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Research Article

To determine if the enhanced glycemic response to epinephrine in patients with insulin-dependent diabetes mellitus (IDDM) is the result of increased adrenergic sensitivity per se, increased glucagon secretion, decreased insulin secretion, or a combination of these, plasma epinephrine concentration-response curves were determined in insulin-infused (initially euglycemic) patients with IDDM and nondiabetic subjects on two occasions: once when insulin and glucagon were free to change (control study), and again when insulin and glucagon were held constant (islet clamp study). During the control study, plasma C-peptide doubled, and glucagon did not change in the nondiabetic subjects, whereas plasma C-peptide did not change but glucagon increased in the patients. The patients with IDDM exhibited threefold greater increments in plasma glucose, largely the result of greater increments in glucose production. This enhanced glycemic response was apparent with 30-min increments in epinephrine to plasma concentrations as low as 100-200 pg/ml, levels that occur commonly under physiologic conditions. During the islet clamp study (somatostatin infusion with insulin and glucagon replacement at fixed rates), the heightened glycemic response was unaltered in the patients with IDDM, but the nondiabetic subjects exhibited an enhanced glycemic response to epinephrine indistinguishable from that of patients with IDDM. In contrast, the FFA, glycerol, and beta-hydroxybutyrate responses were unaltered. Thus, we conclude the following: Short, physiologic increments in plasma epinephrine cause greater increments […]

Enhanced Glycemic Responsiveness to Epinephrine in Insulin-dependent Diabetes Mellitus Is the Result of the Inability to Secrete Insulin

Augmented Insulin Secretion Normally Limits the Glycemic, but Not the Lipolytic or Ketogenic, Response to Epinephrine in Humans

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Abstract

To determine if the enhanced glycemic response to epinephrine in patients with insulin-dependent diabetes mellitus (IDDM) is the result of increased adrenergic sensitivity per se, increased glucagon secretion, decreased insulin secretion, or a combination of these, plasma epinephrine concentration-response curves were determined in insulin-infused (initially euglycemic) patients with IDDM and nondiabetic subjects on two occasions: once when insulin and glucagon were free to change (control study), and again when insulin and glucagon were held constant (islet clamp study). During the control study, plasma C-peptide doubled, and glucagon did not change in the nondiabetic subjects, whereas plasma C-peptide did not change but glucagon increased in the patients. The patients with IDDM exhibited threefold greater increments in plasma glucose, largely the result of greater increments in glucose production. This enhanced glycemic response was apparent with 30-min increments in epinephrine to plasma concentrations as low as 100–200 pg/ml, levels that occur commonly under physiologic conditions. During the islet clamp study (somatostatin infusion with insulin and glucagon replacement at fixed rates), the heightened glycemic response was unaltered in the patients with IDDM, but the nondiabetic subjects exhibited an enhanced glycemic response to epinephrine indistinguishable from that of patients with IDDM. In contrast, the FFA, glycerol, and β-hydroxybutyrate responses were unaltered. Thus, we conclude the following: 1) Short, physiologic increments in plasma epinephrine cause greater increments in plasma glucose in patients with IDDM than in nondiabetic subjects, a finding likely to be relevant to glycemic control during the daily lives of such patients as well as during the stress of intercurrent illness. 2) Enhanced glycemic responsiveness of patients with IDDM to epinephrine is not the result of increased sensitivity of adrenergic receptor-effector mechanisms per se nor of their increased glucagon secretory response; rather, it is the result of their inability to augment insulin secretion. 3) Augmented insulin secretion, albeit restrained, normally limits the glycemic response, but not the lipolytic or ketogenic responses, to epinephrine in humans.

Introduction

Shamoon et al. (1) demonstrated that patients with insulin-dependent diabetes mellitus (IDDM)[1] exhibit an enhanced glycemic response to epinephrine. Studying patients infused with insulin in doses sufficient to produce normal plasma glucose concentrations and glucose turnover rates at base line, they found that prolonged infusion of epinephrine to plasma concentrations of 300–450 pg/ml caused an increase in plasma glucose greater than that which occurred in nondiabetic controls, the result of an enhanced and more sustained increase in glucose production. They suggested that this contributes to the pathogenesis of stress hyperglycemia in IDDM.

The cause of the enhanced glycemic response to epinephrine in patients with IDDM is not known. Shamoon et al. (1) suggested increased sensitivity of the liver per se but acknowledged that a role for insulin deficiency could not be excluded. Further, it is not known if short increments in plasma epinephrine to levels that occur commonly in humans also produce an increased glycemic response in patients with IDDM. Indeed, 90–120 min of substantial epinephrine elevations appeared to be required to demonstrate disparate responses of patients and nondiabetic individuals (1).

The physiologic mechanisms of the hyperglycemic effect of epinephrine are complex. Normally, they involve both direct and indirect (hormone-mediated) actions, are the result of both stimulation of glucose production and limitation of glucose utilization, and are mediated through both β- and α-adrenergic receptors in humans (2–9). α-Adrenergic limitation of insulin secretion is normally an important indirect hyperglycemic action of epinephrine (2–6). On the other hand, there is some increase in insulin secretion, albeit limited, during sustained epinephrine elevations (3, 5); the physiologic relevance of this has not been established previously. The role of epinephrine-stimulated glucagon secretion, observed in some (2, 3, 7) but not all (5, 6) studies, is unclear. Epinephrine-stimulated increments in glucose production clearly occur in the absence of glucagon release (4, 7); Gray et al. (7) concluded that the effect of epinephrine on glucose production is normally independent of glucagon. The direct hyperglycemic actions of epinephrine (4, 8) involve both limitation of glucose utilization and stimulation of glucose production. The former is mediated

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1. *Abbreviations used in this paper:* IDDM, insulin-dependent diabetes mellitus; PEG, polyethylene glycol.

through β-adrenergic mechanisms (4, 8). In humans, direct stimulation of hepatic glucose production is mediated predominantly through β-adrenergic mechanisms (4), although a small direct α-adrenergic component can be demonstrated under some conditions (9).

The mechanisms of the hyperglycemic effect of epinephrine differ in patients with IDDM. To the extent that endogenous insulin secretion is deficient, α-adrenergic limitation of insulin secretion cannot play a role. Among other potential indirect hyperglycemic actions, the glucagon secretory response to epinephrine has been found to be increased in humans with IDDM (10, 11) and in insulin-deficient dogs (12). Evidence that enhanced glucagon secretion contributes to the hyperglycemic response to epinephrine in experimental diabetes has been presented (12, 13). Both the direct hyperglycemic actions of epinephrine (4, 8), discussed earlier, and the glucagon secretory response to epinephrine (4, 7, 10) are mediated through β-adrenergic mechanisms. Whereas β-adrenergic blockade with propranolol reduces the hyperglycemic response to epinephrine minimally (3, 14) in nondiabetic humans, it reduces the response markedly (10, 14) in patients with IDDM. Thus, in contrast to nondiabetic individuals, the response is mediated predominantly through β-adrenergic mechanisms in patients with IDDM.

The enhanced glycemic response to epinephrine in patients with IDDM (1) could be the result of increased responsiveness of adrenergic receptor-effector mechanisms per se. However, the fact that such patients do not have a generalized increase in β-adrenergic receptor density or affinity or in adenylate cyclase sensitivity to a β-adrenergic agonist (15) argues against that possibility. Alternatively, increased glycemic responsiveness to epinephrine could be the result of decreased insulin secretion, increased glucagon secretion, or both in patients with IDDM. To test the latter hypothesis, we measured the responses to physiologic plasma epinephrine increments in patients with IDDM (selected for the absence of adrenergic neuropathy) and nondiabetic subjects on two occasions: once when changes in insulin and glucagon were free to occur and once when the levels of these were held constant. The data indicate that patients with IDDM have increased glycemic responsiveness to plasma epinephrine concentrations that span the physiologic range, and that this is the result of their inability to augment insulin secretion as the plasma glucose concentration rises. They also indicate that insulin secretion, albeit restrained, normally limits the glycemic response to epinephrine in humans.

Methods

Subjects. Eight patients with IDDM (seven men and one woman) and nine nondiabetic persons (five men and four women) gave their written consent to participate in this study, which was approved by the Washington University Human Studies Committee. The mean (±SE) ages of the patients and controls were 26.0±2.7 and 25.0±1.1 yr, respectively. All were within 10% of ideal body weight (101±1% in patients and 103±3% in nondiabetic subjects). The patients, who had IDDM for 14.0±2.8 yr (range 3–25 yr), were selected for the absence of nephropathy (serum creatinine > 1.3 mg/dl and urine protein > 0.3 g/24 h), proliferative retinopathy, symptomatic autonomic neuropathy, postural hypotension (supine to standing decrement in mean blood pressure > 20 mmHg), hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg), overt heart disease (on clinical grounds or by electrocardiogram), anemia, and drug use other than insulin. All had documented histories of diabetic ketoacidosis. The patients were participants in the Diabetes Registry program of the Washington University Diabetes Research and Training Center, St. Louis, MO. Studies were performed at the Washington University General Clinical Research Center, St. Louis, MO.

Experimental design. All subjects were admitted to the General Clinical Research Center the day before study. Each was studied, after a 14-h overnight fast and in the supine position, on two occasions, during a control study and an islet clamp study, separated by at least 2 wk. The design is outlined in Fig. 1. Intermediate or long-acting insulin therapy was last administered 48 h before study in the patients; diabetes was managed with regular insulin thereafter. From 1600 h on the day before study to 0600 h on the day of study euglycemia (~90 mg/dl) was maintained with a variable intravenous insulin infusion, using a dosage algorithm based upon hourly bedside glucose monitoring (16). This is designated "open loop" in Fig. 1. Starting at 0600 h on the day of study, both patients and nondiabetic subjects were handled identically. Using a closed loop system, the Biostator, (Miles Ames Div., Miles Laboratories, Inc., Elkhart, IN), an individualized intravenous insulin infusion dose that produced stable plasma glucose levels of ~75 mg/dl was determined. When this was stable for at least 30 min, and no earlier than 0830 h, the insulin infusion rate was fixed (i.e., insulin infusion was no longer feedback controlled) and held constant at the 0800–0830 h rate throughout the remainder of the study. After an additional 30 min of base-line observations, L-epinephrine (Adrenaline; Parke, Davis & Co., Detroit, MI) was infused over 30-min intervals in doses of 0.1, 0.5, 1.0, 2.0, 3.0, and 5.0 μg/min. Epinephrine infusions were begun no earlier than 0900 h; the start of these infusions is designated 0 min in the presentation of the results. A primed (20 μCi), continuous (0.2 μCi/min) infusion of [3-^{3}H]glucose (11.5 Ci/nmol; New England Nuclear, Boston, MA) was started at 0700 h and continued through the remainder of the study. Observations were made at 10-min intervals for 30 min before and 180 min after the start of epinephrine infusions.

The islet clamp studies were identical to the control studies just described except that somatostatin (Beckman Instruments, Inc., Fullerton, CA) in a dose of 250 μg/h and glucagon (Eli Lilly Co., Indianapolis, IN) in a dose of 1.0 ng/kg^{-1} per min^{-1} were also infused from 0700 h through the end of the study. Thus, this is a modification of widely used clamp techniques (17), particularly the "pancreatic clamp" used extensively in dogs by Cherrington and colleagues (18, 19). A unique feature is the use of the Biostator to determine an insulin infusion dose that is individualized for each study to achieve and maintain a preselected plasma glucose concentration and then fixed at that rate. Because endogenous glucagon (as well as insulin) secretion is suppressed and replaced with exogenous glucagon, circulating glucagon levels are also fixed. This approach permits use of the plasma glucose concentration, as well as glucose kinetics, as response variables. The sequence of control and islet clamp studies was varied.

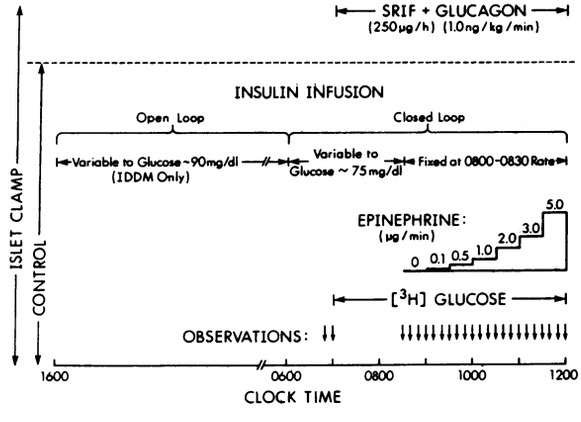

Figure 1. Design of the control and islet clamp studies. See Methods section of text for description. SRIF, somatostatin.

Analytical methods. Plasma glucose was measured with a glucose oxidase method on a glucose analyzer (Beckman Instruments, Inc.). Blood glycerol (20), β-hydroxybutyrate (20), lactate (21), and alanine (22) were measured with microfluorometric techniques, and serum fatty acids with a colorimetric method (23). Plasma epinephrine and norepinephrine were measured with a single isotope derivative method (24), using 50-μl samples. Plasma cortisol (25) and growth hormone (26) were measured with standard radioimmunoassays. To eliminate cross-reacting high molecular weight nonglucagon species (27, 28), plasma glucagon was measured (by double antibody radioimmunoassay using antiserum 30K) after precipitation of these with polyethylene glycol (PEG), using the method of Ensinck (29). Plasma, from blood samples collected in aprotinin (Trasylol, 500 IU/ml), was mixed with an equal volume of 26% PEG at 4°C for 1 h. Glucagon recoveries of 85% were used to correct the final values. For comparison, glucagon was also measured in aliquots of plasma not subjected to PEG precipitation. Without PEG, mean (±SD) plasma "glucagon" levels were 231±245 pg/ml (range 67–908 pg/ml) in base-line samples from nondiabetic subjects. Corresponding values after PEG were 67±28 pg/ml (range 33–114 pg/ml). The two determinations were not correlated ($r = 0.166$). Without PEG, mean plasma "glucagon" levels were 148±28 pg/ml (range 99–187 pg/ml) in base-line samples from the patients with IDDM. Corresponding values after PEG were 48±16 pg/ml (range 24–71 pg/ml). Again, the two determinations were not correlated ($r = 0.560$). Plasma insulin (30) and C-peptide (31) were also measured in the PEG supernatants. Materials for the C-peptide radioimmunoassay were provided by the Novo Research Institute, Copenhagen, Denmark. Standard curves were constructed in 13% PEG. Detection limits and between assay coefficients of variation were 10 pg/ml and 7% for epinephrine, 16 pg/ml and 5% for norepinephrine, 3 μU/ml and 12% for insulin, 0.01 nmol/liter and 10% for C-peptide, 12 pg/ml and 9% for glucagon, 0.5 ng/ml and 10% for growth hormone, and 3.0 μg/dl and 12% for cortisol. Glucose appearance (production) and disappearance (utilization) rates were calculated, as described previously (5, 6, 9).

Statistical methods. Statistical analysis involved fitting polynomial equations to individuals' time-related data for each parameter of interest and performing multivariate analysis of variance on the polynomial coefficients to identify group and condition differences. This approach eliminated the multiple comparison difficulties and lack of independence of observations associated with multiple tests performed at individual time points. The time-related data for each subject under each experimental condition were adjusted to base line by calculating the average for an individual's measured values before and including time 0 and subtracting this average value from all data points. A fourth degree polynomial was fit by least squares regression analysis to the adjusted data for each subject. These polynomial coefficients provided the data used in a multivariate analysis of variance. Four specific contrasts were tested for significance: the difference between the islet clamp study and the control study for patients with IDDM, the difference between the islet clamp study and the control study for nondiabetic subjects, the difference between subjects with IDDM and nondiabetics in the control study, and the difference between subjects with IDDM and nondiabetics in the islet clamp study. All calculations were performed on the Washington University Medical School's VAX 11/780 computer. The polynomial regression analyses were performed using the RS/1 V12.0 software package. The multivariate analyses of variance were performed using VAX SAS 4.07. Between and within group comparisons were then made with a t test for unpaired or paired data. Linear regression analysis was used to calculate correlation coefficients. The coefficient of variation is the standard deviation divided by the mean. Data are expressed as the mean±SE except where the standard deviation is specified.

Results

Base-line data. Base-line data in both the nondiabetic subjects and the patients with IDDM from both the control and islet clamp studies are shown in Table I. These zero time values are from just before initiation of epinephrine infusions, i.e., during fixed, individualized insulin infusions in both groups and both studies and fixed somatostatin and glucagon infusions in the islet clamp studies. They document that the basic objectives of the experimental design were accomplished: 1) plasma glucose concentrations and glucose production and utilization rates were comparable in both groups on both study days. 2) Plasma epinephrine, norepinephrine, free insulin, glucagon, and cortisol levels were comparable at base line in both groups on both study days. 3) Plasma C-peptide and growth hormone were suppressed on the islet clamp days. Further, C-peptide levels were substantially lower in patients with IDDM than in nondiabetic subjects before the control study. Inexplicably, base-line plasma growth hormone levels were somewhat higher in the nondiabetic subjects before the control study.

The base-line concentrations of intermediary metabolites are of particular interest. After 17 h of intravenous insulin infusion resulting in normal plasma glucose concentrations, glucose turnover rates, and reduced blood alanine levels, patients with IDDM exhibited elevated serum FFA and blood glycerol and β-hydroxybutyrate concentrations, despite normal plasma growth hormone and glucagon levels. Patients with IDDM also had higher heart rates and blood pressures.

Plasma catecholamine concentrations. Steady state plasma epinephrine concentrations, along with corresponding plasma norepinephrine concentrations, at the end of each 30-min epinephrine infusion are listed in Table II. Multivariate analysis (Table III) disclosed that plasma epinephrine curves (including all data points) did not differ significantly between groups in either study or between studies in either group. Plasma norepinephrine curves were slightly lower in nondiabetic subjects during the control, but not the islet clamp study; these did not differ between studies in either group (Table III).

Plasma glucose concentrations and kinetics (Fig. 2). During epinephrine infusions plasma glucose concentration, glucose production rate, and glucose utilization rate curves were significantly higher in patients with IDDM than in nondiabetic subjects during the control study, but these did not differ between the two groups during the islet clamp study (Table III). Compared with the control study, plasma glucose concentration and glucose production rate curves were significantly higher in the nondiabetic subjects, but not different in the patients with IDDM, during the islet clamp study (Table III).

During the control study, plasma glucose rose from a zero time value of 80±2 mg/dl to a final value of 116±5 mg/dl ($P < 0.001$) in the nondiabetic subjects and from 79±3 mg/dl to 181±14 mg/dl ($P < 0.001$) in the patients with IDDM in response to epinephrine infusions. Plasma glucose levels were significantly ($P < 0.005$) higher in the patients at epinephrine infusion rates of 0.5–5.0 μg/min. The higher plasma glucose concentrations in the patients with IDDM were largely the result of higher glucose production rates (Fig. 2). During the islet clamp study plasma glucose rose from a zero time value of 77±3 mg/dl to a final value of 205±12 mg/dl ($P < 0.001$) in the nondiabetic subjects and from 73±2 mg/dl to 208±18 mg/dl ($P < 0.001$) in the patients with IDDM in response to epinephrine infusions. The greater glycemic response to epinephrine in nondiabetic subjects during the islet clamp than during the control study was likewise largely due to increased glucose production.

*Table I. Base-line Data (Zero Minutes)**

	Control study			Islet clamp study		
	NL‡	IDDM	*P*	NL	IDDM	*P*
Hemoglobin A_1 (%)	6.3±0.3	10.1±1.2	<0.001	6.1±0.1	9.6±1.1	<0.001
Glucose concentration (*mg/dl*)	80±2	79±3	NS	77±3	73±2	NS
Glucose R_A§ (mg/kg^{-1} *per* min^{-1})	1.24±0.29	1.16±0.20	NS	1.89±0.28	1.53±0.26	NS
Glucose R_D‖ (mg/kg^{-1} *per* min^{-1})	1.10±0.25	1.10±0.17	NS	1.73±0.21	1.51±0.26	NS
Epinephrine (*pg/ml*)	74±18	77±13	NS	66±10	72±8	NS
Norepinephrine (*pg/ml*)	224±39	224±32	NS	203±24	233±41	NS
Insulin (*μU/ml*)	23±4	18±3	NS	18±2	16±3	NS
C-peptide (*nmol/liter*)	0.22±0.06	0.03±0.01	<0.01	0.05±0.01	0.02±0.00	<0.01
Glucagon (*pg/ml*)	67±9	48±6	NS	77±12	66±9	NS
Growth hormone (*ng/ml*)	8.7±3.1	3.8±1.2	<0.001	<0.5	<0.5	NS
Cortisol (*μg/dl*)	20.9±3.1	21.5±3.6	NS	16.9±1.7	16.8±2.7	NS
FFA (*μmol/liter*)	91±12	179±18	<0.01	82±20	140±12	<0.05
Glycerol (*μmol/liter*)	85±14	155±27	<0.05	105±12	166±30	NS
β-Hydroxybutyrate (*μmol/liter*)	90±9	401±94	<0.01	81±7	314±84	<0.02
Lactate (*μmol/liter*)	999±102	925±61	NS	1150±127	905±100	NS
Alanine (*μmol/liter*)	409±44	279±30	<0.05	445±40	335±22	<0.05
Heart rate (*beats/min*)	62±1	69±4	<0.001	60±2	66±3	<0.01
Systolic BP¶ (*mmHg*)	103±3	117±3	<0.001	100±3	109±3	<0.001
Diastolic BP (*mmHg*)	67±2	74±2	<0.01	64±2	73±2	<0.001
Insulin infusion (mU/kg^{-1} *per* min^{-1})	0.15±0.02	0.22±0.05	NS	0.16±0.03	0.12±0.12	NS

* Mean±SE. ‡ NL, nondiabetic subjects. § R_A, rate of glucose appearance (production). ‖ R_D, rate of glucose disappearance (utilization). ¶ BP, blood pressure.

*Table II. Plasma Catecholamine Concentrations**

Time	Epinephrine infusion rate	Control study		Islet clamp study	
		NL‡	IDDM	NL	IDDM
min	*μg/min*	*pg/ml*	*pg/ml*	*pg/ml*	*pg/ml*
Plasma epinephrine					
0	0	74±18	77±13	66±10	72±8
30	0.1	111±27	104±28	130±13	108±15
60	0.5	186±35	164±31	179±16	143±16
90	1.0	266±44	250±26	281±21	257±32
120	2.0	475±115	408±61	445±27	399±12
150	3.0	609±122	561±48	615±38	501±33
180	5.0	708±144	655±53	803±31	662±32
Plasma norepinephrine					
0	0	224±39	224±32	203±24	233±41
30	0.1	203±30	220±37	204±25	247±27
60	0.5	233±36	242±29	195±17	264±37
90	1.0	220±34	243±34	202±17	274±44
120	2.0	234±29	276±44	212±18	283±42
150	3.0	255±34	285±45	233±22	269±46
180	5.0	228±40	300±43	249±30	271±61

* Mean±SE. ‡ NL, nondiabetic subjects.

Table III. Multivariate P Values (Base-line Adjusted Data)

	Group effect (NL* vs. IDDM subjects)		Clamp effect (control vs. clamp studies)	
	Control study	Clamp study	NL subjects	IDDM subjects
Glucose concentration	0.030	0.720	0.004	0.627
Glucose R_A‡	0.034	0.672	0.031	0.480
Glucose R_D§	0.020	0.954	0.565	0.799
Epinephrine	0.630	0.089	0.303	0.783
Norepinephrine	0.041	0.767	0.091	0.622
Insulin	0.399	0.504	0.517	0.386
C-peptide	0.001	0.360	0.001	0.999
Glucagon	0.464	0.227	0.600	0.074
Growth hormone	0.697	0.413	0.144	0.136
Cortisol	0.296	0.099	0.423	0.442
FFA	0.379	0.057	0.630	0.023
Glycerol	0.410	0.680	0.929	0.729
β-Hydroxybutyrate	0.007	0.060	0.999	0.031
Lactate	0.617	0.108	0.182	0.505
Alanine	0.385	0.887	0.155	0.033
Heart rate	0.155	0.120	0.032	0.225
Systolic BP‖	0.811	0.491	0.975	0.658
Diastolic BP	0.163	0.344	0.098	0.415

* NL, nondiabetic subjects.
‡ R_A, rate of glucose appearance (production).
§ R_D, rate of glucose disappearance (utilization).
‖ BP, blood pressure.

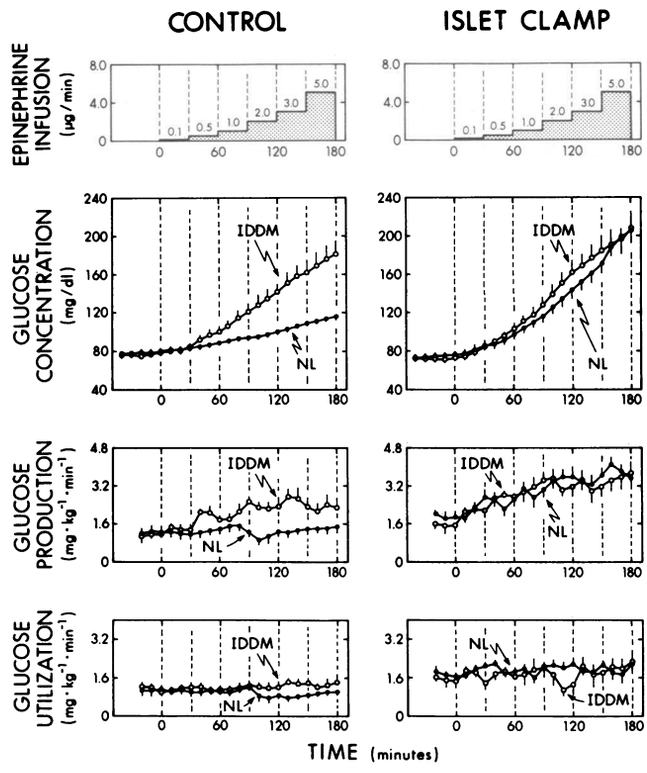

Figure 2. Mean (±SE) plasma glucose concentrations, glucose production rates, and glucose utilization rates during graded epinephrine infusions (top panels). Data from control studies are on the left, those from islet clamp studies on the right. NL, nondiabetic subjects.

The relationship between plasma glucose and epinephrine concentrations in both groups during both studies is shown in Fig. 3. Clearly, patients with IDDM exhibited an increased glycemic response to a given epinephrine level during the control study, and this enhanced glycemic response was produced in nondiabetic subjects during the islet clamp study. Further, increased glycemic responsiveness was demonstrated with short (30 min) increments in plasma epinephrine to levels in the range of 100–200 pg/ml.

Hormones (Fig. 4). During epinephrine infusions, plasma C-peptide curves were significantly higher in nondiabetic subjects than patients with IDDM, during the control study but not during the islet clamp study; C-peptide levels were higher during the control study than during the islet clamp study in the nondiabetic subjects but not in the patients with IDDM (Table III). Although an apparent increase in plasma free insulin from a zero time value of 23±4 μU/ml to a final value of 33±8 μU/ml was not significant, plasma C-peptide rose from 0.22±0.06 nmol/liter to 0.46±0.09 nmol/liter ($P < 0.01$) in nondiabetic subjects during the control study. Neither plasma insulin nor C-peptide levels changed during the control study in patients with IDDM or during the islet clamp study in either group; C-peptide levels remained suppressed throughout the islet clamp study in the nondiabetic subjects.

Plasma glucagon curves did not differ significantly between groups in either the control or islet clamp studies or between studies in either the nondiabetic subjects or the patients with IDDM (Table III). Nonetheless, in response to epinephrine infusions plasma glucagon levels increased (from a zero time value of 48±6 pg/ml to a maximum value of 112±26 pg/ml [$P < 0.05$]) only during the control study in patients with IDDM (Fig. 4). There was no evidence of breakthrough of either insulin or glucagon secretion during the islet clamp studies.

Plasma growth hormone and cortisol curves did not differ between groups in either study or between studies in either group (Table III). Plasma growth hormone decreased from a zero time value of 8.7±3.1 ng/ml to a final value of 1.0±0.4 ng/ml in nondiabetic subjects and from 3.8±1.2 ng/ml to 0.7±0.2 ng/ml ($P < 0.05$) in patients with IDDM during the control study, and remained suppressed to <0.5 ng/ml in both groups throughout the islet clamp study. Plasma cortisol declined in both groups during both studies (Fig. 4).

In response to epinephrine infusions serum FFA concentrations (Fig. 5) rose from a zero time value of 91±12 μmol/

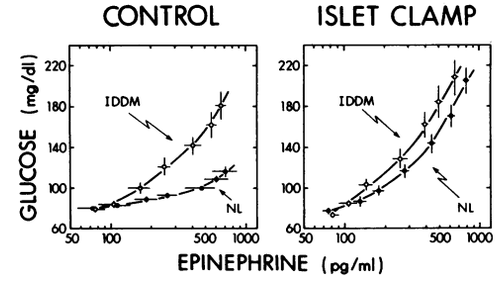

Figure 3. Mean (±SE) plasma glucose concentrations in relation to mean plasma epinephrine concentrations (plotted on a logarithmic scale) at the end of each 30-min epinephrine infusion. Data from control studies are on the left, those from islet clamp studies on the right. NL, nondiabetic subjects.

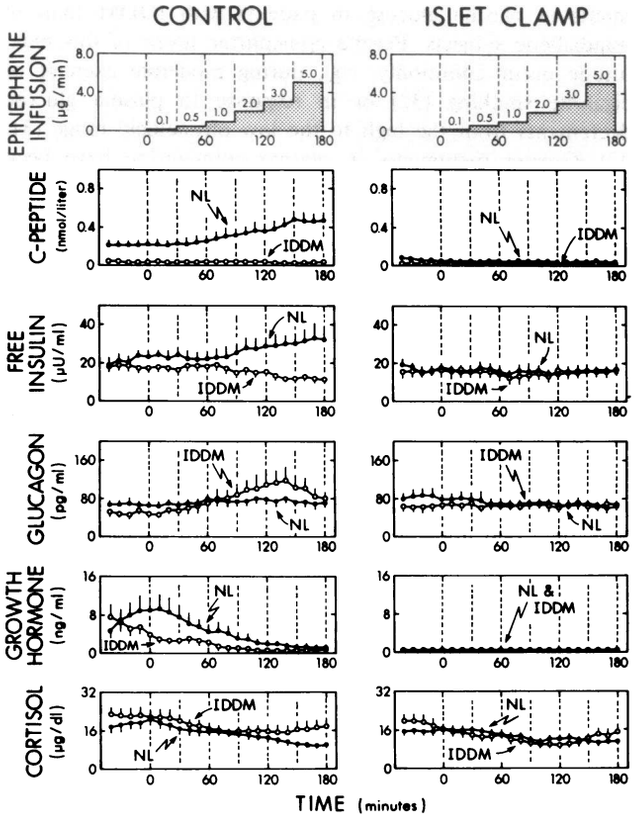

Figure 4. Mean (±SE) plasma C-peptide, free insulin, glucagon, growth hormone, and cortisol concentrations during graded epinephrine infusions (top panels). Data from control studies are on the left, those from islet clamp studies on the right. NL, nondiabetic subjects.

liter to a final value of 178±28 μmol/liter ($P < 0.02$) in the nondiabetic subjects and from 179±18 μmol/liter to 322±50 μmol/liter ($P < 0.05$) in the patients with IDDM during the control study. Similar increments, from 82±20 μmol/liter to 208±43 μmol/liter ($P < 0.01$) in the nondiabetic subjects and from 140±12 μmol/liter to 276±31 μmol/liter ($P < 0.01$) in

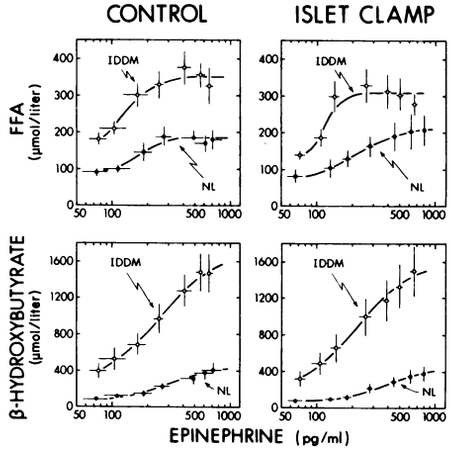

Figure 5. Mean (±SE) serum FFA and blood β-hydroxybutyrate concentrations in relation to mean plasma epinephrine concentrations (plotted on a logarithmic scale) at the end of each 30-min epinephrine infusion. Data from control studies are on the left, those from islet clamp studies on the right. NL, nondiabetic subjects.

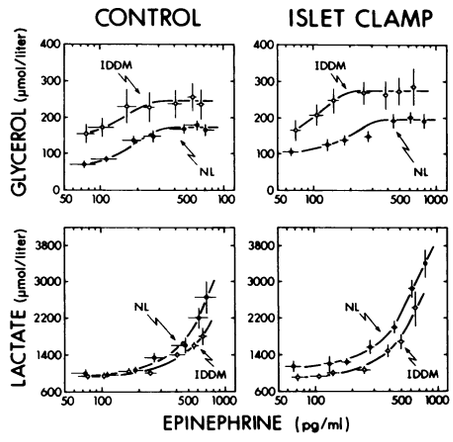

Figure 6. Mean (±SE) blood glycerol and lactate concentrations in relation to mean plasma epinephrine concentrations (plotted on a logarithmic scale) at the end of each 30-min epinephrine infusion. Data from control studies are on the left, those from islet clamp studies on the right. NL, nondiabetic subjects.

the patients with IDDM, occurred during the islet clamp study. Blood glycerol concentrations (Fig. 6) rose from a zero time value of 85±14 μmol/liter to a final value of 166±17 μmol/liter ($P < 0.01$) in the nondiabetic subjects and from 155±27 μmol/liter to a maximum value of 255±40 μmol/liter ($P < 0.05$) in the patients with IDDM during the control study. Similar increments, from 105±12 μmol/liter to 188±22 μmol/liter ($P < 0.01$) in the nondiabetic subjects and from 166±30 μmol/liter to 286±52 μmol/liter (NS) in the patients with IDDM, occurred during the islet clamp study. Blood β-hydroxybutyrate concentrations (Fig. 5) rose from a zero time value of 90±9 μmol/liter to a final value of 412±83 μmol/liter ($P < 0.01$) in the nondiabetic subjects and from 401±94 μmol/liter to 1,467±μmol/liter ($P < 0.001$) in the patients with IDDM during the control study. Similar increments, from 81±7 μmol/liter to 384±84 μmol/liter ($P < 0.01$) in the nondiabetic subjects and from 314±84 μmol/liter to 1,499 μmol/liter ($P < 0.01$) in the patients with IDDM, occurred during the islet clamp study.

Blood lactate curves did not differ significantly between groups in either the control or the islet clamp studies nor between studies in either the nondiabetic subjects or the patients with IDDM (Table III). During epinephrine infusions, blood lactate rose from a zero time value of 999±102 μmol/liter to a final value of 2,671±361 μmol/liter ($P < 0.001$) in the nondiabetic subjects and from 925±61 μmol/liter to 1,794±177 μmol/liter ($P < 0.01$) in the patients with IDDM during the control study. Similar increments, from 1,150±127 μmol/liter to 3,403±317 μmol/liter ($P < 0.001$) in the nondiabetic subjects and from 905±100 μmol/liter to 2,421±376 μmol/liter ($P < 0.01$) in the patients with IDDM, occurred during the islet clamp study. The relationships between lactate and epinephrine levels are also shown in Fig. 6.

When adjusted for differences at base line, blood alanine curves did not differ significantly between groups in either the control or islet clamp studies; blood alanine curves were slightly higher in the patients with IDDM during the islet clamp study (Table III). During epinephrine infusions, blood alanine concentrations did not change significantly (data not shown).

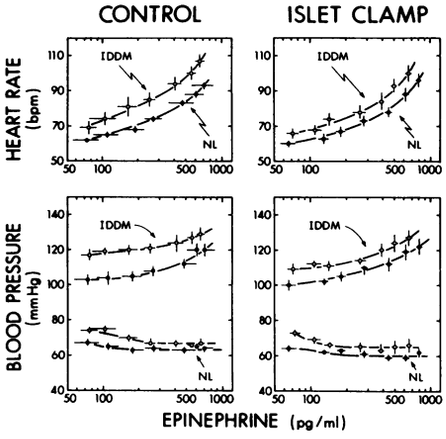

Figure 7. Mean (±SE) heart rates and systolic (*top*) and diastolic (*bottom*) blood pressures in relation to mean plasma epinephrine concentrations (plotted on a logarithmic scale) at the end of each 30-min epinephrine infusion. Data from control studies are on the left, those from islet clamp studies on the right. bpm, Beats per minute; NL, nondiabetic subjects.

Heart rate and blood pressure (Fig. 7). When adjusted for differences at base line, heart rate curves did not differ significantly between groups in either study. The heart rate curve for nondiabetic subjects was slightly lower during the islet clamp study than during the control study (Table III). In response to epinephrine infusions, heart rate increased similarly in both groups in both studies. When adjusted for differences at base line, systolic and diastolic blood pressure curves did not differ significantly between groups in either study or between studies in either group (Table II). In response to epinephrine infusions, systolic blood pressure increased similarly in both groups in both studies. Diastolic blood pressure did not change significantly in either group in either study.

Discussion

These data demonstrate that patients with IDDM, infused with insulin in doses sufficient to produce normal plasma glucose concentrations and glucose turnover rates at base line, exhibit an enhanced glycemic response to epinephrine, results similar to those reported by Shamoon et al. (1). Greater epinephrine-stimulated increments in the plasma glucose concentration are largely the result of greater increments in glucose production in patients with IDDM (present data and reference 1). However, since plasma glucose concentrations are higher in the patients one could reason that glucose utilization rates only slightly greater than those of nondiabetic subjects are inappropriately low and, therefore, that limitation of glucose utilization by epinephrine is also a factor.

The present dose-response data with and without the islet clamp technique extend previous information in that they demonstrate that increased glycemic responsiveness to epinephrine occurs with short increments in plasma epinephrine to levels that span the physiologic range in patients with IDDM, establish that enhanced glycemic responsiveness to epinephrine in patients with IDDM is the result of their inability to augment the secretion of insulin, and indicate that augmented insulin secretion normally limits the glycemic (but not the lipolytic or ketogenic) response to epinephrine.

Short (30 min) increments in epinephrine to plasma concentrations as low as 100–200 pg/ml resulted in greater increments in plasma glucose in patients with IDDM than in nondiabetic subjects. Plasma epinephrine levels of this magnitude occur commonly, e.g., during moderate exercise or cigarette smoking (32) or in response to plasma glucose decrements from the high to the low physiologic range (31, 33). Greater increments in plasma epinephrine have been documented during vigorous exercise, during and after surgery, following myocardial infarction, and during diabetic ketoacidosis as well as in response to hypoglycemia (32). Thus, enhanced glycemic responsiveness to epinephrine is likely to be relevant to glycemic control during the daily lives of patients with IDDM as well as during the stress of intercurrent illness.

In theory, enhanced glycemic responsiveness to epinephrine could be the result of increased sensitivity of cellular adrenergic receptor-effector mechanisms per se in patients with IDDM. The literature concerning the effects of experimental diabetes on adrenergic receptor-effector systems (34–42) includes only one report consistent with increased β-adrenergic sensitivity (41). Increased lipolytic, cAMP, and protein kinase responses to epinephrine in adipocytes from streptozotocin diabetic rats were reported (41), but others found reduced basal and isoproterenal-stimulated adenylate cyclase activity and decreased β-adrenergic receptor density in adipocytes from similar animals (42). In humans with diabetes, reduced adipocyte phosphodiesterase activity (43) and increased pressor responses to norepinephrine (44) have been reported. In an earlier study, we found mononuclear leukocyte β_2-adrenergic receptors and adenylate cyclase sensitivity to agonist to be normal in patients with IDDM selected for the absence of adrenergic neuropathy, and concluded that there is not a generalized increase in β-adrenergic receptors or in the sensitivity of their linked adenylate cyclase systems in such patients (15). To the extent that measurements on circulating cells faithfully reflect adrenergic receptor-effector mechanisms in extravascular target cells, those data did not support the possibility that an increase in these might underlie the enhanced glycemic responsiveness to epinephrine in patients with IDDM. Thus, available evidence indicates that the sensitivity of β-adrenergic receptor-effector mechanisms, relevant to the hyperglycemic effect of epinephrine in patients with IDDM as discussed earlier, is not increased. Therefore, in the present study we tested the alternative hypothesis that increased glycemic responsiveness to epinephrine in patients with IDDM is the result of decreased insulin secretion, increased glucagon secretion, or both.

To test this hypothesis, we compared the responses to epinephrine of patients with IDDM and nondiabetic subjects under conditions in which glucagon and insulin could change (the control study) with the responses to epinephrine under conditions in which endogenous glucagon and insulin secretion were suppressed with somatostatin and glucagon and insulin were replaced by infusions and held constant at levels that produced stable, normal plasma glucose concentrations and glucose kinetics at base line. The latter "islet clamp" study demonstrated that the enhanced glycemic response to epinephrine in the control study was not the result of the increased glucagon secretory response in the patients with IDDM because their enhanced glycemic response was unchanged when glucagon levels were held constant in the islet clamp study. On the other hand, increased glycemic responsiveness was produced in nondiabetic subjects during the islet clamp study; its degree was virtually identical to that in patients with IDDM in both studies. This can only be attributed to the demonstrated

inhibition of insulin secretion in the nondiabetic subjects; if it were the result of another effect of somatostatin the glycemic response of the patients with IDDM should also have been altered. Thus, the data establish that patients with IDDM exhibit enhanced glycemic responsiveness to epinephrine because they cannot augment insulin secretion as the plasma glucose concentration rises.

It should be emphasized that the patients studied were selected for the absence of diabetic adrenergic neuropathy. It is conceivable that chronically decreased catecholamine release could lead to up-regulation of adrenergic receptor-effector systems. Indeed, Hilsted and co-workers (45) have reported recently that patients with diabetic autonomic neuropathy exhibit a somewhat greater glycemic response to epinephrine than those without autonomic neuropathy; both groups, of course, had substantially greater glycemic responses than nondiabetic subjects.

The data also provide insight into physiologic modulation of the glycemic response to epinephrine. Although limitation of insulin secretion contributes to the hyperglycemic effect of epinephrine (2–6), as discussed earlier, it is clear from the present data that an increase in insulin secretion, albeit restrained, normally limits the glycemic response to epinephrine. During the control study in nondiabetic subjects epinephrine infusions resulted in a twofold increase in plasma C-peptide concentrations and a relatively small increase in plasma glucose; undoubtedly restrained by α-adrenergic stimulation, this increase in insulin secretion could have been the result of a rising plasma glucose concentration, β-adrenergic stimulation, or both. However, when these nondiabetic subjects were unable to secrete insulin during the islet clamp study, their glycemic response to epinephrine was increased more than threefold to levels comparable to those of patients with IDDM.

In contrast to the glycemic response, the data indicate that insulin secretion does not normally limit the lipolytic or ketogenic responses to epinephrine and that patients with IDDM have an enhanced ketogenic, but not lipolytic, response to the hormone. Despite 17 h of intravenous insulin in doses sufficient to produce normal plasma glucose concentrations and glucose turnover rates, as well as suppressed blood alanine levels, our patients with IDDM had elevated base-line serum FFA and blood glycerol and β-hydroxybutyrate levels on both study occasions. This pattern has been observed in several (46–48), but not all (49), previous studies using intravenous insulin and in a study using subcutaneous insulin (50) in patients with IDDM. In view of these differences at base line in the two groups, comparisons of the responses with epinephrine must be interpreted with caution. Nonetheless, since absolute increments in serum fatty acids and blood glycerol were similar in both groups, patients with IDDM do not appear to have enhanced lipolytic responsiveness to epinephrine. This contrasts with the greater absolute increases in blood β-hydroxybutyrate during epinephrine infusions in the patients with IDDM compared with the nondiabetic subjects. Thus, the data from the control study confirm other reports (51, 52) that patients with IDDM exhibit an enhanced ketonemic response to epinephrine that is not attributable to enhanced lipolysis and FFA delivery to the liver. Although one cannot exclude limited ketone clearance in patients with IDDM in the absence of kinetic measurements, this would seem an unlikely explanation since the ketonemic response to epinephrine is primarily due to an increase in ketone body production in nondiabetic subjects, even when insulin secretion is suppressed with somatostatin and ketone body concentrations are elevated (53). The data from the islet clamp study clarify but do not explain this observation. Since the enhanced ketonemic response to epinephrine occurred in both the control and islet clamp studies in the patients with IDDM, it is not attributable to increased glucagon secretion; since it was not produced in nondiabetic subjects during the islet clamp study, it is also not attributable to the inability of patients with IDDM to augment insulin secretion. Since the enhanced ketonemic, presumably ketogenic, responsiveness of patients with IDDM to epinephrine is not explicable on the basis of the dominant extracellular regulatory factors (increments in FFA delivery, glucagon, or the glucagon to insulin molar ratio [54]), it would appear to be the result of hepatocellular alterations not corrected by short-term insulin infusion sufficient to produce normal glucose concentrations and turnover rates. We would speculate that this might be depletion of fructose 2,6-bisphosphate, which would favor ketogenesis (54, 55); decreased hepatic fructose 2,6-bisphosphate levels correlate with ketosis and are restored to normal by relatively long-term, but not short-term insulin therapy in streptozotocin diabetic mice (55).

In contrast to the comparison between groups, the physiologic interpretation of the data with respect to the lipolytic and ketogenic responses to epinephrine is straightforward. It is clear from the data from the nondiabetic subjects that augmented insulin secretion during epinephrine infusions does not normally limit the lipolytic or ketogenic responses since increments in circulating FFA, glycerol, and β-hydroxybutyrate were not greater when insulin was held constant during the islet clamp study. This should not, of course, be taken to suggest that basal insulin does not limit the lipolytic and ketogenic responses to epinephrine; there is good evidence that it does (53).

We interpret the data in the physiologic context as follows. Although α-adrenergic restraint of insulin secretion contributes to the glycemic response to epinephrine (3–7), some augmentation of insulin secretion occurs normally in response to a rising plasma glucose concentration, β-adrenergic stimulation, or both. The increment in insulin secretion is relatively small; in the present study plasma C-peptide increased only twofold at the highest epinephrine level. Such insulin increments would be expected to impact largely on hepatic metabolism with minimal peripheral effects, at least with respect to carbohydrate metabolism (56). Thus, increments in insulin during epinephrine elevations are sufficient to limit the epinephrine-induced increase in hepatic glucose production and the glycemic response but are not sufficient to limit the lipolytic response, presumably at peripheral sites, to the hormone. Since they do not limit the lipolytic response, they also do not limit the ketogenic response to epinephrine, which is determined primarily by FFA delivery to the liver (54, 57). From this it follows that patients with IDDM are unable to limit the epinephrine-induced increase in glucose production and the glycemic response because they are unable to augment insulin secretion, whereas the lipolytic response to the hormone is unaltered. However, an enhanced ketogenic response to epinephrine in such patients is unexplained. Lastly, increments in glucagon secretion do not appear to contribute to the glycemic, lipolytic, or ketogenic responses to epinephrine in humans.

From the data presented we draw the following conclusions: 1) Short, physiologic increments in plasma epinephrine cause greater increments in plasma glucose in patients with IDDM

than in nondiabetic subjects, a finding likely to be relevant to glycemic control during the daily lives of such patients as well as during the stress of intercurrent illness. 2) Enhanced glycemic responsiveness of patients with IDDM to epinephrine is not the result of increased sensitivity of adrenergic receptor-effector mechanisms per se nor of their increased glucagon secretory response; rather, it is the result of their inability to augment insulin secretion. 3) Insulin secretion, albeit restrained, normally limits the glycemic response, but not the lipolytic or ketogenic responses, to epinephrine in humans.

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