A Role for Alpha-Adrenergic Receptors in Abnormal Insulin Secretion in Diabetes Mellitus

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ABSTRACT To determine whether endogenous alpha-adrenergic activity contributes to abnormal insulin secretion in nonketotic, hyperglycemic, diabetic patients, alpha-adrenergic blockade was produced in normal and diabetic subjects. The diabetics had a significantly ($P < 0.01$) greater increase in circulating insulin 1 h after an intravenous phentolamine infusion than did the normal subjects. During the phentolamine infusion, there was also a significant augmentation of acute insulin responses to intravenous glucose (20 g) pulses in normal subjects ($P < 0.05$) and diabetics ($P < 0.02$); this augmentation was fivefold greater in the diabetics. Simultaneous treatment with the beta-adrenergic blocking agent, propranolol, did not alter these findings. Thus a role for exaggerated endogenous alpha-adrenergic activity in abnormal insulin secretion of the diabetic subjects is suggested. To determine whether this alpha-adrenergic activity might be related to elevated circulating catecholamines, total plasma-catecholamine levels were compared in normal and nonketotic diabetic subjects given intravenous glucose pulses. These levels were significantly greater ($P < 0.02$) in the diabetic compared to the normal group before the glucose pulse, and increased significantly in both groups ($P < 0.02$ and < 0.001, respectively) after the pulse. These data suggest that excessive catecholamine secretion may lead to an abnormal degree of endogenous alpha-adrenergic activity, which contributes to defective insulin secretion in diabetic subjects.

INTRODUCTION

An intravenous glucose pulse stimulates secretion of insulin within minutes of injection in normal subjects, resulting in dose-related increments in circulating insulin which are usually maximal from 3 to 5 min after injection (1). This immediate release of insulin has been termed the acute insulin response. In contrast to normal subjects, patients with fasting hyperglycemia have markedly diminished or absent acute insulin responses to intravenous glucose stimulation (2). Several years ago, we reported that treatment with the alpha-adrenergic blocking agent, phentolamine, partially restored the acute insulin response in a nonketotic diabetic subject (3). This phenomenon was of particular interest because catecholamine-induced alpha-adrenergic activity inhibits glucose-induced insulin secretion in normal subjects (4), and because circulating plasma catecholamines are elevated in diabetic ketosis (5). It thereby seemed possible that an abnormal alpha-adrenergic state in nonketotic diabetics might be contributing to the absence of the acute insulin response after glucose stimulation.

Consequently, several questions were raised: (*a*) Is there an abnormal degree of endogenous alpha-adrenergic activity in subjects with nonketotic diabetes mellitus?; (*b*) Does endogenous alpha-adrenergic activity contribute to abnormal acute insulin responses in nonketotic diabetics?; and (*c*) Are circulating plasma catecholamine levels in nonketotic diabetics elevated and thereby a source of excessive alpha-adrenergic activity? The answers to these questions were sought in studies of nonketotic diabetic and normal subjects by comparing (*a*) basal insulin levels before and during treatment with the alpha-adrenergic blocking agent, phentolamine; (*b*) acute insulin responses to intravenous glucose pulses before and during treatment with phentolamine; and (*c*) circulating total catecholamine levels before and after an intravenous glucose challenge.

METHODS

Normal subjects and diabetic patients of varying degrees of obesity were studied. The normal subjects had no personal or family history of diabetes mellitus. The diabetic

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TABLE I

Comparison of Circulating Glucose and Insulin Levels before and after a Glucose Pulse (20 g i.v.) in 12 Diabetic and 44 Normal Subjects

Patients	Fasting plasma glucose	Fasting serum insulin	After glucose pulse: Mean Δ3'5' insulin	After glucose pulse: Mean Δ3'5' insulin
	mg/dl	*μU/ml*	*μU/ml*	%
1	272	9	−1	−11
2	291	9	+6	+67
3	234	12	−1	−8
4	254	14	−4	−29
5	304	15	+3	+20
6	321	17	+4	+24
7	132	18	0	0
8	330	21	−4	−19
9	224	22	−1	−5
10	306	26	−4	−15
11	173	34	−11	−32
12	217	47	−5	−11
Mean	257	20	−2	−2
±SD	58	11	4	26
Normal (n = 44)				
mean	90	14	93	686
±SD	5	7	72	393

subjects were all nonketotic, noninsulin-dependent, known to have fasting hyperglycemia (plasma glucose greater than 115 mg/dl), and had received no oral hypoglycemic agents for at least 5 days before the experiments. All studies were conducted after a 12-h fast and at bed rest between the hours of 8 a.m. and 1 p.m. Intravenous 0.85% sodium chloride infusions were begun in both arms 1 h before administration of the various test agents. All blood samples were drawn through three-way stopcocks to avoid additional venepunctures during the infusions.

Alpha-adrenergic blockade was provided by intravenously infusing phentolamine (0.5 mg/min). Glucose stimulation was provided by intravenously injecting 20 g of glucose in less than 30 s. Blood samples were drawn at 15, 30, 45, and 60 min before and after the beginning of the phentolamine infusion, and at 2, 3, 4, 5, 7, 10, 15, 30, 45, 60, and 90 min after the intravenous glucose pulses had been given. Basal insulin levels were calculated as the mean of the four samples drawn 15 min apart before the phentolamine infusions. The acute insulin response (mean 3–5 min Δ immunoreactive insulin [IRI]) [1] was defined as the mean of the 3-, 4-, and 5-min postglucose injection insulin values for a given subject subtracted from the mean of the basal values for that subject. After collection in heparin, blood samples for glucose were kept at 4°C until the end of the study and centrifuged at 4°C; the plasma was frozen for future analysis by an AutoAnalyzer-ferricyanide method (6). Blood samples for insulin were allowed to clot at room temperature and centrifuged; the serum was frozen for future analysis by a modification of the method of Morgan and Lazarow (7). This assay has an intraassay variation of 10% and an interassay variation of 20%. All samples from one subject were analyzed in the same assay. Total plasma catecholamines were analyzed by a double-isotope derivative method described by Engleman et al. (8), and as modified by Christensen (9). This method has a 15% recovery of [^{3}H]norepinephrine and a 10% intraassay variation.

[1] *Abbreviation used in this paper:* IRI, immunoreactive insulin.

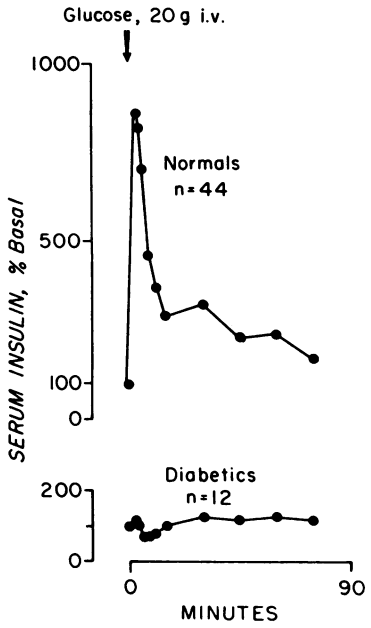

FIGURE 1 Comparison of mean serum insulin responses to an intravenous glucose pulse (20 g) in normal and diabetic subjects.

RESULTS

Acute insulin responses in normal and diabetic subjects. The mean fasting plasma glucose level in the diabetic subjects (Table I) was markedly increased compared to the normal subjects (diabetics, 257±58,

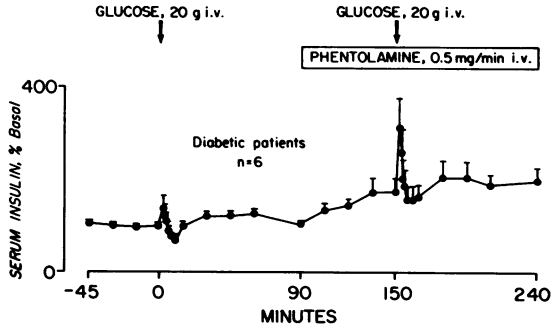

FIGURE 2 The effect of alpha-adrenergic blockade (phentolamine, 0.5 mg/min i.v.) upon serum insulin levels expressed as percent basal (mean±SE) before and during an intravenous glucose pulse (20 g) in diabetic subjects.

TABLE II

Comparison of Circulating Insulin Levels (μU/ml) before and after a 1-h Intravenous Phentolamine Infusion (0.5 mg/min) in Normal and Diabetic Subjects

	Phentolamine		
	Before	After	Δ
Diabetic patients			
1	9	27	18
2	17	34	17
3	21	33	12
4	22	26	4
5	26	29	3
6	47	76	29
Mean±SD	24±13	38±19	14±9
Normal subjects			
1	6	9	3
2	7	12	5
3	7	12	5
4	7	10	3
5	8	13	5
6	9	9	0
7	10	12	2
8	10	10	0
9	11	16	5
10	13	16	3
11	15	18	3
12	16	19	3
Mean±SD	10±3	13±4	3±2

$n = 12$; normal group, 90±5, $n = 44$; mean±SD, mg/dl, $P < 0.001$) but the fasting serum insulin levels were comparable (diabetics, 20±11; normal groups, 14±7; μU/ml). After the glucose pulse all subjects in the normal group had an acute insulin response (Fig. 1), the mean of which was 93±72 μU/ml. There was a significant ($r = 0.50$, $n = 44$, $P < 0.001$) linear correlation between the basal insulin level and the acute insulin response in the normal group. Since this relationship validates expression of acute insulin-response data as percent of basal, the acute insulin response of the normal group can be calculated to be 686±393% of initial basal level. In contrast to the normal group, acute insulin responses in the diabetic group (Fig. 1) were absent (−2±4 μU/ml or −2±26% of initial basal level).

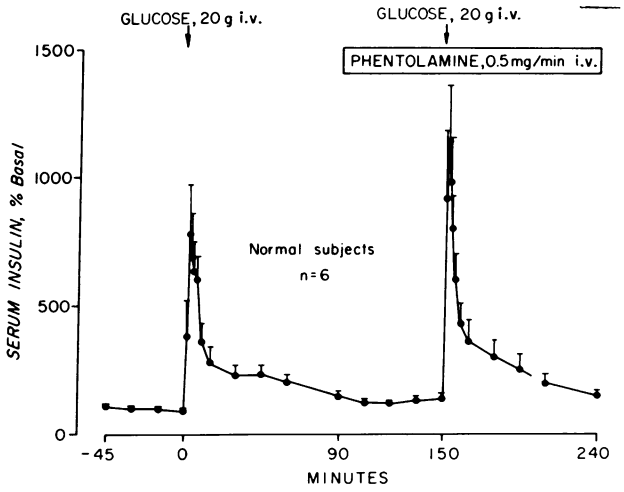

FIGURE 3 The effect of alpha-adrenergic blockade (phentolamine, 0.5 mg/min i.v.) upon serum insulin levels expressed as percent basal (mean±SE) before and during an intravenous glucose pulse (20 g) in normal subjects.

The effect of alpha-adrenergic blockade upon basal insulin levels in diabetic and normal subjects. After 1 h of the phentolamine infusion there was a significantly greater increment in circulating insulin levels in the diabetic patients than in the normal subjects (diabetics, 14±9; normal group, 3±2; insulin increments in microunits per milliliter; $P < 0.01$; Table II). There was no correlation between the magnitude of these increments and the basal insulin levels, nor were there changes in circulating glucose levels during the phentolamine infusion in either the diabetic or the normal groups. Three diabetic subjects were given simultaneous intravenous infusions of both phentolamine and propranolol (80 μg/min intravenously). The increments in circulating insulin at the end of 1 h of the combined infusions were 3, 3, and 9 μU/ml; there was no increase in circulating glucose.

The effect of alpha-adrenergic blockade upon acute insulin responses to glucose in diabetic and normal subjects. Six diabetic patients and six normal subjects were given glucose pulses before, and then during intravenous infusion of phentolamine (Figs. 2 and 3). In all diabetic subjects the acute insulin response to glucose during alpha-adrenergic blockade was greater than that observed before infusion with phentolamine, whether the data (Table III) is expressed in absolute terms (before phentolamine, −1±4 μU/ml; during phentolamine, +10±11 μU/ml; $P < 0.02$) or as percentage basal (before phentolamine, +7±30%; during phentolamine, +44±32%; $P < 0.02$). Two diabetic subjects were given glucose pulses before and then during intravenous infusions of both phentolamine and propranolol (80 μg/min intravenously). In both subjects the acute insulin response to glucose was greater than that observed before infusion of the adrenergic drugs whether expressed as absolute values before, −1 and −4; during, +4 and +2; μU/ml) or as percent of initial basal level (before, 93 and 71%; during, 156 and 172%).

Similarly, in five of the six normal subjects, the acute insulin response was greater ($P < 0.05$) during infusion with phentolamine, whether the data is expressed in absolute terms or as percent basal (Table III). How-

TABLE III

Comparison of Acute Insulin Responses after a Glucose Pulse (20 g i.v.) Both before and during Alpha-Adrenergic Blockade with Phentolamine (0.5 mg/min i.v.) in Normal Subjects and Diabetic Patients

	Mean Δ 3'5' insulin		% Δ 3'5' insulin		
	Initial	During phentolamine	Initial	During phentolamine	
Diabetic patients					
2	+6	+8	+67	+89	
6	+4	+11	+24	+65	
8	−4	+3	−19	+14	
9	−1	+6	−5	+27	
10	−4	0	−15	0	
12	−5	+33	−11	+70	
Mean	−1	+10	+7	+44	% Δ ratio
±SD	4	11	30	32	44/7 = 6.29
Normal subjects					
3	57	82	814	1,171	
4	36	61	514	871	
5	35	60	438	750	
8	117	117	1,170	1,170	
9	67	95	609	864	
12	23	28	144	175	
Mean	56	74	615	834	% Δ ratio
±SD	34	31	350	366	834/615 = 1.36

ever, the ratio of acute responses (expressed as percent Δ 3'-5' IRI) during and before phentolamine was approximately fivefold greater in the diabetic group (6.29 vs. 1.36).

Comparison of circulating total plasma catecholamine levels in diabetic and normal subjects before and during intravenous glucose stimulation. The mean plasma-catecholamine level before glucose injection was significantly greater in the diabetic group compared to the normal group (diabetics, 370±120; normal group, 200 ±60; pg/ml, $P < 0.02$; Fig. 4). After the glucose pulse, both the diabetic and normal subjects had small but significant increases in mean circulating catecholamine levels (diabetics, 70±60, $P < 0.02$; normal group, 50 ±30, $P < 0.001$; pg/ml, mean increment over basal catecholamine level for 15 min). The catecholamine response appeared to be somewhat delayed in the diabetic group compared to the normal group. No diabetic subjects, but all normal subjects, had acute insulin responses to the intravenous glucose pulse.

DISCUSSION

These studies demonstrate that alpha-adrenergic blockade increases basal insulin levels in diabetics to a greater extent than in normal subjects and also partially restores the acute insulin response to intravenous glucose stimulation in diabetics. The simultaneous phentolamine and propranolol infusions in the diabetic subjects were conducted because of the theoretical possibility that alpha-adrenergic blockade during ongoing adrenergic stimulation would result in relative increases in beta-adrenergic effects. The fact that increases in

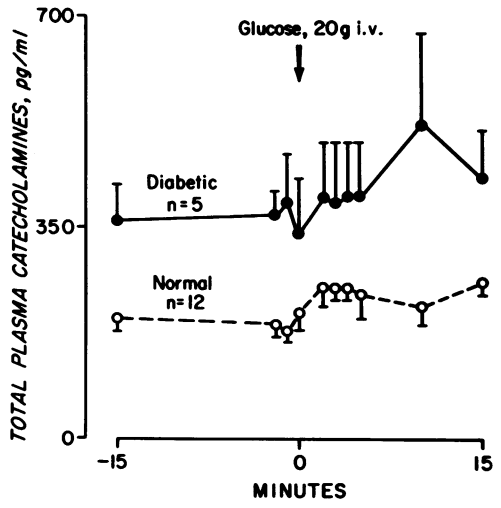

FIGURE 4 Comparison of plasma total catecholamine levels (mean±SE) before and after an intravenous glucose pulse (20 g) in normal and diabetic subjects.

basal insulin levels and improved acute insulin responses to glucose persisted during alpha blockade, despite simultaneous beta blockade, demonstrates that this augmentation of insulin secretion was directly a result of less alpha-adrenergic, and not more beta-adrenergic, stimulation. This distinction is important since beta-adrenergic stimulation itself can cause insulin secretion in both normal and diabetic subjects (2). The data which demonstrate that circulating catecholamine levels, both in the basal state and immediately after glucose stimulation, are greater in diabetics than in normal subjects provide a likely hormonal candidate to explain the observed increases in endogenous alpha-adrenergic activity in these nonketotic diabetic patients.

We have previously proposed that the increases in basal insulin levels in normal subjects caused by alpha-adrenergic blockade are a reflection of the removal of tonic alpha-adrenergic inhibition of basal insulin secretion (10). The data described herein suggest that this phenomenon is exaggerated in nonketotic diabetes mellitus. Independent data which support this viewpoint have been provided by Linde and Deckert, who reported increments in basal insulin during alpha-adrenergic blockade in diabetic but not in normal subjects (11). 9 of the 11 diabetics studied by these investigators were only marginally hyperglycemic and therefore their results are not strictly comparable to ours. However, it is noteworthy that even in their mildly diabetic patients, alpha-adrenergic blockade improved glucose-stimulated insulin responses. The fact that glucose-stimulated insulin responses were augmented by phentolamine in our normal subjects, together with previously reported data (12), indicates that this augmentation is not unique to diabetes, although it may be exaggerated in diabetic patients. The importance of this phenomenon to our understanding of pathophysiology in diabetes, however, lies not in its seemingly quantitatively greater effect in diabetic subjects, but rather in the fact that such a markedly abnormal acute insulin response can be improved at all. Since diabetes is a chronic disease, it is not surprising that a brief 1-h infusion of phentolamine could not completely normalize the acute insulin response. Clearly, an extended trial of oral alpha-adrenergic antagonists, preferably specific for pancreatic alpha-adrenergic effects, would be required before one could determine the maximal degree to which the acute insulin response could be improved.

Christensen has reported that circulating epinephrine and norepinephrine levels are elevated in ketotic diabetics (5). The data reported herein appear to be the first demonstration that circulating catecholamine levels in the basal state are elevated in nonketotic diabetics as well. The observation that an intravenous glucose pulse is accompanied by increases in circulating catecholamine levels in a smaller group of normal subjects has been previously reported by us (13). The current data in an expanded group of normal individuals confirm our previous report and suggest that a similar trend is also present in nonketotic diabetics. In conclusion, these findings suggest that nonketotic diabetics have higher rates of catecholamine secretion both in the basal state and after intravenous glucose stimulation than do normal subjects, and thereby may provide a hormonal explanation for the postulated and exaggerated alpha-adrenergic state in diabetes mellitus, and its untoward effects upon glucose-stimulated insulin secretion.

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