FURTHER STUDIES ON DIAZOXIDE SUPPRESSION OF INSULIN RELEASE FROM ABNORMAL AND NORMAL ISLET TISSUE IN MAN*

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In previous reports,[1,2] we described studies of the effects of diazoxide, given in combination with trichlormethiazide, on plasma insulin and blood glucose levels in healthy subjects and in patients with functioning islet cell tumors of the pancreas. From these studies, we have concluded: (a) that decreased insulin secretion, as well as a failure of appropriate increases in insulin secretion, are involved in the mechanisms by which diazoxide and trichlormethiazide induce hyperglycemia; (b) that these compounds inhibit insulin release induced by the administration of glucose and leucine; and (c) that these benzothiadiazines also increase blood glucose by mechanisms that do not involve alterations in plasma insulin.

Graber and coworkers,[3,4] Seltzer and Allen[5] and Marks and coworkers[6] have also reported that diazoxide decreases plasma levels of insulin in patients with beta-cell tumors of the pancreas,[3,4,6] in healthy subjects[4,5] and in diabetic patients.[3,4] Frerichs and associates,[7] and Howell and Taylor[8] have shown by *in vitro* experiments that diazoxide causes an inhibition of insulin secretion induced by glucose. The studies described in this paper had the following objectives:

1. To extend observations of diazoxide's effect on plasma insulin and blood glucose in patients with pancreatic islet cell tumors.
2. To investigate in these patients the relation between any changes in plasma and urinary levels of catecholamines and changes in levels of plasma insulin during administration of diazoxide.
3. To ascertain the effect of diazoxide on insulin secretion induced by glucagon, tolbutamide and arginine in addition to that induced by glucose and leucine.

The results of these studies indicate that: (a) diazoxide is a potent, rapid, and consistent inhibitor of pancreatic insulin release, (b) diazoxide increases blood glucose by decreasing insulin secretion as well as by activating extrapancreatic mechanisms, (c) these effects of diazoxide on plasma insulin and blood glucose may be observed without concomitant increases in plasma or urinary levels of catecholamines, (d) diazoxide is an effective agent for the alleviation of hypoglycemia due to functioning pancreatic islet cell tumors, and (e) diazoxide is a useful agent for studying possible differences in mechanism by which nutrients, hormones and pharmacologic agents induce the release of insulin.

METHODS

Studies in Patients with Insulin-Secreting Pancreatic Islet Cell Adenomas

Thirteen patients with pancreatic islet cell tumors have been studied. In all of these patients, the diagnosis was confirmed by operative removal of a beta cell

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tumor. Data from a fourteenth patient with a suspected islet cell tumor are also included. The daily dietary carbohydrate intake was 300 grams, and this was kept constant within narrow limits. All patients received a midnight or 10:00 P.M. feeding. Daily determinations of blood glucose and plasma insulin were made before and after removal of the tumor as well as during all testing procedures. Control infusions of saline (300 ml over 60 minutes) were performed before the administration of benzothiadiazines. Intravenous glucose tolerance tests (rapid injection of 25 g of glucose as a 50 percent solution), intravenous tolbutamide tests (injection over 2 minutes of 1 g of sodium tolbutamide* diluted in 20 cc of distilled water), and glucagon tests (intravenous injection over 4 minutes of 1 mg of crystalline glucagon† were performed before diazoxide administration. These tests were also performed on the day of diazoxide infusion, starting 120 to 150 minutes after the beginning of the infusion, or following the oral administration of diazoxide over 5 to 7 days. Trichlormethiazide was given orally in a dose of 1 to 4 mg per day. The majority of patients received 4 mg per day before intravenous diazoxide, and 2 mg per day concomitant with its oral administration. Intravenous infusions of diazoxide‡ (450–600 mg of Hyperstat in 300 ml of saline) were given over 60 minutes to 11 patients. Diazoxide was administered orally to 10 patients in a dose of 100–500 mg per day for 3 to 53 days and to the patient with the suspected islet cell tumor in a dose of 150 mg per day for 7 months.

Studies in Healthy Subjects

Seven healthy young men served as subjects for the studies reported here.

Four subjects received intravenous injections of 1 mg of crystalline glucagon over four minutes immediately after the completion of (a) an infusion of 300 cc of saline over 60 minutes, and (b) an infusion of 600 mg of diazoxide in 300 cc of saline over 60 minutes.

Five subjects received infusions of 30 g of arginine over a 30-minute period immediately after (a) an infusion of 300 cc of saline over 60 minutes, and (b) an infusion of 600 mg of diazoxide in 300 cc of saline over 60 minutes. Trichlormethiazide was given orally in a dose of 2 mg every 6 hours for 2 days before, and then 8 mg immediately preceding, the diazoxide-arginine infusions.

Blood levels of glucose were determined with the Technicon Auto-Analyzer and frequently verified by the Somogyi-Nelson technique.[9] All levels below 35 mg/100 ml were determined by the latter technique. Plasma levels of insulin were determined by the immunoassay of Yalow and Berson[10] as previously described in detail from this laboratory,[11,12] or by the Morgan-Lazarow double antibody technique.[13] Plasma catecholamines were determined by the method of Crout,[14] and urinary catecholamines by the method of Von Euler and Lishajko.[15]

RESULTS

Effect of Diazoxide in Patients With Insulin-Secreting Pancreatic Islet Cell Tumors

Infusion of diazoxide. Diazoxide was administered intravenously to 11 patients. Results obtained during six of these experiments were reported previously.[2] The

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TABLE 1

EFFECT OF INTRAVENOUS ADMINISTRATION OF DIAZOXIDE UPON LEVELS OF BLOOD GLUCOSE AND PLASMA INSULIN IN FIVE PATIENTS WITH INSULIN-SECRETING ISLET CELL TUMORS

Patient	Infusion of Saline or Diazoxide, 0 to 60 Minutes	Blood Glucose at Time in Minutes											
		−30	−15	0	15	30	45	60	75	90	105	120	150
		(mg/100 ml)											
R.Boz.	Saline	56	59	68	68	64	65	63	63	63	63	65	58
	Diazoxide, 450 mg*	69	71	75	85	105	117	123	128	125	122	117	111
R.L.	Saline	61	63	77	71	72	71	70	74	78	76	93	87
	Diazoxide, 600 mg*	68	75	82	83	85	98	106	123	145	156	159	173
H.S.	Saline	48	52	52	53	48	46	46	47	46	47	46	42
	Diazoxide, 500 mg*	58	62	64	62	64	75	95	112	128	126	126	118
J.P.	Saline	60	65	66	63	61		52		43		22	20
	Diazoxide, 600 mg	48	47	46	43	47	49	62	90	108	124	141	151
E.W.	Saline	53	51	53	55	52	46	40	41	45	41	47	52
	Diazoxide, 600 mg*	46		56	69	87	104	111	162	170	186	185	178
		Plasma Insulin at Time in Minutes (μU/ml)											
R.Boz.	Saline	16	13	17	15	15	15	13	13	14	14	16	23
	Diazoxide, 450 mg*	10	15	15	6	5	8	3	13	19	3	13	12
R.L.	Saline	32	35	23	28	29	28	25	39	26	26	30	29
	Diazoxide, 600 mg*	33	40	42	37	34	23	21	21	26	26	33	29
H.S.	Saline	80	81	62	80	73	73	67	73	70	68	70	72
	Diazoxide, 500 mg*	67	76	84	67	46	35	51	55	47	69	82	74
J.P.	Saline	30	25	28	30	36	42	46		53		53	57
	Diazoxide, 600 mg	34	35	44	17	13	9	8	7	5	9	11	10
E.W.	Saline	23†	21	12	17	56	35	12	19	25	12	18	13
	Diazoxide, 600 mg*	19†		10	3	6	3	3	3	7	5	6	3

* Trichlormethiazide, 4 mg/day, had been given orally on preceding 2 days and again 1 hour before infusion.

† Plasma insulin levels determined by Morgan-Lazarow Method.

effects of the infusion of saline or diazoxide upon blood glucose and plasma insulin in the other five patients are given in TABLE 1. As in the previous experiments, blood glucose rose (maximal increases of 53 to 130 mg/100 ml) in the 150 minutes following the beginning of the diazoxide infusion while plasma insulin fell promptly (within 15 minutes in 3 of 5 patients) and significantly (12–49 μU/ml) in each patient. Insulin levels remained depressed for 60 to 150 minutes after the beginning of the diazoxide infusions. An example is shown in FIGURE 1. This patient (J.P.) had not received trichlormethiazide before the diazoxide. In patient H.S., blood levels of glucose rose from 64 to 128 mg/100 ml at 90 to 120 minutes, even though plasma levels of insulin did not fall below 35 μU/ml and were between 47 and 82 μU/ml at the height of the hyperglycemia.

Effect of Orally Administered Diazoxide upon Daily Fasting and Postprandial Levels of Blood Glucose and Plasma Insulin

Diazoxide in combination with trichlormethiazide was administered orally to 10 patients with pancreatic islet cell tumors and to one additional patient with a suspected tumor (H.N.). Results obtained in the first five patients were reported previously.[2] Levels of blood glucose and plasma insulin before, during and after the administration of diazoxide for the six subsequent patients are given in TABLE 2. In all subjects, administration of diazoxide in combination with trichlormethiazide induced significant changes in blood glucose and plasma insulin with return to, or toward, control levels after the drugs were discontinued. In subjects C.M. and T.P., increases in fasting blood glucose from hypoglycemic to normal levels were accompanied by decreases in mean fasting plasma levels of insulin from 20 to 4 and from 41 to 18 μU/ml, respectively. Increases in the levels of blood glucose in the postprandial state were also accompanied by significant decreases

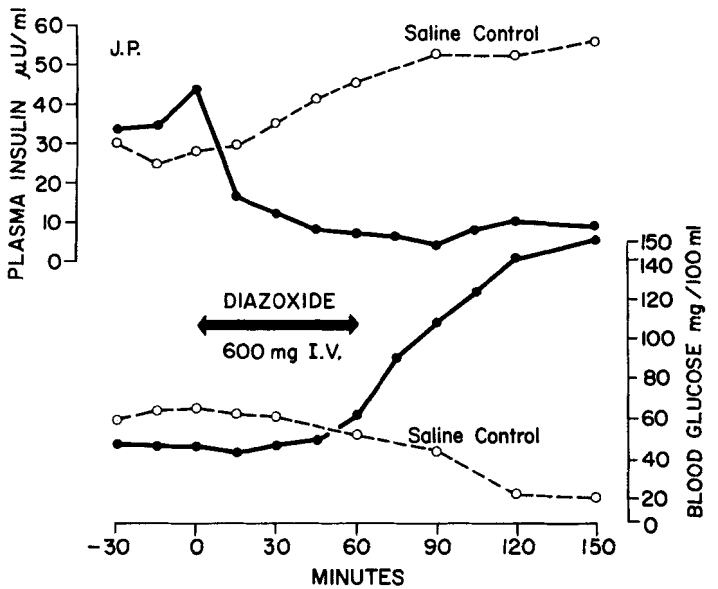

FIGURE 1. Effect of diazoxide on plasma insulin and blood glucose levels in a patient with a functioning pancreatic islet cell tumor.

TABLE 2

EFFECT OF ADMINISTRATION OF DIAZOXIDE IN COMBINATION WITH THRICHLORMETHIAZIDE ON MEAN LEVELS OF BLOOD GLUCOSE AND PLASMA INSULIN IN SIX PATIENTS WITH INSULIN-SECRETING ISLET CELL TUMORS

		7:30 A.M.–8:00 A.M. (Last feeding at 10:00 P.M. or Midnight)				4:00 P.M.			
Patient	Benzothiadiazine Administration	Blood Glucose	P	Insulin	P	Blood Glucose	P	Insulin	P
		mg/100 ml		μU/ml		mg/100 ml		μU/ml	
H.S.	Before (7 days)	59		78					
					< .025				
	During (9 days)•	63	< .001	64					
					< .05				
	After (11 days)	44		87					
C.M.	Before (4 days)	44		20		56		45	
			< .005		< .001				
	During and 1 day after (7)••	76		4		77		17	
			< .025		< .005				
	After (4 days)	47		20		51		31	
T.P.	Before (5 days)	34		41		67		44	
			< .001		< .001				< .001
	During (15 days)†	83		18		94		22	
J.P.	Before (8 days)	49		34		68		41	
	During (11 days) and 1 day after‡	115	< .001	13	< .001	110	< .005	21	< .001
H.N.	Before (11 days)	70		15.6		64		28	
			< .001		< .05		< .001		< .001
	During (8 days)§	98		11.4		95		13	
E.W.	Before (10 days)	54		24		73		77	
			< .001		< .005		< .001		< .005
	During (10 days)¶	131		16		149		23	
			< .001				< .001		
	After (4 days)	82	vs "During"	18		94	vs "During"	29	vs "During"
			< .001				< .001		< .05
	After (next 6 days)	59		21		72		35	

• 2150 mg diazoxide and 23 mg trichlormethiazide over 8 days (includes day diazoxide was given intravenously).
•• 2865 mg diazoxide and 18 mg trichlormethiazide over 7 days.
† 5600 mg diazoxide and 67 mg trichlormethiazide over 33 days.
‡ 16,300 mg diazoxide and 106 mg trichlormethiazide over 54 days (includes day diazoxide was given intravenously).
§ 10,900 mg diazoxide and 114 mg trichlormethiazide over 52 days.
¶ 2500 mg diazoxide and 22 mg trichlormethiazide over 10 days (includes day diazoxide was given intravenously).

in levels of plasma insulin. In subjects J.P. and E.W., increases in mean fasting levels of blood glucose from hypoglycemic levels into the hyperglycemic range (mean of 115 and 131 mg/100 ml, respectively) were associated with significant decreases in plasma insulin from mean fasting levels of 34 (J.P.) and 24 μU/ml (E.W.) to mean levels of 13 and 16 μU/ml, respectively. Changes in blood glucose and plasma insulin were similar in patients H.S. and H.N., although of lesser magnitude in the fasting state.

Patient T.P. received diazoxide and trichlormethiazide for 33 days. During the last 22 days of this period, he received 150 mg of diazoxide and 2 mg of trichlormethiazide per day while an outpatient. He remained completely asymptomatic, and levels of fasting blood glucose ranged between 66 and 91 mg/100 ml. One day after the drugs had been discontinued, the fasting level of blood glucose was 56 mg/100 ml and on the next day, the day of surgery, it was 52 mg/100 ml.

Patient J.P. received diazoxide orally for a total of 54 days. During this period, he received 300 mg of diazoxide (250 mg on 3 days) and 2 mg of trichlormethiazide per day. He was completely asymptomatic, and fasting blood sugar levels ranged between 108 and 136 mg/100 ml. One day after discontinuation of the drugs, fasting blood sugar was 96 mg/100 ml, and on the next day, 59 mg/100 ml.

Since an islet cell tumor was not visualized at celiotomy in patient H.N., 90 percent of the pancreas (tail, body and then part of the head) was removed during the operation. Postoperatively, fasting hypoglycemia recurred, levels of fasting blood glucose being as low as 39 mg/100 ml. The patient has received 150 mg of diazoxide and 2 to 3 mg of trichlormethiazide daily for seven months. This has resulted in good control of fasting levels of blood glucose (69 to 103 mg/100 ml), and the patient has remained asymptomatic. On several occasions, when diazoxide was decreased to 100 mg per day, fasting blood glucose levels were again in the hypoglycemic range (51 and 52 mg/100 ml). Mild ankle edema appeared while she received 2 mg of trichlormethiazide per day, but disappeared when she was given 3 mg of this diuretic benzothiadiazine. A minimal accentuation of a fine downy facial hair growth was apparent after four months of therapy with diazoxide, but has not progressed further in the subsequent three months.

Injection of glucose. Glucose was given intravenously before and after diazoxide to eight patients. The results obtained in five of these experiments were reported previously.[2] Increases in levels of blood glucose and plasma insulin evoked by intravenous administration of glucose 150 minutes after the beginning of saline or diazoxide infusion, or after oral administration of diazoxide, are given in TABLE 3. As in the previous experiments, increases in plasma insulin were greatly depressed after diazoxide (maximal increases 18, 12 and 8 μU/ml, respectively) as compared with the control experiments performed before diazoxide (maximal increases 36, 92 and 64 μU/ml, respectively).

Injection of glucagon. Glucagon was given intravenously before and after oral administration of benzothiadiazines (1965 mg of diazoxide and 12 mg of trichlormethiazide over five days) to patient C.M. Increases in blood glucose and plasma insulin during the two experiments are shown in FIGURE 2. Before diazoxide, the injection of glucagon was followed by maximal increases in blood glucose and plasma insulin of 6 mg/100 ml and 222 μU/ml, respectively. After administration of diazoxide, the glucagon induced maximal blood glucose increases of 60 mg/100 ml and plasma insulin increases of 60 μU/ml.

Injection of tolbutamide. Tolbutamide was given intravenously before and after diazoxide to three patients with insulin-producing beta cell tumors (TABLE 4). In one patient, tolbutamide was administered 150 minutes after the beginning

TABLE 3

INCREASE IN LEVELS OF BLOOD GLUCOSE AND PLASMA INSULIN AFTER RAPID INTRAVENOUS ADMINISTRATION OF 25 GM OF GLUCOSE IN THREE PATIENTS WITH INSULIN SECRETING ISLET CELL TUMORS*

Subject	Experimental Period	Increase in Blood Glucose at Time in Minutes					Increase in Plasma Insulin at Time in Minutes				
		10	20	30	45	60	10	20	30	45	60
		mg/100 ml					mg/100 ml				
R. Boz.	Control	152	135	101	97	63	36	29	21	7	11
	Diazoxide (450 mg I.V. over 60 minutes)	221	179	159	126	113	18	5	2	−4	3
R.L.	Control	132	105	88	64	55	92	52	39	20	35
	Diazoxide (600 mg I.V. over 60 minutes)	119	127	115	89	91	5	6	12	5	4
C.M.	Control	130	100	74	53	42	64	33	7	11	11
	Diazoxide (100–500 mg per day orally for 7 days)	147	143	139	108	86	8	8	7	7	7

* Glucose was given either 150 minutes after the beginning of diazoxide or control infusion, or after oral administration of diazoxide.

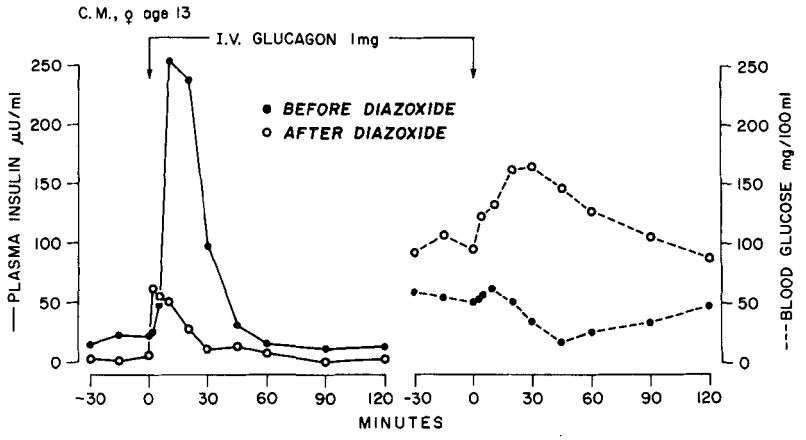

FIGURE 2. Effect of diazoxide on increases in plasma insulin and blood glucose levels induced by intravenous glucagon in a patient with a functioning pancreatic islet cell tumor.

of a 60-minute infusion of 600 mg of diazoxide. In the other two patients, the tolbutamide tests were performed after the oral administration of diazoxide and trichlormethiazide for six and eight days, respectively. In all three patients, diazoxide in combination with trichlormethiazide caused decreases in plasma levels of insulin and increases in blood levels of glucose before the administration of tolbutamide. In patient T.P., increases in plasma insulin were almost identical before and after administration of diazoxide. In patient J.P., increases in plasma insulin were greater after diazoxide, while in patient E.W., they were smaller.

Plasma levels and urinary excretion of catecholamines. Arterial plasma levels of catecholamines were determined before and at the end of, or shortly after the end of, the diazoxide infusion in four patients (TABLE 5). Plasma levels of adrenaline increased in one patient (H.S.) but either did not change or decreased in the other three patients. Plasma levels of noradrenaline increased in one patient (H.S.), changed from a low to a high normal level in a second (R.Boz.), but either did not change or decreased in the other two patients (R.L. and J.P.). In patients R.L. and J.P., plasma levels of insulin were decreased by diazoxide at a time when arterial plasma levels of catecholamines had not changed or were decreased.

Urinary excretion of catecholamines was determined both on a control day and on the day of the diazoxide infusions in nine patients (TABLE 6). Urinary excretion of adrenaline was increased on the morning of the diazoxide infusion in only one patient (H.R.). Urinary excretion of noradrenaline was increased in five patients (A.W., R.B., H.R., J.P, and E. W.). Patient J.P. had received tolbutamide intravenously 150 minutes after the beginning of the diazoxide infusion. Severe symptoms of hypoglycemia were manifest 60 minutes later, and may have elicited this increase in noradrenaline secretion.

While diazoxide was being given orally to six patients, urinary excretion of catecholamines was determined in 24-hour specimens obtained during periods of 6 to 12 days (TABLE 7). Urinary excretion of catecholamines was also determined during periods ranging from 3 to 14 days before and after discontinuation of diazoxide. Although mean levels of blood glucose were elevated and mean levels of plasma insulin were depressed significantly in each patient, mean urinary excretion of adrenaline was not increased in any patient during diazoxide

TABLE 4

EFFECT OF INTRAVENOUS ADMINISTRATION OF TOLBUTAMIDE UPON LEVELS OF BLOOD GLUCOSE AND PLASMA INSULIN BEFORE AND AFTER ADMINISTRATION OF DIAZOXIDE IN THREE PATIENTS WITH INSULIN-SECRETING ISLET CELL TUMORS

Patient	Control or Diazoxide	−30	−15	0	1	3	5	10	15	20	30	33	36	40	50	60
		Blood glucose at time in minutes (mg/100 ml)														
J.P.	Control	58	59	59	58	57	56	48	32	25	25			4		
	Diazoxide*	141		151	147	149	146	139	129	117	97			78		58
T.P.	Control	26	27	37			29	25		19	18			15		
	Diazoxide†	102	105	105			105	99		84	74			60	56	
E.W.	Control	48	47	49		47	46	38	26		5	4	8			
	Diazoxide‡	122	136	152	148	153	150	144	128		87	73	63			
		Plasma insulin at time in minutes (μU/ml)														
J.P.	Control	32§	25	35	45	200	250	385	330	275	122			72		
	Diazoxide*	§		20	96	445	560	675	635	530	305			180		185
T.P.	Control	37	36	35			193	122		90	81			87	80	
	Diazoxide†	10	9	9			168	78		105	91			65	70	
E.W.	Control	15§	17	22		465	635	750	815		265	190	140			
	Diazoxide‡	5§	3	10	23	381	345	500	475		225	150	100			

* Diazoxide, 600 mg, had been given intravenously over 60 minutes, beginning 150 minutes before injection of tolbutamide.

† Diazoxide, 1750 mg, and trichlormethiazide, 13 mg over 6 days had been given orally before injection of tolbutamide.

orally before injection of tolbutamide (100 mg diazoxide and 2 mg trich on first day), and trichlormethiazide, 21 mg over 8 days, had been given

‡ Diazoxide, 1600 mg over 7 days (in addition to 600 mg intravenouslylormethiazide given orally 60 minutes before injection of tolbutamide).

§ Morgan-Lazarow Method.

TABLE 5

ARTERIAL PLASMA LEVELS OF ADRENALINE AND NORADRENALINE BEFORE AND AFTER INFUSION OF DIAZOXIDE TO PATIENTS WITH FUNCTIONING PANCREATIC ISLET CELL TUMORS

Patient, Sex	Amount of Diazoxide Infused Over 60 min.	Timing of Blood Sample in Relation to Start of Infusion	Arterial Plasma Noradrenaline (μg/1)	Arterial Plasma Adrenaline (μg/1)
R.Boz. Female	450 mg	Before	0.06	0.91
		150 Minutes After	0.24	0.64
		Postoperative Control	0.19	0.65
R.L. Male	600 mg	Before	0.21	0.44
		95 Minutes After	0.15	0.31
		Postoperative Control	0.19	0.65
H.S. Female	500 mg	Before	0.19	0.65
		75 Minutes After	0.32	1.04
J.P. Male	600 mg	Before	0.60	2.95
		60 Minutes After	0.30	1.47

TABLE 6

EFFECT OF INTRAVENOUSLY ADMINISTERED DIAZOXIDE ON URINARY EXCRETION OF CATECHOLAMINES IN PATIENTS WITH FUNCTIONING PANCREATIC ISLET CELL TUMORS

Patient, Sex	Experimental Periods*	Urine Collection† (Hours)	Total Free Noradrenaline (μg)	Total Free Adrenaline (μg)
L.M., Male	Saline Control	4	5.0	2.0
	Diazoxide-600 mg I.V.	4	5.1	1.2
	Saline Control	24	14.8	2.0
	Diazoxide-600 mg I.V.	24	16.8	1.5
A.W., Female	Saline Control	6¾	2.5	2.4
	Diazoxide-450 mg I.V.	7	8.4	0.7
R.B., Male	Saline Control	5	10.1	2.9
	Diazoxide-600 mg I.V.	4	16.8	3.4
H.R., Female	Saline Control	4	11.7	2.8
	Diazoxide-500 mg I.V.	4	18.8	6.2
R.Boz., Female	Saline Control	4	4.3	3.8
	Diazoxide-450 mg I.V.	4	2.8	3.2
R.L., Male	Saline Control	4	1.3	0.7
	Diazoxide-600 mg I.V.	4	2.2	0.5
H.S., Female	Saline Control	4	7.2	3.7
	Diazoxide-500 mg I.V.	4	7.2	2.4
J.P., Male	Saline Control	5	10.7	6.0
	Diazoxide-600 mg I.V.	6	17.2	7.6
E.W., Female	Saline Control	4	3.3	1.0
	Diazoxide-600 mg I.V.	4	11.7	1.1

* Saline or diazoxide infused over 60 minutes.

† Beginning 30 minutes before infusions.

TABLE 7

EFFECT OF ORALLY ADMINISTERED DIAZOXIDE UPON FREE URINARY EXCRETION OF CATECHOLAMINES IN PATIENTS WITH FUNCTIONING PANCREATIC ISLET CELL TUMORS

Patient, Sex	Period	Number of Days	Noradrenaline μg/24 Hours		Adrenaline μg/24 Hours	
			Mean ± S.E.M.	P*	Mean ± S.E.M.	P*
C.M. Female	2 days before, 4 days after Diazoxide	6	28.1 ± 2.0		5.8 ± 1.2	
	Diazoxide, 230–500 mg/day	6	35.3 ± 4.1		5.7 ± 0.4	
	Second postoperative week	5	37.2 ± 5.0		5.0 ± 0.9	
T.P., Male	Before	2	38.5		17.5	
	Diazoxide, 100–450 mg/day	12	56.3 ± 4.9	<.05	10.9 ± 0.8	
	From second postoperative week on	9	43.3 ± 2.8		11.6 ± 0.6	
J.P., Male	3 days before and 2 days after	5	38.5 ± 3.8		21.4 ± 2.0	
	Diazoxide, 300 mg/day	8	48.0 ± 3.0		21.8 ± 1.8	
	From 5th day postoperative on	9	55.9 ± 3.5		25.4 ± 2.3	
H.N., Female	Before	8	34.8 ± 2.3	<.05	8.6 ± 1.2	
	Diazoxide, 150 mg/day	5	43.7 ± 3.1		7.1 ± 0.7	
	Diazoxide 2 weeks later, 300 mg/day	2	31.8		16.4	
	After	6	30.3 ± 1.8		22.8 ± 1.8	
H.S., Female	1 day before, 2 days after	3	36.6 ± 3.2	<.005	8.5 ± 0.1	
	Diazoxide, 150–300 mg/day	7	56.6 ± 2.4		7.2 ± 0.8	
E.W., Female	Before	8	30.7 ± 4.3		7.8 ± 1.0	<.001
	Diazoxide, 200 mg/day	10	41.3 ± 10.9	<.005	3.1 ± 0.5	
	After	9	28.9 ± 5.8		3.5 ± 1.0	

* Differences are not significant unless indicated.

TABLE 8

EFFECT OF INTRAVENOUS ADMINISTRATION OF GLUCAGON UPON LEVELS OF BLOOD GLUCOSE AND PLASMA INSULIN AFTER INTRAVENOUS ADMINISTRATION OF SALINE OR DIAZOXIDE IN FOUR HEALTHY SUBJECTS

Subject	Infusion*	Blood glucose at time in minutes (mg/100 ml)																
		−60	−45	−30	−15	0	2	5	10	20	30	40	50	60	75	90	120	150
C.H.	Saline	84	84	84	83	83	83	89	108	128	145	122	103	96		78	84	
	Diazoxide	83	100	99	104	111	115	126	134	161	173	170	166	162		137	117	
D.B.	Saline	85	83	84	84	85	84	89	98	124	125	120	112	104	91	86	73	68
	Diazoxide	82	83	84	85	91	95	108	133	159	166	155	148	143		123	104	88
G.Q.	Saline	92	94		94	92	94	96	117	151	120	100	87	79		78	80	79
	Diazoxide	95	91	92	103	106	111	121	125	139	151	149	142	134		128	113	101
J.J.	Saline	76	78	77	77	73	74	84	104	128	116	106	106	93		77	72	
	Diazoxide	77	81	80	93	101	105	112	122	165	171	174	160	159	150	139	116	106
Mean	Saline	84	85	81	85	84	85	89	107	133	127	112	102	93		80	77	70
Mean	Diazoxide	84	89	89	96	102	107	117	129	155	165	162	154	149		132	112	99
		Plasma insulin at time in minutes (μU/ml)																
C.H.	Saline	11	12	12	12	14	15	81	81	54	40	37	20	18		11	12	
	Diazoxide	9	9	6	3	7	43	63	59	19	18	15	12	16		17	11	
D.B.	Saline	14	9	16	17	13	7	73	70	39	33	29	24	26	16	9	9	12
	Diazoxide	14	6	7	7	7	29	35	27	14	14	14	27	12		11	11	9
G.Q.	Saline	9	7	6	6	11	11	83	85	71	46	25	18	15		11	13	12
	Diazoxide	16	9	10	10	9	66	67	77	29	37	23	38	24		19	22	18
J.J.	Saline	9	8	9	11	9	12	50	50	29	23	18	13	11		10	8	
	Diazoxide	9	9	4	3	5	45	51	31	8	10	11	16	13	19	29	25	13
Mean	Saline	11	9	11	12	12	11	72	71	48	35	28	19	18		10	11	12
Mean	Diazoxide	12	8	7	6	7	46	54	49	18	19	16	23	16		19	17	13

* 300 cc of saline, or 600 mg of diazoxide in 300 cc of saline, were infused from −60 to 0 minutes. Glucagon, 1 mg, was injected over 4 minutes beginning at 0 minutes.

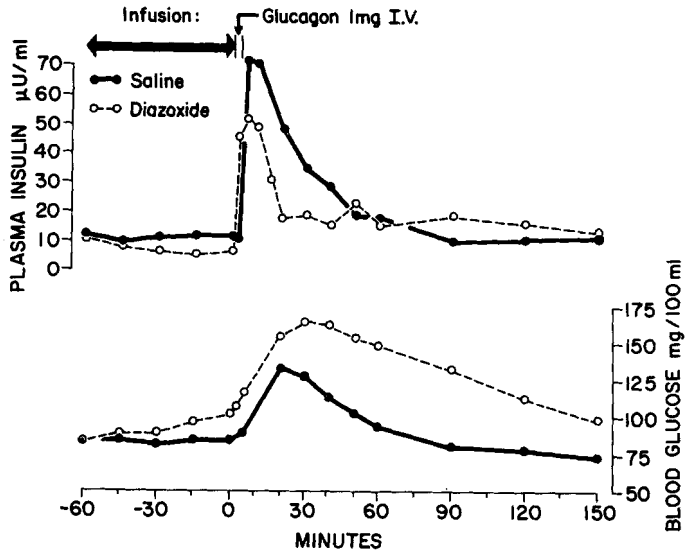

FIGURE 3. Diazoxide's effect on plasma insulin and blood glucose increases in response to intravenous glucagon. (Mean of four paired experiments in four healthy subjects.)

administration. Mean urinary excretion of noradrenaline was increased in four of the six patients (T.P., H.N., H.S., and E.W.) during diazoxide. However, in one of these patients (H.N.), excretion of noradrenaline was not increased when a larger dosage of diazoxide was given during a later period. In the other two of the six patients (C.M. and J.P.), mean urinary excretion of noradrenaline appeared higher during diazoxide than during the days immediately preceding administration of the drug. However, mean urinary excretion of noradrenaline was as great or greater during a postoperative control period.

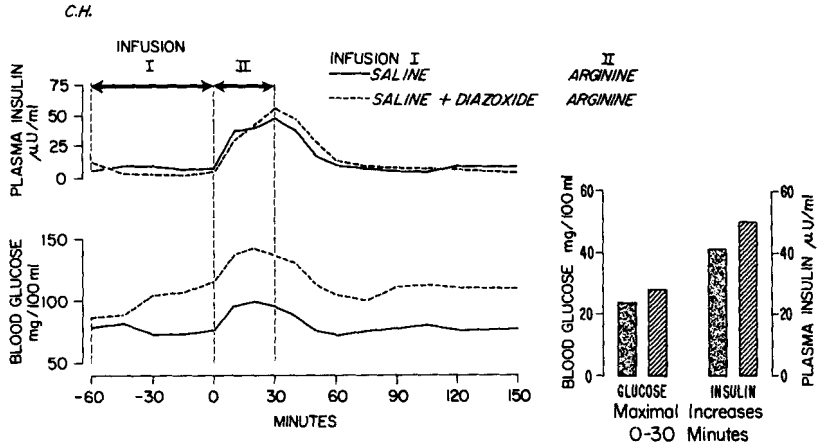

FIGURE 4. Effect of diazoxide and trichlormethiazide on plasma insulin and blood glucose levels before, during and after infusion of 30 g of arginine. (Stippled bars represent maximal increases during saline-arginine infusion; striped bars, during diazoxide-arginine infusion.)

Effect of Diazoxide in Healthy Subjects

Injection of glucagon. Glucagon was given intravenously to four healthy young subjects after infusions of saline and diazoxide. The results of these studies are shown in TABLE 8 and FIGURE 3. During the infusion of diazoxide, plasma levels of insulin decreased and blood glucose increased. The maximal increases in plasma insulin induced by glucagon after diazoxide were 20 to 25 percent smaller than those induced by glucagon after a saline infusion. The levels of plasma insulin two minutes after the beginning of the glucagon injection, however, were higher in each subject when diazoxide had been infused. Blood levels of glucose were from 6 to 28 (mean of 19) mg/100 ml higher at the time of the glucagon administration when diazoxide, rather than saline, had been infused. The hyperglycemic response to glucagon was also greater after diazoxide than after saline.

Intravenous administration of arginine. Five healthy subjects each received infusions of 30 gm of arginine over 30 minute periods on two separate occasions. One arginine infusion was preceded by saline given over 60 minutes. On the other occasion, the subjects received trichlormethiazide orally for two days preceding the i.v. administration of arginine, and an infusion of 600 mg of diazoxide in saline over the 60 minutes immediately beforehand. The details of these experiments will be reported separately. A representative example is shown in FIGURE 4. Diazoxide administered in combination with trichlormethiazide did not inhibit the increases in plasma insulin that accompanied arginine administration.

DISCUSSION

When diazoxide was given intravenously to 11 patients with functioning pancreatic islet cell tumors, prompt decreases in plasma levels of insulin occurred in each instance, followed by, or associated with, increases in blood glucose levels. In the healthy subjects, intravenous diazoxide was also associated with decreases in plasma levels of insulin, although they were of lesser magnitude than those observed in the patients with islet cell tumors. Since acute changes in plasma insulin levels can be interpreted as reflecting corresponding changes in the secretory rate of insulin,[16,17] we can conclude that a decreased release of insulin from abnormal and normal beta cells must have been responsible, at least in part, for the hyperglycemic effect of diazoxide. Our conclusion that diazoxide causes decreased insulin release[2] is further supported by the findings of Seltzer, who has demonstrated decreased levels of insulin in pancreatic venous blood after the administration of diazoxide to dogs,[18] and the findings of Frerichs and associates,[7] and of Howell and Taylor,[8] who have demonstrated a direct effect of diazoxide upon insulin release from pancreatic slices *in vitro*.

The effect of intravenous diazoxide on plasma levels of insulin and blood levels of glucose can be observed without prior administration of trichlormethiazide (see patient J.P., TABLE 1). However, since it has been shown in previous studies that the hyperglycemic effect of diazoxide is greatly accentuated by prior or concomitant administration of the diuretic benzothiadiazines,[19] we have employed the combination of diazoxide and trichlormethiazide in the majority of our studies.

When diazoxide was given orally in combination with trichlormethiazide for more prolonged periods of time to 10 patients with islet cell tumors, increases in fasting levels of blood glucose from the hypoglycemic to the normal, or hyperglycemic, range were also accompanied by significant decreases in fasting plasma insulin levels. Similar changes in blood glucose and plasma insulin were observed in the postprandial state. After the drugs were discontinued, levels of plasma

insulin and blood glucose returned to control values. These studies show that, in patients with islet cell tumors, these agents depress hyperinsulinemia in the fasting and postprandial states, and indicate that this action is responsible, in part, for the elevation of blood glucose levels that are observed.

The present studies provide evidence that the hyperglycemic action of diazoxide, intravenously or orally administered, is produced by one or more mechanisms in addition to that of decreased insulin secretion from the pancreatic islet cell tumors. Following the intravenous administration of diazoxide, there was not a good correlation between the magnitude of plasma insulin decreases and blood glucose increases. In the eight patients (five patients of the present series and three others reported previously[2]) in whom diazoxide was given intravenously without its prior oral administration, increases in blood glucose (42 to 130 mg/100 ml) were not proportional to the decreases in plasma insulin (12 to 49 μU/ml). In three patients, diazoxide was given orally for three days before its intravenous administration. In patient S.L., oral diazoxide over three days had reduced plasma levels of insulin from a mean level of 43 μU/ml to a level of 20 μU/ml on the morning of the diazoxide infusion.[2] Following the infusion, a maximal decrease in plasma insulin of only 9 μU/ml was, nevertheless, accompanied by an increase in blood glucose of 154 mg/100 ml. More importantly, increases of blood glucose that extended into the hyperglycemic range occurred despite persistent hyperinsulinemia. In subject H.S. (TABLE 1), blood levels of glucose rose from 62 to 128 and 126 mg/100 ml 90 and 120 minutes after the beginning of the diazoxide infusion, even though plasma insulin levels did not fall below 35 μU/ml and were between 47 and 82 μU/ml at the height of the hyperglycemia. In patient R.B., blood glucose rose to 117 mg/100 ml 150 minutes after the beginning of the infusion, while plasma insulin had not decreased below 76 μU/ml.[2] After more prolonged oral administration of diazoxide (9 days for H.R., 28 days for R.B.), normal fasting blood sugar levels or hyperglycemic levels were achieved in the presence of marked hyperinsulinemia (mean plasma levels of 46 and 58 μU/ml, respectively).[2]

Diazoxide, particularly in combination with trichlormethiazide, has proved to be an effective agent for the alleviation of hypoglycemia due to functioning pancreatic islet cell tumors. Patients T.P. and J.P. received diazoxide in doses of 150 or 300 mg per day for 33 and 54 days, respectively. Both remained completely asymptomatic. Similar results for a period of 28 days were reported previously for patients R.B.[2] Hypoglycemia did not recur until the drugs were discontinued. In patient H.N., hypoglycemia has been well controlled for seven months by a dose of 150 mg of diazoxide per day. An increase in trichlormethiazide dosage from 2 to 3 mg per day has prevented edema due to the sodium-retaining action of diazoxide.

In the patients with islet cell tumors, increases in plasma insulin induced by intravenous glucose were greatly suppressed by diazoxide. In these, as well as in the healthy subjects, increases in blood glucose induced by the diazoxide infusion were accompanied not by increases, but by decreases in plasma insulin levels (FIGURE 3).[2] Thus, it can be concluded that glucose-induced increases in insulin secretion are inhibited by diazoxide.

Increases in plasma insulin levels following intravenous glucagon were partially inhibited by a previous administration of diazoxide. This was demonstrated in a patient with a functioning pancreatic islet cell tumor and in healthy subjects. In the latter, however, plasma insulin levels were higher two minutes after the beginning of the glucagon injection when diazoxide, rather than saline, had been

previously infused. The higher blood levels of glucose at the time of the glucagon injection, and following the diazoxide, may account for this, since it has been shown by others that the hyperinsulinemic effect of glucagon is enhanced by a raised blood glucose concentration.[20] The subsequent hyperglycemic response to glucagon was also greater after diazoxide than after saline. Thus, the total insulinogenic response to glucagon was less after diazoxide, in spite of higher blood glucose levels during this entire period. Irrespective of the direct effect of glucagon on insulin release, the ratio of the sum of plasma insulin increases to the sum of blood glucose increases during the first 60 minutes immediately following the glucagon injection was 1.2 following the saline infusion while it was 0.57 following the diazoxide infusion.

On the other hand, the insulin response to intravenous tolbutamide was not inhibited by prior administration of diazoxide and trichlormethiazide in patients with islet cell tumors. In one patient, increases in plasma insulin following tolbutamide were almost identical before and after the benzothiadiazine drugs. In the other two patients with islet cell tumors, increases in plasma insulin following a tolbutamide injection that was preceded by the benzothiadiazine compounds were greater in one and smaller in the other. The differences in the last two patients were most likely due to spontaneous variations in the pathologically exaggerated insulin response to tolbutamide observed in patients with islet cell tumors. In experiments in dogs, Seltzer has also demonstrated that diazoxide infusion does not inhibit tolbutamide-induced insulin release.[18] Similarly, it has been demonstrated *in vitro* that diazoxide has no effect on tolbutamide-induced insulin release.[7]

We have previously reported that diazoxide in combination with trichlormethiazide inhibits leucine-induced insulin release.[2] In contrast, the present studies show that this combination does not inhibit arginine-induced insulin release. These findings are consonant with our previous demonstration that the mechanism by which leucine induces the release of insulin differs from that by which arginine or other amino acids induce its release from the pancreatic beta cells.[21,22] The findings that diazoxide inhibits insulin release induced by glucose, glucagon and leucine but not that induced by tolbutamide or arginine also indicate that it is a useful agent for studying possible differences in mechanism by which nutrients, hormones and pharmacological agents induce insulin release.

The mechanism by which diazoxide decreases the secretory capacity of normal and pathological pancreatic beta cells is not known. Tabachnick and associates,[23,24] Kvam and Stanton,[25] Wolff and associates[26,27] and Graber and associates[4] have postulated that increased release of catecholamines or increased catecholamine activity may play an important role in diazoxide-induced suppression of insulin release. Seltzer and Crout have challenged the view that increased release of catecholamines plays a role in diazoxide hyperglycemia on the basis of experiments performed in dogs.[28] Administration of adrenaline and noradrenaline have been shown by Porte and coworkers[29,30] to be associated with a reduction in plasma levels of insulin in response to the insulinogenic stimuli of hyperglycemia, glucagon and tolbutamide. Therefore, we have studied the relation between possible changes in plasma and urinary levels of catecholamines and changes in levels of plasma insulin during diazoxide administration in patients with functioning islet cell tumors. In many experiments, the effects of diazoxide on plasma insulin and blood glucose were discernible either without concomitant increases or with actual decreases in plasma or urinary levels of catecholamines. In patient H.S., increases in arterial plasma levels of adrenaline and noradrenaline following

diazoxide infusion were not associated with increases in urinary levels of catecholamines. This suggests that elevations in plasma levels of catecholamines were of very short duration. Thus, the diazoxide-induced suppression of insulin release, and hyperglycemia, cannot be mediated solely via increased release of catecholamines. The fact that the administration of catecholamines suppresses tolbutamide-induced insulin release,[29,30] while diazoxide does not, also suggests that diazoxide and catecholamines suppress insulin release by different mechanisms.

However, most likely because of its hypotensive action, diazoxide causes increased secretion of catecholamines in some patients, and, in them, this may contribute to diazoxide-induced inhibition of insulin release and hyperglycemia.

Somewhat more is known about the extrapancreatic effects by which diazoxide and the diuretic benzothiadiazines increase blood glucose. Senft and coworkers have demonstrated by *in vivo* and *in vitro* experiments that diazoxide increases levels of adenosine 3′, 5′ cyclophosphate (3′, 5′-AMP) by directly inhibiting 3′, 5′-AMP phosphodiesterase.[31] Increased levels of 3′, 5′-AMP increase blood glucose by enhancing the activity of enzymes that increase glycogenolysis and decrease glycogenesis. The action of catecholamines in increasing the concentration of 3′, 5′-AMP is potentiated by diazoxide secondary to its inhibiting effect on 3′, 5′-AMP phosphodiesterase. Diazoxide's effect on 3′, 5′-AMP phosphodiesterase is independent of, and in addition to, its effect of decreasing insulin secretion. Decreased insulin activity is followed by decreased activity of 3′, 5′-AMP phosphodiesterase and thus by an increase in the concentration of 3′, 5′-AMP. Senft and coworkers have presented evidence to demonstrate that the diuretic benzothiadiazines also may inhibit the activity of 3′, 5′-AMP phosphodiesterase,[32] and, in addition, may potentiate the effect of diazoxide by more than one other mechanism.[33,34]

The experimental findings reported in this paper, and in others referred to briefly, indicate that diazoxide, particularly when given in combination with trichlormethiazide, produces its hyperglycemic effect not by a single mechanism, but by several mechanisms. The decreased insulin secretion induced by diazoxide, as well as the effects of diazoxide and trichlormethiazide that are not mediated via alterations in insulin secretion, contribute to the total hyperglycemic effect of these compounds.

Summary and Conclusions

Diazoxide, with or without trichlormethiazide, was given to 14 patients with functioning pancreatic beta cell tumors and to healthy subjects. The following conclusions can be drawn from these studies:

1. Diazoxide is a potent, rapid, and consistent inhibitor of pancreatic insulin release.

2. Diazoxide causes increases in blood glucose by decreasing insulin secretion as well as by activating one or more extrapancreatic mechanisms.

3. Diazoxide is an effective agent for the alleviation of hypoglycemia due to functioning pancreatic islet cell tumors.

4. The effects of diazoxide on plasma insulin and blood glucose may be observed without concomitant increases in plasma or urinary levels of catecholamines. However, under some circumstances, increased release of catecholamines may contribute to diazoxide-induced inhibition of insulin release and hyperglycemia. In either case, diazoxide's potentiation of catecholamine activity may contribute to its extrapancreatic effects.

5. Diazoxide inhibits glucose and glucagon-induced insulin release. Diazoxide

does not inhibit tolbutamide-induced insulin release. Diazoxide inhibits leucine but not arginine-induced insulin release. These findings are consonant with our previous demonstration that the mechanisms by which arginine and leucine induce insulin release differ. Thus, diazoxide is a useful agent for studying possible differences in mechanism by which nutrients, hormones and pharmacologic agents induce insulin release.

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DISCUSSION OF THE PAPER

P. G. WALFISH (*New Mount Sinai Hospital, Toronto, Ontario, Canada*): We have studied the effects of chronic diazoxide therapy in two proven insulinoma cases. During the control period, fasting blood sugars in patient B were quite low. Plasma immuno-reactive insulin levels were within the normal range. However, when these values were expressed as an IRI (μU/ml)/blood sugar (mg%) ratio, they were clearly elevated above the normal fasting range of 0.18 to 0.22 recently reported by Conn.*

When patient B was given diazoxide therapy, the absolute insulin values did not change as much as the fasting IRI/blood sugar ratio, which clearly declined toward the normal range. Subsequently, on combined diazoxide and trichlormethiazide therapy, ratios returned to normal.

In patient A, control IRI/blood sugar ratios were quite high, as were the absolute insulin levels. On combined diazoxide and trichlormethiazide, ratios were almost normal, and were associated with no further hypoglycemic attacks.

It was also of interest that the control 100 gram oral glucose tolerance test on patient B showed that the elevated fasting IRI/blood sugar ratios paradoxically declined with glucose, but rebounded to high values after three hours, correlating with the onset of hypoglycemic symptoms.

Our studies on these two insulinoma patients, similar to those of Dr. Fajans and coworkers, showed that the IRI/blood sugar ratios were elevated in the untreated state, occasionally in the absence of markedly elevated absolute IRI values. However, following chronic therapy, diazoxide alone, or in combination with trichlormethiazide, restored these ratios to normal values.

DR. FAJANS: There should be a change in the ratio of plasma insulin to blood glucose after administration of diazoxide to a patient who has hyperinsulinism and hypoglycemia, whatever the plasma level of insulin is at the beginning of therapy. However, I would like to point out that we have now studied 14 patients with pancreatic islet cell tumors, and in every patient we have been able to demon-

* Conn, J. W. 1965. "Special Article"–Hypertension, The Potassium Ion, and Impaired Carbohydrate Tolerance, New Engl. J. Med. **273**: 1135.

strate absolute decreases in plasma insulin after the administration of diazoxide. We have had no difficulty in showing that plasma levels of insulin fall after diazoxide, and we don't have to resort to ratios to demonstrate this effect.

H. SELTZER (*Veterans Administration Hospital, Dallas, Tex.*): With regard to your point about the extrapancreatic effect of diazoxide: In every case where you give diazoxide, insulin decreases and the blood sugar rises. In one case mentioned here, it went from 100 or so units down to 75. There was still hyperinsulinemia, but the blood sugar went up, so you got hyperglycemia in the presence of hyperinsulinemia. Perhaps these are related phenomena.

If a patient with an insulinoma has 4-plus hyperinsulinemia, and you reduce it to 2-plus with your injection of diazoxide, you may be getting rebound hyperglycemia, which might later drift down within the normal range. Perhaps the hyperglycemia remained after several days of oral diazoxide, giving higher than normal levels of blood glucose, even though the depressed insulin values were still excessive. If so, persistently elevated insulin levels would certainly suggest a nonpancreatic, hyperglycemic effect of diazoxide.

DR. FAJANS: There were apparent disparities between plasma glucose and plasma insulin in more than one patient during the administration of diazoxide. In several patients, hyperglycemia persisted while plasma levels of insulin were still greatly elevated for prolonged periods of time. Perhaps insulin resistance, which may exist in these patients, was still present, so that even though there was a total reduction in plasma insulin, hyperglycemia developed.

What might cause such insulin resistance? Is it caused by increased levels of growth hormone? Not likely. Growth hormone levels in these patients are either not elevated at all or are not sufficiently elevated to explain it. Adrenalin? No. Hydrocortisol? No. We haven't measured glucagon levels, but we have no evidence to suggest that glucagon could be responsible either.

Our suggestion that benzothiadiazines exert a non-pancreatic effect is supported by the fact that in one or two of our patients not reported here, hyperglycemia continued for a period of three days after diazoxide was withdrawn, although plasma insulin was not suppressed during that time.

R. LEVINE (*New York Medical College, New York, N. Y.*): Dr. Tabachnick has demonstrated a hyperglycemic response to diazoxide in the pancreatectomized dog. Therefore, there must be some extrapancreatic effect.

DR. FAJANS: The extrapancreatic effects of diazoxide have been demonstrated *in vivo* as well as *in vitro*. I don't think there can be much doubt that they exist.