Endocrine, *vol. 13, no.3, 273–280, December 2000* 0969–711X/00/13:273–280/$12.00

Diabetogenic Role of Insulin's Counterregulatory Hormones in the Isletectomized, Diabetic Goby

James T. Haigwood, Robert M. Flores, Reza Mazloumi, Gilbert Ngan, and Kevin M. Kelley

Endocrinology Laboratory, Department of Biological Sciences, California State University at Long Beach, Long Beach, CA 90840

In an experimental model of insulin-dependent diabetes mellitus (IDDM) in the teleost fish, the goby *Gillichthys mirabilis*, an isletectomy procedure completely removes the pancreatic endocrine tissue without affecting the exocrine acini or other essential tissues. Interestingly, isletectomized (Ix) gobies do not exhibit a significant hyperglycemia until 10–15 d after this procedure, suggesting a lack of initial diabetogenic actions of a pancreatic factor(s). Administering exogenous glucagon in otherwise nonsymptomatic 7-d Ix gobies, however, induces a hyperglycemic state comparable to that in severely diabetic rats or gobies (after 20 d post-Ix). The spontaneously arising hyperglycemia observed between 10 and 15d post-Ix, on the other hand, is significantly correlated with increasing serum cortisol concentrations, with both exhibiting sustained elevated levels (approx 23 mmol/L and >100 ng/mL, respectively) at 20- and 25-d post-Ix. Exogenous cortisol treatment also significantly induced hyperglycemia in nonsymptomatic, 7-d Ix gobies. By contrast, growth hormone (GH) had no detectable diabetogenic effect in 7-d Ix gobies. Serum levels of ammonia, the principal nitrogenous waste in this species, were not affected by glucagon treatment but were reduced slightly by GH treatment (30% reduction; $p < 0.05$). Cortisol treatment, on the other hand, increased ammonia levels twofold, suggesting that the glucocorticoid induces a negative nitrogen balance. These results indicate that the counterregulatory hormones—glucagon and cortisol—are effective diabetogenic factors in the Ix goby, capable of driving metabolic imbalance in this model of IDDM.

Received May 11, 2000; Revised June 13, 2000; Accepted June 13, 2000.
Author to whom all correspondence and reprint requests should be addressed: Dr. Kevin M. Kelley, Endocrinology Laboratory, Department of Biological Sciences, California State University at Long Beach, CA 90840. E-mail: kmkelley@csulb.edu

Key Words: Insulin-dependent diabetes mellitus; counterregulators; teleost fish; nonmammalian alternative disease models.

Introduction

In insulin-dependent diabetes mellitus (IDDM), both in that arising spontaneously and in experimental models such as the streptozotocin (STZ)-injected diabetic rat, the insulin-producing pancreatic β-cells are lost in a specific manner, leaving behind the other pancreatic endocrine cells *(1–4)*. This means that insulin's primary pancreatic counterregulator, glucagon, persists within the diabetic state during the onset period of IDDM. This early pancreatic release and action of glucagon, in the face of declining insulin levels, is considered an important mechanism underlying the expression of hyperglycemia and other diabetic imbalances during the onset period of IDDM *(3–7)*.

In total pancreatectomy in mammals, however, an acute expression of diabetic hyperglycemia is observed, despite an absence of pancreatic glucagon *(8–12)*. This implies that extrapancreatic factors also play an important role in driving the diabetic symptomatology in this experimental model. In part, gut-derived glucagon and glucagon-like peptides could drive glucose production, although the experimental evidence for this has not been consistent *(13–16)*; for example, experimentally reducing gut production of glucagon was not effective in reducing blood glucose in totally pancreatectomized dogs *(13)*. Early diabetogenic effects of glucocorticoids and catecholamines are also possible, particularly because the complex of conditions resulting from pancreatectomy cause significant physiologic stress in the animals *(17–23)*. In addition to a tricky replacement therapy with the metabolic hormones, malabsorption is typical in pancreatectomized mammals, and they must be provided pancreatic enzymes orally, given the ablation of pancreatic exocrine tissue. Despite certain shortcomings, pancreatectomy has nonetheless served as an important experimental model for studies on the interac-

tions of glucagon, insulin, and other metabolic hormones *(3–7,10,11,24–29)*. With the exception of pancreatectomy, there have been no other available experimental IDDM models described in which the glucagon-producing pancreatic α-cells are removed simultaneously with insulin-producing β-cells.

More recently, a nonmammalian model of IDDM was identified in which it is possible to remove specifically all the pancreatic endocrine cells, providing an opportunity to assess the diabetogenic role of glucagon similar to that of pancreatectomy but without concomitant ablation of exocrine and other tissues *(30)*. In the teleost fish, the goby *Gillichthys mirabilis*, the entire pancreatic endocrine cell population is contained within a single and well-defined islet organ that is not associated with exocrine acini, as in mammals, or with other important structures or tissues. This anatomical feature allows a relatively simple surgical isletectomy (Ix) to be performed, which cleanly removes the pancreatic endocrine cells and results in a full-blown diabetic state comparable with that of severe IDDM in mammals *(30–33)*.

In earlier studies on the Ix goby, it was noted that expression of the diabetic metabolic imbalances was delayed substantially relative to that expected in IDDM onset in mammals, requiring up to 30 d before maximal hyperglycemia (25 mmol/L glucose) was observed. By comparison, diabetic hyperglycemia in the STZ-injected rat (insulin loss alone) usually occurs within 12–24 h *(1,2,6)*. This difference in the Ix goby led to the hypothesis that the delayed onset of a diabetic symptomatology may be related to the absence of pancreatic glucagon, because in the IDDM models in which only β-cells are absent, glucagon is present to exert its well-established counterregulatory actions.

In the present study, the diabetogenic influences of glucagon, as well as cortisol and growth hormone (GH), were investigated in the Ix goby model. In mammals as well as in fishes, cortisol *(34–39)* and GH *(34,39–45)* have a variety of actions that are counterregulatory to those of insulin. A substantial diabetogenic role of glucocorticoids in mammals was recently highlighted by studies in the STZ-diabetic rat in which hyperglycemia and other diabetic imbalances were ameliorated after adrenalectomy, but returned with corticosteroid replacement therapy *(36,37,46)*. We provide evidence that glucagon as well as cortisol have significant diabetogenic roles in the Ix goby, whereas for GH no diabetogenic effect was evident in this model.

Results

The Ix goby exhibited a very late onset of diabetic hyperglycemia compared with that in STZ-injected rats (Fig. 1). In the STZ-injected rats, the expression of a significant hyperglycemia occurred within 12 h after treatment (14.8 vs 5.5 mmol/L in nondiabetic controls, $p < 0.05$), and maximal hyperglycemia (~24 mmol/L) was reached after 24 h. By contrast, significant elevations in serum glucose concentration in the Ix goby were not observed until 15 d after Ix, and a maximal diabetic hyperglycemia (~23 mmol/L) was not reached until 20 d post-Ix.

Immediately following the Ix procedure, serum glucose tended to be slightly elevated ($p = 0.11$ at the 12-h timepoint), but this subsided by 48 h, and it was not until after 10 d post-Ix that the sustained diabetic hyperglycemia was evident (Fig. 1). Similar initial transient increases in serum glucose concentration were observed in the sham-Ix fish ($p < 0.05$ at 12 h post-Ix). In contrast to the Ix goby and STZ-injected rats, sham-Ix gobies did not again show elevated glucose levels throughout the rest of the 25-d experiment. Hepatosomatic index (HSI), an index of relative hepatic fuel storage (principally glycogen and fat), was decreased in a progressive manner after Ix, significantly so by 10 d post-Ix (Fig. 2); by 25 d post-Ix, HSI was half that in sham-Ix and intact control groups ($p < 0.05$).

In earlier studies, we had noted high serum cortisol levels in 30-d Ix gobies (data not shown), and, therefore, it was of interest to determine the degree to which endogenous glucocorticoid levels may be associated with the diabetic symptomatology through the onset period of IDDM. Serum cortisol concentrations measured by radioimmunoassay (RIA) (Fig. 3) spiked in the immediate post-Ix period (~80 ng/mL in the 12-h and 1-d groups, $p < 0.05$), subsided to control levels by 5 and 10 post-Ix (<20 ng/mL), but then showed a sustained increase at 15 d and thereafter, surpassing 100 ng/mL in the 20- and 25-d post-Ix groups ($p < 0.01$). By contrast, the sham-Ix gobies also showed a temporal spike in serum cortisol levels in the first 24 h posttreatment ($p < 0.05$), but did not show any subsequent increases. Among the Ix animals, there was a significant positive correlation between serum cortisol concentration and serum glucose concentration ($r^2 = 0.77$, $p < 0.05$).

The lack of expression of a diabetic hyperglycemia for up to a 2-wk period in the Ix goby provided for an opportunity to test the degree to which certain counterregulatory hormones may play a diabetogenic role in the onset of IDDM. In this study, exogenous treatments of glucagon, cortisol, and GH, all known to exert anti-insulin effects in both fishes and mammals, were tested. Injection of 7-d Ix nonsymptomatic gobies with glucagon had a significant hyperglycemic effect (Fig. 4). Treatment of Ix gobies with either 1.0 or 5.0 μg/g of glucagon significantly increased serum glucose concentration over that in all other groups ($p < 0.01$), reaching 18–19 mmol/L (2.8-fold increase over sham-Ix controls). Similarly, cortisol treatment of 7-d Ix gobies at the same doses resulted in significant elevations in serum glucose over that of the other groups (Fig. 5), reaching 21–24 mmol/L (3.4-fold increase over sham-Ix controls; $p < 0.01$). By contrast, the injection of GH pro-

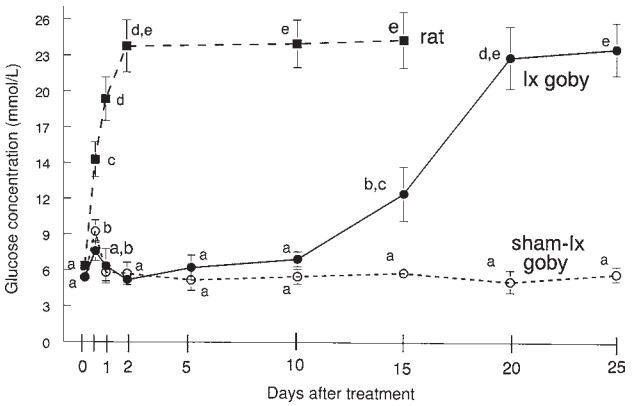

Fig. 1. Temporal change in serum glucose concentration after treatment in the isletectomized (Ix) goby (d), sham-Ix goby (s), and streptozotocin-injected diabetic rat (u). Data shown are mean ± SE of individual millimoles/liter glucose values at 12 and 24 h and 2, 5, 10, 15, 20, and 25 d, in which n =12–15 (gobies) or 7 to 8 (rats) per point. Different superscript letters indicate significantly different values ($p < 0.05$).

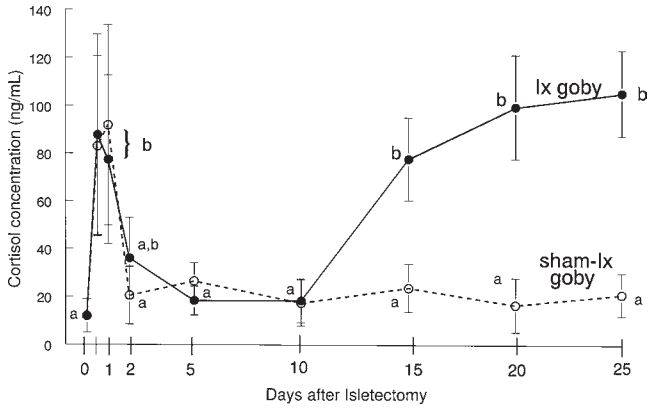

Fig. 3. Temporal change in serum cortisol concentration in Ix (d) and sham-Ix (s) gobies. Cortisol concentrations were measured by RIA with the data expressed as mean ± SE of individual nonograms/milliliter values at 12 and 24 h and 2, 5, 10, 15, 20, and 25 d posttreatment, in which n = 12–15 per point. Different superscript letters indicate significantly different values ($p < 0.05$).

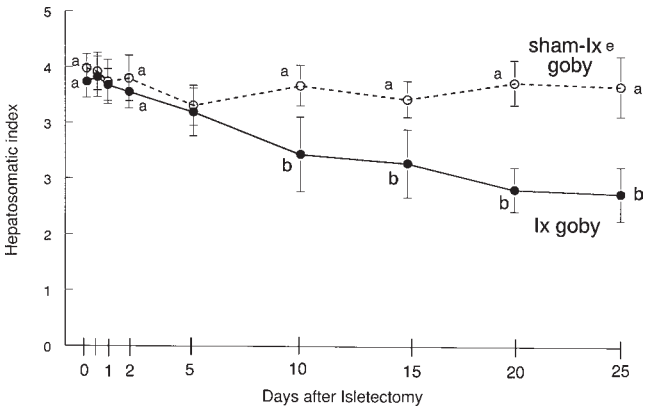

Fig. 2. Temporal change in HSI in Ix (d) and sham-Ix (s) gobies. HSI is calculated as grams of liver weight/grams body weight × 100, with the data expressed as mean ± SE at 12 and 24 h and 2, 5,10,15, 20, and 25 d posttreatment, in which n =12–15 per point. Different superscript letters indicate significantly different values ($p < 0.05$).

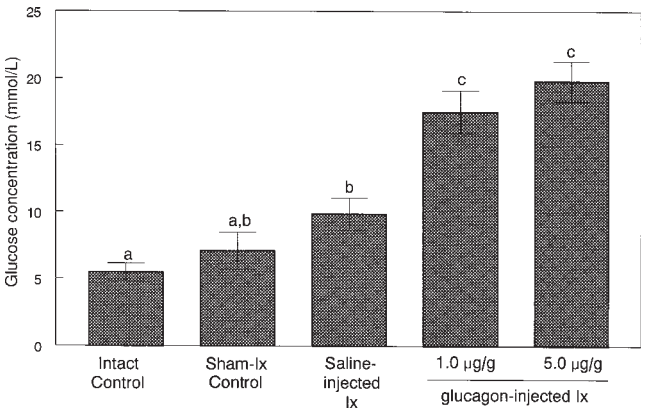

Fig. 4. Effect of glucagon treatment in 7-d Ix gobies on serum glucose concentration. Ix gobies injected with 1.0 or 5.0 µg of glucagon/g of body weight were compared with saline-injected Ix, sham-Ix, and intact controls. Data shown are mean ± SE of millimoles/liter glucose values (n =12–15 per point) compiled over three separate experiments. Bars with different superscript letters are significantly different from each other ($p < 0.05$).

duced no detectable changes in serum glucose level in the Ix goby (Fig. 6).

Initial experiments determined that the nitrogenous waste compounds, urea and uric acid, were below the limit of detection in control and Ix goby serum, as is true for the majority of fish species (data not shown). Ammonia (Fig. 7), on the other hand, was present in serum of the control animals at concentrations between 200 and 225 µmol/L and showed a significant (40%) elevation in the saline-injected Ix group ($p < 0.05$). Experiments on the effect of each hormone at its highest tested dose indicated that cortisol was the only hormone to increase serum ammonia concentrations, by greater than twofold over that in all other groups ($p < 0.01$). By contrast, glucagon had no detectable effect, whereas GH resulted in a significant (30%) decrease in serum ammonia concentration compared with that in the control groups ($p < 0.05$).

Discussion

In the Ix goby, the entire pancreatic endocrine tissue is removed without collateral damage to the exocrine acini or other important tissues and structures, and it results in a severely diabetic state like that of untreated IDDM in mammals. However, the development of the metabolic imbalances is significantly delayed by more than 10 d in the Ix goby, in contrast to the STZ-injected diabetic rat, in which hyperglycemia occurs within hours of treatment (Fig. 1). This temporal discrepancy may be explained by differences

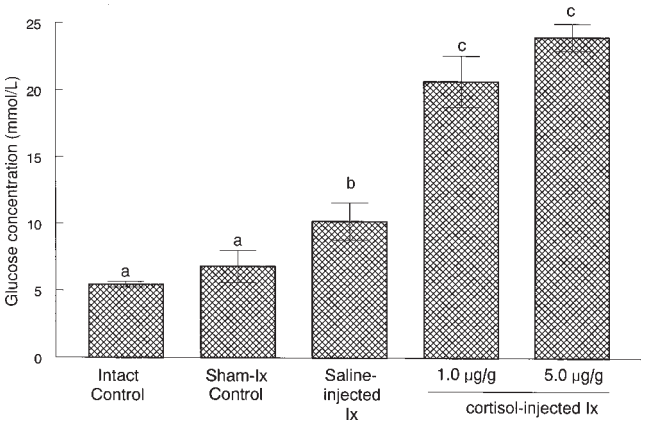

Fig. 5. Effect of cortisol treatment in 7-d Ix gobies on serum glucose concentration. Ix gobies injected with 1.0 or 5.0 μg of cortisol/g of body weight were compared with saline-injected Ix, sham-Ix, and intact controls. Data shown are mean ± SE of millimoles/liter glucose values (n =11–15 per point) compiled over two separate experiments. Bars with different superscript letters are significantly different from each other ($p < 0.05$).

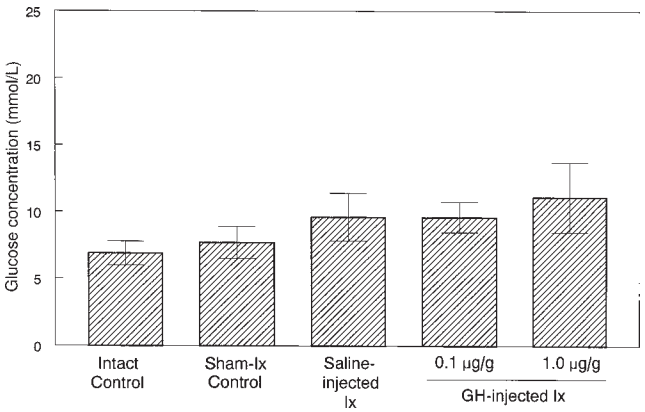

Fig. 6. Effect of GH treatment in 7-d Ix gobies on serum glucose concentration. Ix gobies injected with 0.1 or 1.0 μg GH/g of body weight were compared with saline-injected Ix, intact, and sham-Ix controls. Data shown are mean ± SE of millimoles/liter glucose values (n = 10–11 per point) compiled over three separate experiments. No significant differences were found among all groups.

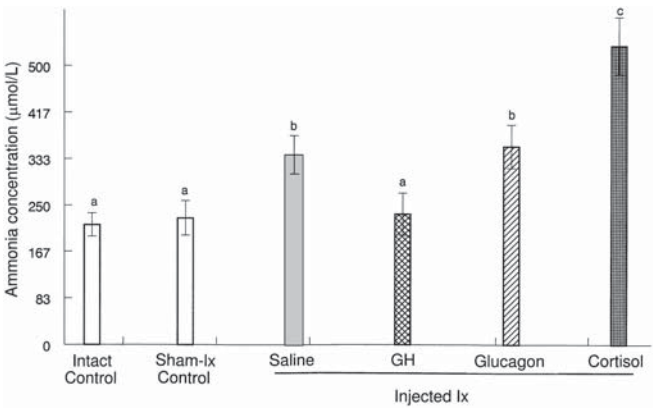

Fig. 7. Serum levels of ammonia (major form of nitrogenous waste in goby) in 7-d isletectomized Ix gobies treated with GH, glucagon, or cortisol, as compared with that in controls (saline-injected Ix, intact, and sham-Ix gobies). Data shown are mean ± SE of millimoles/liter glucose values (n = 10–15 per point) compiled over two separate experiments. Injection doses were 1.0, 5.0, and 5.0 μg of hormone/g of body weight for GH, glucagon, and cortisol, respectively. Bars with different superscript letters are significantly different from each other ($p < 0.05$).

in the methodology utilized to induce insulin deficiency between the two models, because in the STZ-diabetic rat (as well as in spontaneous IDDM in humans and other animals), the insulin-producing β-cells are lost in a specific manner whereas the α-cells remain and release glucagon *(1–4,47,48)*. Unchallenged in insulin's absence, this pancreatic glucagon, released directly into the hepatic portal vein, is hypothesized as a primary factor driving hepatic glucose release and hyperglycemia in diabetic mammals. Indicating its potentially important diabetogenic role in the Ix goby, glucagon replacement therapy was found to produce a significant hyperglycemia within 48 h in otherwise nonsymptomatic 7-d Ix individuals (Fig. 4). Indeed, significant glucose-raising effects of glucagon in 7 to 10-d Ix gobies has been observed after periods as short as 6 h *(49)*, indicating that the kinetics of the metabolic responses in the goby are relatively fast, despite the fact that they are ectotherms.

The diabetogenic effects of glucagon likely include hepatic glycogenolytic mechanism, as inferred from studies in the goby, in other fish species, as well as in mammals *(3,4,35,49–51)* in which the hormone has been demonstrated to reduce hepatic glycogen content, activate glycogen phosphorylase activity, and/or stimulate hepatic glucose release. Altough direct hepatic glucoregulatory actions are evident, glucagon as yet has not been found to be associated with changes in nonprotein nitrogen (e.g., ammonia) (Fig. 7), triglycerides, or glycerol *([49]*; unpublished data) in the Ix goby, suggesting that changes in some of the other metabolic pathways during IDDM may be driven by additional counterregulatory hormones (e.g., cortisol).

In contrast to the diabetogenic effects of glucagon, there were no detectable hyperglycemic effects of GH in the Ix goby (Fig. 6) at concentrations that otherwise are highly effective in stimulating somatic growth *(31,50)*. An earlier study also could not establish any glucose-raising effects of GH treatment in 30-d Ix gobies *(30)*, although the serum glucose levels were already at >20 mmol/L when the hormone was tested. In the present study, normoglycemic Ix individuals were studied and an additional 10-fold higher GH concentration was also tested (Fig. 6), again without effect. Although GH did not produce hyperglycemia in either study, its metabolic actions are nonetheless apparent

in the present study because it increased ammonia levels (Fig. 7), suggesting that, as in mammals, the hormone may cause a shift toward a positive nitrogen balance.

An important role for GH in the symptomatology of IDDM in humans and other mammals was suggested as early as the 1930s, when it was discovered that hyperglycemia and other metabolic abnormalities could be ameliorated by hypophysectomy *(52–56)*. Several studies have since shown that GH has significant hyperglycemic effects in humans as well as in a variety of vertebrates, including fishes *(34,41,43,57,58)*. Believed to occur largely through its induction of a peripheral resistance to insulin action, GH decreases postprandial glucose uptake peripherally and causes glucose intolerance generally, resulting in an elevation in circulating glucose levels *(3,59,60)*. These peripheral metabolic actions of GH are believed to be responsible for the "dawn phenomenon" in patients with IDDM, in which nocturnal spikes in its endogenous pituitary GH release elevate serum glucose levels *(4,43,61–64)*. Thus, GH's inhibitory effects on insulin's peripheral actions appear to be an important mechanism accounting for increased serum glucose under circumstances in which insulin is present (e.g., in normal individuals or in diabetics persons treated with insulin to attain metabolic control). However, the degree to which GH is diabetogenic in the absence of insulin is not clear.

When tested in the Ix goby, GH had no apparent diabetogenic effect, whereas glucagon and cortisol induced significant hyperglycemia. These results suggest that the latter two hormones, which stimulate the production of glucose, may be more fundamental driving forces underlying the diabetic symptomatology. However, despite GH's inability to cause hyperglycemia in the Ix goby, it cannot be excluded as a contributing factor, not only via possible insulin-antagonistic actions but also through additional metabolic actions. For example, GH's known lipolytic effects *(1,34,44,65)* could support gluconeogenesis and ketogenesis. Such potentially important mechanisms of action of GH remain to be characterized in the Ix goby model.

While the amelioration of diabetic symptoms by hypophysectomy *(40,42,52,54–56)* has often been used as evidence supporting a fundamental diabetogenic role for pituitary GH, recent studies have demonstrated that a similar amelioration occurs after adrenalectomy *(36,37,45)*. These results would suggest that the relieving effect of hypophysectomy is less related to GH loss and is more a result of ablation of corticosteroid production (via loss of adrenocorticotropic hormone–stimulated corticosteroid production). In support of a lesser "driving" role of GH in the diabetic symptomatology, it is notable that serum GH levels are decreased in the diabetic rat but increased in diabetic humans, and yet the IDDM symptomatology is otherwise highly similar, suggesting the involvement of more fundamental factors, such as corticosteroids.

In the Ix goby, a significant diabetogenic role for cortisol was supported by two lines of evidence from the present study. The first is that the spontaneous increases in serum glucose occurring between 10 and 20 post-Ix were found to be significantly and positively correlated with increasing serum concentrations of cortisol, with both glucose and cortisol maintained at elevated levels for the remainder of the experiment (Figs. 1 and 3). These data indicate that, as in mammals, endogenous cortisol production is strongly associated with the diabetic symptomatology in the Ix goby. Indeed, a positive relationship between cortisol and glucose was even observed in the transient, coincident peaks in serum cortisol (Fig. 3) and glucose (Fig. 1) in the 24-h period immediately following Ix or sham-Ix; such responses are typical of stress responses in fishes as well as in mammals *(66)* and were presumably a result of the Ix procedure itself.

The second line of evidence supporting the diabetogenic role of cortisol is the experiments demonstrating its metabolic actions. In 7-d Ix individuals with control levels of glucose and cortisol, treatment with cortisol significantly induced hyperglycemia (Fig. 5) and increased serum ammonia concentrations (Fig. 7). Because the diet given to the gobies is protein-rich and carbohydrate-poor (*Artemia salina*), it is hypothesized that the cortisol-induced hyperglycemia may result from an enhancement of the rate of gluconeogenesis from diet and/or internally derived amino acids—hence the increase in levels of nitrogenous waste as ammonia. In carnivorous fishes, metabolism is typically dependent on gluconeogenic processes for providing glucose to the general circulation *(34,67–69)*. In addition to its potential gluconeogenic effects in the Ix goby, cortisol could activate peripheral lipolysis (*34*). Whether gluconeogenesis or lipolysis is enhanced in Ix gobies, particularly in hormone-treated Ix gobies, is not understood and is a subject of continuing studies in this model.

In conclusion, glucagon and cortisol, but not GH, were determined to have significant diabetogenic actions when tested in the Ix goby. In this model of IDDM, the ablation of the entire pancreatic endocrine tissue is associated with a delay in the onset the diabetic symptomatology by up to 15 d, suggesting that diabetogenic pancreas-derived factors are missing. Glucagon replacement therapy of nonsymptomatic 7-d Ix gobies resulted in a hyperglycemic state comparable with that in severely diabetic rats or gobies, implicating glucagon as a significant pancreatic diabetogenic hormone. Cortisol was also implicated as a diabetogenic factor in the present study, as treatment with the steroid also induced hyperglycemia in 7-d Ix gobies, while the spontaneously arising hyperglycemia (after 15 d of Ix) was significantly correlated with increasing endogenous cortisol levels. Glucagon and cortisol were implicated as effective diabetogenic factors in the Ix goby, but the relative contribution of other counterregulatory factors, such as epi-

nephrine, glucagon-like peptides, and enteric glucagons, remains to be determined. The results of this study underscore the multifactorial nature of IDDM, because insulin's absence does not appear to be the sole cause of a diabetic symptomatology; rather, insulin's counterregulators appear to play an important role in driving the metabolic imbalance characterized by IDDM.

Materials and Methods

Animals and Surgical Isletectomy

Adult gobies (longjaw mudsucker, *G. mirabilis*) weighing between 20 and 30 g and measuring 10–15 cm long were obtained from International Bait and Supply (San Diego, CA) and housed in a temperature (15 ± 2°C)- and photoperiod (12 h of light; 12 h of dark)-controlled room at the University Animal House in Long Beach. Fish were maintained in a 1 × 8 m runway supplied with recirculated, filtered natural seawater (4 cm water depth on runway, with 1000-L capacity reservoir) and were fed daily to satiation with brineshrimp (*Artemia salina*) purchased fresh weekly from Bayou Aquatics (Ontario, CA). Fish utilized in the isletectomy experiments were transferred to 0.5 × 1.0 m plastic aquaria (6–10 individuals/tank) containing seawater supplemented with antibiotic (Maracyn; Mardel Villa Park, IL); water was changed daily throughout the experimental period. Fish were allowed to adjust to their surroundings for 7–10 d prior to the beginning of all experiments.

Surgical isletectomy was carried out as previously described *(7)*. All procedures used in this study are in accordance with National Institutes of Health (NIH) guidelines and were approved by our institutional animal care and use committee. Briefly, gobies were anesthetized for 4–6 min in water containing 0.3% (w/v) methanetricanesulfonate (MS-222), a ≤1-cm incision was made on their ventral surface immediately posterior to the left pectoral fin, and the upper intestine was gently pulled through until the single pancreatic endocrine ("islet") organ became visible. The islet organ was then excised, the intestine was returned to the peritoneal cavity, and the incision was closed with three sutures using 4-0 monofilament nylon (Ethicon, Somerville, NJ). Sham-Ix control fish were subjected to the same procedure with the exception of islet organ excision. Post surgically, the animals typically resumed normal swimming activity and feeding behavior within 24 h; by 7 d, the edges of the incisions were fused. IDDM was induced in Sprague-Dawley rats by a single injection of 100 mg/kg of streptozotocin (STZ, Sigma, St. Louis, MO).

IDDM Onset Time-Course Experiments

In three separate experiments Ix gobies at the following time points post-Ix were investigated: 12 h; 24 h; and 2, 5, 10, 15, 20, and 25 d (total of 9–15 individuals per time point). The same time-course experiments were carried out for sham-Ix gobies, serving as controls along with intact untreated gobies. After anesthetization, gobies were weighed to the nearest 0.5 g, and the blood was collected from the severed dorsal artery, allowed to clot on ice for 15 min, and then centrifuged at 3500 rpm for 5 min. The serum was saved and stored until analysis at –80°C. Livers were then removed, weighed to the nearest 0.05 g, and stored at –80°C; liver weight values were used to calculate HSI (g of liver weight/g of body weight × 100), an indicator of relative hepatic fuel (glycogen, fat) storage in fishes (*30,41*). As a mammalian comparison, blood samples from the STZ-injected rats were collected at the following time intervals: 12 h; 24 h; and 2,10, and 15 d (n = 7 to 8 per group).

Individual goby and rat serum samples were measured in duplicate for glucose concentration using an enzymatic glucose oxidase method (Sigma); absorbancies at 450 nm were analyzed on a microplate spectrophotometer (Spectramax 250; Molecular Devices, Sunnyvale, CA). Serum concentrations of cortisol were measured in triplicate using an RIA (Diagnostic Systems, Webster, TX). ^{125}I-labeled cortisol binding in standards (0–60 μg/dL) and in serum samples was counted, with the concentrations in experimental samples determined from a standard curve using calculated B/B_0 values. The intraassay coefficient of variation for this RIA is 6.1%, and the interassay coefficient is 8.0%. Mean glucose and cortisol concentrations for each treatment group were calculated from a final number of animals between 9 and 16 per group.

Hormone Injection Experiments

Given that significant elevations in serum glucose concentrations were not expressed until 15 d post-Ix (Fig. 1), all hormone injection experiments commenced at d 7 post-Ix, a time when diabetic hyperglycemia was not yet evident. Prior to all injections, animals were anesthetized by the addition of 0.3% MS222 (w/v) to the back of the aquarium (in order not to disturb the animals). They were then injected intraperitoneally with 0.8% NaCl (saline-injected Ix controls) or with hormone dissolved in 0.8% saline on d-7 post-Ix and then again 24 h later. Twelve hours after the second injection, tissue samples were collected as described above. Porcine glucagon (pancreatic, HPLC-purified; Sigma) and cortisol (hydrocortisone; Sigma) were tested at doses of 1.0 and 5.0 μg/g of body weight. Ovine GH (NIDDK-oGH-15; NIH, Bethesda, MD) was tested at doses of 0.1 and 1.0 μg/g. All experiments were repeated on two to three separate occasions, with injected Ix animals directly compared against intact and sham-Ix controls (n = 7–15 compiled per group). Serum glucose concentrations and HSI were measured as described above. Serum concentrations of ammonia (as well as urea and uric acid in initial studies) were measured using colorimetric assays (all available from Sigma) in a microplate format as above.

Data Analyses

The data illustrated in Figs. 1–7 are compiled from two to three repeated experiments. Significance among groups ($p < 0.05$) was determined using one-way analysis of variance followed by Bonferroni's multiple comparisons analysis, using CRISP version 3.05A software (Crunch Software, San Francisco, CA).

Acknowledgments

We wish to thank the following persons from California State University at Long Beach for their assistance and advice in this study: K. England, E. Shimizu, K. E. Schmidt, G. S. Nicholson, M. Perez, L. Berg, P. Desai, S. A. Arope, J. T. Roth, V. Desai, and the students of the undergraduate laboratory in animal physiology research (BIOL345L). This research was supported through National Science Foundation grants IBN-9600783 and DUE-9651189.

References

1. Chang, A. Y. and Diani, A. R., (1985). In: *The diabetic pancreas*, Volks, B. W. and Wellmand, K. F. (eds.) Plenum: New York.
2. Wilson, G. L. and Leiter, E. H. (1990). *Curr. Top. Microbiol. Immunol.* **156,** 27–54.
3. Lager, I. (1991). *J. Intern. Med. Suppl.* **735,** 41–47.
4. Cryer, P. E. (1993). *Am. J. Physiol.* **264,** E149–E155.
5. De Feo, P., Perriello, G. Torlone, E., Fanelli, C. Ventura, M. M., Santeusanio, F., Brunetti, P., Gerich, J. E., and Bolli, G. B. (1991). *Am. J. Physiol.* **260,** E203–E212.
6. Smith, U., Attvall, S., Eriksson, J., Fowelin, J., Lonnroth, P., and Wesslau, C. (1993). *Adv. Exp. Med. Biol.* **334,** 169–180.
7. Raskin, P. and Unger, R. H. (1978). *Med. Clin. North Am.* **62,** 713–722.
8. Barnes, A. J., Bloom, S. R., Mashiter, K., Alberti, K. G., Smythe, P., and Turnell, D. (1977). *Diabetologia* **13,** 71–75.
9. Barnes, A. J., Bloom, S. R., George, K., Alberti, G. M., Smythe, P., Alford, F. P., and Chisholm, D. J. (1977). *N. Engl. J. Med.* **296,** 1250–1253.
10. Barnes, A. J. and Bloom, S. R. (1976). *Lancet* **1,** 219–221.
11. Yasugi, H., Mizumoto, R., Sakurai, H., and Honjo, I. (1976). *Am. J. Surg.* **132,** 577–580.
12. Mizumoto, R., Yano, T., Sekoguchi, T., and Kawarada, Y. (1986). *Int. J. Pancreatol.* **1,** 185–193.
13. Matsuyama, T., Tanaka, R., Shima, K., and Tarui, S. (1978). *Endocrinol. Jpn.* **25,** 529–532.
14. Lussier, B. and Hetenyi, G. Jr. (1986). *Can. J. Physiol. Pharmacol.* **64,** 1440–1442.
15. Trevisan, R., Marescotti, C., Avogaro, A., Tessari, P., del Prato, S., and Tiengo, A. (1989). *Diabetes Res.* **12,** 57–62.
16. Tiengo, A., Bessioud, M., Valverde, I., Tabbi-Anneni, A., Delprato, S., Alexandre, J., and Assan, R. (1982). *Diabetologia* **22,** 25–32.
17. Higuchi, A., Yasugi, H., Yokota, T., Tobe, T., and Mizumoto, R. (1979). *Gastroenterol. Jpn.* **14,** 316–326.
18. Gabel, H., Bitter-Suermann, H., Henriksson, C., Save-Soderbergh, J., Lundholm, K., and Brynger, H. (1985). *Horm. Metab. Res.* **17,** 275–280.
19. Yano, T. (1985). *Nippon Geka Gakkai Zasshi* **86,** 470–481.
20. Wilson, J. D., Dhall, D. P., Simeonovic, C. J., and Lafferty, K. J. (1986). *Aust. J. Exp. Biol. Med. Sci.* **64,** 489–500.
21. Stump, K. C., Swindle, M. M., Saudek, C. D., and Strandberg, J. D. (1988). *Lab. Anim. Sci.* **38,** 439–443.
22. Wenger, J. M. Meyer, P., Morel, D. R., Costabella, P. M., and Rohner, A. (1990). *J. Surg. Res.* **49,** 361–365.
23. Dresler, C. M., Fortner, J. G., McDermott, K., and Bajorunas, D. R. (1991). *Ann. Surg.* **214,** 131–140.
24. Polonsky, K. S., Herold, K. C., Gilden, J. L., Bergenstal, R. M., Fang, V. S., Moossa, A. R., and Jaspan, J. B. (1984). *Diabetes* **33,** 1112–1119.
25. Mizumoto, R., Kawarada, Y., Goshima, H., Tamaki, H., Sekoguchi, T., and Tomikawa, I. (1982). *Am. J. Surg.* **143,** 237–243.
26. Yvi-Jarvinen, H., Kiviluoto, T., and Taskinen, M. R. (1986). *Metabolism* **35,** 718–727.
27. Stevenson, R. W., Williams, P. E., and Cherrington, A. D. (1987). *Diabetologia* **30,** 782–790.
28. Vigili de Kreutzenberg, S., Maifreni, L., Lisato, G., Riccio, A., Trevisan, R., Tiengo, A., and del Prato, S. (1990). *J. Clin. Endocrinol. Metab.* **70,** 1023–1029.
29. Giacca, A., Fisher, S. J., McCall, R. H., Shi, Z. Q., Vranic, M. (1997). *Endocrinology* **138,** 999–1007.
30. Kelley, K. M. (1993). *Endocrinology* **132,** 2689–2695.
31. Kelley, K. M., Gray, E. S., Siharath, K., Nicoll, C. S., and Bern, H. A. (1993). *Endocrinology* **132,** 2695–2701.
32. Kelley, K. M., Desai, P., Roth, J. T., Haigwood, J. T., Arope, S. A. Flores, R. M., Schmidt, K. E., Perez, M., Nicholson, G.S., and Song, W. W. (2000). In: *Recent advances in marine biotechnology,* vol. 4, Fingerman, M., Thompson, M.-F., and Nagabhushanam, R. (eds.). Oxford and IBH Publishing: New Delhi.
33. Kelley, K. M., Johnson, T. R., Ilan, J., and Moskowitz, R. W. (1999). *Am. J. Physiol.* **276,** R1164–*R1171.
34. Sheridan, M. A. (1986). *Gen. Comp. Endocrinol.* **64,** 220–238.
35. Mommsen, T. P., Danulat, E., and Walsh, P. J. (1992). *Opsanus beta. Gen. Comp. Endocrinol.* **85,** 316–326.
36. Rodgers, B. D., Lau, A. O. T., and Nicoll, C. S. (1994). *Proc. Soc. Exp. Biol. Med.* **206,** 220–226.
37. Rodgers, B. D., Strack, A. M., Dallman, M. F., Hwa, L., and Nicoll, C. S. (1995). *Diabetes* **44,** 1420–1425.
38. Bates, R. W. and Garrison, M. M. (1971). *Endocrinology* **88,** 1429–1436.
39. Chernick, S. S., Clark, C. M. Jr., Gardiner, R. J., and Scow, R. O. (1972). *Diabetes* **21,** 946–954.
40. Holly, J. M. P., Amiel, C. S., Sandhu, R. R., Rees, L. H., and Wass, J. A. H. (1988). *J. Endocrinol.* **118,** 353–364.
41. Leung, T. C., Ng, T. B., and Woo, N. Y. (1991). *Comp. Biochem. Physiol. A.* **99,** 633–636.
42. Sönkson, P. H., Russell-Jones, D., and Jones, R. H. (1993). *Horm. Res.* **40,** 68–79.
43. Cryer, P. E. (1996). *Horm. Res.* **46,** 192–194.
44. Leena, S., Shameena, B., and Oommen, O. V. (1999). *Endocr. Res.* **25,** 341–355.
45. Albissier, A. M., Nomura, M., Schulz, M., Bahoric, A., Kubota, M., and Holland, F. J. (1987). *Horm. Metab. Res.* **19,** 348–352.
46. Untermann, T. G., Jentell, J. J., Oehler, D. T., Lacson, R. G., and Hofert, J. T. (1993). *Endocrinology* **133,** 2531–2539.
47. Gerich, J. E. (1988). *Diabetes*, **37,** 1608–1617.
48. Kroncke, K. D., Fehsel, K., Sommer, A., Rodriquez, M. L., and Kolb-Bachofen, V. (1995). *Biol. Chem. Hoppe Seyler* **376,** 179–185.
49. Haigwood, J. T. and Kelley, K. M. (1998). *Amer. Zoologist* **37,** 180A.
50. Gray, E. S. and Kelley, K. M. (1991). *J. Endocrinol.* **131,** 57–66.
51. Umminger, B. L., Benziger, D., and Levy, S. (1975). *Comp. Biochem. Physiol.* **51C,** 111–115.
52. Houssay, B. A. and Biasotti, A. (1930). *Rev. Soc. Argent. Biol.* **6,** 251–296.
53. Lyall, A. and Innes, J. A. (1935). *Lancet,* **1,** 318–319.

54. Kollind, M., Adamson, V., Lins, P., and Efendic, S. (1987). *Horm. Metab. Res.***19,** 156–159.
55. Kelley, K. M., Russell, S. M., Matteucci, M. L., and Nicoll, C.S. (1993). *Diabetes* **42,** 463–469.
56. English, D. E., Barnum, C. L., Russell, S. M., and Nicoll, C. S. (1993). *Endocr. J.* **1,** 73–78.
57. McKeown, B. A., Leatherland, J. F., and John, T. M. (1975). *Comp. Biochem. Physiol.* **B50,** 425–430.
58. Clarke, W. C. and Bern, H. A. (1980). In: *Hormonal proteins and peptides, vol. 8*, Li, C. H. (eds.) Academic: New York.
59. Press, M., Tambourlane, W. V., and Sherwin, R. S. (1984). *N. Engl. J. Med.* **315,** 810–815.
60. Press, M., Tambourlane, W. V., and Sherwin, R. S. (1988). *Metabolism* **35,** 956–959.
61. Campbell, P. J., Bolli, G. B., Cryer, P. E., and Gerich, J. E. (1985). *N. Engl. J. Med.* **312,** 1473–1479.
62. Periello, G., DeFeo, P., Torlone, E., Fanelli, C., Santeusania, F., Brunetti, P., and Bolli, G. B. (1990). *Diabetologia* **33,** 52–59.
63. Boyle, P. J., Avogaro, A., Smith, L., Shah, S. D., Cryer, P. E., and Santiago, J. V. (1992). *Diabetologia* **35,** 372–379.
64. Pal, B. R., Phillips, P. E., Matthews, D. R., and Dunger, D. B. (1992). *Diabetologia* **35,** 542–549.
65. Moller, N., Jorgensen, J. O., Moller, J., Orskov, L., Ovesen, P., Schmitz, O., Christiansen, J. S., and Orskov, H. (1995). *Metabolism* **44,** 33–36.
66. Schreck, C. B. (1993). In: *The endocrinology of growth, development, and metabolism of vertebrates*. Schreibman, M. P., Scanes, C. G., and Pang, P. K. T. (eds.) Academic: San Diego.
67. Murat, J. C., Plisetskaya, E. M., and Woo, N. Y. S. (1981). *Comp. Biochem. Physiol.* **68A,** 149–158.
68. Petersen, T. D. P., Hochachka, P. W., and Suarez, R. K. (1987). *J. Exp. Zool.* **243,** 173–180.
69. Suarez, P. K. and Mommsen, T. P. (1987). *Can. J. Zool.* **65,** 1869–1882.