Diazoxide Effects on Biphasic Insulin Release: "Adrenergic" Suppression and Enhancement in the Perifused Rat Pancreas

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ABSTRACT An in vitro system for perifusion of rat pancreas has been used to investigate the effects of diazoxide on glucose-induced insulin release. Administration of diazoxide with a stimulating concentration of glucose produced a dose-dependent suppression of insulin release. This effect was partly reversed by phentolamine. In the presence of nonstimulatory concentrations of glucose, diazoxide plus phentolamine, but neither alone, stimulated a biphasic release of insulin similar to that observed with 1-isopropyl norepinephrine. A prior period of perifusion with a low concentration of diazoxide enhanced the primary component of subsequent glucose-stimulated insulin release, an effect inhibited by addition of either phentolamine or propranolol to the diazoxide during this "prestimulation" period. These effects are similar to those observed with epinephrine. By contrast with epinephrine however, increasing the concentration of diazoxide during the period before glucose stimulation enhanced both the primary and secondary components of subsequent glucose-induced insulin release. These data suggest that at least some of the direct effects of diazoxide on the pancreas are mediated through α- and β-adrenergic receptor mechanisms.

INTRODUCTION

Diazoxide, an antihypertensive benzothiadiazine (1), produces hyperglycemia in vivo (2–9) and is presently used in the management of intractable hypoglycemia in man (5). It would appear that this hyperglycemic action is exerted through both pancreatic (6–9) and extrapancreatic effects (10, 11). The extrapancreatic effects have been attributed to diazoxide-induced increase in circulating catecholamines (12–14). This conclusion is supported by the reported inhibition of diazoxide hyperglycemia by prior adrenalectomy and by the administration of adrenergic receptor–blocking agents (11, 15–17), although other extrapancreatic effects not mediated via the catecholamine response may exist (18).

Increases in circulating catecholamines could thus contribute to the observed reduction of insulin levels in vivo (19) during diazoxide administration. However, such reduction has been observed in man in the absence of increased circulating catecholamines (18). Furthermore, in vitro evidence indicates that diazoxide also exerts a direct inhibitory effect on insulin release (20, 21). The present study was undertaken in order to define the relationship between the action of diazoxide on insulin release and the activity of adrenergic α- and β-receptor mechanisms of B-cells, utilizing a dynamic system for insulin release in which the interactions of catecholamines and adrenergic-blocking agents have been defined previously (22).

METHODS

The apparatus and procedures have been detailed elsewhere (22–25). In brief, pancreases were removed under ether anesthesia from male Wistar rats weighing 175–200 g and fasted overnight. Pancreas segments of 100 mg each were weighed and cut under buffer into pieces approximately 1 mm in diameter, then placed into a perifusion chamber. The fragments then were continuously perifused (suprafused) with Krebs' Ringer bicarbonate buffer, containing bovine serum albumin 0.5 g/100 ml and trasylol 500 IU/ml. The buffer pH was maintained at 7.4 by continuous gassing with CO_2 5%, O_2 95%. The temperature of buffers and chambers was kept at 37.4°C by means of a thermostat-controlled water jacket system. In all experiments, a prestimulation pe-

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TABLE I

The Effect of Diazoxide and of Phentolamine on Glucose-Stimulated Biphasic Insulin Release

Experiment	Stimulation			n	IRI release			
	Glucose	Diazoxide	Phentolamine		Primary	P*	Secondary	P*
	mg/100 ml	*μg/ml*	*μg/ml*		*ng, mean ±SEM*			
a.	300	—	—	10	3.8 ±0.2		172 ±9	
b.	300	1	—	6	2.7 ±0.4	0.02 (a)	172 ±16	NS (a)
c.	300	10	—	6	3.2 ±0.2	0.05 (a)	98 ±9	0.01 (a)
d.	300	45	—	8	2.7 ±0.2	0.05 (a)	37 ±2	0.01 (a)
e.	300	45	20	6	7.4 ±0.4	0.01 (a, d)	130 ±7	0.01 (a, d)
f.	300	—	20	6	3.9 ±0.1	NS (a)	156 ±15	NS (a)
g.	50	—	—	6	2.4 ±0.2		38 ±2	
h.	50	—	20	4	1.8 ±0.3	NS (g)	35 ±6	NS (g)
i.	50	10	—	4	2.3 ±0.3	NS (g)	35 ±4	NS (g)
j.	50	10	20	9	5.2 ±0.6	0.01 (g)	57 ±8	0.01 (g)

In all experiments a 35 min prestimulation period, in which tissue was perifused with buffer containing 50 mg/100 ml glucose, preceded the 60 min period of stimulation.

* *P* values represent the significance of differences between test and control conditions. The letter(s) of the control experiment(s) to which each test is compared appear(s) in parentheses after the *P* value.

NS = not significant. n = number of separate experiments.

riod of 35 min preceded the test or stimulation period of 60 min. Glucose, 50 mg/100 ml (a nonstimulating concentration), was added to all prestimulation buffers. In the stimulation buffers, glucose concentration was either 50 or 300 mg/100 ml.

In the first series of experiments, diazoxide was added to the stimulation buffer with or without phentolamine and at both glucose concentrations; no drugs were added during prestimulation. In the second series of experiments, diazoxide with or without phentolamine or propranolol was added to the prestimulation buffer, but not to the stimulation buffer, again with both glucose concentrations being used for stimulation. Control experiments in the absence of added diazoxide were performed for each experimental design.

Samples were collected at 1 min intervals for the latter 5 min of the prestimulation period and the latter 54 min of the stimulation period, and at 30-sec intervals for the first 6 min of the stimulation period. Immunoreactive insulin content of the samples was assayed by a modification of the method of Hales and Randle (26).

RESULTS

As has been described previously for our system (22, 24, 25), constant stimulation with 300 mg/100 ml glucose produces a biphasic insulin release profile. The amount of insulin released during the primary and secondary components of the insulin release profiles were determined by simple addition for the primary response; all insulin released over this period was collected. The duration of this response was taken from the onset of stimulation to the first nadir of the insulin release profile. The secondary response commencing with this nadir, was calculated from the area subtended by the release rate: time plot. For series in which no definite biphasic patterns were observed (control series), the phases were arbitrarily defined by reference to the appropriate test response. Diazoxide at a concentration of 1 μg/ml, incompletely but significantly inhibited the primary but not the secondary phase of this glucose-induced insulin release; at a concentration of 10 μg/ml, approximately 50% inhibition of both phases was obtained, and at concentrations of 45 μg/ml, inhibition was practically complete (Table I). Addition of 20 μg/ml phentolamine with 45 μg/ml diazoxide partly reversed the diazoxide-induced inhibition of the secondary component of glucose-induced insulin release and enhanced the primary component of this response (Table I and Fig. 1).

When either diazoxide, 10 μg/ml, or phentolamine, 20 μg/ml, was added in the presence of a nonstimulatory concentration of glucose, no stimulation resulted. When both substances were added together, however, a biphasic insulin release response was observed, a primary spike being followed by a low-grade secondary phase (Fig. 2). Both components of this response were significantly greater than those observed in the presence of glucose 50 mg/100 ml, alone or with either diazoxide 10 μg/ml or phentolamine 20 μg/ml (Table I).

Prestimulation with diazoxide, 1 μg/ml, resulted in an enhanced primary insulin release response to subsequent glucose challenge, whereas prestimulation with 10 or 45 μg/ml diazoxide produced enhancement of both primary and secondary components of the response to glucose (Table II and Fig. 3). Addition of either phentolamine, 20 μg/ml, or propranolol, 20 μg/ml, with the diazoxide to the prestimulation buffer abolished this enhancement of the primary component at all three concentrations of diazoxide. The enhancement of the

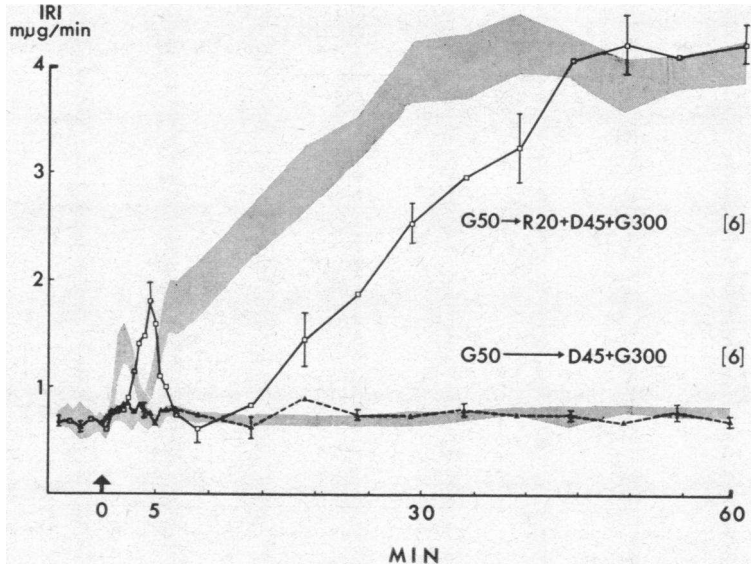

FIGURE 1 The effect of diazoxide (D) and phentolamine (R) plus diazoxide on glucose (G)-induced insulin release. The shaded areas represent the response (mean ±SEM) to stimulation with glucose alone at 50 mg/100 ml (G50, lower profile) and at 300 mg/100 ml (G300, upper profile), beginning at zero time (arrow) following a 35 min prestimulation period, the last 5 min of which are shown. The response with the addition of 45 μg/ml diazoxide (D45 + D300, broken line) or with the further addition of 20 μg/ml phentolamine (R20 + D45 + G300, solid line) is indicated. Mean ±SEM are shown. Numbers in parentheses indicate the number of separate experiments.

secondary insulin release component due to diazoxide prestimulation (at 10 and 45 μg/ml) was abolished by the further presence of propranolol during prestimulation. By contrast, phentolamine added with the diazoxide during prestimulation caused greater enhancement of the secondary insulin release phase (Table II and Fig. 4) than did diazoxide alone. Prestimulation with a high concentration (5 μg/ml) of epinephrine in contrast with the effect of low (0.25–0.5 μg/ml) epinephrine concentrations previously reported (22) did not enhance either component of glucose-induced insulin release (primary response 2.18 ±0.14 ng, secondary response 57.8 ±6.0 ng).

The separate addition of diazoxide, propranolol, or phentolamine to the prestimulation buffer was without effect on subsequent (basal) insulin release in the presence of 50 mg/100 ml glucose. Similarly, the separate addition of phentolamine, 20 μg/ml, or propranolol, 20 μg/ml, during prestimulation did not modify the subsequent insulin release response to 300 mg/100 ml glucose (Table II).

DISCUSSION

It is recognized that diazoxide may exert a direct suppressive effect on glucose-induced insulin release in vitro (20). The present studies confirm and extend these observations. The inhibitory effect of diazoxide was reversed by adrenergic α-receptor blockade with phentolamine, which suggests that this inhibitory effect was mediated through activation of α-adrenergic receptor activity in the pancreatic B-cell. Further, in the presence of nonstimulatory concentrations of glucose, the simultaneous action of diazoxide and phentolamine produced a biphasic insulin release pattern similar to that observed with low concentrations of isoproterenol (22), indicating that diazoxide might stimulate β-adrenergic receptor mechanisms. That is, diazoxide appears to share some of the demonstrated properties of physiological adrenergic compounds in its actions upon insulin release from the pancreas.

Further similarities between diazoxide and catecholamines were demonstrated in the results of prestimulation experiments. Diazoxide, added during prestimulation at a concentration smaller than that required for the suppression of glucose-induced insulin release during stimulation, produced a significant enhancement of the primary component of the biphasic insulin release response to subsequent glucose challenge. This effect, previously described for low concentrations of epinephrine (22), was reduced or abolished by adding either an α- or a

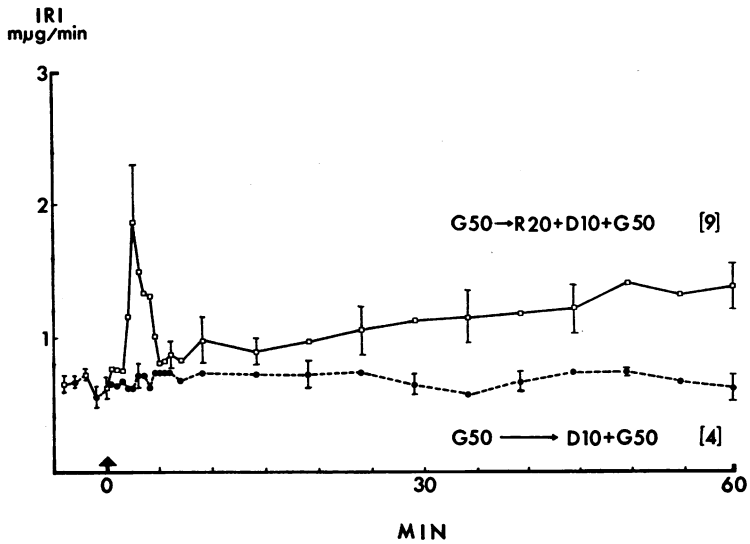

FIGURE 2 Insulin release in the presence of 50 mg/100 ml (G50) glucose plus 10 μg/ml diazoxide (D10 + G50, broken line) and to the further addition of 20 μg/ml phentolamine (R20 + D10 + G50, solid line). Data are presented as in Fig. 1.

β-adrenergic receptor–blocking agent together with the diazoxide. The dependence on the integrity of both α- and β-receptor mechanisms for the expression of diazoxide action may not be unique to the B-cell since it also appears to obtain for mediation of diazoxide-induced renin release in vivo (27).

TABLE II

The Effect of Prestimulation with Diazoxide and Adrenergic-Blocking Agents on the Subsequent Basal and Glucose-Stimulated Biphasic Insulin Release

Experiment	Prestimulation				Stimulation	n	IRI release			
	Glucose	Diazoxide	Phentolamine	Propranolol	Glucose		Primary	P	Secondary	P
	mg/100 ml	*μg/ml*	*μg/ml*	*μg/ml*	*mg/100 ml*		*ng, mean ±SEM*			
a.	50	—	—	—	50	6	2.4 ±0.2		38 ±2	
b.	50				300	10	3.8 ±0.2	0.01 (a)	172 ±9	0.01 (a)
c.	50	1	—	—	300	7	8.6 ±0.6	0.01 (b)	166 ±12	NS (b)
d.	50	10	—	—	300	7	9.6 ±0.9	0.01 (b)	244 ±17	0.01 (b)
e.	50	45	—	—	300	7	5.7 ±0.6	0.01 (b)	205 ±15	0.01 (b)
f.	50	—	20	—	300	6	3.9 ±0.1	NS (b)	161 ±4	NS (b)
g.	50	—	—	20	300	6	3.6 ±0.1	NS (b)	169 ±4	NS (b)
h.	50	1	20	—	300	6	4.3 ±0.3	NS (b)	255 ±9	0.01 (b)
								0.01 (c)		0.01 (c)
i.	50	1	—	20	300	8	5.6 ±0.4	0.02 (b)	182 ±11	NS (b)
								0.01 (c)		NS (c)
j.	50	10	20	—	300	6	3.6 ±0.3	NS (b)	289 ±9	0.01 (b)
								0.01 (d)		0.05 (d)
k.	50	10	—	20	300	9	4.4 ±0.3	NS (b)	190 ±15	NS (b)
								0.01 (d)		0.05 (d)
l.	50	10	—	—	50	6	2.0 ±0.3	NS (a)	26 ±3	NS (a)
m.	50	—	20	—	50	6	2.3 ±0.3	NS (a)	30 ±4	NS (a)
n.	50	—	—	20	50	6	2.1 ±0.2	NS (a)	29 ±3	NS (a)

Data are presented as in Table I.

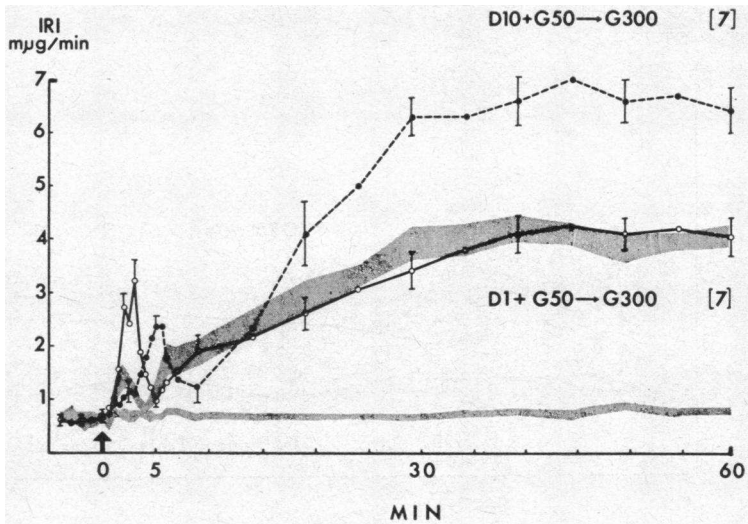

FIGURE 3 The effect of prestimulation with diazoxide 1 μg/ml (D1 + G50, solid line) and 10 μg/ml (D10 + G50, broken line) on the subsequent insulin response to glucose stimulation. The shaded areas represent the mean ±SEM response to glucose at 50 mg/100 ml (lower profile) and 300 mg/100 ml (upper profile). Data are presented as in Fig. 1.

Preperifusion of pancreas fragments with diazoxide concentrations 10 and 45 times greater than those which selectively enhanced the primary component of subsequent glucose-induced biphasic insulin release resulted in enhancement of both components of the subsequent glucose-induced insulin release. At these concentrations,

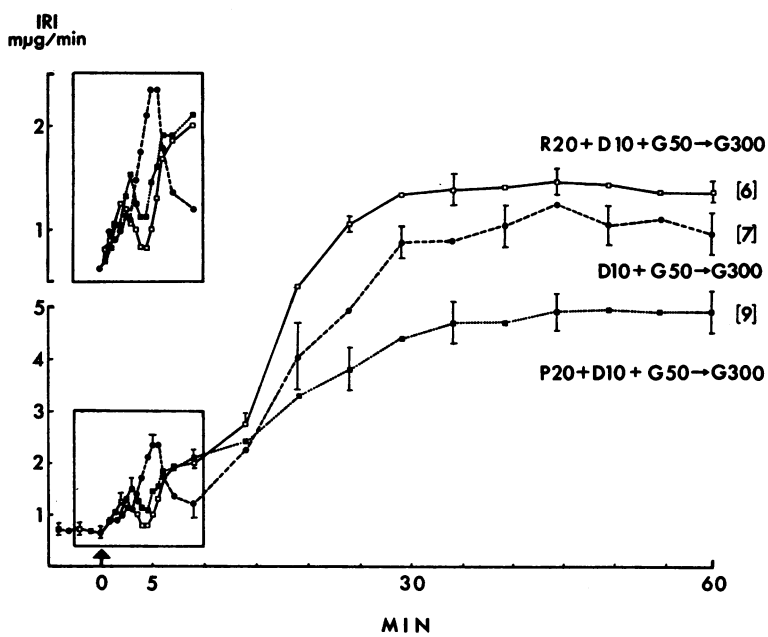

FIGURE 4 The effect of prestimulation with 10 μg/ml diazoxide in the absence (D10 + G50, broken line) plus presence of adrenergic-blocking agents, on subsequent insulin response to glucose 300 mg/100 ml (G300). The influence of addition of 20 μg/ml phentolamine (R20 + D10 + G50, solid line) or of 20 μg/ml propranolol (P20 + D10 + G50, dotted line) on the enhancement of response due to diazoxide is shown. Primary response is shown with an expanded scale in the upper box. Data are presented as in Fig. 1.

diazoxide produced approximately 50 and 100% inhibition of glucose-induced insulin release in our system. Similar enhancement of the insulin response to glucose challenge after prior diazoxide treatment has been reported in vivo in a patient with insulinoma (28) and for in vitro perfused pancreas removed from rats which had been treated with the agent (21). In both of these instances glucose challenge had been delayed until many hours after the last dose of diazoxide, a factor which may be critical in producing such an effect. It has been suggested (21) that this phenomenon may result from increased insulin stores secondary to continued synthesis in the face of reduced release. It is unlikely, however, that increased insulin stores could be solely responsible for the enhancement observed in the present study. Preincubation with diazoxide lasted only 35 min, which are unfavorable conditions in the presence of only 50 mg/100 ml glucose, for a significant addition to insulin stores through synthesis. Propranolol inhibition of the diazoxide-induced enhancement of the secondary component of subsequent glucose-induced insulin release resembles the effect of propranolol on epinephrine prestimulation (22), and suggests to us that mechanisms similar to those postulated for epinephrine-induced enhancement of insulin release (22) may be involved in this diazoxide action.

The enhancement by diazoxide prestimulation of *both* phases of glucose-induced insulin release is an evident departure from the parallelism of diazoxide and epinephrine effects on insulin release. In the present studies, 10 μg/ml diazoxide was required to produce this effect, i.e. a dose which, when added *during* stimulation, resulted in 40–50% inhibition of glucose-induced insulin release in our system, an inhibition comparable with that previously obtained with 0.5 μg/ml of epinephrine (22). By contrast, when this "equivalent" concentration of epinephrine was used during prestimulation, it induced only a selective enhancement of the primary component of glucose-induced biphasic insulin release (22). Further, increase of the concentration of diazoxide to 45 μg/ml in the prestimulation buffer, confirmed the enhancement of both phases of the glucose response, whereas a 10-fold greater prestimulation concentration of epinephrine than that which selectively enhanced the primary response to glucose (22) resulted in loss of any stimulation in our system. Whereas it is not possible to exclude differences in the tissue clearance of the effective concentrations of these agents or their metabolites as an explanation for this apparent difference in their actions, the data raise the possibility that the apparent β-adrenergic receptor–stimulating activity of diazoxide, although blocked by propranolol, may be mediated in part through different pathways than the similar effect of epinephrine. In support of this contention is that diazoxide enhanced, whereas epinephrine inhibited potassium-induced insulin release in vitro (29).

Of interest was the observation that the addition of phentolamine with diazoxide during prestimulation, while inhibiting the diazoxide-induced enhancement of the primary response to glucose, further enhanced the effect of diazoxide prestimulation on the secondary component of glucose induced insulin release. This effect of phentolamine had been suggested, but not confirmed statistically, in similar experiments utilizing epinephrine as a prestimulating agent (22).

In conclusion, these data support previous suggestions of both direct inhibitory and direct stimulatory effects of diazoxide on insulin release mechanisms. The similarity of these effects to those of epinephrine and norepinephrine (22), particularly in terms of their interactions with α- and β-adrenergic receptor–blocking agents, suggests that diazoxide may directly affect α- and β-adrenergic receptor mechanisms in the B-cell. A similar in vitro effect of diazoxide on adipose tissue has been reported (13), although a direct epinephrine-like effect of this agent on hepatic metabolism has not been demonstrated (30).

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