Br J clin Pharmac 1994; **38**: 427–432

Reduced sensitivity to β-adrenoceptor stimulation and blockade in insulin dependent diabetic patients with hypoglycaemia unawareness

T. S. TROVIK[1], R. JAEGER[1], R. JORDE[2] & G. SAGER[1]
[1]Department of Pharmacology, Institute of Medical Biology, University of Tromsø, N-9037 Tromsø and [2]Medical Department, University Hospital of Tromsø, N-9038 Tromsø, Norway

1 Nine IDDM-patients with hypoglycaemia unawareness, seven IDDM-patients with hypoglycemia awareness and a control group of nine healthy persons were included in this study. The patients were recruited from the medical out-patients' department of the University Hospital of Tromsø.

2 The pathophysiological changes which cause hypoglycaemia unawareness are today not clear. Reduced peripheral tissue sensitivity to catecholamines is suggested as one of several mechanisms which may contribute.

3 For further investigation of β-adrenergic sensitivity an isoprenaline/metoprolol sensitivity test was performed. Isoprenaline and metoprolol were administered intravenously, and the effects on heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and plasma levels of adrenaline (ADR) and noradrenaline (NA) were measured. All subjects were given the same doses of isoprenaline (0.25–8 μg) and metoprolol (0.5–8 mg). Metoprolol was given together with the dose of isoprenaline which increased heart rate by 25 beats min^{-1}.

4 The dose/response curves of both isoprenaline/HR and metoprolol/HR were significantly shifted to the right in IDDM-patients with hypoglycaemia unawareness compared with controls and IDDM-patients with hypoglycaemia awareness ($P < 0.05$).

5 Reduced sensitivity of isoprenaline stimulation has also been shown before, whereas reduced sensitivity of a blocking agent has not earlier been shown.

6 These findings support the hypothesis of reduced β-adrenergic sensitivity as one pathophysiological component in hypoglycaemia unawareness.

Keywords IDDM hypoglycaemia unawareness β-adrenergic sensitivity isoprenaline sensitivity test metoprolol sensitivity test

Introduction

Studies of hypoglycaemia unawareness report frequencies of the condition varying from 8 to 70%. In a review of 15 studies by Gerich *et al.* [1] the weighted average prevalence of hypoglycaemia unawareness in populations of diabetic patients was estimated to be 25%. The term hypoglycaemia unawareness refers to a condition with absence of autonomic warning symptoms or lack of ability to recognize them, before neuroglycopenia develops. The autonomic warning symptoms, such as sweating, tremor, palpitations, hunger and anxiety, are usually the first to be elicited when the blood glucose level decreases. They are aroused by increased release of adrenaline, noradrenaline and acetylcholine, and most often they appear at a blood glucose level of about 3.4 mM. When blood glucose concentration falls below 2.8 mM, neurologic symptoms appear [2, 3]. No single pathophysiological mechanism can explain the condition of hypoglycaemia unawareness. The aim of this study has been to investigate the changes in peripheral tissue sensi-

Correspondence: Dr Thor S. Trovik, Department of Pharmacology, Institute of Medical Biology, University of Tromsø, N-9037 Tromsø, Norway

tivity to β-adrenergic agents as one possible mechanism which may contribute to hypoglycaemia unawareness. Other authors have shown that IDDM-patients with hypoglycaemia unawareness have a reduced sensitivity of β-adrenoceptor stimulators [4, 5]. In this study we have also performed sensitivity tests of the β_1-adrenergic receptor blocker metoprolol.

Methods

Subjects

The study was performed on three groups of age and sex matched test persons: 1) The control group which comprised nine healthy volunteers, five females and four males, mean age 29.1 years (age range 23–40 years). 2) Seven IDDM-patients with hypoglycaemia awareness, four males and three females, mean age 30.1 years (age range 23–42 years). 3) Nine IDDM-patients with hypoglycaemia unawareness, three females and six males, mean age 31.4 years (age range 21–41). The patients were selected among IDDM-patients asked by a questionnaire about the frequency and severity of their hypoglycaemic episodes, their symptoms and need of assistance during hypoglycaemia. The duration of disease was significantly longer ($P < 0.01$) in the unaware-group (mean 18 years, range 7–30 years) than in the aware-group (mean 5 years, range $\frac{1}{2}$–12 years). All patients were on multiinjection insulin treatment. The test persons gave a written informed consent to take part in the study. The study was approved by the Ethics Committee of the Norwegian Health Region V.

Protocol

The isoprenaline/metoprolol sensitivity test was carried out in a quiet room between 09.00 and 13.00 h. It has been found that the period of day in which the isoprenaline sensitivity test is performed does not influence the result of the test [6]. There was no extraordinary dietary restrictions or intervention in insulin therapy.

The isoprenaline sensitivity test was performed when the test person had rested in bed for 30 min. Bolus injections of isoprenaline (isoprenaline 0.2 mg ml^{-1}, Hydro Pharma, Oslo, Norway) were given intravenously in a forearm vein through a three-way valve. Blood samples were obtained from arterialized venous blood in the other forearm through another three-way valve. Both the intravenous catheter for injections and the intravenous catheter for blood sampling were continuously perfused at a low rate with 0.9% NaCl during the test. The injection valve and intravenous catheter were flushed with 5 ml 0.9% NaCl after each injection. From each blood sample the first 5 ml were discarded.

The starting isoprenaline dose was 0.25 μg according to George *et al* [6]. This was doubled until heart rate had increased at least 30 beats min^{-1} above the basal level. The maximum isoprenaline dose given was 8 μg. The next isoprenaline injection was given 2–3 min after the heart rate had returned to its basal level. This procedure gave an injection interval of 5–10 min.

The metoprolol sensitivity test was performed after the isoprenaline sensitivity test. Together with the dose of isoprenaline which increased heart rate with 25 beats min^{-1} (TEST I_{25}), increasing doses of metoprolol (Seloken® 1 mg ml^{-1}, Hässle, Mölndal, Sweden) were injected. Starting dose of metoprolol was 0.5 mg and this was doubled until heart rate no longer increased above the basal level. Maximum metoprolol dose was 8 mg. The isoprenaline dose was kept constant during the metoprolol sensitivity test. Methods of injections and blood sampling were identical with the isoprenaline sensitivity test.

Heart rate was registered with an instantaneous rate meter (Propaq® 104EL, Protocol® systems, INC., Beaverton, Oregon 97006 USA). The same apparatus was used on all test persons. Blood pressures were measured (Propaq® 104EL) and blood samples were obtained before start of the test and on peak isoprenaline effect after each isoprenaline or isoprenaline/metoprolol injection (50–70 s after injection).

Catecholamine and isoprenaline concentrations

The following chemicals were employed: (±)-Adrenaline bitartrate, noradrenaline bitartrate, (±)-isoprenaline hydrochloride and (±)-dihydroxybenzylamine hydrobromide from Sigma Chemical Corp., St. Louis, MO, USA. All other chemicals were of analytical grade. Blood with heparin (4 iu ml^{-1}), reduced glutathione (4.5 nM) and EGTA (5 mM) was kept on ice before centrifugation at 1.000 *g* for 20 min at 4° C. The samples were stored at −20° C awaiting analysis. Standards and samples were prepared as described previously [7]. Plasma concentrations of catecholamines and isoprenaline were measured by h.p.l.c.

The high performance liquid chromatography setup of catecholamines and isoprenaline analysis included: 590 Solvent delivery module, U6K injector, 460 electrochemical detector and M740 data module (Millipore Corp., Waters Chromatography Division, Milford, MA, USA). A Clin-Rep® column and eluent (Pharma Vertriebs GmbH & Co KG, Munich, FRG) were employed for the chromatography performed at ambient temperature. Eluent flow was 1.0 ml min^{-1}.

Metoprolol concentrations

The following chemicals were employed: Metoprolol and H93/47 (AB Hässle, Mölndal, Sweden), dichloromethane and diethyl ether (E. Meck, Darmstadt, FRG), triethylamine and acetonitrile of HPLC-grade (Rathburn Chemicals, Walkerburn, UK. Blood with heparin (4 iu ml^{-1}) was kept on ice before centrifugation at 1.000 g for 20 min at 4° C. The samples were stored at −20° C awaiting analysis on h.p.l.c.

H.p.l.c. setup of metoprolol analysis: The samples and standards were essentially prepared according to [8] with following modifications: H93/47 (50 μl of 10 μl ml^{-1}) was used as internal standard, tri-

ethylamine was used instead of Physics solvent delivery, model SP 8770 (Spectra Physics, Santa Clara, CA), Rheodyne 7125 injector (Rheodyne, Cotati, CA) with a 100 µl loop and chromatographed on a Supelcosil LC-8-DB (15 cm × 4.6 mm, tp 35° C) column with a Supelguard LC-8-DB (2 cm × 4.6 mm) guard column (Supelco, Bellefonte, PA) and a Shimadzu UV detector (Shimadzu, Kyoto, Japan) 220 nm/0.002 AUFS and the mobile phase comprised (Acetonitrile/0.02 M $KHPO_4$-buffer (22.5:77.5) and 100 µl triethylamine added/1, final pH 3.15.

Plasma cyclic AMP concentrations

The concentrations of cAMP were determined by a radioimmunoassay (Skomedal 80).

HbA_{1c} and blood glucose

HbA_{1c} was assayed by h.p.l.c. (Diamat, Bio-Rad). Blood glucose (BG) was determined by glucose oxidase assay (Kodak ektachem 700 XR).

Calculations

From the dose response curves of isoprenaline and metoprolol regression lines were constructed. I_{25}, the isoprenaline dose that increased heart rate with 25 beats min^{-1}, and $M_{-12.5}$, the metoprolol dose that inhibited the TEST I_{25} with 50% or 12.5 beats per minute, of each case were calculated from regression lines. I_{25} and $M_{-12.5}$ can be used as determinants of isoprenaline and metoprolol sensitivity.

Statistical analyses

Repeated measures analysis of variance, students *t*-tests and regression analyses were performed by the computer program Statgraphics (STSC, Inc., 2115 East Jefferson Street, Rockville, Maryland 20852, U.S.A.). *P* values < 0.05 were considered significant.

Results

Basal values

Table 1 shows that the basal values of heart rate, blood pressure, plasma cAMP and plasma catecholamine levels did not differ between the groups. There was no significant difference in blood glucose or HbA_{1c} between the groups of diabetic patients.

Isoprenaline sensitivity test

The pharmacodynamics of isoprenaline have been measured by its effect on the variables heart rate, blood pressure, blood level of glucose and plasma levels of adrenaline and noradrenaline. With the exceptions of diastolic blood pressure and the plasma level of adrenaline, all variables showed a significant correlation to the dose of isoprenaline given (results not shown).

The changes of heart rate (Table 2b, Figure 1) and blood levels of glucose were significantly different in the three groups of test persons. For heart rate the slopes of all dose-response curves were equal, but the dose-response curves of the IDDM-patients with hypoglycaemia unawareness were significantly shifted to the right compared with controls and IDDM-patients with hypoglycaemia awareness. Other authors [4, 5, 9, 10] use I_{25} as a standard of isoprenaline sensitivity. In our study we find a

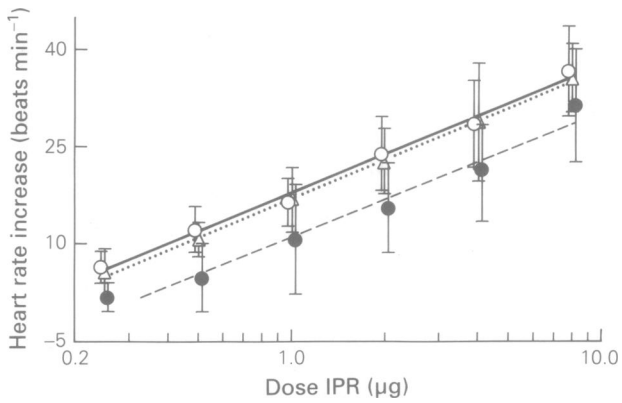

Figure 1 Isoprenaline sensitivity test. Isoprenaline sensitivity is measured as isoprenaline effect on heart rate. Repeated measures analysis of variance shows that the dose-response curve of isoprenaline/HR is sigificantly shifted to the right in IDDM-patients with hypoglycaemia unawareness (●) ($P < 0.05$) compared with controls (○) and IDDM-patients with hypoglycaemia awareness (△). Calculation of I_{25} show a significant increase in IDDM-unaware compared with controls ($P < 0.025$) and with IDDM-aware ($P < 0.05$). There is no difference in I_{25} between controls and IDDM-aware ($P > 0.05$). *P* levels regarding I_{25} are estimated by Student's *t*-test.

Table 1 Basal values of the measured variables

	Control (n = 9)		*IDDM-aw* (n = 7)		*IDDM-unaw* (n = 9)	
	Mean	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*
HbA_{1c} (%)	–	–	8.6	0.8	8.7	1.6
HR (beats min^{-1})	69	9	77	10	79	13
SBP (mm Hg)	120	13.3	123	7.5	125	11.4
DBP (mm Hg)	69	8.2	70	5.8	74	6.7
BG (mmol l^{-1})	5.27	0.82	11.54	3.77	9.76	4.03
cAMP (pmol l^{-1})	5.67	1.27	5.02	0.71	6.51	2.20
ADR (nmol l^{-1})	0.28	0.08	0.19	0.08	0.26	0.20
NA (nmol l^{-1})	1.38	0.55	1.34	0.77	1.30	0.81

P values were estimated by Student's *t*-test. There were no significant differences in the basal values of any measured variable.

Table 2 Isoprenaline sensitivity test

	Control (n = 9)		*IDDM-aw* (n = 7)		*IDDM-unaw* (n = 9)	
	Mean	*s.e. mean*	*Mean*	*s.e. mean*	*Mean*	*s.e. mean*
a: Slope						
HR	20.28	1.30	21.19	1.76	20.02	1.90
SBP	23.43	1.97	22.68	2.68	19.69	2.81
DBP	1.14	1.70	2.01	1.63	−1.52	0.95
BG	0.10	0.05	−0.30*	0.12	−0.55*	0.29
ADR	−0.009	0.02	−0.52	0.45	0.09	0.05
NA	0.34	0.10	0.31	0.12	0.15	0.08
b: Ordinate intercept						
HR	17.74	0.69	17.17	0.94	11.39*	1.02
SBP	7.54	1.06	11.18	1.44	8.50	1.51
DBP	−1.54	0.91	−0.97	0.87	−2.99	0.51
BG	0.03	0.03	−0.41	0.06	−0.07	0.15
ADR	−0.009	0.02	0.53	0.24	0.04	0.03
NA	0.04	0.05	0.03	0.06	0.04	0.04

Dose-response curves of the different variables described by slopes and y-intercepts of their regression lines. Slopes and y-intercepts were estimated by Simple Regression Analysis – Linear model: Y = a+bX. *P* levels were estimated by Repeated Measures Analysis of Variance. An asterisk in the 'intercept' table means significant horizontally shift of the dose/response curve. An asterisk in the 'slope' table means significant different changes in the current variable over time. Slopes of the variables are shown in Table 2a, ordinate intercept in Table 2b.

Table 3 $I_{25}/M_{-12.5}$

	Control (n = 9)		*IDDM-aw* (n = 7)		*IDDM-unaw* (n = 9)	
	Mean	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*
I_{25} μg	2.6	1.2	3.0	1.4	5.7*	3.4
$M_{-12,5}$ mg	1.14	0.75	1.15	0.56	2.17*	1.06

I_{25} and $M_{-12.5}$ were increased in IDDM-patients with hypoglycaemia unawareness.
* = $P < 0.05$.
P levels were estimated by Student's *t*-test.

significantly increased I_{25} in the IDDM-patients with hypoglycaemia unawareness (Table 3).

The slopes of the glucose dose-response curves of both IDDM-groups were significantly different from the slope of the dose-response curve of the controls (Table 2a). We observed a fall in the blood level of glucose in both IDDM-groups, whereas a rising glucose level was observed in the controls. All changes of blood levels of glucose were significantly correlated to the dose of isoprenaline given.

Metoprolol sensitivity test

The pharmacodynamic effects of metoprolol have been measured by the same variables as in the isoprenaline sensitivity test. Heart rate and systolic blood pressure showed a significant negative correlation to the dose of metoprolol. There were no significant changes in diastolic blood pressure, blood levels of glucose, plasma levels of adrenaline or noradrenaline (results not shown).

Reduced effect of metoprolol on heart rate was demonstrated in IDDM-patients with hypoglycaemia unawareness by right-shifted dose-response curves (Figure 2), compared with controls. There were no significant differences in metoprolol response

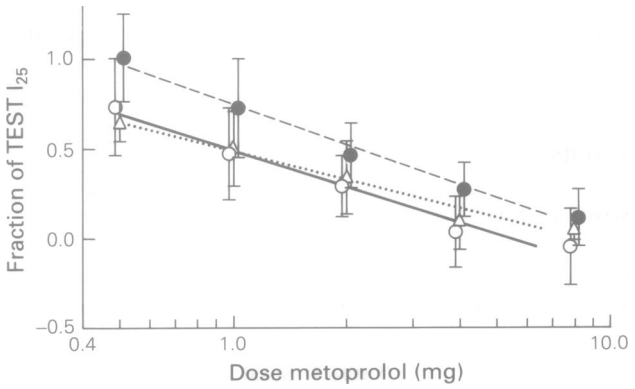

Figure 2 Metoprolol Sensitivity Test. Metoprolol effect is measured as fractional reduction of TEST I_{25}. Repeated measures analyses of variance shows that the dose-response curve of metoprolol/HR is significantly shifted to the right in IDDM-patients with hypoglycaemia unawareness (●) compared with controls (○) and IDDM-patients with hypoglycaemia awareness (△) ($P < 0.05$). Calculation of $M_{-12.5}$ show a significant increase in IDDM-unaware compared with controls ($P < 0.05$) and with IDDM-aware ($P < 0.05$). There is no difference in $M_{-12.5}$ between controls and IDDM-aware ($P > 0.05$). *P* levels regarding $M_{-12.5}$ are estimated by Student's *t*-test.

between controls and IDDM-patients with hypoglycaemia awareness. $M_{-12.5}$ can be used as a standard of metoprolol sensitivity. In Table 3 it is shown that $M_{-12.5}$ was significantly increased in IDDM-patients with hypoglycaemia unawareness.

Plasma levels of isoprenaline and metoprolol

The plasma levels of isoprenaline were measured in blood samples obtained after each injection of 8 μg. In both IDDM-groups the plasma levels of isoprenaline were significantly higher than in controls (IDDM-aware: 3.01 nM, s.d. 1.42 nM, IDDM-unaware: 4.9 nM, s.d. 2.91 nM, control: 1.19 nM, s.d. 0.76 nM). Plasma levels of metoprolol were measured in blood samples obtained after every metoprolol injection. There were no significant differences in plasma levels of metoprolol between the tested groups (results not shown).

Discussion

We have demonstrated a reduced β-adrenergic sensitivity in IDDM-patients with hypoglycaemia unawareness, both to β-adrenergic stimulation and β_1-adrenoceptor blockade. Displacement of dose-response curves has been calculated by repeated measures analysis of variance. It is also possible to use the I_{25} and $M_{-12.5}$ as determinants of adrenergic sensitivity. The reduction in β-adrenergic sensitivity in IDDM-patients with hypoglycaemia unawareness was evident by right-shifts of both the dose-response curves of isoprenaline/HR and metoprolol/HR, and by increased I_{25} and $M_{-12.5}$. Reduced sensitivity to stimulation has also been shown in earlier reports [4, 5], whereas reduced sensitivity to a blocking agent has not earlier been shown.

Other authors [4] have found reduced mean resting plasma levels of adrenaline in IDDM-patients with hypoglycaemia unawareness compared with IDDM-patients with hypoglycaemia awareness. Our study showed no such differences.

We observed significantly higher plasma levels of isoprenaline in both IDDM-groups compared with controls. This may reflect a slower distribution rate of isoprenaline. A delay in the arrival of isoprenaline to the target tissue may mimic a reduced sensitivity, but the importance of this remains to be shown. No such difference was observed regarding metoprolol.

Events of hypoglycaemia are side effects of insulin therapy in diabetes. The pathophysiological changes which lead to hypoglycaemia unawareness are today not fully understood. Some authors stress reduced catecholamine secretion as one possible causative component in hypoglycaemia unawareness [11, 12]. Reduced biological activity of catecholamines due to changed protein binding, has been suggested as one possible mechanism in hypoglycaemia unawareness. Protein binding of some adrenergic agents has been investigated in a not sub-grouped population of diabetic patients. However, no change was found in protein binding of catecholamines [13].

Different kinds of tissue adaptation to hypoglycaemia are also suggested as pathophysiological mechanisms of hypoglycaemia unawareness. That is adaptation of hypothalamus cells to low blood glucose levels with the consequence that initiation of counterregulation is delayed [1] and adaptation of β-adrenoceptor complexes to increased catecholamine levels [4, 5]. As far as we know, the β-adrenoceptor status has not been determined in patients with hypoglycaemia unawareness. Two studies showed no difference in β-adrenoceptor density on mononuclear leucocytes from not sub-grouped IDDM-patients compared with control [14, 15]. The hypothesis of changed G-protein function as a cause of reduced β-receptor sensitivity is put forward [16]. A change in G-protein function may explain the reduced sensitivity of isoprenaline, by reduced coupling. It may also explain the reduced sensitivity for metoprolol, if G-protein subunits play a role in the competitive interaction of agonist and antagonist for the binding site. Some reports show that IDDM-patients with poor glycaemic control require less decrease in blood glucose to elicit autonomic warning symptoms [3, 17, 18]. In our study the glycaemic control, expressed by HbA_{1e}, seems to be equal in the two groups of diabetic patients. However, HbA_{1c} is only a measure of the average blood glucose levels during the past 10 weeks. In spite of equal levels of HbA_{1c} there may be important differences in the frequency of hypoglycaemic episodes between the aware- and unaware-group. In this material there is a significantly longer disease duration in the group of patients with hypoglycaemia unawareness compared with patients with hypoglycaemia awareness. The importance of this is uncertain. Several reports show that intensified insulin therapy prevents development of the long-term complications retinopathy, nephropathy and neuropathy in IDDM-patients [19–21]. On the other hand intensified insulin therapy is also reported to weaken the catecholamine response to hypoglycaemia [22]. Patients treated with intensified insulin therapy with the intention to achieve near-normal blood glucose levels, go through severe hypoglycaemic episodes more often than patients on traditional insulin therapy [23]. It is shown that hypoglycaemia unawareness in IDDM patients on intensified insulin therapy, may be reversed by increasing their HBA_{1c} and careful prevention of hypoglycaemic episodes [24].

The findings of this study support the theory of reduced β-adrenergic sensitivity in peripheral tissue as a part of the pathophysiological changes associated with hypoglycaemia unawareness. On this background there might be a conflict between the important task of avoiding long-term complications and hypoglycaemia unawareness and increased risk of severe hypoglycaemic events.

Further investigation of the effect of insulin therapy on adrenergic sensitivity must be important. The isoprenaline and metoprolol sensitivity tests have shown to be useful tools in this work.

We thank The Norwegian Council on Cardiovascular Diseases and the Norwegian Diabetic Association for financial support, Dr P.-O. Lagerstrøm for the advice on the metoprolol analysis and the gifts H93/47 and metoprolol, Dr B. Straume for help and advice on statistical matters.

References

1 Gerich JE, Mokan M, Veneman T, Korytkowski M, Mitrakou A. Hypoglycemia unawareness. *Endocrine Reviews* 1991; **12**: **4**, 356–371.

2 Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, Durrant J, Cryer P, Gerich J. Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, cerebral dysfunction. *Am J Physiol* 1991; **260**: E67–E74.

3 Widom B, Simonson D. Glycemic control, neuropsycologic function during hypoglycemia in patients with insulin-dependent diabetes. *Ann intern Med* 1991; **112**: 904–912.

4 Berlin I, Grimaldi A, Landault C, Zoghbi F, Thervet F, Puech AJ, Legrand JC. Lack of hypoglycemic symptoms and decreased β-adrenergic sensitivity in insulin-dependent patients. *J clin Endocrin Metab* 1988; **66**: 273–278.

5 Berlin I, Grimaldi A, Payan C, Sachon C, Bosquet F, Thervet F, Peuch AJ. Hypoglycemic symptoms and decreased β-adrenergic sensitivity in insulin-dependent diabetic patients. *Diabetes Care* 1987; **10**: 742–747.

6 George CF, Conolly ME, Fenyvesi T, Briant R, Dollery CT. Intravenously administered isoproterenol sulfate dose-response curves in man. *Arch intern Med* 1972; **130**: 361–364.

7 Sager GT, Slørdal L, Jaeger R, Prytz P, Brox J, Reikerås O. Catecholamine binding and concentrations in the acute phase plasma after surgery. *Scand J clin Lab Invest* 1988; **48**: 419–424.

8 Balmèr K, Zang Y, Lagerstrøm PO, Persson BA. Determination of Metoprolol and two major metabolites in plasma and urine by colomn liquid chromatography and fluorimetric detection. *J Chromatogr* 1987; **417**: 357–365.

9 Arnold JMO, McDevitt DG. Standardised isoprenaline sensitivity tests – A comparison of existent methods. *Br J clin Pharmac* 1983; **15**: 167–172.

10 Berlin I, Grimaldi A, Bosquet F, Puech AJ. Decreased β-adrenergic sensitivity in insulin-dependent diabetic subjects. *J clin Endocrinol Metab* 1986; **63**: 262–265.

11 Hoeldtke RD, Boden G, Shuman CR, Owen OE. Reduced epinephrine secretion and hypoglycemia unawareness in diabetic autonomic neuropathy. *Ann intern Med* 1982; **96**: 459–462.

12 Adamson U, Lius P, Efendic S, Hamberger B, Wajngot A. Impaired counterregulation of hypoglycemia in a group of insulin dependent diabetics with recurrent episodes of severe hypoglycemia. *Acta med Scand* 1984; **216**: 215–222.

13 Trovik TS, Jaeger R, Jorde R, Ingebretsen O, Sager G. Plasma protein binding of catecholamines, prazosin and propranolol in diabetes mellitus. *Eur J clin Pharmac* 1992; **43**: 265–268.

14 Serusclat P, Rosen SG, Smith EB, Shah SD, Clutter WE, Cryer PE. Mononuclear leucocyte β_2-adrenergic receptors and adenylate cyclase sensitivity in insulin-dependent diabetes mellitus. *Diabetes* 1983; **32**: 825–829.

15 Sager G, Slørdal L, Huseby N-E, Florholmen J. β-Adrenoceptor regulation in insulin-dependent diabetes mellitus. *Scand J clin lab Invest* 1991; **51**: 283–288.

16 Lynch CJ, Exton JH. Alterations in G-protein-mediated cell signaling in diabetes mellitus. In *G-proteins. Signal, Transduction and Disease*. eds Milligan G and Wakelam M, Academic Press Limited, London. 1992: 87–108.

17 Mokan M, Mitrakou A, Raptis G, Platanisiotis D, Grannakopoulos F, Korytkowski M, Cryer P, Gerich J. Differential effects of duration of diabetes and glycemic control on thresholds and magnitudes of response to hypoglycemia. *Diabetes* 1991; **40** [Supl 1]: 556A.

18 Boyle P, Schwartz N, Shah S, Clutter W, Cryer P. Plasma glucose concentrations at the onset of hypoglycemic symptoms in patients with poorly controlled diabetes and in nondiabetics. *New Engl J Med* 1988; **318**: 1487–1492.

19 Dahl-Jørgensen K, Bjøro T, Kierulf P, Sandvik L, Bangstad HJ, Hanssen KF. Long term glycemic control and kidney function in insulin dependent diabetes mellitus. *Kidney Int* 1992; **41**: 920–923.

20 Brinchmann-Hansen O, Dahl-Jørgensen K, Sandvik L, Hanssen KF. Blood glucose concentrations and progression of diabetic retinopathy; the seven years results of the Oslo study. *Br med J* 1992; **304**: 19–22.

21 Reichard P, Berglund B, Britz A, Nilsson BY Cars, Rosenqvist U. Intensified conventional insulin treatment retards the microvascular complications of insulin dependent diabetes mellitus (IDDM). *New Engl J Med* 1993; **321**: 304–309.

22 Simonson DC, Tamborlane WV, DeFronzo RA, Sherwin RS. Intensive insulin therapy reduces counterregulatory hormone responses to hypoglycemia in patients with type I diabetes. *Ann intern Med* 1985; **103**: 184–188.

23 The Diabetes Control and Complications Trial Research Group. Epidemiology of severe hypoglycemia in the diabetes control and complications trial. *Am J Med* 1991; **90**: 450–459.

24 Fanelli CG, Epifano L, Rambotti AM *et al.* Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 1993; **42**; 11: 1683–1689.

(Received 20 December 1993, accepted 24 June 1994)