Secretory Protein 7B2 Response to Oral Glucose Loading and Intravenous Glucagon Injection in Patients with Diabetes Mellitus

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Secretory Protein 7B2 Response to Oral Glucose Loading and Intravenous Glucagon Injection in Patients with Diabetes Mellitus

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Summary Serum 7B2 concentrations in control subjects and patients with diabetes mellitus were measured following a 75 g oral glucose load and following intravenous glucagon infusion. In response to oral glucose, serum 7B2 levels increased in the controls (n=10) and in the diabetic patients (n=7). The increment of the serum 7B2 level was smaller in the diabetic patients than the controls. During the 75 g oral glucose tolerance test (75g OGTT), serum 7B2 levels were significantly positively correlated with serum C-peptide levels. In contrast, following intravenous glucagon infusion, serum 7B2 levels increased only in diabetic patients treated with oral hypoglycemic agents (n=20) and did not increase in controls (n=5): the group having the highest insulin secretion activity in the present study, nor in diet or insulin-treated diabetic patients. No correlation between serum 7B2 levels and serum CPR levels was observed in the intravenous glucagon infusion study. These data suggest that an extra-pancreatic source which produces the observed serum 7B2 increase following oral glucose intake can not be excluded and that 7B2 may not be secreted concomitantly with insulin from the pancreatic beta cell in response to intravenous glucagon injection.

Introduction

7B2 is a secretory protein originally isolated from porcine and human anterior pituitary glands[5),13)]. Cloning of human 7B2 cDNA revealed that this protein consists of 186 amino acids (molecular weight 20,793) and possesses an 18 or 26 amino acid signal peptide[10)]. 7B2 is found in neuroendocrine or endocrine cells of diverse tissues including the hypothalamus, pituitary glands, thyroid gland, pancreatic islet, adrenal medulla and gastrointestinal tract[7)14)]. Recent investigation indicates that 7B2 is confined in the secretory granules of pancreatic beta and alpha cells[1),2)], and that this peptide interacts with prohormone convertase PC2 to prevent with premature activation of this enzyme in the regulated secretory pathway[3)]. However, it is still unclear whether this protein is secreted concomitantly with insulin from the pancreatic beta cell. The present investigation, therefore, was designed to evaluate 7B2 secretion in response to oral glucose loading or intravenous glucagon infusion in patients with non-insulin-dependent diabetes mellitus (NIDDM) and their age-matched control subjects.

Subjects and methods

Subjects

Eighty Kyushu University Hospital patients with NIDDM or borderline diabetes mellitus (BDM) and 15 age-matched control were included in this study. NIDDM and BDM were diagnosed according to the criteria recommend-

Table 1 Clinopathologic characteristics of experimental subjects in the 75 g oral glucose tolerance test (OGTT) and the intravenous glucagon infusion test

a. 75 g OGTT	Control	Borderline diabetes mellitus	NIDDM
n	10	19	7
Age (years)	43.1±5.8	50.7±2.7	48.3±6.1
Sex (M/F)	6/4	10/9	2/5
Body mass index (kg/m²)	24.4±1.6	22.3±0.9	26.8±2.0
Fasting blood glucose (mg/dl)	68.4±3.8	80.2±4.2	117.0±11.9

b. Intravenous glucagon infusion test	Control	NIDDM		
		Diet	OHA	Insulin
n	5	21	20	13
Age (years)	60.6±7.0	49.8±3.2	56.4±2.9	55.5±2.7
Sex (M/F)	2/3	10/11	11/9	11/2
Body mass index (kg/m²)	20.4±1.9	25.2±0.3	20.9±0.8	19.7±1.1
Fasting blood glucose (mg/dl)	84.0±1.6	116.1±8.2	154.9±10.0	187.7±23.8
Age at diagnosis (years)	/	44.7±4.0	46.9±3.2	47.6±3.6
Duration (years)	/	4.8±1.5	8.9±1.9	8.9±1.8

ed by the Japan Diabetes Society[9]. All patients with associated liver cirrhosis, chronic renal failure, and hyperthyroidism or other endocrine disorders were excluded. Clinical characteristics of the experimental subjects are shown in Table 1. Ten control subjects, 19 BDM patients and 7 NIDDM patients were administered the 75 g oral glucose tolerance test (OGTT). During this protocol, peripheral venous blood was drawn from a catheter placed in the antecubital vein at 0 min, 30 min, 60 min and 120 min following oral glucose administration (Table 1, a). A separate set of subjects consisting of five non-diabetic control subjects, 21 NIDDM patients treated with diet alone, 20 NIDDM patients treated with oral hypoglycemic agents (OHA), and 13 NIDDM patients treated with insulin injections were given a 1 mg intravenous bolus of glucagon (Novo Glucagon, Novo Pharmaceutical Co. Tokyo, Japan). Peripheral blood was drawn at 0 min, 6 min and 10 min following glucagon infusion (Table 1, b).

Blood glucose concentrations were measured by a Beckman glucose analyzer 2 which utilizes a glucose oxidase method. Serum immunoreactive insulin (IRI) and serum C-peptide (CPR) were measured using commercially available kits: insulin riabead (Dainabot Radioisotope Laboratory, Tokyo, Japan) for insulin measurement[6] and a C-peptide radioimmunoassay kit (Daiichi Radioisotope Laboratory, Tokyo, Japan) for C-peptide measurement[12].

7B2 Radioimmunoassay

Serum 7B2 was measured using 7B2 radioimmunoassay as described by Iguchi, et al.[7]. In brief, a fragment of 7B2 (7B2 23-39) and 125 I-labeled 7B2 were used to provide a standard and a radioactive tracer, respectively. The antibody against 7B2 was kindly provided by

Dr. S. R. Bloom (Hammersmith Hospital, London, United Kingdom). The minimal sensitivity of this assay was 20 pg/ml, and the mean intra- and interassay coefficients of variation were 6.9% and 12.9%, respectively (n=15). All samples for 7B2 measurement were assayed in duplicate.

All data are expressed as the mean±SE. Statistical analyses were performed using the paired or non-paired Student's t-test. P values less than 0.05 were accepted as statistically significant. Correlation analyses performed by the least square method.

Results

75 g OGTT Protocol

Baseline levels of serum 7B2 did not differ significantly between NIDDM patients, BDM patients, or age-mahtced controls (Fig. 1, Table 2). However, baseline serum 7B2 levels in BDM patients tended to be higher than those in the NIDDM patients or controls. In addition, serum 7B2 concetrations in NIDDM patients tended to be lower than those in controls. In each group, serum 7B2 increased in response to the 75 g oral glucose load (Fig. 1, Table 2). The magnitude of the increment of serum 7B2 level in the 75 g OGTT did not differ significantly between the three experimental groups except at 30 min following glucose load where the increment of the serum 7B2 level from baseline was significantly smaller in the NIDDM group than in the control group ($P<0.05$)(Table 2). The serum 7B2 response in NIDDM patients was lower than it was in the other two groups. In fact, in NIDDM patients, a significant increase in

Table 2 Changes in 7B2, CPR, IRI and BG in response to an oral glucose load

		0 min	30 min	60 min	120 min
Control (n=10)	7B2 (pmol/l)	49.1±7.7	61.8±4.9 **	61.8±4.2 **	62.9±4.2 **
	CPR (ng/ml)	1.3±0.2	5.8±0.8 **	5.5±0.6 **	5.0±0.4 **
	IRI (μU/ml)	8.8±1.2	80.2±22 **	48.8±11 **	41.7±8 **
	BG (mg/dl)	69±4	111±8 **	107±6 **	88±7 **
	Δ7B2		12.7±2.5	12.4±2.4	13.8±3.8
	ΔCPR		4.5±0.7	4.3±0.5	3.6±0.4
BDM (n=19)	7B2 (pmol/l)	55.9±3.7 ns	66.6±4.0 ** ns	67.7±5.2 ** ns	72.1±4.8 ** ns
	CPR (ng/ml)	1.6±0.2 ns	5.3±0.4 ** ns	7.5±0.7 ** ns	7.8±0.6 **&&
	IRI (μU/ml)	11.3±1.5 ns	55.7±7.4 ** ns	69.0±8.5 ** ns	68.5±8.7 ** ns
	BG (mg/dl)	80±4 ns	155±8 **&&	168±11 **&&	132±8 **&&
	Δ7B2		10.7±2.4 ns	11.8±2.8 ns	16.2±2.8 ns
	ΔCPR		3.7±0.4 ns	5.9±0.7 ns	6.2±0.5 &&
NIDDM (n=7)	7B2 (pmol/l)	41.3±4.8 ns	43.7±5.5 NS &	51.1±6.7 * ns	51.8±6.7 NS ns
	CPR (ng/ml)	2.0±0.4 ns	3.4±0.6 **&	4.8±1.0 ** ns	7.1±1.5 ** ns
	IRI (μU/ml)	13.2±3.1 ns	31.2±6.9 ** ns	47.0±9.1 ** ns	82.1±25.5 * ns
	BG (mg/dl)	117±12 &&	209±25 **&&	261±21 **&&	261±19 **&&
	Δ7B2		2.4±2.6 &	9.8±3.5 ns	10.4±5.2 ns
	ΔCPR		1.4±0.4 &&	2.8±0.7 ns	5.1±1.2 ns

NS: not significant vs 0 min (paired t test)
*: $P<0.05$ vs 0 min (paired t test)
**: $P<0.01$ vs 0 min (paired t test)
ns: not significant vs Control (non-paired t test)
&: $P<0.05$ vs Control (non-paired t test)
&&: $P<0.01$ vs Control (non-paired t test)
Δ7B2=7B2 concentration at each time-7B2 concentration at 0 min
ΔCPR=CPR concentration at each time-CPR concentration at 0 min
BG: Blood glucose
BDM: Borderline diabetes mellitus
NIDDM: Non-insulin dipendent diabetes mellitus

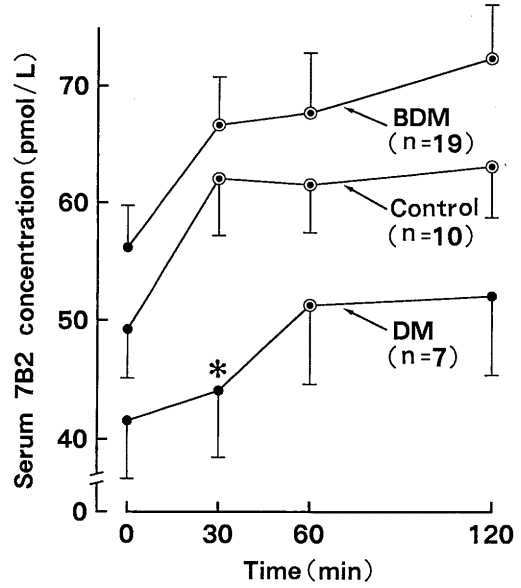

Fig. 1 Serum 7B2 response to a 75 g oral glucose load in normal subjects, patients with borderline diabetes mellitus (BDM) and patients with diabetes mellitus.
Values are given as mean±SEM.
⊙: P<0.05 vs 0 min (paired t test)
*: P<0.05 vs control (non-paired t test)

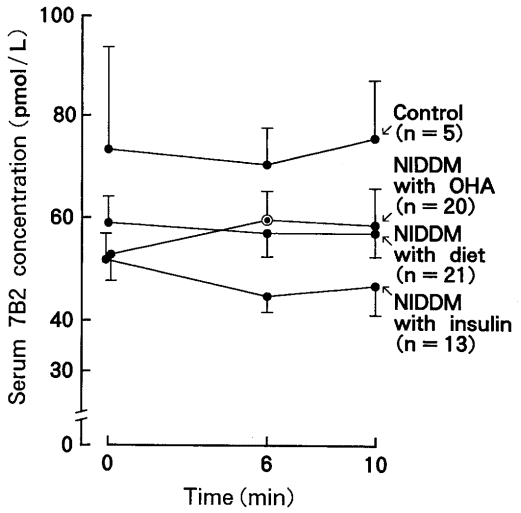

Fig. 2 Serum 7B2 response to intravenous glucagon injection in NIDDM subgroups treated with diet alone, oral hypoglycaemic agents (OHA) or insulin injection.
Values are given as mean±SEM.
⊙: P<0.05 vs 0 min (paired t test)

serum 7B2 was found only at 60 min following glucose loading (Fig. 1, Table 2).

The correlation between serum 7B2 levels and serum CPR levels following oral glucose load was examined in all subjects of the three experimental groups combined. As shown in Fig. 3, a significantly positive correlation was observed between serum 7B2 levels and serum CPR levels ($r=0.414$, $P<0.001$, $n=144$). In addition, a positive correlation was found between serum 7B2 levels and serum IRI levels in the 75 g OGTT ($r=0.197$, $P<0.05$, $n=144$).

Intravenous Glucagon Infusion Protocol

In order to determine whether increased serum 7B2 levels correlate with endogenous insulin secretion, serum 7B2 levels were measured in control subjects and NIDDM patients following intravenous glucagon infusion (Fig. 2). The increase of serum CPR levels over baseline at 6 min following glucagon infusion was significantly lower in NIDDM patients than in controls ($P<0.01$)(Table 3). Baseline serum 7B2 levels in these diabetic patients tended to be lower, but were not statistically different from those in the controls (Fig. 2, Table 3). In controls, serum 7B2 levels showed no significant change following glucagon infusion. However, in the OHA-treated NIDDM patients, serum 7B2 levels increased significantly ($P<0.01$) at 6 min after glucagon infusion (Fig. 2, Table 3). Also, the correlation between serum 7B2 levels and serum IRI or CPR levels following glucagon infusion was examined. No significant correlation was observed between serum 7B2 levels and serum CPR levels (Fig. 3) nor serum IRI levels (data not shown).

Discussion

The present study demonstrates that serum 7B2 concentrations increased in response to an oral glucose load. This response proved to be lower in the diabetic patients than in the control subjects. In addition, serum 7B2 levels significantly correlated with serum CPR levels in the 75 g OGTT. This correlation, however, does

Table 3 Responses of 7B2, CPR, IRI and blood glucose to intravenous glucagon injection

		0 min	6 min	10 min	15 min
Control (n=5)	7B2 (pmol/l)	73.5±20.5	70.7±6.2 NS	75.5±11.3NS	69.6±8.5 NS
	CPR (ng/ml)	1.2±0.3	5.1±0.8 **	5.2±0.9 **	4.8±0.9 **
	IRI (μU/ml)	7.0±1.9	70.4±14.3 **	61.5±14.4 *	44.2±12.1 *
	BG (mg/dl)	84±2	108±6 *	116±8 *	122±5 **
	Δ7B2		−2.8±4.7	2.0±4.9	−3.9±9.2
	ΔCPR		3.9±0.5	4.0±0.6	3.6±0.5
NIDDM (n=54)	7B2 (pmol/l)	54.8±3.2	54.9±2.9 NS	54.7±3.5 NS	
	CPR (ng/ml)	1.4±0.1	3.1±0.2 **	2.9±0.2 **	
	BG (mg/dl)	148±8	162±9 **	180±14 **	
	Δ7B2		0.1±2.6 ns	−0.1±3.0 ns	
	ΔCPR		1.7±0.1 &&	1.5±0.1 &&	
NIDDM with diet (n=21)	7B2 (pmol/l)	58.8±5.1	56.9±4.3 NS	56.7±4.4 NS	
	CPR (ng/ml)	1.7±0.2	3.9±0.3 **	3.6±0.3 **	
	IRI (μU/ml)	24.2±13.6	58.4±17.5 **	46.5±16.4 **	
	BG (mg/dl)	116±8	132±9 **	143±10 **	
	Δ7B2		−1.9±1.9 ns	−2.1±1.8 ns	
	ΔCPR		2.2±0.2 &&	1.9±0.2 &&	
NIDDM with OHA (n=20)	7B2 (pmol/l)	52.9±5.5	59.2±5.7 **	58.1±7.5 NS	
	CPR (ng/ml)	1.5±0.2	3.4±0.3 **	3.2±0.4 **	
	IRI (μU/ml)	7.9±1.2	32.5±3.9 **	27.7±4.1 **	
	BG (mg/dl)	155±10	168±10 **	175±8 **	
	Δ7B2		6.4±1.5 &	5.2±2.9 ns	
	ΔCPR		1.9±0.2 &&	1.8±0.2 &&	
NIDDM with insulin (n=13)	7B2 (pmol/l)	51.4±5.6	44.8±3.3 NS	46.5±5.6 NS	
	CPR (ng/ml)	0.8±1.9	1.4±0.3 **	1.3±0.3 **	
	BG (mg/dl)	188±24	202±25 **	248±50 NS	
	Δ7B2		−6.7±3.1 ns	−4.9±3.2 ns	
	ΔCPR		0.6±0.2 &&	0.5±0.2 &&	

*: P<0.05 vs 0 min
**: P<0.01 vs 0 min
NS: not significant vs 0 min
&: P<0.05 vs Normal Group
&&: P<0.01 vs Normal Group
ns: not significant vs Normal Group
Δ7B2=7B2 concentration at each time-7B2 concentration at 0 min
ΔCPR=CPR concentration at each time-CPR concentration at 0 min
BG: Blood glucose
NIDDM: All NIDDM patients
NIDDM with diet: NIDDM patients treated with diet alone
NIDDM with OHA: NIDDM patients treated with oral hypoglycemic agent
NIDDM with insulin: NIDDM patients treated with insulin

not prove co-secretion of 7B2 and insulin from pancreatic beta cells. Therefore, in order to study the possibility that serum 7B2 response represents endogenous insulin responsiveness, we measured 7B2 response to intravenous glucagon in three diabetic subgroups with different levels of insulin reserve, which could be determined by glucagon-stimulated serum CPR increases. In contrast to the findings in the 75 g OGTT, no serum 7B2 response to glucagon infusion was found in controls or diabetic patients. Moreover, there was no significant correlation between serum 7B2 levels and CPR levels in the glucagon infusion test. Only in the OHA-treated NIDDM subgroup, serum 7B2 levels increased at 6 min after glucagon injec-

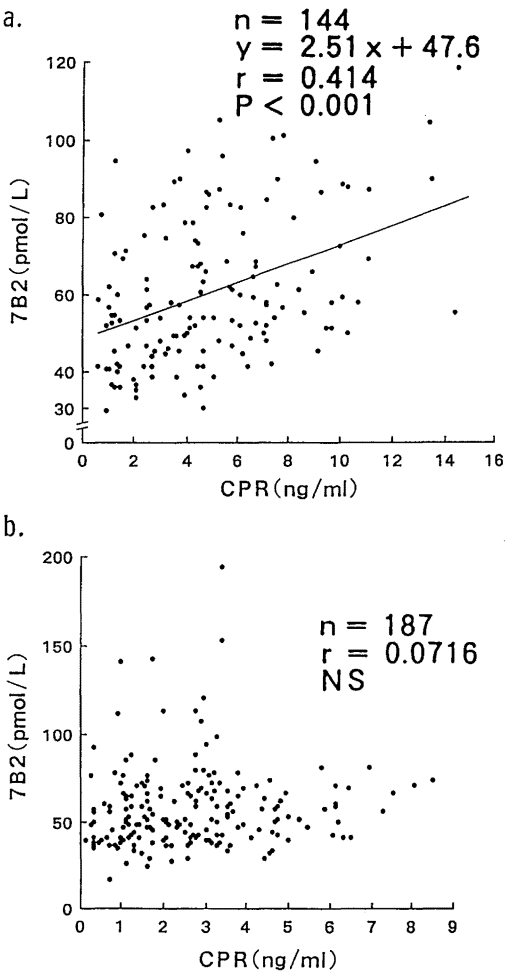

Fig. 3 Correlation analysis between serum 7B2 and serum CPR in the 75 g OGTT and the intravenous glucagon infusion test.
a : 75 g OGTT
b : intravenous glucagon infusion test

tion. The reasons for this response are unclear. Although the effect of intravenous glucagon as an insulin secretagogue is considered to be more specific and direct than that of oral glucose, the effect of glucagon on 7B2 secretion was not substantial. Although the present data could not clearly indicate the source of the oral glucose-induced 7B2 response, it may arise from extrapancreatic sites such as the pituitary glands[11),13)] or gastrointestinal tract[4)]. Alternatively, this response could be due to indirect islet cell stimulation by gut hormones released following a glucose load.

High levels of serum 7B2 has been noted in patients with insulinomas or other islet cell tumors and may be of diagnostic value[14)]. We also found that baseline serum 7B2 was elevated to 1041±1786 pmol/l in 13 patients with an islet cell tumor and that, in addition, in 3 cases with non-functioning islet cell tumor, the elevated 7B2 levels were normalized following surgical removal of the tumor[8)]. Therefore, we conclude that although the determination of serum 7B2 may have some value in the diagnosis of neoplastic pancreatic islet lesions, this has little clinical use as an indicator of insulin secretion from the pancreatic beta cell, both in healthy subjects and in patients with diabetes mellitus.

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(和文抄録)

75 g ブドウ糖負荷とグルカゴン静注負荷による血清 7 B 2 の変動

―― 正常耐糖能者，境界型糖尿病，糖尿病における検討 ――

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久富昭孝・名取省一・井口東郎・大橋昌夫

梅田文夫・名和田新

7B2 はブタおよびヒトの下垂体前葉より単離された分泌蛋白である．ヒト 7B2 はシグナルペプチドを有するアミノ酸 186 個からなる分泌蛋白（分子量 20,793）であり，視床下部，下垂体，甲状腺，膵ランゲルハンス氏島，副腎，消化管などの内分泌細胞に存在することが知られている．最近，7B2 は膵ラ氏島 α, β 細胞の分泌顆粒内に局在し，生理的役割を果たしていると考えられている．しかし，7B2 が膵ラ氏島ホルモン，とくにインスリンと同時に分泌されるか否かについては明らかではない．そこで，コントロール群（n＝10），境界型糖尿病群（n＝19），糖尿病群（n＝7）の 3 群で経口ブドウ糖負荷による 7B2 分泌を，コントロール群（n＝5），糖尿病食事療法群(n＝21)，糖尿病経口剤治療群（n＝20），糖尿病インスリン治療群（n＝13）の 4 群でグルカゴン静注負荷による 7B2 分泌を比較検討した．

経口ブドウ糖負荷により血清 7B2 は，コントロール群にて基礎値 49.1±7.7 pmol/L より 120 分後に頂値 62.9±4.2 pmol/L に増加した．糖尿病群でも同様に 7B2 の反応が認められたが，基礎値からの増加分は糖尿病群ではコントロール群に比較して低い傾向がみられた．ブドウ糖負荷時の血清 7B2 と血清 C ペプチドの相関は r＝0.414（P＜0.001）と有意であった．一方，グルカゴン静注負荷では，血清 7B2 は糖尿病経口剤治療群をのぞくすべての群で無反応であり，また，血清 7B2 と血清 C ペプチドの間に有意の相関は認められなかった．

今回の成績では，経口ブドウ糖負荷では血清 7B2 の増加反応が見られたのに対して，より直接的な膵 β 細胞分泌刺激と考えられるグルカゴン静注負荷では血清

7B2 は無反応であった．この成績からは経口ブドウ糖負荷による血清 7B2 の分泌源は不明であるが，下垂体，消化管などの膵外臓器由来の可能性あるいは経口糖負荷で分泌された何らかの消化管ホルモンによる二次的な膵 β 細胞刺激による可能性が考えられる．膵ラ氏島細胞腫瘍の症例では手術前の血清 7B2 の異常高値と術後の正常化が報告されており，血清 7B2 の診断的意義が認められているが，今回検討した正常耐糖能者や糖尿病症例では膵内分泌機能検査としての臨床的価値は少ないと考えられる．