Our data support the view that antidepressants occupy sites in synapses in which 5HT and other putative neurotransmitters regulate transaction of information. The antidepressant binding sites may be binding sites for cotransmitters involved in the modulation of the properties of the recognition sites of a number of different putative neurotransmitters. The occupancy of these sites by the antidepressants could result in the down-regulation of transmitter recognition sites and thereby relieve some symptoms of depression. If this is the case, certain depressive states may be due to a deficit in cotransmitter modulation of transmitter detectors.

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Hyperglycemia of Diabetic Rats Decreased by a Glucagon Receptor Antagonist

Abstract. *The glucagon analog [1-N$^{\alpha}$-trinitrophenylhistidine, 12-homoarginine]-glucagon (THG) was examined for its ability to lower blood glucose concentrations in rats made diabetic with streptozotocin. In vitro, THG is a potent antagonist of glucagon activation of the hepatic adenylate cyclase assay system. Intravenous bolus injections of THG caused rapid decreases (20 to 35 percent) of short duration in blood glucose. Continuous infusion of low concentrations of the inhibitor led to larger sustained decreases in blood glucose (30 to 65 percent). These studies demonstrate that a glucagon receptor antagonist can substantially reduce blood glucose levels in diabetic animals without addition of exogenous insulin.*

The central role of relative or absolute deficiency of insulin in the development of diabetes has been recognized since the experiments of von Mering and Minkowski in 1889 (*1*) and the identification of insulin by Banting and Best in 1922 (*2*). The development of radioimmunoassay techniques for measuring glucagon in the late 1960's revealed a relative or absolute excess of circulating glucagon in virtually all forms of human and animal diabetes (*3*). These findings led to the "bihormonal" concept of diabetes (*3*), the disorder being attributed to a lack of insulin effect and an excess of glucagon.

The discovery of somatostatin (*4*) and its ability to suppress both insulin and glucagon secretion (*5*, *6*) provided two more lines of evidence supporting the importance of glucagon in glucose homeostasis. When the secretion of insulin and glucagon was suppressed by infusion of somatostatin, there was a fall in the blood glucose concentration (*6*) that could be reversed by concomitant infusion of glucagon (*7*, *8*). Furthermore, infusion of somatostatin to inhibit secretion of endogenous glucagon reduced the hyperglycemia of diabetic patients (*9*, *10*).

In view of the evidence implicating excessive glucagon secretion as a contributing factor in the metabolic abnormalities of diabetes mellitus, several investigators have tried to develop analogs of glucagon that would be specific antagonists (inhibitors) of glucagon effects on target tissues (*11–13*) and thus provide direct evidence for glucagon's role in diabetes mellitus. Recently we have prepared the highly purified glucagon analog [1-N^{α}-trinitrophenylhistidine, 12-homoarginine]glucagon (THG, Fig. 1) and several related analogs that are potent antagonists of the glucagon-stimulated adenylate cyclase system in rat liver membranes in vitro. The present study was undertaken to determine whether THG was effective in vivo in reducing the hyperglycemia of diabetic animals. We report that THG causes a marked reduction in glucose levels in streptozotocin-induced diabetic rats. These results provide evidence that blocking endogenous glucagon with a glucagon receptor antagonist can significantly lower glucose concentrations in diabetic animals without added exogenous insulin.

The synthesis and purification of THG was carried out as described (*12*) except that the scale of the synthesis was expanded five to ten times. This scale-up caused no significant changes in purity as assessed by previously reported methods (*12*).

Male Wistar rats weighing 310 to 360 g were made diabetic by intravenous infusion of streptozotocin (50 mg/kg) through a tail vein (*14*). The animals were placed in individual metabolic cages to permit measurement of daily urinary glucose excretion. After the amount of glycosuria had stabilized (1 to 5 weeks), individual rats were anesthetized with intraperitoneally administered pentobarbital (65 mg/kg). A jugular vein was catheterized for repetitive blood sampling and infusion of THG.

Infusion of a bolus of THG (1.0 mg/kg dissolved in 0.2 ml of saline) produced a rapid decrease in the mean concentration of blood glucose (Fig. 2). In this particular experiment, 5 minutes after administration of THG the mean blood glucose concentration decreased 28 percent below baseline levels. Glucose levels returned to normal in about 10 to 20 minutes and remained stable up to 1 hour (Fig. 2).

In a second group of experiments anesthetized rats were given a THG bolus of 1.0 mg/kg followed immediately by a continuous infusion of THG, 33 μg per

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kilogram per minute for 60 minutes. Glucose from tail blood samples decreased to 67 percent of baseline at 5 minutes and remained 30 to 55 percent below baseline for greater than 90 minutes. Comparable decreases in blood glucose were achieved with one-half (0.5 mg/kg bolus, 17 μg/kg-min for 60 minutes) and one-tenth (0.1 mg/kg bolus, 3.4 μg/kg-min) of this dose (Fig. 3).

Lower concentrations of THG (1/25 and 1/100 the highest dose) had no discernible effect when compared to infusion of an equal volume of saline (0.2-ml bolus followed by 0.34 ml given over a 60-minute infusion). Saline infusion caused an 18 percent decrease in blood glucose 5 minutes after the bolus injection. Within 10 minutes the blood glucose had returned to baseline values and remained stable for the remainder of the study period (105 minutes).

The rapid and substantial lowering of blood glucose concentrations in diabetic rats by the glucagon antagonist THG supports the concept that glucagon is an important contributing factor in the hyperglycemia of uncontrolled diabetes. Although some previous studies have suggested that the effect of exogenous glucagon on blood glucose is short-lived (*15*), other studies have demonstrated a prolonged increase in hyperglycemia (*16*). The results with THG indicate that, in vivo, the inhibition of the activity of endogenous glucagon receptors with a glucagon antagonist can produce a sustained decrease in blood glucose concentration for at least 105 minutes. The rebound of blood glucose within 10 to 20 minutes after a single bolus of THG was probably due to removal of the antagonist from hepatic receptors rather than any decrease in intracellular hepatic response.

The magnitude of the decrease in blood glucose concentration in THG-treated animals (approximately 50 percent) is larger than that produced by inhibition of glucagon secretion with somatostatin (*7, 17*). This may be due to the lack of suppression of insulin secretion with THG. Insulin in the peripheral plasma of the diabetic rats was undetectable by radioimmunoassay. Nevertheless, even low concentrations of insulin in the portal venous blood may normally counterbalance glucagon-stimulated hyperglycemia. Since somatostatin inhibits release of both insulin and glucagon, the insulin effect may be attenuated, leading to a lesser decline in blood glucose.

The field of peptide hormone analogs is still in its infancy. Few studies have been performed on peptides as large as glucagon in an effort to determine the effect of minor changes in structure on hormonal function. As a peptide analog that can act as an "antihormone" or hormone antagonist, THG may be the prototype for a new kind of endocrine pharmacology. THG appears to offer great promise for study of the diabetic state. Further studies will be necessary to determine whether THG or other specific glucagon antagonists might be of benefit in the treatment of patients with diabetes.

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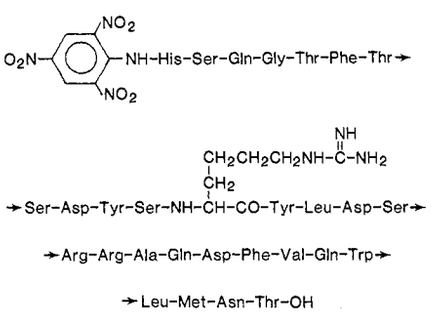

Fig. 1. The primary structure of the glucagon inhibitor [1-N^{α}-trinitrophenylhistidine, 12-homoarginine]glucagon (THG) showing the specific structural modifications of the N^{α}-amino group of His-1 and the ε-amino group of Lys-12.

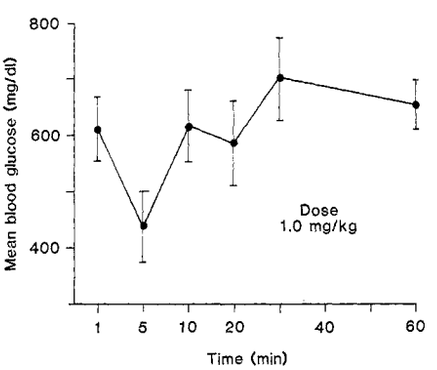

Fig. 2. The effect of an intravenous bolus injection of THG on the blood glucose concentration of streptozotocin-induced diabetic rats. The rats were tested 1 to 5 weeks after they received streptozotocin (50 mg/kg) intravenously. Results are expressed as the means (± standard error) for four rats.

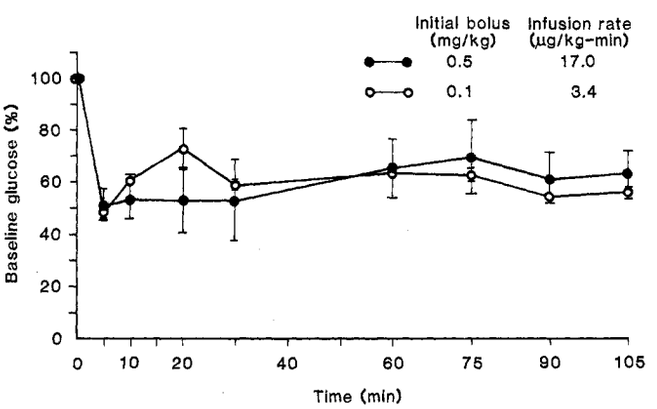

Fig. 3. Effect of an intravenous bolus injection of THG followed by continuous infusion for 60 minutes on the blood glucose concentration of streptozotocin-induced diabetic rats. Two different dosage schedules were used as shown. Results are expressed as means (± standard error) for four rats in each dosage schedule.

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