Catecholamine Response During Human and Pork Insulin-Induced Hypoglycemia in IDDM Patients

THOMAS LINGENFELSER, MD
WALTER RENN, PHD
CHRISTIAN PLONZ, BSC
MAHMUD RADJAIPOUR, PHD
MANFRED EGGSTEIN, MD
BERNHARD JAKOBER, MD

OBJECTIVE — To evaluate the catecholamine response during human and pork insulin-induced hypoglycemia.

RESEARCH DESIGN AND METHODS — Ten insulin-dependent diabetes mellitus (IDDM) patients without any signs of autonomic neuropathy received either human or pork insulin in a randomized crossover fashion on 2 nonconsecutive days. The glucose clamp technique was applied to achieve stable glycemic plateaus of 5.6, 3.3, 2.2, and 1.7 mM.

RESULTS — The effect of both types of insulin on glucose metabolism and circulating catecholamines was almost identical. There was a sharp rise of both epinephrine ($P < 0.05$) and norepinephrine ($P < 0.02$) during hypoglycemia, which did not depend on the type of insulin applicated. Symptom awareness increased significantly during the decrease of blood glucose concentration. Only during developing hypoglycemia (3.3-mM plateau), was this effect more pronounced (cumulative symptom score 2 vs. 26, $P < 0.05$) with pork insulin.

CONCLUSIONS — An attenuated catecholamine secretion seems not to be the putative mechanism of a reduced awareness of human insulin-induced hypoglycemia.

The phenomenon of a reduced awareness of hypoglycemia after application of human insulin preparations to insulin-dependent diabetes mellitus (IDDM) patients remain the issue of a controversial debate. Berger et al. (1) claim an attenuation of autonomic symptoms as a putative mechanism for the diminished ability of diabetic patients on human insulin to recognize that they are hypoglycemic. If this observation is true, pork insulin should be a more intensive stimulus to the sympathoadrenal system, which, indeed, is the main inductor of autonomic symptoms (2). Therefore, the physiological basis for a loss of warning symptoms of patients on human insulin could be an impaired catecholamine response to hypoglycemia (3). Earlier observations seem to confirm this possibility (4). Heine et al. (5) provided further support by means of a controlled study demonstrating higher norepinephrine and higher (albeit not significant) epinephrine responses when hypoglycemia was induced by pork insulin. These studies were conducted in healthy subjects. Because hormonal counterregulation is remarkably different in IDDM (6), one must be careful to extrapolate the aforementioned differences of sympathoadrenal response to IDDM patients. The results of one study, having been performed in IDDM patients, are limited due to diverging glucose levels and lack of free insulin determinations (7).

RESEARCH DESIGN AND METHODS

— To elucidate the physiological mechanism involved in the prevention of hypoglycemia, we studied 10 IDDM patients (6 men, 4 women) without measurable C-peptide secretion, mean ± SE age 29.9 ± 3.2 yr, with manifestations of diabetes for 8.6 ± 1.8 yr, HbA_1 8.1 ± 0.4% (normal <7.8%), and who were 100.0 ± 3.6% of ideal body weight. Autonomic neuropathy was unlikely in the face of normal cardiovascular reflexes, such as 30:15 ratio, heart rate variation on breathing, Vasalva maneuver, and postural hypotension. All patients were on intensified conventional therapy (ICT) with retarded human insulin preparations twice daily (14.9 ± 2.0 U in the morning and 16.2 ± 2.1 U in the evening) and additional bolus injections of human regular insulin as needed (20.8 ± 3.2 U/day). Hypoglycemic epi-

FROM THE DEPARTMENT OF MEDICINE, DIVISIONS OF ENDOCRINOLOGY AND CHEMICAL PATHOLOGY, EBERHARD-KARLS-UNIVERSITY TUEBINGEN, TUEBINGEN, GERMANY.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO THOMAS LINGENFELSER, MD, DEPARTMENT OF MEDICINE, EBERHARD-KARLS-UNIVERSITY, SCHNARRENBERG, D-7400 TUEBINGEN, GERMANY.

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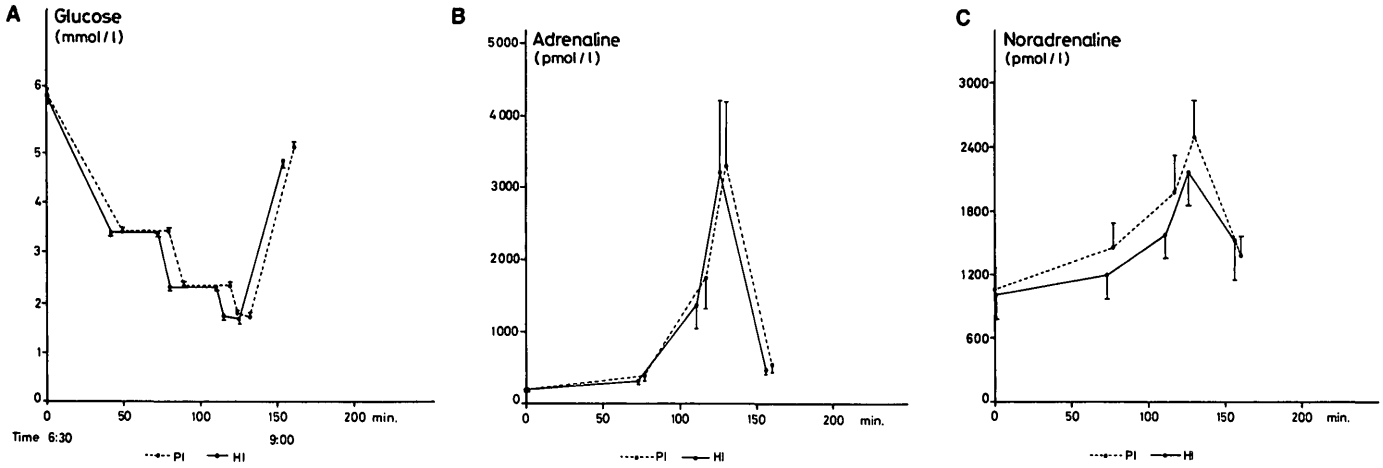

Figure 1—*Mean peak plasma adrenaline (B) and noradrenaline (C) concentrations at end of each glycemic plateau (3.3, 2.2, 1.7 mM) during stepwise blood glucose fall (A).*

sodes were familiar to the patients, but they never experienced so-called hypoglycemia unawareness, nor did they ever need external help during these episodes. Furthermore, patients had to avoid any episode of hypoglycemia the last 3 days before study. The study was approved by the local ethical committee according to the Declaration of Helsinki.

Indwelling catheters were inserted into an antecubital and two dorsal hand veins, and their fasting blood glucose concentration was clamped at ~5.6 mM overnight with a glucose-controlled insulin-infusion system (Biostator, Life Science, Miles, Elkhart, IN). The following morning (0600), blood glucose concentration was lowered stepwise wise to 3.3, 2.2, and 1.7 mM by external infusion of regular insulin (Velasulin-H or Velasulin, Nordisk, Copenhagen) at a constant rate of 2 mU · kg^{-1} · min^{-1} and held constant for 30 min at 3.3 and 2.2 mM or 10 min at 1.7 mM. Human or pork insulin were allocated in a double-blind crossover and randomized fashion. Blood (arterialized by heated chamber which was verified by O_2 saturation) for hormone analysis was drawn during the last 5 min of each plateau (pooled sample). The time interval between two studies in an individual patient was at least 2 days and did not exceed 7 days (median 4 days).

Blood glucose concentrations were measured by a glucose dehydrogenase method (Eppendorf ACP 5040, Hamburg, Germany), free insulin with enzyme-linked immunosorbent assay (Boehringer Diagnostica, Mannheim, Germany), and catecholamines by high-performance liquid chromatography in plasma with the ClinRep analysis kit (Recipe Pharma, Munich, Germany).

A structured and standardized questionnaire on the appearance of hypoglycemia symptoms was applied to evaluate symptom awareness during decreasing blood glucose concentrations. Hypoglycemia symptoms had to be scored by the patients according to their intensity at the preselected glycemic plateaus (0, not experienced; 10, severe). These symptoms were classified (D.A. Hepburn, unpublished observations) as being primarily autonomic (sweating, trembling, nervousness, shakiness, blurred vision) or neuroglycopenic (tiredness, lack of concentration). Some could not be clearly attributed to either group (hunger, headache, feeling different in any way). Dummy questions were included to detect stereotype answering due to neuroglycopenia.

Values are means ± SE. Effects on plasma epinephrine, plasma norepinephrine, serum insulin, and glucose requirements were assessed by analysis of variance with repeated-measures design containing the factors glucose level and type of insulin. A nonparametric test (Wilcoxon's signed-rank test) was applied for the analysis of symptom awareness.

RESULTS — Glucose requirements to maintain hypoglycemic clamps (coefficients of variation of blood glucose concentrations at clamp plateaus varied between 0.7 and 3%) were not significantly different (3.3 mM, 335 ± 28 [human insulin] vs. 297 ± 23 [pork insulin] mg · min^{-1}; 2.2 mM, 324 ± 29 vs. 283 ± 29 mg · min^{-1}; 1.7 mM, 19 ± 2 vs. 10 ± 1 mg · min^{-1}, respectively). This resulted in similar blood glucose levels throughout the study (Fig. 1*A*), signifying identical metabolic potency of the insulins. Due to constant infusion of either human or pork insulin free insulin levels were not significantly different during both euglycemia and hypoglycemia (1.7 mM) (170 ± 39

Table 1—*Catecholamine response during human and pork insulin-induced hypoglycemia*

	Glucose (mM)				Hypoglycemia
	5.6	3.3	2.2	1.7	
Human insulin	197 ± 49	317 ± 33	1370 ± 333	3221 ± 999	
Epinephrine (pM)					$P < 0.05$*
Pork insulin	191 ± 33	388 ± 76	1747 ± 442	3309 ± 890	
Human insulin	993 ± 230	1176 ± 219	1548 ± 213	2134 ± 307	
Norepinephrine (pM)					$P < 0.02$*
Pork insulin	1040 ± 201	1436 ± 225	1944 ± 337	2459 ± 343	

Values are means ± SE.
*Euglycemia vs. hypoglycemia.

to 831 ± 53 pM vs. 216 ± 62 to 737 ± 46 pM, respectively). There was a significant increase of both adrenaline ($P < 0.05$) and noradrenaline ($P < 0.02$) during hypoglycemia, which did not depend on the species of insulin used (Table 1; Fig. 1, *B* and *C*).

The cumulative symptom score increased significantly ($P < 0.05$) during decreasing blood glucose concentrations (3.3 to 2.2 mM, 2.2 to 1.7 mM), independent of the type of insulin used (Fig. 2). This rise in symptom score was mainly caused by the autonomic symptoms of sweating, shakiness and nervousness and the neuroglycopenic symptom tiredness. Only at the 3.3-mM plateau (developing hypoglycemia) was the cumulative symptom score significantly different, depending on the insulin type (2 vs. 26, human vs. pork insulin, respectively, $P < 0.05$; Fig. 2). The difference in symptom awareness was most striking for the symptom nervousness.

CONCLUSIONS— We have shown that the response of the sympathoadrenal system to overt hypoglycemia induced by human or pork insulin (as assessed from circulating catecholamines) is not different in IDDM patients. We minimized β-error in this study by appropriate application of the glucose clamp technique and within-subject comparison. The question arises as to whether the observed differences in the catecholamine response of healthy nondiabetic and diabetic patients could not be attributed to a generally blunted hormonal response in the latter group. This appears not to be the case because catecholamine levels have been comparable to normal levels in our study group, and deficiencies typically occur late in the disease (6).

Catecholamine secretion is thought to be activated by central glucopenia (8). Because glucose influx into the brain depends on the characteristics of the blood-brain barrier and is modified by insulin (9), it may be that well-known central adaptation phenomena in diabetic patients attenuate the different insulin effects on the catecholamine response.

In our study, insulin-induced hypoglycemia caused autonomic and neuroglycopenic symptoms to emerge and to increase in intensity with time and decreasing blood glucose concentrations. But only during developing hypoglycemia was this effect more pronounced when using pork insulin, mainly due to increased reporting of nervousness. Differential effects on excitement or arousal during early hypoglycemia in the study by Kern et al. (10) indicate a comparable influence on symptom awareness. Nevertheless, the differential insulin effects van-

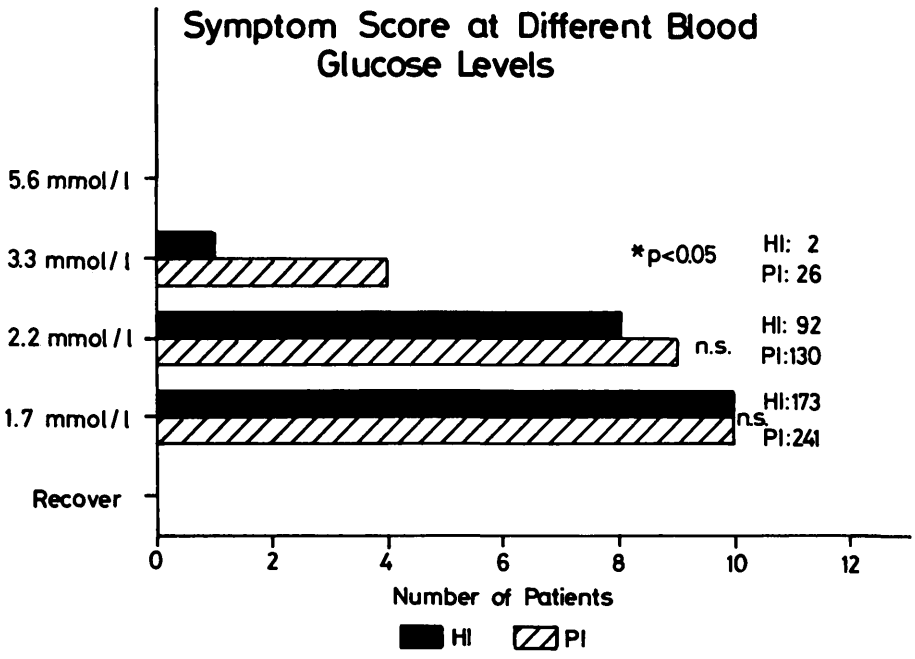

Figure 2—*Number of patients from study group aware of hypoglycemia symptoms at each glycemic plateau. Right, cumulative symptom score of hypoglycemia symptoms (*$P < 0.05$ at 3.3 mM).*

ished during established hypoglycemia. We argue that subtle differential insulin effects on CNS function persist during significant hyperinsulinemia accompanied by just-developing hypoglycemia, whereas, during established hypoglycemia the limited glucose supply to the brain with consecutive central glucopenia superimposes the pure insulin effect (11). Indeed, there is a reduced awareness of human insulin–induced hypoglycemia compared with pork insulin during developing hypoglycemia. This phenomenon vanishes during established hypoglycemia and cannot be attributed to different levels of circulating catecholamines. An alternative approach to study brain glucose deprivation by electrophysiological techniques is promising (10).

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