.

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