PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **179**, 32–37 (1985)

Increased Glucagon Receptors in Chronically Hypersomatotrophic and Hyperglucagonemic Rats (42060)

JOSEPH C. DUNBAR, PATRICIA BROWN, AND SIGRID DIXON

Wayne State University, School of Medicine, Detroit, Michigan 48201

Abstract. The effect of increased levels of growth hormone on glucagon binding by isolated hepatocytes and on the cellular cyclic AMP response to glucagon was evaluated in rats bearing growth hormone-secreting tumor (Mt-T-W15) and in rats treated with rat growth hormone. An increased binding, due to an increased number of receptors, was observed in both groups of animals. Glucagon binding did not correlate with plasma glucagon levels, suggesting a failure of down regulation, possibly due to an effect of growth hormone and insulin on the number of receptors. Tumor-bearing and growth hormone-treated rats had larger hepatocytes so that, when hormone binding was expressed in terms of square micrometer of membrane surface, it appeared decreased. When the tumor was removed the increase in the number of glucagon receptors per cell persisted, even though the average cell size returned toward normal. It is suggested that this retention of the receptors may have been the result of continuing hyperinsulinism. Basal cAMP levels were elevated in hepatocytes of tumor-bearing and growth hormone-treated animals, possibly due to cell hypertrophy. On the other hand, the maximum cAMP response to glucagon was not altered by the experimental procedures. A negative effect of insulin on cAMP accumulation may explain this apparent paradox. Indeed, hepatocytes isolated from rats following tumor removal, but with continuing hyperinsulinemia, had a lower maximum cAMP response, even though the glucagon binding per cell or per unit of cell surface was increased.

Recently, we have reported that hepatic membranes prepared from rats with high serum growth hormone, insulin, and glucagon levels due to a transplantable Mt-T-W15 tumor have a decreased glucagon and insulin binding capacity (1). Although this loss did not correlate directly with the serum glucagon level, it did correlate with the serum glucagon/insulin ratio. Thus, it was interpreted as additional evidence that glucagon down regulates its own receptor (2–4).

Other investigators reported that the hepatocytes of hypersomatotrophic rats were hypertrophic (5) and that the liver was enlarged and contained hemopoietic cells (6). For this reason we decided to reexamine the problem by measuring the binding of glucagon to intact hepatocytes rather than to cell membranes. We also sought to determine if the changes produced by the tumor would disappear after its removal or could be duplicated in normal rats by treatment with growth hormone. Glucagon binding was further correlated with the basal cyclic AMP content of the cells and its response to glucagon stimulation.

Materials and Methods. Female Wistar–Furth rats purchased from Harlan Laboratories (Indianapolis, Ind.) received a subcutaneous transplant of three or four 1–2 mm^3 pieces of Mt-T-15 tumor tissue derived from a tumor-bearing animal. Normal rats of the same age and weight were used as controls. All animals were kept in individual cages with food and water *ad libitum*. Four to five weeks after transplantation, the well-encapsulated tumors were removed from some of the animals, all of whom were observed for 2 additional weeks. Another group of rats received subcutaneous injections of rat growth hormone (NIH; 100 μg/rat/day) for 2 weeks.

At the time of sacrifice, the rats were anesthetized with sodium pentobarbital (35 mg/kg). The abdomen was opened and a blood sample was collected from the inferior vena cava into a tube containing aprotinin (1000 IU/ml; Sigma Chemical Co., St. Louis, Mo.). The serum was separated and frozen for future determinations of glucose, insulin, glucagon, and growth hormone. Hepatocytes, isolated by collagenase digestion, using minor modifications of the method of Feldhoff *et*

0037-9727/85 $1.50

TABLE I. SERUM GLUCOSE, INSULIN, GLUCAGON, AND GROWTH HORMONE CONCENTRATIONS IN CONTROL, TUMOR-BEARING, AND GROWTH HORMONE-TREATED RATS AND IN RATS 2 WEEKS AFTER REMOVAL OF THE TUMOR

	Glucose (mg/dl)	Insulin (μU/ml)	Glucagon (pg/ml)	Growth hormone (ng/ml)
Control	55 ± 5 (20)	17 ± 1.82 (20)	150 ± 20 (15)	140 ± 20 (17)
Tumor bearing	40 ± 3* (10)	90 ± 4.5** (10)	408 ± 67** (10)	3170 ± 330** (12)
Growth hormone treated	79 ± 10* (10)	29 ± 5.5* (10)	156 ± 37 (8)	571 ± 21** (8)
After tumor removal	49 ± 4 (8)	55 ± 7.8** (8)	178 ± 12 (8)	108 ± 19 (8)

* $P < 0.05$.
** $P < 0.01$.

al. (7), were shown to have a 90–95% viability by the trypan blue exclusion test. They were counted using a Neubauer counting chamber and their size distribution profile was determined using a Coulter counter. Isolated cells were suspended in Krebs Ringer bicarbonate buffer and used for ^{125}I-glucagon-binding studies. These were carried out using a mod-

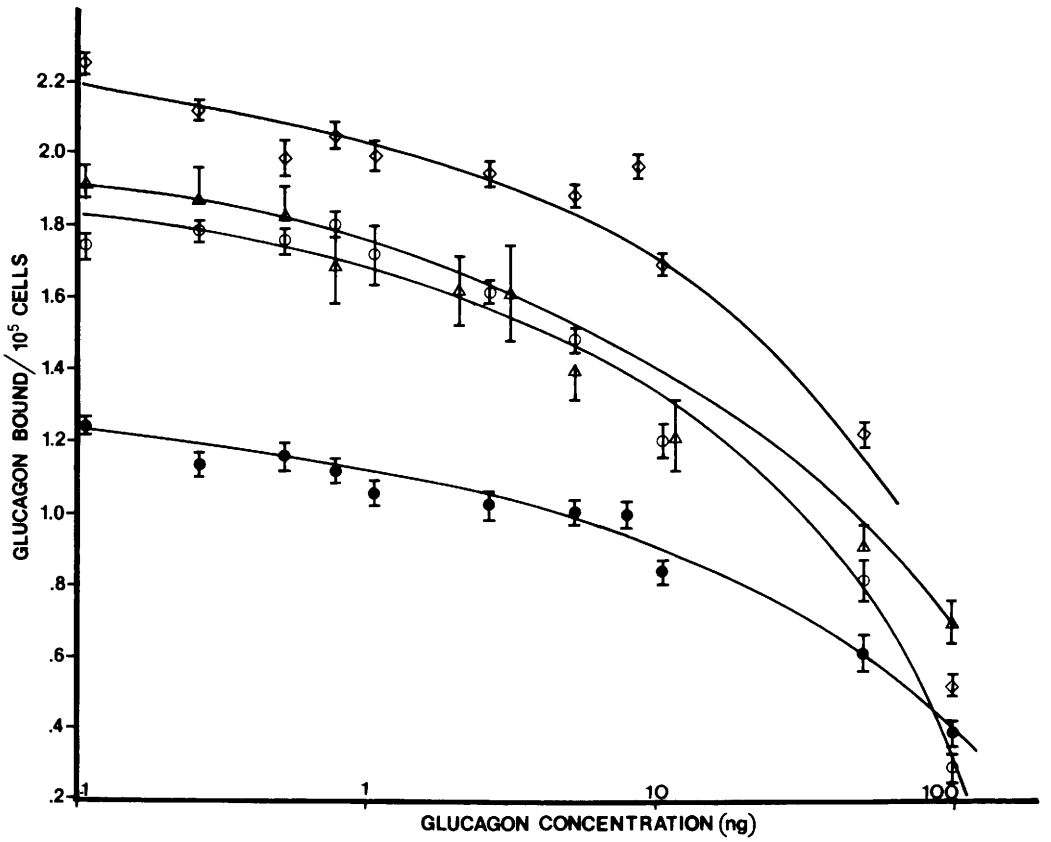

FIG. 1. Glucagon receptor binding (percentage bound/10^5 cells) to hepatocytes of control ●; tumor-bearing ◇; growth hormone-treated ○ rats; and of rats 2 week after removal of the tumor △.

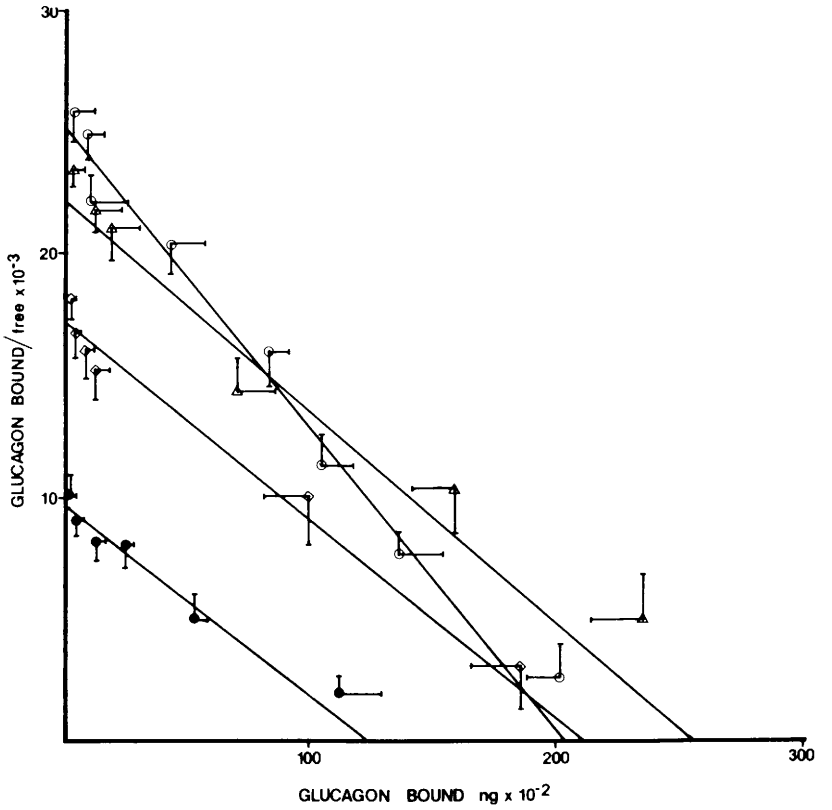

FIG. 2. Scatchard plots of ^{125}I-glucagon binding; straight line calculated by least-squares linear regression of the mean values of B/f and glucagon bound (ng) for each group. High affinity receptor population only. Control ●; tumor bearing ◇; growth hormone-treated ○ rats; and of rats 2 weeks after removal of the tumor △.

ification of the method of Holst (8), as described earlier (1).

The cAMP content of the hepatocytes was measured according to Steiner *et al.* (9) and its response to glucagon was evaluated as previously described (1) using cells suspended in Krebs Ringer bicarbonate buffer containing 10 mg/ml BSA.

Serum glucose was determined using the GOD-period enzymatic method (Auto-test; Boehringer-Mannheim Co., Mannheim, West Germany); insulin using the Autopak-12 Test Delivery System (Micromedic, Horham, Pa.); pancreatic glucagon and growth hormone were assayed using the method of Foa *et al.* (10) and of Birge *et al.* (11), respectively.

Results. Serum glucose was lower in tumor-bearing and higher in growth hormone-treated than in control rats. Insulin and growth hormone levels were higher in both groups of experimental animals, while glucagon was significantly elevated only in the tumor-bearing rats. After removal of the tumor, serum glucose and growth hormone levels returned to values that were not significantly different from those found in control rats (Table 1).

Hepatocytes of tumor-bearing and of growth hormone-treated animals bound more glucagon than hepatocytes of control rats (Fig. 1). Two to three weeks following tumor removal, glucagon binding remained elevated above control levels. Scatchard analysis (Fig. 2) suggests that this phenomenon was due to an increase in the number of receptors rather than in their affinity for the hormone.

The hepatocytes of growth hormone-

TABLE II. HEPATOCYTE SIZE, NUMBER OF RECEPTORS PER CELL AND PER SQUARE MICROMETER OF CELL SURFACE IN CONTROL, TUMOR-BEARING AND GROWTH HORMONE TREATED RATS AND IN RATS 2 WEEKS AFTER REMOVAL OF THE TUMORS

	Average hepatocyte size (μm^3)	Average size range (μm^3)	Number of receptors/cell	Number of receptors/μm^2
Control	25.1 ± 0.72 (10)	19–34	210,993 ± 13,764 (10)	107.3 ± 3.8 (10)
Tumor bearing	38.0 ± 4.2 (6)	16–46	360,600 ± 17,255** (6)	79.5 ± 11* (6)
Growth hormone treated	35.1 ± 3.8 (8)	20–42	343,800 ± 29,249** (8)	92.0 ± 9.9 (8)
After tumor removal	28.0 ± 0.50 (4)	20–36	432,600 ± 37,920** (4)	220.6 ± 3.9* (4)

* $P < 0.05$.
** $P < 0.01$.

treated and of tumor-bearing rats were significantly larger than those of the controls, as indicated by their average size and by the increased number of the larger cells (Table II), suggesting that this was a major cause of the overall hepatic hypertrophy observed in these animals. Following tumor removal the values decreased, but not to control levels. Further analysis demonstrated that the number of glucagon receptors per cell was greater in the tumor-bearing and growth hormone-treated rats than in the controls and remained significantly elevated after tumor removal (Table II). On the other hand, the number of receptors per unit of cell surface was significantly smaller in the tumor-bearing, but not in the growth hormone-treated animals. The number of receptors per square

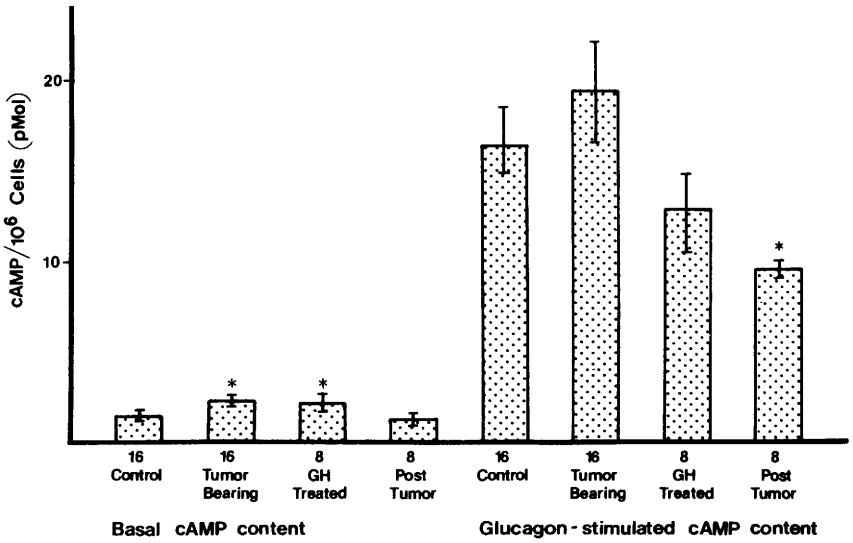

FIG. 3. Basal cyclic AMP content and response to glucagon (100 nM) in control, tumor-bearing, growth hormone-treated rats, and rats 2 weeks after removal of the tumor. *$P < 0.05$.

micrometer was increased significantly after tumor removal. The basal cAMP content of the hepatocytes was higher in tumor-bearing and in growth hormone-treated than in control rats (Fig. 3), and returned to control values in rats whose tumor had been removed. However, the cAMP response to glucagon stimulation was significantly smaller than that in controls only in the animals that had their tumors removed.

Discussion. We have shown that hepatocytes from tumor-bearing rats have greater glucagon binding capacity than in control hepatocytes, in spite of an elevated plasma glucagon level that might have caused down regulation of the glucagon receptors (2–4). This finding is contrary to our previously reported decrease in binding in the same model (1), a discrepancy which we attribute to the fact that, in the present study, we calculated glucagon binding per hepatocyte, rather than per milligram of membrane protein. We and other investigators have observed that the liver and hepatocytes of tumor-bearing rats are larger than those of control rats (1, 5), and therefore contain more membranes and membrane protein. Thus, when cell number, rather than membrane surface or membrane protein, is used as reference, the resulting values are larger and may more adequately reflect total liver binding, the significant parameter for the economy of the organism.

In this study we found also that repeated injections of growth hormone increase glucagon binding by isolated hepatocytes in a manner similar to that observed in the tumor-bearing rats, that is, by increasing the size of the hepatocytes, while decreasing the number of receptors and therefore the binding per square micrometer of cell membrane surface. These findings support our hypothesis that the observed results were due to growth hormone and not to other substances secreted by the tumor.

When we removed the tumors and allowed the rat to recover for 2 weeks, we found that glucagon binding was still elevated and the liver was still enlarged, even though the average cell size had returned almost to normal. Thus, increased glucagon binding coupled with small differences in cell size translated into an increase, not only in the number of glucagon receptors per cell, but also in the number of glucagon receptors per unit of membrane surface. In view of our previous finding that either growth hormone or insulin can increase the number of glucagon receptors (1, 12), this residual effect could be explained by the high insulin levels still present in the rats after removal of the tumors. Indeed, the Mt-T-W15 tumor-bearing rats can be used as a model of hyperinsulinism.

Basal cAMP content was elevated in the hepatocytes from tumor-bearing and growth hormone-treated rats, but had returned to normal values 2 weeks after removal of the tumor. In tumor-bearing rats, this increase may have been the result of the prevailing hyperglucagonemia. However, this explanation does not hold for the growth hormone-treated animals, in which the elevated basal cAMP may simply reflect the increased cell size. The maximum cAMP response to glucagon was the same in hepatocytes of tumor-bearing, growth hormone-treated, and control rats, but was decreased in hepatocytes of rats whose tumor had been removed. Again this may have been the result of the inhibitory effect of hyperinsulinism on the accumulation of cAMP (13–15).

1. Walsh MF, Dunbar JC. Glucagon binding to liver membranes of Mt-T-W15 tumor bearing and hypophysectomized rats. A possible role of insulin and growth hormone. Diabetes **33**:978–983, 1984.
2. Bhathena SJ, Voyles NR, Smith S, Recant L. Decreased glucagon receptors in diabetic rat hepatocytes. J Clin Invest **61**:1488–1497, 1978.
3. Santos A, Blazquez E. Direct evidence of a glucagon dependent regulation of the concentration of glucagon receptors in the liver. Eur J Biochem **121**:671–677, 1981.
4. Srikant CB, Freemand D, McCorkle KM, Unger RH. Binding and biologic activity of glucagon in liver membranes of chronically hyperglucagonemic rats. J Biol Chem **252**:7434–7436, 1977.
5. Davidson AB, Melmed S. Hepatocyte insulin binding and action in rats with somatomammotrophic tumors. Diabetologia **25**:60–65, 1983.
6. Peschle C, Rapport IA, Sasso GF, Condorelli M. Mechanism of growth hormone (GH) action on erythropoiesis. Endocrinology **91**:511–517, 1972.
7. Feldhoff RC, Taylor JM, Jefferson LS. Synthesis and secretion of rat albumin in vivo, in perfused liver

and in isolated hepatocytes. J Biol Chem **252**:3611–3618, 1977.

8. Holst JJ. A radioreceptor assay for glucagon: Binding of enteroglucagon to liver and plasma membranes. Diabetologia **11**:211–219, 1975.
9. Steiner AL, Kipnis DM, Utiger R, Parker C. Radioimmunoassay for the measurement of adenosine 3',5'-cyclic phosphate. Proc Natl Acad Sci **64**:367–373, 1969.
10. Foa PP, Matsuyama T, Foa NL. Radioimmunoassay of glucagon. In: Abraham GE, ed. Handbook of Radioimmunoassay. New York, Marcel Dekker, p299, 1977.
11. Birge CA, Peake GT, Mariz IK, Daughaday W. Radioimmunoassayable growth hormone in the rat pituitary gland: Effect of dye sex and hormonal state. Endocrinology **81**:195–204, 1967.
12. Dunbar JC, Cremonesi P. Regulation of the sensitivity of cultured hepatocytes to glucagon by glucagon and insulin. In: Pozza A, Micossi P, Catapano A, Paolett R, eds. Diet Diabetes and Atherosclerosis. New York, Raven Press, p1, 1984.
13. Illiano G, Cuatrecasas P. Modulation of adenylate cyclase activity in liver and fat cell membranes by insulin. Science (Washington, DC) **175**:906–908, 1972.
14. Zinman B, Hollerberg C. Effect of insulin and lipolytic agents on rat adipocyte low Km cyclic adenosine 3',5'-monophosphate phosphodiesterase. J Biol Chem **249**:2182–2187, 1974.
15. House PDR, Poulis P, Weidemann MJ. Isolation of a plasma-membrane subfraction from rat liver containing an insulin sensitive cyclic-AMP phosphodiesterase. Eur J Biochem **24**:429–437, 1972.

Received September 14, 1984. P.S.E.B.M. 1985, Vol. 179.
Accepted January 10, 1985.