Rapid Publication

The Dynamic Insulin Secretory Response of Isolated Pancreatic Islets of the Diabetic Mouse

Evidence for a Gene Dosage Effect on Insulin Secretion

JOSEPH M. MOLINA, FRANCIS H. PREMDAS, ROBERT E. KLENCK, GEOFFERY EDDLESTONE, SUSAN B. OLDHAM, AND LOREN G. LIPSON

SUMMARY

Expression of the autosomal recessive (*db*) gene in homozygous (*db*/*db*) C57BL/KsJ mice results in a severe and eventually fatal diabetic syndrome. Many studies of the diabetic mouse have used lean littermates (+/?) as controls despite evidence suggesting a gene dosage effect in heterozygous animals. In order to study the gene dosage effect of the diabetes (*db*) gene on insulin release in the heterozygote, perifusion experiments were performed on isolated islets of Langerhans of diabetic (*db*/*db*), heterozygous (+/*db*), and normal (+/+) control mice. Islets of normal controls exhibited a fivefold greater increase in insulin release than did those of diabetics in response to 16.7 mM D-glucose. The insulin secretory response of islets of heterozygotes to glucose was intermediate, being twofold greater than that of diabetics but only about half of that of normal controls. Biphasic insulin release in response to glucose was observed only in islets of normal controls. Islets of all three genotypes exhibited biphasic insulin release in response to 10 mM D-glyceraldehyde; however, overall insulin release in both heterozygotes and diabetics remained diminished as compared with the response of normal controls. This is in contrast to the situation we have previously reported in islets of fasted or aging rats in which, though manifesting defects in glucose-stimulated insulin release, the islets are able to respond normally to 10 mM D-glyceraldehyde in respect to both the dynamic secretory pattern and quantity of insulin released. Our data suggest a gene dosage effect of the (*db*) gene on glucose-stimulated insulin release in heterozygous (+/*db*) C57BL/KsJ mice. DIABETES 1984; 33:1120–23.

The diabetes (*db*) gene is an autosomal recessive gene with complete penetrance that results in obesity, hyperglycemia, polyuria, and glycosuria in homozygous (*db*/*db*) mice of the C57BL/KsJ strain. Islets of Langerhans in these diabetic mice undergo hypertrophy and hyperplasia of the beta cells until the animals are 16–20 wk of age, at which time the islets undergo a progressive loss of beta cells that correlates with falling plasma insulin levels, increasing hyperglycemia, and a course of rapid weight loss culminating in death at the age of 24–32 wk.[1] In vitro studies of insulin release from isolated islets or the perfused pancreas from these homozygous (*db*/*db*) mice have demonstrated a progressive age-related decrease in glucose-stimulated insulin release.[2,3] The effect of the (*db*) gene on the dynamics of insulin release has not been studied previously in the heterozygote.

Many studies of the diabetic (*db*/*db*) mouse have used lean littermates (+/?) as controls despite several studies that have suggested both a physiologic and metabolic gene dosage effect of the diabetes gene in heterozygous mice. Heterozygous (+/*db*) C57BL/KsJ mice have a prolonged survival when fasted as compared with normal (+/+) controls; this may be related to the heterozygote's increased ability to utilize acetone as a precursor in gluconeogenesis.[4] Male heterozygotes are significantly heavier and exhibit a mildly abnormal response to an intraperitoneal injection of glucose compared with normal controls, thus suggesting that the normal allele (+) is not completely dominant and that the presence of the diabetes (*db*) allele confers a mild form of glucose intolerance.[5] Altered insulin release from islets of heterozygotes may account in part for this impaired ability to dispose of a glucose load.

In vitro studies demonstrate an age-related impairment of glucose-stimulated insulin release in diabetic (*db*/*db*) mice in which the first phase is blunted and overall insulin release is diminished. Similarly, in islets of normal fasted or aging rats, both the first and second phases of insulin release in response to glucose are blunted. Islets of fasted or aging rats respond to D-glyceraldehyde with biphasic insulin release comparable to that of normal control rats.[6–9]

In the present study we have examined glucose- and

From the Division of Diabetes and Clinical Nutrition, Department of Medicine, University of Southern California School of Medicine, Los Angeles, California 90033.

Address reprint requests to Loren G. Lipson, M.D., Research Building 1, U.S.C. School of Medicine, 2025 Zonal Avenue, Los Angeles, California 90033.

Received for publication 27 April 1984 and in revised form 6 August 1984.

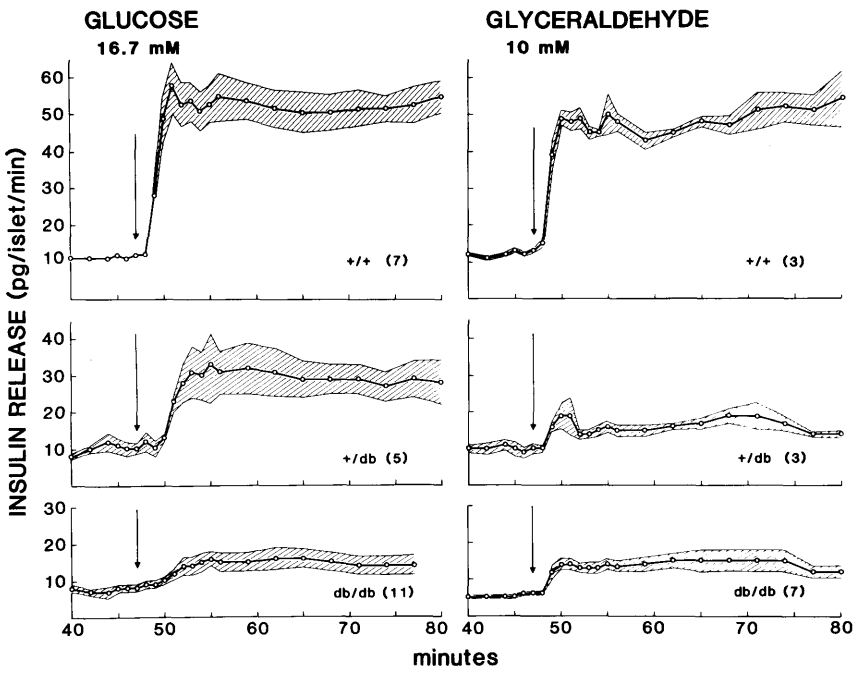

FIGURE 1. Dynamic insulin release from islets of (+/+), (+/*db*), and (*db*/*db*) mice all at 12 wk of age. The islets were perifused for 47 min with 2.8 mM D-glucose; then, the three groups of islets were stimulated for 31 min with either 16.7 mM D-glucose or 10 mM D-glyceraldehyde in the presence of 2.8 mM D-glucose. Data are shown as the mean (central line) ± SEM (shaded areas about the central line), with the number of experiments in parentheses.

glyceraldehyde-stimulated insulin release from isolated islets of Langerhans obtained from 12-wk-old male diabetic (*db*/*db*) C57BL/KsJ mice and 12-wk-old male heterozygotes (+/*db*) or normal (+/+) controls to study the effect of the diabetes gene on insulin secretion in the heterozygous mouse.

METHODS

Simultaneous experiments were performed on islets of Langerhans isolated under identical conditions from 12-wk-old male diabetic C57BL/KsJ mice (Jackson Laboratory, Bar Harbor, Maine) and from 12-wk-old male heterozygotes or normal controls. The gene for the coat color misty (*m*) is closely linked to the gene for diabetes (*db*), allowing identification of normal controls (+*m*/+*m*) as lean and gray, heterozygotes (+*m*/*db*+) as lean and black, and diabetics (*db*+/*db*+) as obese and black.[1] Hemoglobin A_{1c} levels were similar in both normal and heterozygous mice (5.5 ± 1.07%, N = 3 versus 5.05 ± 0.6%, N = 8), but hemoglobin A_{1c} levels in diabetics (8.0 ± 0.76%, N = 12) were significantly elevated when compared with those of (+*m*/+*m*) and (+*m*/*db*+) ($P < 0.001$). Normal control mice were euglycemic (87 ± 10 mg/dl). The diabetic mice were hyperglycemic (368 ± 36 mg/dl) and exhibited significant hyperinsulinemia when compared with normal controls (normal, 39 ± 7 μU/ml, N = 6; diabetics, 62 ± 8 μU/ml, N = 7; $P < 0.001$). Both normal controls and heterozygotes weighed about half as much as diabetic mice.

The mice were decapitated in the fed state, and blood was collected from the neck in heparinized tubes for subsequent determination of hemoglobin A_{1c}, plasma glucose, and plasma insulin. Islets were isolated by the collagenase digestion method of Lacy and Kostianovsky.[10] Perifusion experiments were undertaken to study dynamic insulin release from intact islets from the diabetic mice, heterozygotes, and normal control mice. Twenty-five islets were perifused at

TABLE 1
Dynamic insulin release during perifusion experiments

Genotype	Basal insulin release to 2.8 mM D-glucose (pg/islet-min ± SEM)	Insulin release above basal to 16.7 mM D-glucose (pg/islet ± SEM)	% Increase over basal release	Insulin release above basal to 2.8 mM D-glucose + 10 mM D-glyceraldehyde (pg/islet ± SEM)	% Increase over basal release
+/+	12.7 ± 0.6 (N = 10)	1291 ± 141 (N = 7)	410%	1141 ± 89 (N = 3)	281%
+/*db*	10.5 ± 1.1 (N = 8)	556 ± 116 (N = 3)	166%	195 ± 59 (N = 5)	63%
db/*db*	6.7 ± 0.7	192 ± 13 (N = 11)	82%	274 ± 44 (N = 7)	166%
+/+ vs. +/*db*	NS	$P < 0.001$		$P < 0.001$	
+/*db* vs. *db*/*db*	$P < 0.01$	$P < 0.001$		NS	
+/+ vs. *db*/*db*	$P < 0.001$	$P < 0.001$		$P < 0.001$	

Twenty-five islets were perifused simultaneously in each of four chambers. Initial basal insulin release to 2.8 mM D-glucose is calculated on a pg/islet-min basis. Insulin release to both elevated glucose and glyceraldehyde concentrations is expressed as pg/islet over the 31-min exposure to these test agents. N = number of experiments.

37°C in each of four conical chambers of 0.07-ml capacity under conditions described previously.[6] Islets from the diabetic and heterozygote or normal control mice were exposed to 2.8mM D-glucose for 47 min and then subsequently exposed to 16.7 mM D-glucose or to 10 mM D-glyceraldehyde in the continued presence of 2.8 mM D-glucose for 31 min. Insulin content of the perifusion effluent samples and plasma samples was determined by conventional radioimmunoassay.[11] Values for insulin release were calculated from the areas under the curves shown in Figure 1. Plasma glucose determinations were made by autoanalyzer, employing the glucose-oxidase method. Hemoglobin A_{1c} was determined by the method of Trivelli et al.[12] Statistical analysis was performed using Student's *t*-test for unpaired data.

RESULTS

Dynamics of glucose-stimulated insulin release. Perifusion experiments were performed simultaneously on islets from diabetic (*db*/*db*), heterozygote (+/*db*), and normal control (+/+) mice. Insulin release in response to either 16.7 mM D-glucose or to 10 mM D-glyceraldehyde in the continued presence of 2.8 mM D-glucose is shown in Figure 1.

Basal release of insulin in the presence of 2.8 mM D-glucose appeared to be slightly lower in the heterozygotes than in the normal controls (normal, 12.7 ± 0.6 pg/islet-min, N = 10; heterozygotes, 10.5 ± 1.1 pg/islet-min, N = 8), but this difference was not statistically significant ($P < 0.1$). In contrast, basal release of insulin from diabetic mice (6.7 ± 0.7 pg/islet-min, N = 18) was significantly lower when compared with either normal controls ($P < 0.001$) or heterozygotes ($P < 0.01$). Insulin release in response to 16.7 mM D-glucose from the normal controls (1291 ± 141 pg/islet, N = 7) was significantly greater than the response of the diabetics (192 ± 13 pg/islet, N = 11; $P < 0.001$). The response of the islets from the heterozygotes (556 ± 116 pg/islet, N = 3) was intermediate between normal controls and diabetic mice, being significantly less than normal controls ($P < 0.001$) but greater than the diabetic group ($P < 0.001$). When expressed as a percentage increase over basal insulin release, it can be seen (Table 1) that the response of the heterozygotes was twofold greater than that of the diabetics (166% versus 82%), and the response of the normal controls was fivefold greater (410% versus 82%). Only the islets from the normal control mice exhibited biphasic release to glucose; first-phase insulin release was severely blunted in the heterozygote and the diabetic.

Dynamics of insulin release to glyceraldehyde. When islets were exposed to 10 mM D-glyceraldehyde in the presence of 2.8 mM D-glucose, mice of all three genotypes responded with biphasic insulin release. There was considerable variation in the magnitude of the first phase in the diabetic mice, resulting in some blunting of the first phase when data from all the diabetic mice are pooled. As with D-glucose, islets from normal controls exhibited significantly greater insulin release to D-glyceraldehyde (1141 ± 89 pg/islet, N = 3) than did the heterozygotes (195 ± 59, N = 5; $P < 0.001$) or diabetics (274 ± 44 pg/islet, N = 7; $P < 0.001$). The response to 10 mM D-glyceraldehyde was similar to the response to 16.7 mM D-glucose in the normal controls, but in both the heterozygotes and the diabetics it was considerably less. Insulin release from the heterozygotes in response to D-glyceraldehyde was not significantly different from that of the diabetics. Results of these experiments are summarized in Table 1.

DISCUSSION

In vitro studies of insulin release in diabetic mice have revealed diminished glucose-stimulated insulin release with marked impairment of first-phase release that progresses with age.[2,3] This study indicates that the defect in the first phase of insulin release in response to glucose extends to mice heterozygous for the (*db*) gene as well. Overall glucose-stimulated insulin release from heterozygotes is intermediate between those of normal controls and diabetic mice. In addition, basal insulin release in the heterozygotes appeared to be intermediate between that of normal controls and of diabetics, but with the number of animals studied, the difference was not statistically significant. This study supports the hypothesis that the diabetes allele confers a mild defect in glucose-stimulated insulin release in the heterozygous state and is evidence for a gene dosage effect on insulin secretion in the C57BL/KsJ mouse. Many previous studies of the diabetic mouse have used lean littermates (+/?) as controls, but our data, along with that of others,[4,5] imply that there are physiologic and metabolic differences between heterozygotes (+/*db*) and normal (+/+) controls that should be considered when selecting control animals for experiments. It should be noted that the (+/+) misty control differs from the original C57BL/KsJ strain in that the genes for coat color and diabetes are maintained in repulsion, allowing identification of the genotype from the phenotype.[1] What effect, if any, the misty allele (*m*) has on insulin secretion has not been studied.

The heterozygous mouse appears to have an impaired ability to dispose of a glucose load acutely, but the normal hemoglobin A_{1c} values found in the animals we studied indicate that it is able to compensate in the unstressed state to maintain overall euglycemia. The inability to dispose of a glucose load in a normal fashion may be due in part to the impaired ability of the islets to respond appropriately to glucose. The blunting of the first phase of insulin release is important because of its postulated role in the pathogenesis of human type II diabetes.[13] In islets of the fasted or aging rat, the first phase of glucose-stimulated insulin release is blunted, and overall insulin secretion is diminished, which is similar to our findings in the heterozygous and diabetic mouse. In the fasted or aging rat, D-glyceraldehyde restores first-phase insulin release and elicits overall insulin secretion that is similar to that of normal controls. This supports the hypothesis that D-glyceraldehyde acts in the rat by bypassing a defect in the major rate-limiting step in stimulus-secretion coupling before the metabolism of the trioses. The finding that D-glyceraldehyde restored first-phase insulin release without restoring normal overall insulin secretion in the diabetic and heterozygous mouse was surprising because the pattern of their glucose-stimulated insulin secretory response is similar to that of islets of fasted or aging rats. This finding indicates that the diabetes gene confers a defect in both glucose- and glyceraldehyde-stimulated insulin release with a gene dosage effect. Furthermore, the defect respon-

sible for the altered glucose-stimulated insulin release in heterozygous and diabetic mice is probably different from that responsible for the altered response to glucose in the fasted or aging rat.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Southern California Affiliate of the American Diabetes Association and the Los Angeles County Medical Association Auxiliary.

REFERENCES

[1] Herberg, L., and Coleman, D. L.: Laboratory animals exhibiting obesity and diabetes syndromes. Metabolism 1977; 26:59–99.

[2] Boquist, L., Hellman, B., Lernmark, A., and Taljedal, I.: Content of adenosine 3′-5′-cyclic monophosphate in the pancreatic islets of mice with hereditary defect of insulin secretion. Biochem. Biophys. Res. Commun. 1974; 60:1391–96.

[3] Berglund, O., Frankel, B. J., and Hellman, B.: Development of the insulin secretory defect in genetically diabetic (*db/db*) mouse. Acta Endocrinol. 1978; 87:543–51.

[4] Coleman, D. L.: Acetone metabolism in mice: increased activity in mice heterozygous for obesity genes. Proc. Natl. Acad. Sci. USA 1980; 77:290–93.

[5] Chick, W. L., Lavine, R. L., and Like, A. A.: Studies in the diabetic mutant mouse. V. Glucose tolerance in mice homozygous and heterozygous for the diabetes (*db*) gene. Diabetologia 1970; 6:257–62.

[6] Lipson, L. G., Siegel, E., Wollheim, C. B., and Sharp, G. W. G.: Insulin release during fasting. Endocrinology 1979; 105:702–707.

[7] Premdas, F. H., Molina, J. M., and Lipson, L. G.: Restoration of normal dynamic insulin secretion in aging rats. Physiologist 1983; 26:A60, 32.1.

[8] Molina, J. M., Premdas, F. H., and Lipson, L. G.: Insulin release in aging: dynamic response of isolated islets of Langerhans of the rat to D-glucose and D-glyceraldehyde. In press. Endocrinology 1985.

[9] Premdas, F. H., Molina, J. M., and Lipson, L. G.: Insulin release in aging: the role of glyceraldehyde. Acta Endocrinol. 1983; 103:539–43.

[10] Lacy, P. E., and Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from rat pancreas. Diabetes 1967; 16:35–39.

[11] Herbert, V., Lan, K. S., Gottlieb, C. W., and Bleicher, S. J.: Coated charcoal immunoassay for insulin. J. Clin. Endocrinol. Metab. 1965; 25:1375–84.

[12] Trivelli, L. A., Ranney, H. M., and Lai, H. T.: Hemoglobin components in patients with diabetes mellitus. N. Engl. J. Med. 1971; 284:353–57.

[13] Pfeifer, M. A., Halter, J. B., and Porte, D.: Insulin secretion in diabetes mellitus. Am. J. Med. 1981; 70:579–88.