Effect of α_2 -adrenoceptor antagonist on platelet activation during insulin-induced hypoglycaemia in Type 2 (non-insulin-dependent) diabetes mellitus

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Summary. The role of epinephrine in platelet activation and the effect of an α_2 -adrenoceptor antagonist, midaglizole, during insulin-induced hypoglycaemia in Type 2 (non-insulin-dependent) diabetes mellitus were examined. The action of midaglizole as a platelet α_2 -antagonist was confirmed by in vitro studies using platelet-rich plasma and washed platelet suspension. Hypoglycaemia was induced by a bolus injection of short-acting insulin in 24 diabetic patients. They were divided into two groups, a control group ($n=12$) and an α_2 -group ($n=12$), and midaglizole was administered orally 60 min before insulin injection in the latter. Blood glucose and plasma C-peptide levels were significantly decreased ($p<0.005$) by insulin injection in both groups. Counter-regulatory hormones, including epinephrine, and

arginine vasopressin were similarly increased at the hypoglycaemic nadir compared with the levels at 0 min in both groups. Plasma β -thromboglobulin was increased at the hypoglycaemic nadir (165.5 ± 12.6 ng/ml) compared with the level at 0 min (121.0 ± 11.5 , $p<0.005$) in the control group, whereas no significant increase was demonstrated in the α_2 -group. These results suggest that plasma epinephrine plays an important role in platelet activation during hypoglycaemia in Type 2 diabetes mellitus, and that the platelet activation is prevented by α_2 -adrenoceptor antagonist.

Key words: Adrenergic α receptor blockers, platelet aggregation, β -thromboglobulin, hypoglycaemia, diabetes mellitus, midaglizole.

It is well known that diabetic complications such as retinopathy develop more rapidly in poorly controlled diabetic patients than in well controlled subjects, and that increased platelet functions, e.g. aggregation, adhesion, thromboxane A_2 synthesis, and β -thromboglobulin (β TG) release, are usually seen in such cases [1–4]. However, retinopathy shows frequent deterioration even with intensive insulin therapy when the subjects experience recurrent hypoglycaemic attacks [5, 6]. Platelet abnormalities may be responsible for such deterioration, because the main lesions are known to be cotton-wool spots or soft exudates due to micro-infarcts in the retinal capillaries [5, 6]. This hypothesis is further supported by the presence of increased platelet aggregability [6] and plasma β TG increment [7] in Type 1 (insulin-dependent) diabetes mellitus during hypoglycaemia. Therefore, we postulated that epinephrine, which is a counter-regulatory hormone, might be the causal factor; and subsequently demonstrated that an α_2 -adrenoceptor antagonist, mianserin, normalised these abnormalities in normal subjects, al-

though this agent also has anti-serotonergic effects [8]. In the meantime, Trovati et al. [9] reported that phenolamine, which is a non-selective α -antagonist, normalised the increased platelet sensitivity to several agonists during hypoglycaemia in normal subjects. On the other hand, Grant et al. [10] have investigated the effect of arginine vasopressin (aVP) as well as epinephrine on haemostasis during hypoglycaemia in Type 1 diabetes mellitus.

Recently, a novel α_2 -selective antagonist, midaglizole (DG-5128), has been developed as an oral hypoglycaemic agent. This agent is known to be α_2 -selective on pancreatic B cells [11–14], whereas its action on platelets is not fully understood. Therefore, the present study was designed to investigate the effects of midaglizole on the synergism between near-threshold concentrations of epinephrine and other agonists in platelet aggregation in vitro [15–17], and in platelet activation during insulin-induced hypoglycaemia in Type 2 (non-insulin-dependent) diabetes mellitus in vivo.

Subjects and methods

Subjects

Venous blood collected from 8 healthy men aged 25 to 40 years was used for platelet aggregation studies on the effect of midaglizole *in vitro* using platelet-rich plasma. Twenty-four Type 2 diabetic patients, diagnosed according to the criteria of the National Diabetic Data Group [18], were subjected to *in vitro* studies using washed platelet suspension and to insulin-induced hypoglycaemic studies *in vivo*. They were divided into two groups, an α_2 -group given midaglizole treatment, and an untreated control group. The clinical characteristics of the patients are shown in Table 1. Assay methods for blood glucose, serum total cholesterol, triglycerides and HDL-cholesterol have been described elsewhere [1]. HbA_{1c} was determined by affinity chromatography (Glyc-Affin system, ISOLAB Inc., Akron, Ohio, USA). Aspirin and ticlopidine (5-(2-chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine) were withdrawn for at least one week, and other drugs which affect platelets for at least 24 h before the start of the study. Informed consent was obtained from all subjects and the study was approved by the Ethical Committee of Kumamoto University.

Reagents

Adenosine 5'-diphosphate (ADP), epinephrine and collagen were purchased from Chronolog Co. (Havertown, Penn, USA), aVP from Sigma Chemical Co. (St. Louis, Mo, USA), serotonin-creatinine sulphate from Merck (Darmstadt, FRG), and prostacyclin sodium salt from Funakoshi Pharmaceutical Co. (Tokyo, Japan). Powder and 100-mg tablets of midaglizole were kindly donated by Daiichi Pharmaceutical Co. (Tokyo, Japan). Other reagents were of analytical grade.

Platelet aggregation *in vitro* using platelet-rich plasma

The effects of midaglizole as a platelet α_2 -antagonist were examined by *in vitro* studies on platelet aggregation induced by epinephrine with or without ADP, collagen, aVP or serotonin. Methods for preparation of platelet-rich plasma and determination of platelet aggregation have been described elsewhere [8]. Briefly, platelet-rich plasma was prepared by centrifuging citrated venous blood obtained from 8 normal subjects at 150 g for 15 min at room temperature, and the platelet count was adjusted to $25\text{--}30 \times 10^4/\text{mm}^3$. An aliquot of the platelet-rich plasma (450 μl) was preincubated with 12.5 μl of midaglizole (final concentration, 2.5 $\mu\text{mol/l}$) or 0.9% sodium chloride for 5 min, then with 12.5 μl of epinephrine (0.1 $\mu\text{mol/l}$) for 1 min at 37°C in a Chronolog lumiaggregometer. Platelet aggregation was induced by incubating the platelet-rich plasma with 25- μl aliquots of various agonists. The maximum aggregation was assessed by measuring the highest peak of aggregation curve during 5 min and expressed the percentage transmission based on 0% of platelet-rich plasma and 100% of platelet-poor plasma. Prior to the study, the concentration of ADP, collagen, aVP or serotonin (0.01 $\mu\text{mol/l}$ or 0.01 $\mu\text{g/ml}$) was increased 2-fold in each successive assay until a synergistic effect with epinephrine (0.1 $\mu\text{mol/l}$) appeared, and the minimum concentration required for a synergistic effect was used as the near-threshold concentration of the aggregant. In the case of the near-threshold concentration of epinephrine, the minimum concentration which induced more than 10% platelet aggregation was selected.

The effects of insulin and midaglizole were examined by *in vitro* studies on thrombin-induced platelet activation with or without epinephrine in washed platelet suspension obtained from 8 diabetic patients. Preparation of washed platelet suspension was based on the method of Siess et al. [19, 20], with slight modification. Blood was anticoagulated with 0.15 volume of acid-citrated dextrose buffer (85 mmol/l trisodium citrate, 111 mmol/l dextrose, 71 mmol/l citric acid, pH 5.5), and platelet-rich plasma was obtained by centrifugation at 200 g for 20 min. Prostacyclin (300 ng/ml) was added to platelet-rich plasma and platelets were centrifuged for 15 min at 800 g. Platelets were resuspended in a modified Tyrode-Hepes buffer 1 (134 mmol/l NaCl, 12 mmol/l NaHCO₃, 2.9 mmol/l KCl, 0.36 mmol/l NaH₂PO₄, 1 mmol/l MgCl₂, 5 mmol/l Hepes, 5 mmol/l dextrose, 1 mmol/l EGTA, pH 7.4). The platelets were washed twice in buffer containing 300 ng/ml of prostacyclin and centrifuged for 10 min at 600 g. After centrifugation, the platelets were finally resuspended in a modified Tyrode-Hepes buffer 2 (1 mmol/l CaCl₂ in modified Tyrode-Hepes buffer 1 instead of 1 mmol/l EGTA). The platelet count was adjusted to $25\text{--}30 \times 10^4/\text{mm}^3$. An aliquot of the platelet suspension (400 μl) was preincubated with 25 μl of insulin (final concentration, 100 $\mu\text{U/ml}$) or 0.9% sodium chloride for 5 min, then with 25 μl of midaglizole (2.5 $\mu\text{mol/l}$) or 0.9% sodium chloride for 5 min, and finally with 25 μl of epinephrine (0.02 $\mu\text{mol/l}$) for 1 min at 37°C in a Chronolog lumiaggregometer. Platelet aggregation was induced by incubating the platelet suspension with 25 μl aliquots of near-threshold concentration of thrombin or epinephrine at 37°C. The maximum aggregation was assessed by measuring the highest peak of aggregation curve during 5 min which was monitored as the percentage transmission based on 0% of platelet suspension and 100% of modified Tyrode-Hepes buffer 2. After monitoring the platelet aggregation, the reaction mixture was transferred to another tube containing theophylline (3 mmol/l), EDTA (9 mmol/l) and indomethacin (20 $\mu\text{g/ml}$), gently mixed, and cooled down to 4°C in an ice bath. Concentrations of βTG and thromboxane B₂ (TXB₂) in the supernatant were determined as described elsewhere [8].

Platelet activation *in vitro* using washed platelet suspension

Insulin-induced hypoglycaemia

Insulin-induced hypoglycaemia

The *in vivo* effects of midaglizole on the plasma levels of βTG , counter-regulatory hormones, and various other substances during insulin-induced hypoglycaemia were studied in 24 Type 2 diabetic

Table 1. Clinical data for the 24 diabetic patients

	Control group	α_2 -group
Numbers		
Total	12	12
Male/female	5/7	6/6
Insulin/drug/diet	4/4/4	4/5/3
Age (years)	54.6 $\pm$ 3.9	63.3 $\pm$ 3.5
Duration (years)	7.7 $\pm$ 2.2	8.2 $\pm$ 2.7
Body mass index (kg/m ²)	22.8 $\pm$ 1.3	21.9 $\pm$ 1.5
Fasting blood glucose (mmol/l)	10.0 $\pm$ 0.9	9.4 $\pm$ 0.9
HbA _{1c} (%)	12.0 $\pm$ 1.1	13.1 $\pm$ 1.0
Blood pressure (mmHg)		
Systolic	132.3 $\pm$ 6.0	129.2 $\pm$ 5.8
Diastolic	73.7 $\pm$ 2.5	72.0 $\pm$ 2.7
Total cholesterol (mg/dl)	206.3 $\pm$ 11.2	205.1 $\pm$ 14.4
Triglycerides (mg/dl)	126.7 $\pm$ 12.3	117.3 $\pm$ 10.4
HDL-cholesterol (mg/dl)	50.4 $\pm$ 3.5	48.6 $\pm$ 3.8

Values are means $\pm$ SEM. α_2 -group: group treated with α_2 -adrenoceptor antagonist

Fig. 1. Effects of α_2 -adrenoceptor blocker on blood glucose and serum C-peptide during insulin-induced hypoglycaemia in Type 2 (non-insulin-dependent) diabetes mellitus. Open circles (O) indicate the values obtained without α_2 -blocker, and closed circles (●) those with α_2 -blocker. Values are means $\pm$ SEM. H, values at hypoglycaemic nadir. * $p < 0.005$, difference from the value at 0 min

patients. After an overnight fast and a 60-min rest period, twelve patients in the control group were administered a bolus injection of short-acting insulin (Actrapid Human, Novo, Copenhagen, Denmark). An amount (0.1 U/kg) of insulin was injected for the patient with 4.5–5.5 mmol/l of fasting blood glucose level, and the amount of insulin was successively increased to 0.15 U/kg for 5.6–8.3 mmol/l and to 0.20 U/kg for 8.4–11.1 mmol/l. Venous blood samples were drawn from the antecubital vein before the insulin injection and at 20, 40, 60 and 120 min after injection. The same experiment was carried out for the α_2 -group after they had taken midaglizole (300 mg orally) 60 min before the insulin injection. The mean values of injected insulin doses were 9.6 ± 1.0 U/subject in the control group and 10.0 ± 1.4 U/subject in the α_2 -group. Blood glucose, plasma C-peptide, β TG, TXB₂, and counter-regulatory hormones such as catecholamines, glucagon, growth hormone and cortisol were measured as described previously [8]. Plasma glucagon, growth hormone, cortisol and aVP were measured in nine patients in each group. Plasma aVP was assayed by radioimmunoassay using [¹²⁵I]-aVP after extraction based on the method of Ysewijn-Van Brussel et al. [21]. Briefly, a 1-ml aliquot of plasma was acidified with 1 ml of 0.1 N hydrochloride, applied to a Sep-pak C₁₈ column, washed with 10 ml of 4% acetic acid, extracted with 1.5 ml of methanol, dried under a nitrogen stream, and redissolved in 1 ml of phosphate buffer. The extraction level using this method was $89.2 \pm 3.9\%$ ($n = 5$), and the coefficient of variance was 5.6% ($n = 15$). The plasma values were stable for up to 60 days when stored at -80°C .

Table 2. Inhibition by midaglizole of the potentiating effect of epinephrine on the platelet aggregation induced by near-threshold concentrations of various agonists in platelet-rich plasma obtained from normal subjects

Epinephrine ($\mu\text{mol/l}$)	Agonists (concentration)	<i>n</i>	Change in light transmission (%)		Inhibition (%)
			Control	Midaglizole	
0.31 ± 0.07	None	8	20.4 ± 4.8	8.4 ± 1.2^a	53.0 ± 4.7
0.1	ADP (0.88 ± 0.20)	8	46.6 ± 8.8	11.3 ± 1.8^b	69.7 ± 7.1
0.1	Collagen (0.44 ± 0.13)	8	51.9 ± 7.9	15.9 ± 6.3^c	74.0 ± 7.0
0.1	aVP (0.03 ± 0.01)	6	13.5 ± 1.1	4.6 ± 1.7^b	66.0 ± 12.9
0.1	Serotonin (10.0 ± 0.00)	6	28.3 ± 12.2	21.4 ± 8.7	14.1 ± 8.1

Values are means $\pm$ SEM. Midaglizole concentration, 2.5 $\mu\text{mol/l}$. aVP, arginine vasopressin. Units of aggregants: ADP = $\mu\text{mol/l}$; Collagen = $\mu\text{g/ml}$; aVP = $\mu\text{mol/l}$; Serotonin = $\mu\text{mol/l}$. ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, differences from the control group

Table 3. Effects of insulin and midaglizole on platelet activation induced by near-threshold concentration of thrombin with or without epinephrine in washed platelet suspension obtained from diabetic patients

Agonists (concentration)	Addition	TXB ₂ (ng/ 10^9 P)	β TG ($\mu\text{g}/10^9$ P)	Aggregation (%)
Thrombin (23.1 ± 3.9 U/l)	0.9% NaCl (control)	90.5 ± 30.6	5.81 ± 0.59	68.5 ± 6.5
	Insulin (100 $\mu\text{U/ml}$)	72.5 ± 25.5^a	6.28 ± 1.10	62.5 ± 7.7^a
	Midaglizole (2.5 $\mu\text{mol/l}$)	54.2 ± 21.3	4.99 ± 0.51^b	62.1 ± 7.8^a
Thrombin (11.9 ± 1.3 U/l) + Epinephrine (0.02 $\mu\text{mol/l}$)	0.9% NaCl (control)	36.4 ± 10.2	4.15 ± 0.73	51.1 ± 5.8
	Insulin (100 $\mu\text{U/ml}$)	32.5 ± 10.1	3.74 ± 0.61	44.6 ± 6.7
	Midaglizole (2.5 $\mu\text{mol/l}$)	29.9 ± 9.5^a	2.95 ± 0.67^b	30.5 ± 6.5^c

Values are means $\pm$ SEM ($n = 8$). 10^9 P, 10^9 platelets. ^a $p < 0.05$, ^b $p < 0.01$, differences from the controls. ^c $p < 0.01$, difference between insulin and midaglizole treatments

Fig. 2. Effects of α_2 -blocker on various counter-regulatory hormones and arginine vasopressin during insulin-induced hypoglycaemia. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, differences from the value at 0 min

Statistical analysis

All results are expressed as means $\pm$ SEM. Statistical analysis was performed using the paired Student's t-test for the *in vitro* experiments (Tables 2–3), and the Wilcoxon's rank sum test for the *in vivo* experiments (Figs. 1–3).

Results

Platelet aggregation *in vitro* using platelet-rich plasma

The effects of midaglizole as a platelet α_2 -antagonist on platelet aggregation induced by epinephrine with or without ADP, collagen, aVP or serotonin in platelet-rich plasma obtained from 8 normal subjects are shown in Table 2. This agent significantly suppressed platelet aggregation induced by near-threshold concentrations of epinephrine ($0.31 \pm 0.07 \mu\text{mol/l}$) in comparison with the control subjects. The concentration of midaglizole ($2.5 \mu\text{mol/l}$) was as low as that seen in human blood for 2 to 3 h following oral administration [14]. Furthermore, the agent strongly suppressed the synergistic effect of epinephrine ($0.1 \mu\text{mol/l}$) with near-threshold concentrations of ADP ($0.88 \pm 0.20 \mu\text{mol/l}$), collagen ($0.44 \pm 0.13 \mu\text{g/ml}$) or aVP ($0.03 \pm 0.01 \mu\text{mol/l}$). However, midaglizole failed to inhibit platelet aggregation induced by serotonin with epinephrine. Platelet aggregation induced by ADP ($2\text{--}10 \mu\text{mol/l}$), collagen ($2\text{--}10 \mu\text{g/ml}$) aVP ($0.2\text{--}1.0 \mu\text{mol/l}$) or serotonin ($1\text{--}20 \mu\text{mol/l}$) alone was not affected by midaglizole ($2\text{--}10 \mu\text{mol/l}$). In addition,

Fig. 3. Effects of α_2 -blocker on plasma β -thromboglobulin during insulin-induced hypoglycaemia. * $p < 0.005$

the same concentrations of aVP or serotonin alone induced only a weak reversible aggregation less than 10%.

Platelet activation *in vitro* using washed platelet suspension

The effects of insulin and midaglizole on platelet activation induced by near-threshold concentration of thrombin with or without epinephrine in washed platelet suspension obtained from 8 diabetic patients are shown in Table 3. Insulin ($100 \mu\text{U/ml}$) slightly but significantly ($p < 0.05$) suppressed TXB_2 production

and platelet aggregation induced by thrombin alone (23.1 ± 3.9 U/l), whereas it did not affect the β TG release. However, midaglizole ($2.5 \mu\text{mol/l}$) slightly but significantly ($p < 0.01$) suppressed the β TG release as well as the platelet aggregation without significant effect on TXB_2 production. On the other hand, the TXB_2 synthesis, β TG release and platelet aggregation induced by synergistic effect of epinephrine ($0.02 \mu\text{mol/l}$) with thrombin (11.9 ± 1.3 U/l) were not significantly suppressed by insulin, whereas these were significantly inhibited by midaglizole ($p < 0.05$).

Insulin-induced hypoglycaemia

The time courses of blood glucose and plasma C-peptide after insulin injection are shown in Figure 1. The blood glucose level before insulin injection (7.93 ± 0.90 mmol/l) was decreased to 2.68 ± 0.34 mmol/l at the hypoglycaemic nadir and to 4.21 ± 0.40 mmol/l at 120 min in the control group. Similar results were obtained in the α_2 -group. The hypoglycaemic nadir occurred at 56.7 ± 9.8 min in the control group and at 68.3 ± 11.4 min in the α_2 -group. The plasma C-peptide level was decreased from 0.46 ± 0.09 nmol/l at 0 min to 0.29 ± 0.07 at the hypoglycaemic nadir in the control group, and from 0.87 ± 0.17 nmol/l to 0.61 ± 0.10 in the α_2 -group. In addition, the blood glucose and plasma C-peptide levels before midaglizole administration in the α_2 -group were 8.1 ± 0.2 mmol/l ($n = 12$) and 0.60 ± 0.13 nmol/l ($n = 8$), respectively, which were not significantly different from those in the control groups.

Responses of various counter-regulatory hormones and aVP during insulin-induced hypoglycaemia are shown in Figure 2. Plasma epinephrine was increased from 0.23 ± 0.03 nmol/l at 0 min to 4.28 ± 1.32 nmol/l at the hypoglycaemic nadir, and it was still higher at 120 min (2.48 ± 0.91) than the level at 0 min in the control group. The α_2 -group demonstrated almost identical responses to those in the control group. Plasma norepinephrine, glucagon, growth hormone and cortisol were also increased at the hypoglycaemic nadir and/or at 120 min in both groups, the responses being quite similar. The levels of plasma aVP, as well as those of the various counter-regulatory hormones, were increased at the hypoglycaemic nadir in both groups. However, the aVP level at the hypoglycaemic nadir was about 1,000-fold lower than the epinephrine level in the α_2 -group as well as the control group (3.27 ± 0.97 pmol/l vs 3.14 ± 0.64 nmol/l).

As shown in Figure 3, the plasma β TG level in the control group was increased from 121.0 ± 11.5 ng/ml at 0 min to 165.5 ± 12.6 ng/ml at the hypoglycaemic nadir. However, in the α_2 -group, the level at the hypoglycaemic nadir (105.7 ± 13.1) as well as that at 120 min demonstrated no significant difference compared with that at 0 min (99.0 ± 9.6). On the other

hand, plasma TXB_2 levels demonstrated no significant change in either group (data not shown). When these groups were each divided into two subgroups with or without retinopathy, neuropathy or nephropathy, there was no significant difference in these parameters between the subgroups.

Discussion

The present study demonstrated that plasma β TG is increased during insulin-induced hypoglycaemia in Type 2 diabetes mellitus and that this increment was almost abolished by administration of an α_2 -adrenoceptor antagonist, midaglizole, which did not affect any response of counter-regulatory hormones including epinephrine. These results indicate that plasma epinephrine plays an important role in platelet activation during hypoglycaemia in Type 2 diabetes mellitus. The importance of plasma epinephrine in platelet activation was also demonstrated by its synergistic action with other agonists *in vitro*.

Diabetic retinopathy sometimes shows deterioration as a result of recurrent hypoglycaemic attacks during intensive insulin therapy, the main lesions being cotton-wool spots or soft exudates due to microinfarcts in the retinal capillaries [5, 6]. Therefore, activation of platelets during hypoglycaemia may be responsible for the resulting deterioration of diabetic retinopathy. Disorders of the retinal capillaries, such as decreased prostacyclin synthesis or other kinds of endothelial cell damage, basement membrane thickening, etc., due to deposition of sorbitol [22], glycosylated collagen or albumin [23] may be necessary factors in the basic microangiopathy, because such forms of diabetic retinopathy are rarely seen in patients with non-diabetic atherosclerotic diseases in spite of increased platelet function.

Midaglizole seems to be a potent α_2 -selective antagonist from the results reported by some investigators [11–14] and the present data. Recently, the clinical importance of the synergistic action of epinephrine with other agonists, e.g., ADP, collagen, arachidonic acid, thrombin and platelet activating factor, etc., has received considerable attention [9, 15–17]. Such synergism is usually produced by very low concentrations of epinephrine (0.05 to $0.5 \mu\text{mol/l}$) and other agonists (0.1 to $1.0 \mu\text{mol/l}$). Trovati et al. [9], in normal subjects, demonstrated potentiation of platelet sensitivity to ADP, thrombin or platelet-activating factor by quite low concentrations of epinephrine (approximately 2.7 nmol/l), which are observed in the human blood stream during hypoglycaemia. These results suggest that the plasma epinephrine concentration during hypoglycaemia may be sufficient to stimulate platelets through its synergistic effects with other agonists. Lanza et al. [17] demonstrated that non-selective α -antagonist suppressed such synergism between epine-

phrine and other agonists in vitro. The present data further clarified that α_2 -antagonist is effective in platelet aggregation induced not only by epinephrine alone, but also by epinephrine plus other agonists.

The plasma C-peptide levels in the α_2 -group were relatively higher throughout the in vivo experiment than those in the control group. The difference is probably due to the effect of midaglizole on pancreatic B cells, because the plasma C-peptide levels before midaglizole administration in the α_2 -group demonstrated no difference with the control group. Recently, Hiramatsu et al. [24] reported that platelet aggregation induced by ADP, epinephrine or collagen ex vivo was significantly improved by an euglycaemic insulin clamp with 100 μ U/ml for 4 h. Therefore, the question may be raised that the high plasma insulin level may be responsible for the plasma β TG suppression in the α_2 -group. However, in the present study in vitro, insulin (100 μ U/ml) did not significantly suppress β TG release in washed platelet suspension stimulated by thrombin with or without epinephrine. Secondly, in the cross-over experiments in 6 patients from the two subgroups, three patients with high plasma C-peptide levels (0.96–2.35 nmol/l) and three patients with the low C-peptide levels (0.06–0.50 nmol/l) demonstrated similar β TG response at the hypoglycaemic nadir in the study with or without α_2 -antagonist. In addition, the plasma insulin levels in the present hypoglycaemic study estimated from the C-peptide levels are very low as compared with those produced by high doses of insulin injection, and there was no difference in the injected insulin doses between the control group and α_2 -group. These data suggest that the plasma β TG suppression in the α_2 -group might be the effect of the α_2 -antagonist on platelets, although the precise mechanisms of insulin action on the β TG release remained to be clarified.

The role of aVP, without epinephrine, in haemostasis during hypoglycaemia has been reported by Grant et al. [10]. However, plasma aVP level at the hypoglycaemic nadir was only about 1/1,000 of the epinephrine level in both groups. The present studies on platelet aggregation in vitro demonstrated that aVP is not as potent as epinephrine, and that its synergistic effect with epinephrine was strongly suppressed by α_2 -antagonist. These results suggest that aVP plays quite a minor role in platelet activation during hypoglycaemia. On the other hand, further investigations may be necessary in order to interpret the plasma TXB₂ responses observed in the present study.

Although there are some reports describing impaired epinephrine responses [25, 26] and decreased numbers of platelet α_2 -receptors [27, 28] in diabetes mellitus with severe autonomic neuropathy, the present data do not suggest the presence of such phenomena. The reason for this may be that none of the diabetic patients studied had severe neuropathy. This study also revealed that platelet responses to both

epinephrine and α_2 -antagonist do not differ in subgroups with or without either retinopathy or nephropathy. However, the possibility of different responses in terms of the severity of diabetic microangiopathy was not fully elucidated here.

In conclusion, the present data suggest that plasma epinephrine plays an important role in platelet activation during hypoglycaemia in Type 2 diabetes mellitus, and that platelet abnormality is prevented by α_2 -adrenoceptor antagonist. These results further suggest that deterioration of diabetic retinopathy due to hypoglycaemia might be prevented by midaglizole administration. Further studies are currently in progress to evaluate the clinical usefulness of midaglizole for diabetic patients.

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