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The Relationship between Glucagon Receptors and Metabolic Profile in Two Strains of Rats: Wistar–Furth and Sprague–Dawley (42486)

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Abstract. We studied glucagon and insulin binding to isolated hepatocyte receptors in Wistar–Furth (WF) and Sprague–Dawley (SD) rats, using ^{125}I-labeled hormones. Hepatocytes from WF rats bound more glucagon than hepatocytes from SD rats. There were no differences in insulin binding. These observations prompted us to investigate other strain differences. Fasting and nonfasting serum glucose, glucagon, insulin, and growth hormone were measured. WF animals had a lower fasting glucose and higher fasting glucagon than SD animals, while SD rats had higher nonfasting insulin levels and a higher hepatic glycogen content. Total hepatic glucose production in response to glucagon (10^{-8} *M*) was greater in WF than in SD rats, while glucagon-stimulated gluconeogenesis from alanine was the same in the two groups of animals. We concluded that the decreased glucagon binding does not play a significant role in the maintenance of serum glucose or in the gluconeogenetic response glucagon, and that neither these responses nor the serum glucagon levels appears to be correlated with the number of glucagon receptors. We conclude further that different animal strains of the same species may differ in their biologic responses.

Hormone binding to its receptors is commonly used as an index of tissue sensitivity and responsiveness (1, 2). In preliminary studies using Sprague–Dawley (SD) and Wistar–Furth (WF) rats, we noted that while fasting blood glucose was higher in the SD animals and glucagon binding by the hepatocytes was consistently greater in the WF rats, the insulin binding was the same. Thus the relative hyperglycemia of the SD rats could have been the result of an increased glucose production due to elevated hepatic sensitivity to glucagon, of a decreased sensitivity to insulin, or of other factors that regulate glucose production (3). Alternatively, the difference between the two strains of rats could have been due to decreased glucose production or increased glucose utilization by the WF rats. To extend these observations, we sought to determine if there were any correlation between plasma hormone levels, insulin and glucagon receptor binding, hepatic glucose production, and glycogen content between the two strains.

Materials and Methods. SD and WF female rats, weighing approximately 150–175 g, were purchased from Harlan Laboratories (Indianapolis, IN), kept two to a cage, and allowed food and water *ad libitum*. On the day of the experiment, 24-hr fasted or nonfasted rats were anesthetized with sodium pentobarbital (35 mg/kg) and blood samples were collected from the inferior vena cava for subsequent glucose, insulin, glucagon and growth hormone determinations. Hepatocytes were isolated by collagenase digestion using minor modifications of the method of Feldhoff *et al.* (4). Briefly, these consisted of *in situ* perfusion of the liver with a Ca^{2+}-free Krebs–Ringer bicarbonate (KRB) buffer, pH 7.4, for 20 min followed perfusion with KRB buffer containing Ca^{2+} and collagenase for 30 min. At the end of the perfusion the liver was removed from the rat, minced, and gently shaken for 15 min. The digest was then filtered and the cells were collected and tested for viability using the trypan blue exclusion test. The viability rate turned out to be 85–95%. Isolated cells were resuspended in Krebs buffer containing 1.0% bovine serum albumin (BSA) and were used for the radioreceptor assay. Liver glycogen was determined using the method of Seifter *et al.* (5).

The radioreceptor assays were carried out according to a variation of the method of Holst *et al.* (6). Assay buffer (25 m*M* Tris + 10 m*M* CaCl, containing 0.3% BSA and 1.0% bacitracin), unlabeled glucagon standard, and labeled hormone (0.7, 0.1, and 0.1 ml, respectively) were added to the assay tubes and mixed. Hepatocytes (5×10^5 cells/0.1 ml) were then

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added to each tube and incubated for 1.0 hr at 25°C. The assay was terminated by the addition of 2.5 ml of ice-cold buffer (25 m*M* Tris + 10 m*M* CaCl, containing 0.1% BSA). The samples were centrifuged, and the sediment was washed twice with termination buffer and its radioactivity was counted. The radioreceptor assay for insulin was carried out in the same manner except that the cells were incubated for 18–24 hr. The results of the assay were corrected for the degradation of glucagon and insulin by hepatocytes during the incubation, estimated by precipitation of ^{125}I-labeled glucagon and ^{125}I-labeled insulin with equal volumes of 10% trichloroacetic acid. Labeled glucagon and insulin were prepared in our laboratory using a modification of the choloramine T method of Greenwood and Hunter (7). Their specific activities were approximately 120 and 140 $\mu c/\mu g$.

Serum glucose was measured using the GOD–Period enzymatic method (autotest; Boehringer Mannheim Co., Mannheim, West Germany), serum insulin with the Auto Pak-12 Test delivery system (Micromedic, Horsham, PA), and glucagon with a modification of the method of Foà *et al.* (8) using an antiserum (AGS BBB) which cross-reacts only with the carboxyl terminal of glucagon. Rat growth hormone was determined using the double antibody assay of Birge *et al.* (9).

Glucose production from alanine (10 m*M*) in response to glucagon was studied in hepatocytes from SD and WF rats using the method of Zahlten *et al.* (10) with modifications. To isolate hepatocytes the liver was perfused *in situ* with a solution of 0.018% collagenase (Worthington, CLS III) and 0.080% hyaluronidase (Sigma) in Ca^{2+}-free Krebs-Henseleit bicarbonate buffer containing 1.0% BSA, 5 m*M* pyruvate, 5 m*M* glutamate, and 11 m*M* glucose, constantly gassed with a mixture of O_2 (95%) and CO_2 (5%). After 20 min, the liver was removed, minced, and shaken with 20 ml of the same solution for another 15 min and filtered through a nylon mesh. The cells were washed three times with Krebs–Henseleit bicarbonate buffer containing 1.5% gelatin and resuspended in the same buffer containing 10 m*M* uniformly labeled [^{14}C]alanine for incubation. The final suspension resulted in a cell concentration of 0.75 to 1.25 $\times$ 10^6 cells/ml. The incubation was carried out in plastic vials at 37°C for 40 min with constant shaking in an atmosphere of $0_2/CO_2$ (95/5%). The experiment was terminated by the addition of perchloric acid to a 0.33 *N* concentration. The samples were centrifuged and the pellet and supernatant were stored at −20°C. The conversion of alanine to glucose was determined by the method of Exton and Park (11), with the following modifications. Aliquots (0.5 ml) of each sample were neutralized to pH 7.0 with KOH and 100 μl was added to 100 μl of phosphate buffer (5 m*M*, pH 7.0), applied to a column of Dowex mixed bed resin (5 $\times$ 60 mm) and eluted with deionized H_2O. The eluate was counted in a liquid scintillation spectrophotometer. Rates of incorporation of ^{14}C-labeled substrate into glucose were expressed as micromoles of alanine converted to glucose per gram of liver, assuming that there were 9.8 $\times$ 10^7 cells/g.

Results. The fasting blood glucose concentration of WF rats was significantly lower than that of the SD animals (Table I), while the nonfasted values were comparable in the two strains of animals. Fasting serum insulin levels

TABLE I. SERUM GLUCOSE, INSULIN, GLUCAGON, AND GROWTH HORMONE IN FASTED AND NONFASTED WISTAR-FURTH AND SPRAGUE-DAWLEY RATS

	Glucose (mg/dl)		Insulin (μU/ml)		Glucagon (pg/ml)		Growth hormone (ng/ml)	
	Fasted	Nonfasted	Fasted	Nonfasted	Fasted	Nonfasted	Fasted	Nonfasted
Wistar–Furth	75 ± 6 (11)	164 ± 10 (16)	4.0 ± .42 (10)	27 ± 3 (16)	156 ± 23 (10)	50 ± 5 (15)	141 ± 23 (7)	100 ± 28 (17)
Sprague–Dawley	103 ± 12* (6)	189 ± 9 (9)	3.5 ± .20 (5)	48 ± 7* (6)	62 ± 9* (5)	50 ± 11 (6)	94 ± 14 (4)	46 ± 12 (7)

* $p < 0.05$.

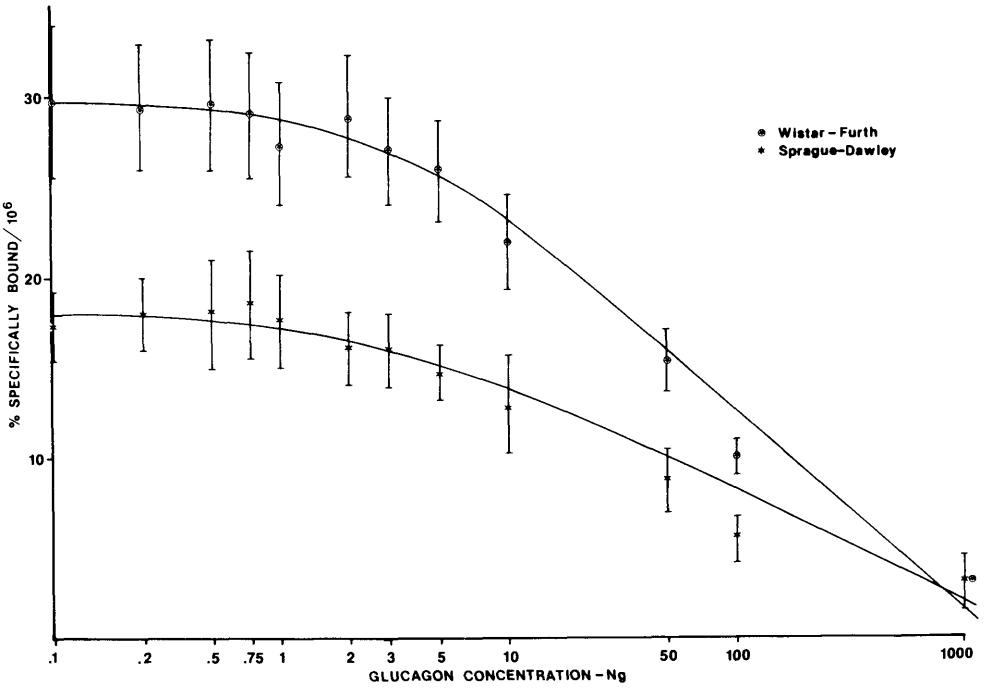

FIG. 1. ^{125}I-glucagon binding by hepatocytes isolated from Wistar–Furth and Sprague–Dawley rats; n = 7.

were the same, while nonfasted SD rats had significantly higher insulin levels. Fasting serum glucagon levels were higher in the WF group while the nonfasted values were the same in the two groups. No significant differences were noted in the growth hormone levels of the two groups, in either the fasted or the nonfasted state.

Hepatocytes isolated from WF rats bound significantly more glucagon than hepatocytes from SD rats (Fig. 1). Further analysis of these data showed that the decrease in binding was due to a decrease in receptor number (Fig. 2). There were no significant differences in insulin binding between the two groups (Fig. 3).

The basal glucose production was lower in the hepatocytes of WF rats and was stimulated significantly by glucagon (Fig. 4). The increase amounted to 33 ± 3.1 and 20 ± 3.4% for hepatocytes of WF and SD animals, respectively. The conversion of [^{14}C]alanine to glucose was the same in the two groups of animals (Fig. 5), and both groups responded equally to glucagon. Liver glycogen content was significantly higher in the SD rats than in the WF rats (194 ± 8.4 and 127 ± 10.2 μmol glucose/g).

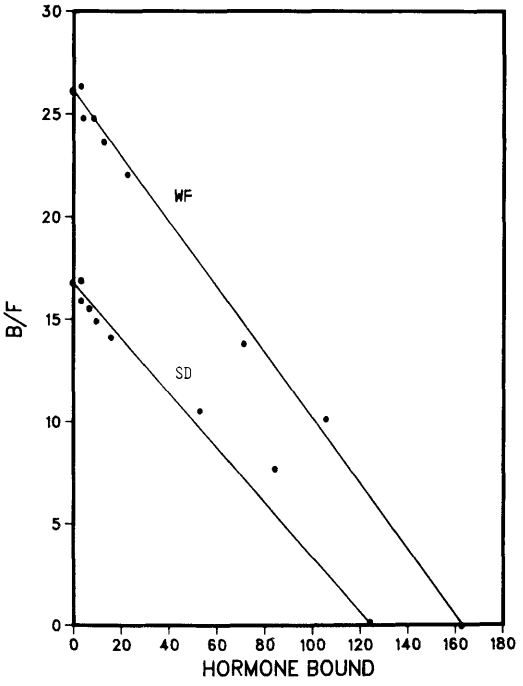

FIG. 2. Scatchard plot of ^{125}I-glucagon binding by hepatocytes isolated from Wistar–Furth and Sprague–Dawley rats; n = 7.

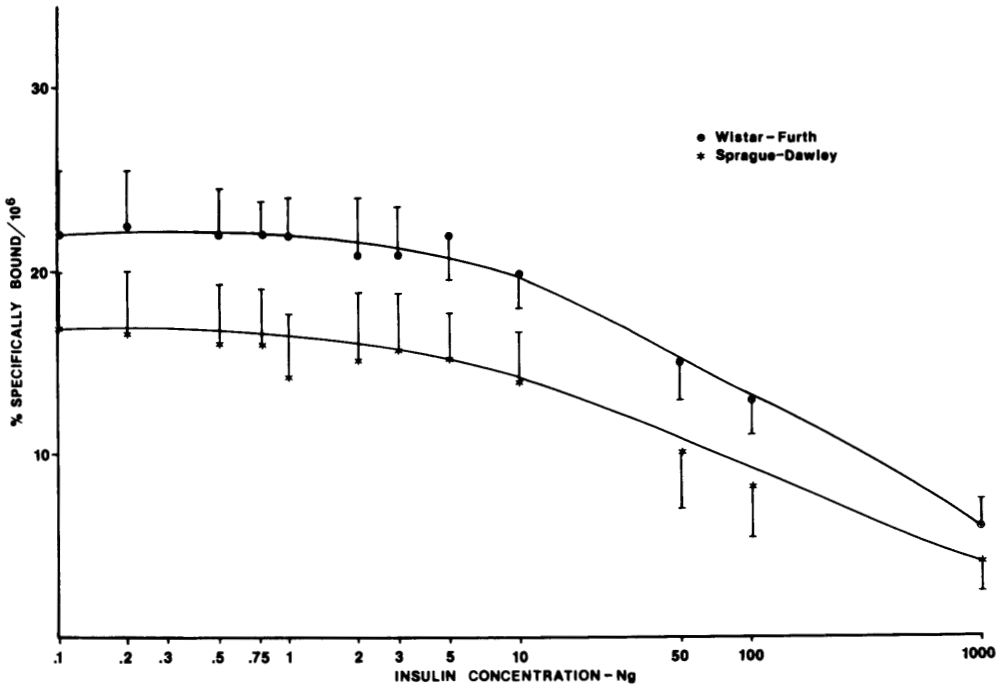

FIG. 3. ^{125}I-insulin binding by hepatocytes isolated from Wistar–Furth and Sprague–Dawley rats; $n = 7$.

Discussion. Hormonal and metabolic differences between different strains of animals have been reported previously (12, 13). In this study we have evaluated plasma hormone levels, serum glucose, liver glycogen, insulin and glucagon receptor binding, and glucose pro-

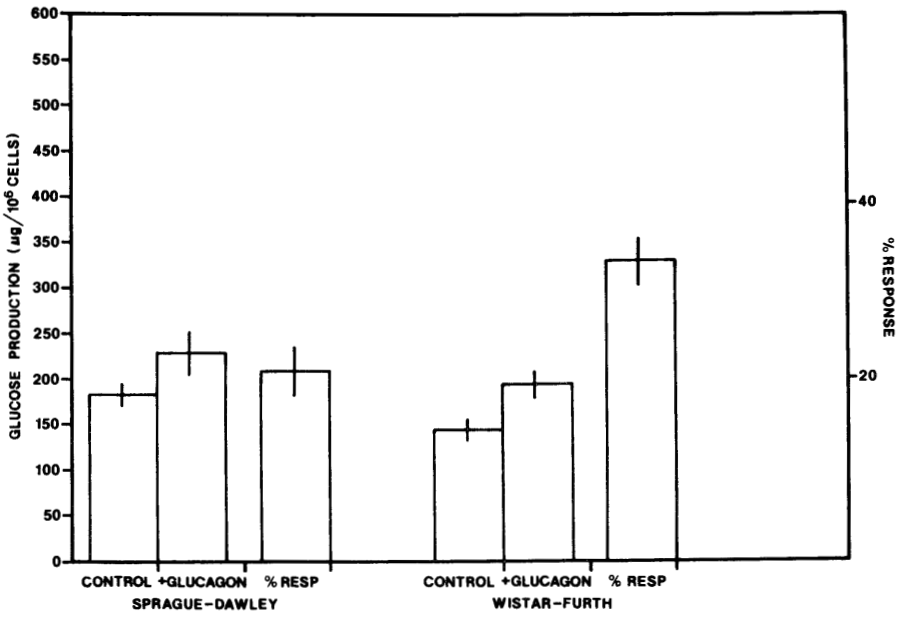

FIG. 4. Basal and glucagon-stimulated (3.5 µg/ml) glucose production by hepatocytes (µg/10^6 cells) in Wistar–Furth and Sprague–Dawley rats; $n = 15$.

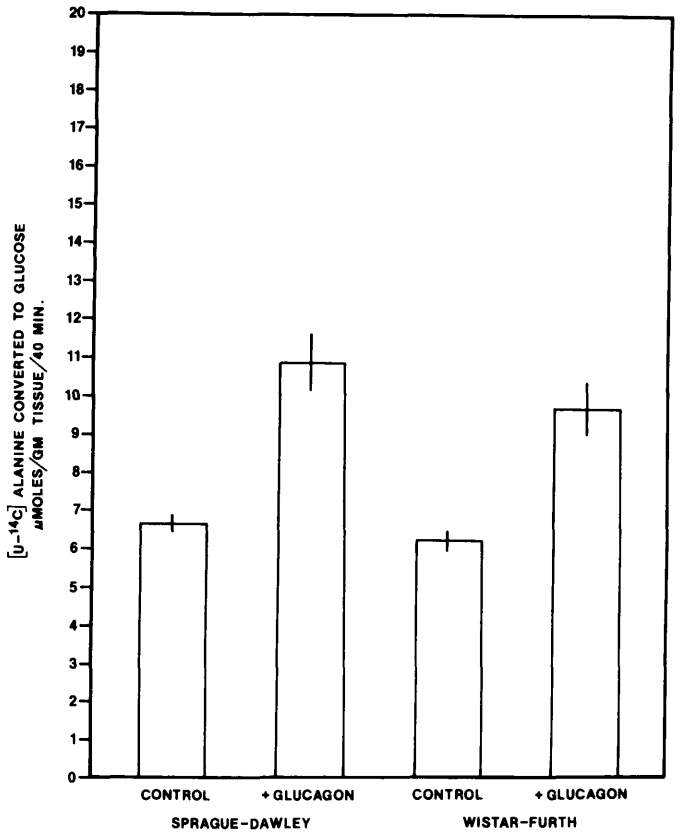

FIG. 5. Glucagon stimulation of [^{14}C]alanine (2 mM) conversion to glucose in hepatocytes isolated from Wistar–Furth and Sprague–Dawley rats; n = 15.

duction by the hepatocytes of two strains of laboratory rats. Our initial observation that, when fasted for 24 hr, SD rats were able to maintain a higher plasma glucose than WF rats (14) was confirmed. However, plasma glucose levels were not different when the animals were not fasted. In addition, we observed that SD rats were able to store more glycogen than WF rats, a possible reason for their ability to maintain a higher blood glucose during fasting.

The nonfasting serum insulin level was higher in SD rats, possibly because of their higher glucose levels. The fasting serum glucagon levels were higher in WF rats, probably a direct reflection of the lower plasma glucose levels. Thus a glucose level of 75 mg/dl in the WF rats may have been sufficient to stimulate glucagon secretion (15), while an average plasma glucose of 103 mg/dl noted in the SD rats may not have been. There were no differences in serum growth hormone levels between the two groups, either in the fasted or in the nonfasted state.

Glucagon binding to hepatocytes isolated in the nonfasted state was greater in the WF animals. The greater receptor population was not what could have been expected on the basis of the plasma hormone levels (16), since the higher glucagon level, especially in the fasted state, should have caused greater down-regulation in the WF group (17–20). An explanation for this apparent paradox could be that the WF rats have a higher basal glucagon receptor population than the SD rats. If so, the phenomenon did not seem to apply to the insulin receptor.

Glucose production from heptocytes was comparable in the two strains of rats and so was the basal rate of gluconeogenesis from alanine and its response to glucagon. Although we did not measure food consumption, our

supplier (Harlan Laboratories) informed us that SD rats grow faster and larger than WF rats, suggesting that food consumption may play a role in the observed differences.

Although it is tempting to correlate metabolic profile to hormonal secretion, plasma hormone level to receptor population, and receptor binding to tissue sensitivity, these systems do not appear to be tightly coupled under all conditions (17, 18, 21). Thus, in our experiments, the higher fasting glucagon levels of WF rats did not translate into a greater receptor down-regulation, nor did the combination of high glucagon level and high number of receptor translate into greater hormone effectiveness, at least as evaluated by the rate of glucose production. Other strain differences in endocrine responses have been reported (22, 23).

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