Insulin Secretion In Vitro and Insulin Binding to Isolated Hepatocytes in Congenic Mice with Different H-2 Complexes

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SUMMARY

Congenic male mice with differences in the H-2 complex have been used to investigate insulin secretion in vitro, insulin binding to isolated hepatocytes, plasma glucose, and serum insulin. Plasma glucose and serum insulin did not show consistent differences in the B10.BR, B10.D_2, B10.A, B10.G, B10.M, B10.S, C57/10SCSN, and C_3H.OH strains. Isolated islets of Langerhans responded to stimulation with 400 mg/dl glucose with a 3–5-fold increase in insulin secretion rates ($2P < 0.01$): B10.BR > B10.M > C57BL/10SCSN > B10.G > C_3H.OH, B10.D_2, B10.A, B10.S. The biphasic pattern of insulin secretion was less distinct in B10.M, B10.G, and C_3H.OH mice.

The high-affinity constants of insulin binding to isolated hepatocytes at 37°C varied between 4.5×10^7 L · mol^{-1} and 4.5×10^8 L · mol^{-1} ($2P < 0.01$): B10.A > B10.BR > C57BL/10SCSN, B10.S, B10.D_2 > B10.M, B10.G.

The glucose-stimulated insulin secretion from isolated islets of Langerhans and the binding of insulin to isolated hepatocytes correlate to the H-2 complex independently. DIABETES 1985; 34:256–59.

The HLA-system in man and the H-2 complex in mice have been discovered as transplantation antigens. The main histocompatibility complexes are located on chromosome 6 in man and on chromosome 17 in mice. The incidence of certain diseases has been correlated to different HLA-antigens. In type I diabetic subjects, there is an increased rate of DR-3 and DR-4 antigens, while DR-2 antigens are rare.[1-3] In mice, some metabolic properties are associated to the H-2 system, such as cAMP or testosterone levels.[4-7] Strains with defined H-2 properties on an identical genetic background have been raised in mice and are termed congenic strains. We have selected some strains with a homogenous sequence of H-2 antigens to investigate whether there is a functional relationship to the insulin secretory pattern of isolated islets of Langerhans and to the insulin binding of isolated hepatocytes. None of these strains was predisposed to develop diabetes.

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MATERIALS AND METHODS

Inbred male mice (8–10 wk old) were obtained from OLAC, Bicester, United Kingdom (B10.BR, B10.D_2, B10.A, B10.G, B10.M, B10.S, C57BL/10SCSN) and from the Max-Planck-Institute, Freiburg, FRG (B10.D_2, B10.A, C_3H.OH). C_3H.OH mice are not a congenic strain. Each lot of mice was adapted to the laboratory conditions for at least 2 wk before entering the study at an age of 11–13 wk. Obese mice were not observed; the body weights of mice used are listed in Table 1. The mice were fed with Altromin Standard Diet (Altromin, Lage, FRG), containing 23.5% (wt/wt) protein, 6% fat, 47.5% carbohydrates, 5% fiber, 6.5% minerals, and 12.5% water. Blood samples were collected from the retro-orbital sinus. Plasma glucose was measured (glucose-oxidase method) with a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, California). Insulin was estimated by RIA (Phadebas Insulin Test, Pharmacia Diagnostics, Uppsala, Sweden) using a mouse insulin standard (Novo, Copenhagen, Denmark). The DNA content of the islets was determined by fluorometry.[8] Islets of Langerhans were isolated with collagenase[9] and subsequent microdissection or by microdissection alone.[10] Twenty islets were perifused in Krebs-Ringer bicarbonate buffer with Hepes 0.02% and BSA 0.25% at 100 mg/dl and 400 mg/dl glucose (flow 0.1 ml/min). After a run-in period of 20 min, insulin secretion was measured for 20 min at 100 mg/dl glucose and subsequently for 18 min at 400 mg/dl glucose. The experimental setup allowed simultaneous runs of secretion experiments using the same buffer, gas, and temperature conditions. Secretion experiments done in parallel were compared statistically.

Hepatocytes were isolated after perifusion of the livers with Ca^{2+}-free buffer (containing 820 mg/dl NaCl, 50 mg/dl KCl,

120 mg/dl Hepes, and 18 mg/dl EGTA, pH 7.35) for 6 min (flow 1.0 ml/min). After removal of the gallbladder, the livers were cut with scissors and kept for 10 min in the same medium at room temperature. Debris was removed by a nylon sieve. The cells were washed three times at 21 × *g*. Cells (400,000–950,000) were incubated in Krebs-Ringer-Hepes buffer with 150 mg/dl glucose for 20 min at 37°C. Each tube contained 10 μU/ml mono-^{125}I-(Tyr A14) human insulin (Eli Lilly and Company, Indianapolis, Indiana) and increasing concentrations of unlabeled insulin. Insulin degradation was determined by precipitation with 12% TCA. The binding curves were analyzed according to Scatchard[11] using a computer program. The data were verified by graphic analysis. Two specific and one nonspecific binding site were assumed.

Student's *t*-test was used for statistical analysis of the binding data. Student's *t*-test for paired differences was used for parallel runs of insulin secretion experiments. Areas under the curves were calculated according to the equation:

$$\sum_{n=1}^{N-1} \frac{(x_{n+1} - x_n)(y_{n+1} + y_n)}{2}.$$

Results are expressed as mean ± SEM.

RESULTS

Body weights, plasma glucose values, and serum insulin concentrations in congenic male mice with different H-2 complexes at an age of 11–13 wk are shown in Table 1. No animals were excluded from the study because of the plasma glucose and serum insulin measurements.

Table 1. There were no significant differences between the different strains tested with respect to their plasma glucose and serum insulin levels either in the fasted state or in the fed state. Plasma glucose and serum insulin levels did not correlate. The measurements of the DNA content of the islets (Table 1) did not show significant differences between the different strains of mice, indicating that the amount of tissue per islet was comparable.

Figure 1. The basal and the glucose-stimulated insulin secretion of isolated islets of Langerhans from the congenic strains of mice with different H-2 complexes are shown in Figure 1.

There were no significant differences in the insulin secretion at 100 mg/dl glucose in the medium after a run-in period of 20 min. Isolated islets of Langerhans from all strains investigated consistently showed an increase in insulin secretion after stimulation with 400 mg/dl glucose. After an initial increase of insulin secretion, a significant decrease from 24 to 26 min (Student's *t*-test for paired differences) was observed in the B10.BR, B10.D_2, and B10.A strains. This biphasic pattern of insulin secretion was not found in the B10.M and B10.G strains of mice. Different levels of insulin secretion were obtained after stimulation with 400 mg/dl glucose from 26 to 38 min of the experiment. The stimulated insulin secretion of isolated islets was higher in B10.BR than in B10.D_2 ($2P < 0.01$) and B10.A ($2P < 0.05$). Islets from B10.M mice secreted more insulin than those of B10.A mice ($2P < 0.05$), and islets of B10.G mice secreted more than those of B10.D_2 mice ($2P < 0.05$) ($N = 8$).

A second series of insulin secretion experiments with B10.BR, B10.D_2, and B10.G mice ($N = 8$) of the same supplier yielded essentially the same results as shown in Figure 1. Additional B10.A, B10.D_2, and C_3H.OH (not congenic) mice were obtained from a different supplier ($N = 11$). These B10.A and B10.D_2 mice showed the same patterns of insulin secretion (Figure 1, panel D). Islets of Langerhans from C_3H.OH mice did not show the initial peak of insulin secretion after stimulation with 400 mg/dl glucose, similar to the results with B10.M and B10.G mice. Isolated islets were also prepared, by microdissection without using collagenase, from B10.BR and B10.D_2 mice fasted for 12 h. These islets did not show any increase of insulin secretion after stimulation with 400 mg/dl glucose. The basal insulin secretion level of B10.BR was higher than that of B10.D_2 ($2P < 0.05$, $N = 7$).

Isolated hepatocytes were prepared from individual fasted mice by Ca^{2+} depletion without using enzymatic digestion. The preparation yielded comparable amounts of hepatocytes in the congenic strains tested. Cells (400,000–950,000 per tube) were incubated for 20 min at 37°C. The insulin binding curves were analyzed according to Scatchard[11] with the assumption of two specific and one nonspecific binding site. The binding data obtained from individual mice ($N = 94$) of the different congenic strains were correlated to the number of cells per tube. There was a linear correlation between the number of cells per tube and the nonspecific binding ($r = 0.87$). The insulin degradation determined by precipitation with 12% TCA was dependent on the insulin

TABLE 1
Body weight, DNA content of isolated islets of Langerhans, plasma glucose, and serum insulin in congenic strains of mice with different H-2 complexes

Strain	H-2 complex	Body wt (g) fed (N = 14)	DNA content (ng/islet) (N = 9)	Plasma glucose (mg/dl)		Serum insulin (μU/ml)	
				Fasted (N = 14)	Fed (N = 14)	Fasted (N = 14)	Fed (N = 14)
A	H-2^a	19.6 ± 0.2	9.5 ± 1.0	67.1 ± 9.8	153.9 ± 15.0	9 ± 9	39 ± 13
BR	H-2^k	20.3 ± 0.1	13.9 ± 1.8	69.9 ± 6.5	150.4 ± 14.5	9 ± 9	39 ± 13
D_2	H-2^d	18.9 ± 0.2	12.8 ± 1.3	49.6 ± 5.8	139.8 ± 11.5	5 ± 13	24 ± 12
G	H-2^q	19.1 ± 0.3	12.8 ± 2.0	55.2 ± 13.5	138.0 ± 20.0	21 ± 9	29 ± 12
M	H-2^f	19.4 ± 0.2	10.6 ± 2.0		139.5 ± 13.3	3 ± 12	14 ± 12
S	H-2^s	18.8 ± 0.1	13.1 ± 2.9	45.4 ± 7.1	156.1 ± 25.2	9 ± 9	41 ± 13
SCSN	H-2^b	20.1 ± 0.2	15.4 ± 1.9	54.9 ± 8.9	139.3 ± 4.1	11 ± 9	46 ± 14

The DNA content was measured by fluorometry after isolation of the islets with collagenase and subsequent microdissection. Plasma glucose and serum insulin were determined at 8 a.m. with free access to food or after a 12-h fast. Mean ± SEM.

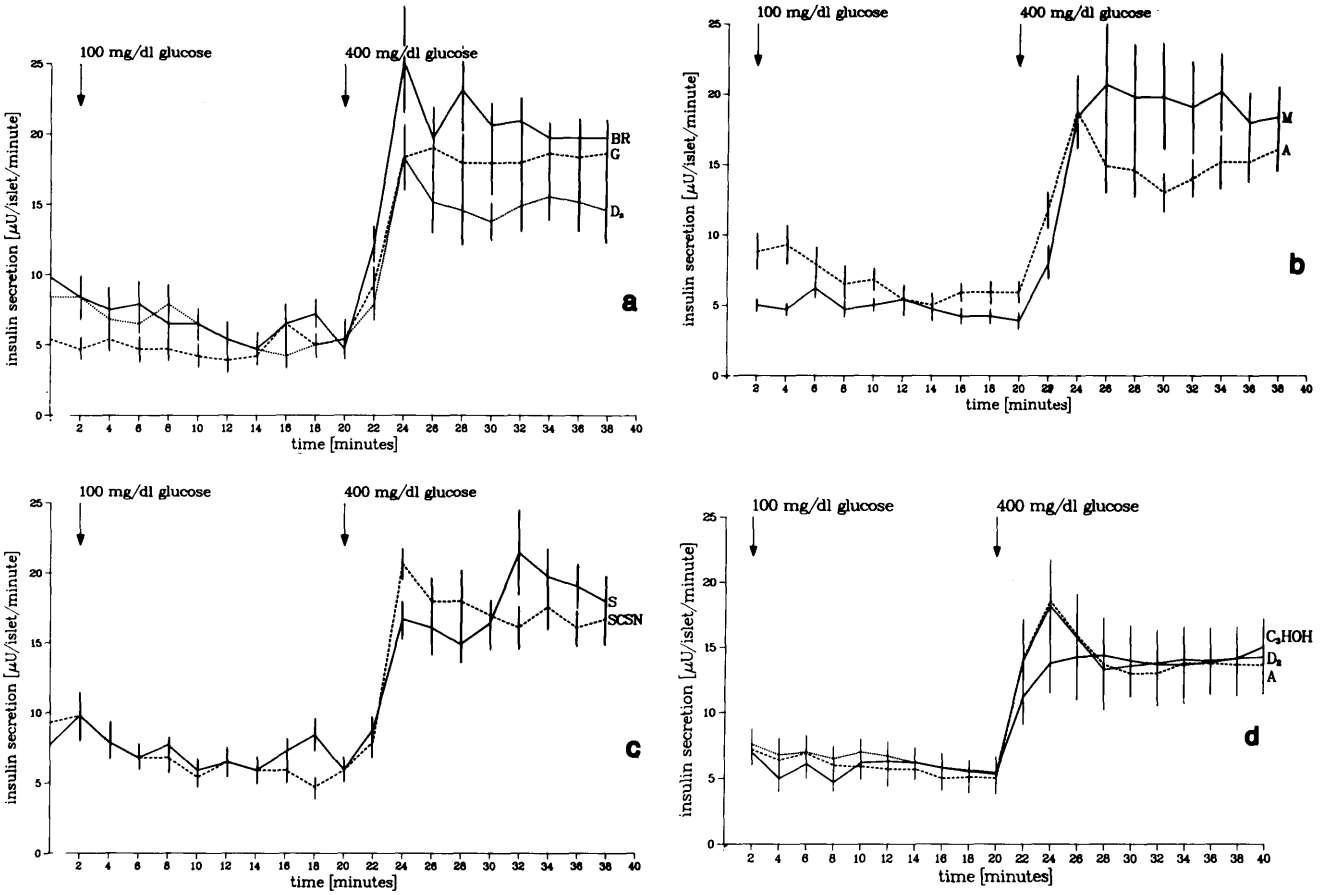

FIGURE 1. Insulin secretion in vitro at 100 mg/dl glucose (2–20 min) and 400 mg/dl glucose (20–38 min) of 20 isolated islets of Langerhans prepared from mice with free access to food and water. Panels A, B, and C show results from seven congenic strains (N = 8 each). Panel D shows secretion experiments with B10.A, B10.D_2, and C_3H.OH mice from a different supplier (N = 11). Mean ± SEM.

concentration in the incubation medium. No significant differences were found in the insulin degradation rate per hepatocyte between the different strains of mice. The binding curves were evaluated with and without taking into account the reduction of free insulin by degradation. The binding data are summarized in Table 2.

Table 2. The high-affinity binding constants varied 10-fold between the different strains of mice. This was also true for the low-affinity binding constants and for the number of high- and low-affinity binding sites per cell. In general, a high affinity corresponded to a low number of binding sites per cell. Significantly different high-affinity binding constants were obtained: B10.BR > B10.D_2 ($2P < 0.05$); B10.A > B10.D_2 ($2P < 0.01$); B10.A, B10.BR > B10.G ($2P < 0.01$); and B10.D_2, B10.S, C57BL/10SCSN (NS).

Strains with a high level of insulin secretion in vitro do not consistently have low insulin binding constants. For instance, islets from B10.BR mice released more insulin than did islets from B10.D_2 mice, and isolated hepatocytes from B10.BR mice had also a higher insulin binding affinity. Isolated islets from B10.A and B10.D_2 mice produced almost identical secretion patterns. The high-affinity insulin binding constant of

TABLE 2
Insulin binding to isolated hepatocytes from congenic strains of mice with different H-2 complexes at 37°C

Strain	H-2 complex	N	K_1 ($L \cdot mol^{-1}$)	R_1/cell	K_2 ($L \cdot mol^{-1}$)	R_2/cell
A	H-2^a	12	4.5×10^8	1.9×10^4	4.9×10^6	2.3×10^6
BR	H-2^k	22	3.4×10^8	7.7×10^4	4.4×10^6	5.1×10^6
D_2	H-2^d	17	1.6×10^8	4.1×10^4	5.1×10^6	2.1×10^6
G	H-2^q	11	4.5×10^7	4.9×10^5	2.9×10^5	2.8×10^7
M	H-2^f	5	5.1×10^7	1.1×10^6	8.7×10^5	2.2×10^7
S	H-2^s	11	1.7×10^8	1.2×10^5	6.2×10^6	3.4×10^6
SCSN	H-2^b	16	2.3×10^8	6.2×10^4	3.8×10^6	5.0×10^6

The binding data are derived from binding curves of the mean values of the measurements at different insulin concentrations in the individual experiments. Free insulin has been corrected for insulin degradation. High-affinity (K_1) and low-affinity (K_2) binding constants and receptors per cell (R_1 and R_2) are tabulated.

isolated hepatocytes was higher in the B10.A than in the B10.D_2 strains. An almost monophasic pattern of glucose-stimulated insulin secretion in vitro was observed in B10.G mice. This strain had the lowest high-affinity insulin binding constant.

DISCUSSION

Several immunologic features have been related to the H-2 complex in mice.[4,13] Metabolic investigations have been made with regard to cyclic AMP levels,[5-7] testosterone,[7] glucagon,[5,6] and insulin binding.[5]

We have investigated variations of insulin secretion and insulin binding that are compatible with a normal metabolic state. To confirm the relation of the differences to the H-2 locus, insulin secretion experiments have been repeated in the B10.BR, B10.D_2, and B10.G strains of mice.

The in vitro secretion experiments show that the pancreatic islets of mice have H-2-related metabolic properties. The mechanism that couples the glucose stimulus to the insulin secretion is not completely clear. For a better understanding, it would be useful to examine metabolic processes that are related to insulin secretion, such as oxygen consumption or calcium or glucose metabolism in congenic strains of mice.

The level of the insulin secretory response differs between mice with an identical genetic background and different H-2 complexes. On one hand, the strains exhibit different levels of insulin secretion at stimulation with 400 mg/dl glucose in the medium; on the other hand, some strains show an initial peak of insulin secretion while others do not. This shows that secretory properties of the islets of Langerhans are inherited together with H-2 properties. Changes in the insulin secretion pattern are frequently observed in type II diabetic subjects. The secretion curves seen in type II diabetes that do not have a pronounced initial insulin peak resemble these secretory patterns. In spite of the inheritance of type II diabetes, associations with the HLA system have not been found in man.

The analysis of the binding properties of hepatocytes isolated by Ca^{2+} depletion without using collagenase confirms earlier results[5] that there is a relationship between hormone binding and the H-2 complex. A discrepancy with regard to B10.A mice may be accounted for by use of a lower temperature and isolated cell membranes in the former study.[5] Studies with monoclonal anti-receptor antibodies could differentiate whether the character of the observed receptor properties is structural or functional.

Correlating the level of glucose-stimulated insulin secretion in vitro and the insulin binding properties of isolated hepatocytes, one would anticipate an inverse relationship because of the downregulation of insulin receptors.[14,15] Our results do not show an inverse relationship between the characteristics of insulin secretion and insulin binding. The data were, however, collected under conditions that are not suitable for this purpose. The mice were fasted for insulin binding studies and the insulin secretion was measured in fed animals. Second, the glucose stimulus in vitro was beyond the range of physiologic glucose concentrations in healthy mice. Differences in the secretory response in vitro were only observed with the high-glucose stimulus, but not at a glucose concentration of 100 mg/dl.

Both the insulin secretion in vitro and the insulin binding to isolated hepatocytes show significant differences in congenic strains of mice with different H-2 complexes, correlating to the H-2 complex independently. With respect to human diabetes, these data imply that different patterns of insulin secretion as well as binding characteristics are inherited that could predispose for resistance, more or less, to diabetogenic loci.

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