Interleukin 1 Pretreatment Reduces the Insulin Response to Glucose in Chronic Sucrose-Fed Rats (43463)

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Abstract. **The present studies were undertaken to determine whether interleukin 1β ([IL-1] 1.0 μg/kg, ip) pretreatment for 3 days impairs the adaptive response to sucrose feeding in rats. One week after the last IL-1 injection, when no differences in plasma glucose and serum immunoreactive insulin (IRI) levels were observed, sucrose feeding was started. Sucrose feeding for 4 weeks did not affect basal glucose levels, whereas basal IRI levels were increased in sucrose-fed rats without IL-1 pretreatment. Eight weeks later, plasma glucose levels were increased before and at 15 min after intravenous bolus of 0.5 g/kg of glucose in sucrose-fed rats with IL-1 pretreatment. Only in IL-1-treated sucrose-fed rats were basal and glucose-stimulated IRI levels significantly reduced, compared with those levels in sucrose-fed vehicle-treated rats. IL-1 decreased pancreatic IRI contents at 1 and 9 weeks after the injection. These data suggest that pancreatic damage by IL-1 attenuated insulin response to glucose stimulation after long-term sucrose feeding.** [P.S.E.B.M. 1992, Vol 200]

The abnormalities of acute insulin secretion after glucose stimulation are associated with noninsulin-dependent diabetes mellitus. In addition, the early insulin response to intravenous glucose challenge is already lost in patients with impaired glucose tolerance (1, 2). From these observations, some investigators suggested that a primary defect may begin as abnormal β-cell function with subsequent impairment of insulin secretory capacity in some patients with noninsulin-dependent diabetes mellitus (3, 4). Furthermore, it has been found that glucose-induced insulin secretion is impaired even in the very early stage of insulin-dependent diabetes mellitus, when hyperglycemia is not obvious (5, 6).

Recently, Leahy *et al.* (7) demonstrated that mild chronic hyperglycemia resulting from the reduction of β-cell mass by a 60% pancreatectomy is a critical determinant of impaired β-cell function. Interleukin 1, one of the cytokines, physiologically exists in the human body (8) and is involved in pancreatic islet damage and inflammations such as viral infection. With *in vitro* experiments, long-term exposure to recombinant human interleukin 1β (IL-1) suppressed insulin release and the proinsulin biosynthesis of pancreatic islets (9, 10). The present studies were undertaken to determine whether exogenously administered interleukin 1 impairs the adaptive response to sucrose feeding in rats.

Materials and Methods

Animals. Male Wistar rats weighing about 100 g were obtained from Imai Animal Laboratories (Saitama, Japan). The animals were housed in a temperature-controlled (23 ± 1°C) room with a 12:12-hr light:dark cycle. The animals were allowed free access to Purina laboratory chow pellets (Oriental Yeast Co., Tokyo, Japan).

Preparation of Recombinant Human IL-1. The IL-1 was kindly provided by Otsuka Pharmaceuticals, Ltd. (Tokushima, Japan). The IL-1 was expressed in *Escherichia coli* and consisted of 153 amino acids; its mol wt was 17,376. The biological activity of IL-1 is indistinguishable from cell-culture-derived material when

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measured in a standard bioassay system. The material has been judged to exhibit a purity of at least 99% based on analysis by high performance liquid chromatography and polyacrylamide gel electrophoresis (11). The endotoxin content of IL-1 preparation was below 0.1 ng/mg of protein, as measured by a limulus test. The IL-1 has been found to contain the identical amino acid sequence predicted by the cDNA sequence (12).

Experimental Protocol. The animals received an intraperitoneal injection of IL-1β (1.0 μg/kg, dissolved in saline) or saline as vehicle for 3 consecutive days. One week after IL-1 treatment, an intravenous glucose tolerance test was performed. Blood was collected in the jugular vein before and at 5 and 15 min after intravenous bolus of 0.5 g/kg body wt of glucose under pentobarbital anesthesia (40 mg/kg, ip). Plasma glucose and serum immunoreactive insulin (IRI) levels were measured. In the second experiment, half of the rats in each of IL-1- and vehicle-pretreated groups ingested 10% sucrose in place of drinking water for 8 weeks from 1 week after IL-1 pretreatment. Four and 8 weeks after the start of sucrose ingestion, an intravenous glucose tolerance test was repeated under pentobarbital anesthesia. On sacrifice before and at 8 weeks after the start of sucrose ingestion, pancreatic IRI content was measured by the method of Grodsky and Forsham (13). Plasma glucose levels were measured by automatic glucose analyzer (model 23A; YSI Yellow Spring, OH). Serum IRI levels were assayed by a radioimmunoassay kit (Amersham Japan, Tokyo, Japan).

Statistical Analysis. The data are expressed as mean ± SE. The statistical analysis was performed by analysis of variance, followed by Duncan's multiple range test for the individual comparisons of the means.

Results

In both sucrose-fed and only-chow-fed groups, IL-1 pretreatment did not affect body weight gain for 8 weeks after the start of sucrose feeding (Table I). Daily chow pellet intake was decreased in sucrose-fed rats due to an increase of caloric intake by sucrose feeding. Therefore, sucrose feeding did not affect the average daily caloric intake (Table I). In addition, IL-1 pretreatment did not change the average of daily total caloric intake in chow- and sucrose-fed animals.

As shown in Table II, IL-1 reduced pancreatic IRI contents by 63.7% of vehicle-treated rats at 1 week after the injection. But no obvious invasion of inflammatory cells was observed (our unpublished observation). The reduction of pancreatic IRI content by IL-1 remained by 8 weeks after the start of sucrose feeding. Pancreatic IRI content tended to be increased by sucrose feeding in both vehicle- and IL-1-treated groups.

Changes in plasma glucose and serum IRI levels after intravenous bolus of glucose are presented in Tables III and IV. Both plasma glucose and serum IRI levels before and after the intravenous bolus of glucose were not changed between vehicle- and IL-1-treated animals at 1 week after the start of sucrose feeding.

Vehicle-treated, chow-fed rats demonstrated a significant increase of basal IRI levels after the initial 4 weeks. Sucrose feeding for 4 weeks failed to show any changes in plasma glucose levels before and after intravenous bolus of glucose among four groups. Although basal IRI levels were increased only in sucrose-fed rats without IL-1 treatment, no obvious differences in glucose-stimulated insulin response were observed.

In contrast, sucrose feeding for 8 weeks increased basal levels of plasma glucose in sucrose-fed rats. Plasma glucose levels of sucrose-fed rats with IL-1 pretreatment (8.84 ± 0.24 mmol/liter) increased significantly compared with those of sucrose-fed rats without IL-1 (7.78 ± 0.26 mmol/liter). At 15 min after intravenous bolus of glucose, plasma glucose levels of sucrose-fed rats without IL-1 pretreatment were decreased to the same levels as chow-fed animals. However, plasma glucose levels at 15 min were significantly higher in sucrose-fed rats with IL-1 pretreatment than in the other three groups (water + vehicle group: 12.88 ± 0.43 mmol/liter; water + IL-1 group: 13.65 ± 0.83 mmol/liter; sucrose vehicle group: 12.78 ± 1.37 mmol/liter; sucrose + IL-1 group: 17.58 ± 0.44 mmol/liter). Although sucrose feeding increased basal IRI levels in the rats without IL-1 (151.7 ± 18.3 pmol/liter), IL-1 pretreatment blocked an increase of basal IRI levels by sucrose feeding (sucrose + IL-1 group: 107.9 ± 7.5 pmol/liter). At 15 min after an intravenous bolus of

Table I. Body Weight Gain and Average of Chow Pellet Intake and Total Calorie Intake[a]

	n	Body wt gain (g/8 weeks)	Chow pellet intake (g/day)	Total caloric intake (kcal/day)
Water + vehicle	7	327.7 ± 13.5	17.4 ± 0.7	101.2 ± 1.1
Water + IL-1	7	313.0 ± 14.7	18.1 ± 1.5	106.0 ± 0.7
Sucrose + vehicle	7	339.5 ± 25.7	27.5 ± 0.3[b]	105.7 ± 3.1
Sucrose + IL-1	7	358.6 ± 12.3	28.8 ± 0.2[b]	104.5 ± 2.6

[a] Data presented are for body weight gain and average chow pellet intake and total caloric intake for 8 weeks after the start of sucrose ingestion in water- or sucrose-fed rats with 1.0 μg/kg of recombinant human IL-1β pretreatment. All values are expressed as mean ± SE.
[b] $P < 0.01$ compared with water + vehicle group.

Table II. Pancreatic Immunoreactive Insulin Contents

	Time after IL-1 injection[a]	
	1 Week	9 Weeks
Water + vehicle	106.7 ± 10.8 (7)	95.4 ± 12.8 (7)
Water + IL-1	68.0 ± 8.0[b] (7)	45.6 ± 7.9[c] (7)
Sucrose + vehicle	—	134.0 ± 20.4 (7)
Sucrose + IL-1	—	70.7 ± 9.1[b] (7)

[a] Data are for pancreatic IRI contents before (1 week after 1.0 μg/kg of recombinant human IL-1β injection) and after the start of sucrose feeding for 8 weeks (9 weeks after IL-1 injection). All data are expressed as mean ± SE (ng/g wet tissue wt). Values in the parentheses represent the number of each group.
[b] $P < 0.05$ compared with the value of vehicle-treated rats.
[c] $P < 0.01$ compared with the value of vehicle-treated rats.

glucose, the serum IRI levels of sucrose-fed rats without IL-1 pretreatment were still higher than those of the other animals (water + vehicle group: 161.1 ± 16.5 pmol/liter; water + IL-1 group: 136.5 ± 14.1 pmol/liter; sucrose vehicle group: 260.5 ± 21.9 pmol/liter; sucrose + IL-1 group: 161.6 ± 18.0 pmol/liter). IL-1 pretreatment attenuated an increase in serum IRI levels by sucrose feeding.

Discussion

The present studies demonstrate that exogenous IL-1 administration for 3 consecutive days reduced pancreatic insulin content at 1 and 9 weeks after the last administration. Recently, electromicroscopic studies by Wogensen *et al.* (14) demonstrated that endothelial damage, intercellular edema, and "microvillous" β-cell surfaces, but no obvious β-cell lysis, were observed after 5 days of intraperitoneal treatment with 4.0 μg/kg/day of IL-1. In addition, our preliminary observation shows the intraperitoneal treatment with 1.0 μg/kg of IL-1 for 3 days did not cause obvious invasion of inflammatory cells in the islets at 1 week after the last IL-1 injection. These data may imply that exogenous administration of a relatively large dose of IL-1 reduces pancreatic insulin content, resulting from IL-induced inhibition of insulin biosynthesis in the islets, while no obvious β-cell lysis may be found.

On the other hand, sucrose feeding for 8 weeks tended to increase pancreatic insulin content in both the vehicle- and IL-1-treated groups, although sucrose ingestion did not affect total daily caloric intake. These results may indicate that IL-1 does not influence the ability of sucrose to stimulate insulin production.

Sucrose-fed rats without IL-1 pretreatment showed basal and glucose-stimulated hyperinsulinemia, which indicates enhanced peripheral insulin resistance. In contrast, IL-1 pretreatment completely attenuated an increase in serum IRI levels by sucrose feeding. Recently, Leahy and colleagues (7) reported similar findings after sucrose feeding for 8 weeks of 60% pancreatectomized rats: Reduced β-cell mass by 60% pancreatectomy did not affect the remaining β-cell function, and mild hyperglycemia caused by sucrose feeding for 8 weeks reduced the insulin response to glucose stimulation. Their findings are compatible with our present results. Chronic hyperglycemia impairs the β-cell function (15, 16). Mild chronic hyperglycemia caused by sucrose diets has been reported to decrease glucose tolerance in normal rats, resulting in enhanced basal and glucose-stimulated insulin secretion and increased pancreatic insulin content (17, 18). In the present experiments, an enhanced insulin response to sucrose was reduced in rats with pancreatic damage by IL-1. It appears that prolonged insulin resistance caused by sucrose feeding provided the additional stress to uncover the β-cell lesions induced by IL-1.

The data obtained herein suggest that the decrease

Table III. Changes in Plasma Glucose Levels[a]

	0 min	5 min	15 min
Before			
Vehicle	7.41 ± 0.47	17.11 ± 0.53	15.20 ± 0.86
IL-1	7.44 ± 0.24	17.02 ± 0.33	14.13 ± 0.47
4 Weeks			
Water + vehicle	7.26 ± 0.50	15.54 ± 0.46	11.84 ± 0.73
Water + IL-1	7.47 ± 0.25	16.25 ± 0.39	11.16 ± 1.00
Sucrose + vehicle	7.17 ± 0.62	15.79 ± 0.50	11.98 ± 0.98
Sucrose + IL-1	6.71 ± 0.44	15.09 ± 0.81	12.08 ± 0.36
8 Weeks			
Water + vehicle	6.80 ± 0.12	16.96 ± 0.82	12.88 ± 0.43
Water + IL-1	6.94 ± 0.28	15.86 ± 0.32	13.65 ± 0.83
Sucrose + vehicle	7.78 ± 0.26[b]	20.05 ± 0.58[b]	12.78 ± 1.37
Sucrose + IL-1	8.84 ± 0.24[b,c]	22.10 ± 0.49[b,d]	17.58 ± 0.44[b,c]

[a] Data are for changes in plasma glucose levels after intravenous bolus of 0.5 g/kg of glucose before and after the start of sucrose feeding in rats pretreated with 1.0 μg/kg of recombinant human IL-1β. All data are expressed as mean ± SE (mmol/liter).
[b] $P < 0.01$ compared with water-ingested rats.
[c] $P < 0.01$ compared with vehicle-treated rats.
[d] $P < 0.05$ compared with vehicle-treated rats.

Table IV. Changes in Serum Immunoreactive Insulin Levels[a]

	0 min	5 min	15 min
Before			
Vehicle	64.8 ± 12.2	187.9 ± 36.0	202.3 ± 45.4
IL-1	61.2 ± 6.5	255.6 ± 62.6	270.7 ± 88.6
4 Weeks			
Water + vehicle	93.6 ± 15.1	242.1 ± 15.2	177.3 ± 25.1
Water + IL-1	131.5 ± 17.3	378.3 ± 93.1	98.7 ± 26.5
Sucrose + vehicle	151.3 ± 10.7[b]	300.2 ± 41.8	223.1 ± 27.7
Sucrose + IL-1	112.5 ± 11.9[c]	332.3 ± 29.6	189.5 ± 36.9
8 Weeks			
Water + vehicle	110.8 ± 11.5	208.8 ± 42.3	161.6 ± 16.5
Water + IL-1	89.8 ± 11.7	185.0 ± 23.1	136.5 ± 14.1
Sucrose + vehicle	151.7 ± 18.3[b]	302.0 ± 50.1	260.5 ± 21.6[d]
Sucrose + IL-1	107.9 ± 7.5[c]	208.7 ± 20.1	161.6 ± 18.0[e]

[a] Data are for changes in serum immunoreactive insulin levels after intravenous bolus of 0.5 g/kg of glucose before and after the start of sucrose feeding in rats pretreated with 1.0 μg/kg of recombinant human IL-1β. All data are expressed as mean ± SE (pmol/liter).
[b] $P < 0.05$ compared with water-ingested rats.
[c] $P < 0.05$ compared with vehicle-treated rats.
[d] $P < 0.01$ compared with water-ingested rats.
[e] $P < 0.01$ compared with vehicle-treated rats.

in β-cell mass resulting from IL-1 treatment is responsible for the diminished insulin secretion after long-term sucrose feeding.

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