Role of Sympathetic Nervous System in Glucagon Response to Insulin Hypoglycemia in Normal and Diabetic Rats

D. G. PATEL

SUMMARY

The effects of adrenergic blockers on the glucagon response to insulin hypoglycemia were investigated in diabetic (10–15 days poststreptozocin [STZ] injection) and age-matched control rats. α- (Phentolamine nonspecific but predominantly α_1), α_2- (yohimbine), or β- (propranolol) adrenergic blockers alone or in combination did not affect plasma glucose levels or plasma glucagon concentrations, in the basal state, in either control or diabetic rats. None of these adrenergic blockers, alone or in combination, inhibited the glucagon response to insulin hypoglycemia in control or diabetic rats. On the contrary, in control rats, the β-adrenergic blocker alone or in combination with an α-adrenergic blocker and in diabetic rats, the α-adrenergic blocker alone significantly stimulated the glucagon response to insulin hypoglycemia.

Second, the effects of yohimbine on the glucagon response to epinephrine infusion were studied in both young and old rats. Recently, Cherksey et al. (Proc. Soc. Exp. Biol. Med. 1982; 171:196–200) have reported that the adrenergic receptors on rat pancreatic islet cells are of the α_2-subtype. Yohimbine (α_2-adrenergic blocker) completely blocked the glucagon response to epinephrine infusion in both young and old rats, but had no inhibitory effect on the glucagon response to insulin hypoglycemia in control and short-term diabetic rats. From these observations, it could be inferred that the lack of glucagon response to insulin hypoglycemia in long-term diabetic rats is unlikely to be explained by an impairment of an adrenergic function.

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Several studies have demonstrated impaired glucagon response to an insulin hypoglycemia in type I and type II diabetic subjects and experimental diabetic rats,[1-9] but normal or greater than normal glucagon release during amino acid infusion. The blunted glucagon response to insulin hypoglycemia is mostly associated with autonomic neuropathy in diabetic human studies.[4-7] Whether their glucagon secretory defect represents an abnormality of the parasympathetic or sympathetic nervous system, or both, is unclear.

There is considerable evidence that the autonomic nervous system plays a regulatory role in glucagon secretion. By electron microscopy, cholinergic, and possible adrenergic, nerve endings on the surface of the α-cells of Langerhans have been demonstrated.[10,11] The sympathetic nervous system may play a role in glucagon secretion. Electrical stimulation of the splanchnic nerves in the unconscious calf produced a rapid rise in plasma glucagon levels.[12] Epinephrine, norepinephrine, and a variety of stresses have been shown to stimulate glucagon secretion.[13-17] In clinical diabetic autonomic neuropathy, the sympathetic mechanisms of glucagon release may be impaired. Christensen showed catecholamine depletion in diabetic autonomic neuropathy.[18] Lozovsky et al.[19] have demonstrated a 30–35% increase in dopamine receptors in striatal membranes of STZ-diabetic rats. This laboratory has also reported a decrease in dopamine levels of whole brain in STZ-diabetic rats.[20] From these studies, it seems possible that first abnormalities occur in catecholamines, which, in turn, affect the glucagon response. However, this might not be the main defect in diabetic subjects, as some of the recent studies have indicated that there is no effect on glucagon response to insulin hypoglycemia of adrenergic blockade, adrenalectomy, or sympathectomy.[21-25] In recent studies,[26] this laboratory was able to dissociate the lack of glucagon response and the blunted catecholamine response to insulin hypoglycemia in STZ-diabetic rats with prolonged insulin treatment. In most of the reported studies, either α- or β-adrenergic blockers were used to demonstrate the role of adrenergic mechanism in the glucagon response to insulin hypoglycemia. Recently, Cherksey et al.[27] have demonstrated that the adrenergic receptors on rat pancreatic islet cells are of α_2 subtype. Therefore, yohimbine (α_2-adrenergic blocker)

From the Barbara Kopp Research Center, Auburn, New York, and the Department of Pharmacology, Upstate Medical Center, Syracuse, New York.
Address reprint requests to Department of Pharmacology at Meharry Medical College, 1005 18th Avenue North, Nashville, Tennessee 37208.
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TABLE 1
Plasma glucose and plasma glucagon levels in response to epinephrine infusion in young and old rats

Test drugs		Body wt (g)	Plasma glucose (mg/dl)		Plasma glucagon (pg/ml)	
			Fasting	20 Min after epinephrine	Fasting	20 Min after epinephrine
None	(6)	276 ± 22	128 ± 22	151 ± 38	47 ± 12	120 ± 26
Yohimbine (0.2 μg/kg, s.c.)	(7)	284 ± 20	126 ± 17	138 ± 17	43 ± 5	42 ± 9
None	(6)	392 ± 17*	122 ± 21	148 ± 18	85 ± 21*	164 ± 28
Yohimbine (0.2 μg/kg, s.c.)	(5)	395 ± 13*	124 ± 7	147 ± 9	93 ± 8*	97 ± 8

All results are expressed as mean ± SD. Number of observations is in parentheses. Yohimbine (0.2 μg/kg) was injected s.c. 30 min before infusion of epinephrine. Epinephrine (0.2 μg/kg/min) was infused via jugular vein.
*$P < 0.05$ compared with young rats.

has been used to investigate the glucagon release in response to insulin hypoglycemia in normal and short-term diabetic rats (10–15 days post-STZ injection). The effects of α- and β-adrenergic blockers on the glucagon response to insulin hypoglycemia in normal and short-term diabetic rats have also been studied. The results, described in this paper, suggest that the sympathetic nervous system does not play an important role in glucagon release in response to insulin hypoglycemia in either normal or STZ-diabetic rats.

MATERIALS AND METHODS

Animals. Sprague-Dawley male, adult rats weighing 275–300 g were used in this investigation. All animals were housed three per plastic cage in animal quarters maintained at constant temperature, humidity, and light cycles. Animals were fed Purina Rat Chow and water ad libitum.

Experimental protocol. All rats were divided into two weight-matched groups. Rats in group I (experimental) were fasted for 18 h with free access to water and injected with streptozocin (STZ) 65 mg/kg, i.v. (freshly prepared in ice-cold 0.05 M citrate buffer, pH 4.5). The animals in group II (control) were fasted for 18 h with free access to water and injected i.v. with ice-cold 0.05 M citrate buffer alone (freshly prepared, pH 4.5). All rats were then offered food immediately. All rats in the experimental group showed glycosuria 24 h after STZ injection.

The experimental rats were studied for glucagon release in response to insulin hypoglycemia at 10–15 days post-STZ injection. Age-matched control rats were also used.

Rats were fasted for 18 h with free access to water. They were anesthetized with chloral hydrate (350 mg/kg, i.p.). Polyethylene cannulae were inserted into the jugular vein (for infusion) and the carotid artery (for blood sampling). Rats were allowed to stabilize for about 30 min after surgery. At that time, a 2.0-ml blood sample was collected from the carotid cannula with a heparinized syringe and transferred to an ice-cold tube containing 100 μl of Trasylol (10,000 U/ml) and EDTA (2.4 mg/tube) mixture. All blood samples were

TABLE 2
Initial (I) and hypoglycemic (H) plasma glucose levels and total insulin units infused to reach nadir plasma glucose levels in normal and diabetic (10–15 days post-STZ injection) male rats with and without adrenergic blockers

Test drugs	Control rats			Diabetic rats		
	Plasma glucose (mg/dl)		Total insulin required to obtain hypoglycemia (U)	Plasma glucose (mg/dl)		Total insulin required to obtain hypoglycemia (U)
	I	H		I	H	
No blocker	151 ± 12 (8)	34 ± 3 (8)	1.8 ± 0.4 (8)	392 ± 77* (7)	30 ± 3 (7)	4.9 ± 1.4* (7)
α-Blocker (phentolamine)	139 ± 20 (6)	34 ± 3 (6)	2.1 ± 0.6 (6)	383 ± 78* (7)	29 ± 5 (7)	5.0 ± 0.7* (7)
α_2-Blocker (yohimbine)	126 ± 18 (8)	33 ± 3 (8)	1.8 ± 0.5 (8)	313 ± 60* (7)	32 ± 3 (7)	4.4 ± 0.7* (7)
β-Blocker (propranolol)	122 ± 20 (10)	30 ± 2 (10)	1.7 ± 0.3 (10)	404 ± 68* (5)	29 ± 4 (5)	4.6 ± 0.7* (5)
α + β-Blocker (phentolamine + propranolol)	115 ± 13 (6)	34 ± 2 (6)	1.5 ± 0.5 (6)	409 ± 40* (7)	33 ± 3 (7)	5.1 ± 1.5* (7)
α_2 + β-Blocker (yohimbine + propranolol)	111 ± 12 (8)	30 ± 3 (8)	1.6 ± 0.3 (8)	297 ± 54* (6)	33 ± 2 (6)	4.1 ± 0.9* (6)

All results are expressed as mean ± SD. Numbers of observations are in parentheses.
Phentolamine 0.2 mg/kg, i.p., as bolus and 0.05 mg/kg/h infused i.v. for 1.5 h; or propranolol 5 mg/kg, i.p., as bolus and 0.5 mg/kg/h infused i.v. for 1.5 h; or combination of phentolamine and propranolol infused i.v. for 1.5 h; or yohimbine 1 mg/kg, s.c., as bolus alone (30 min); or combination of yohimbine, 1 mg/kg, and propranolol, 5 mg/kg, s.c., as bolus alone (30 min).
*$P < 0.01$ compared with respective values of control group.

TABLE 3
Plasma glucose and glucagon levels before and after adrenergic blocking drugs in control rats

Drugs	Plasma glucose (mg/dl)		Plasma glucagon (pg/ml)	
	Basal	After drugs	Basal	After drugs
Phentolamine (8)	122 ± 23	135 ± 52	19 ± 8	18 ± 9
Propranolol (8)	135 ± 20	136 ± 21	18 ± 7	18 ± 4
Yohimbine (8)	129 ± 19	128 ± 12	61 ± 24	51 ± 18
Yohimbine + propranolol (8)	120 ± 18	119 ± 9	73 ± 39	65 ± 48

All results are expressed as mean ± SD. Numbers of observations are in parentheses.
Phentolamine 0.2 mg/kg, i.p., as bolus and 0.05 mg/kg/h infused i.v. for 1.5 h; or propranolol 5 mg/kg, i.p., as bolus and 0.5 mg/kg/h infused i.v. for 1.5 hr; or yohimbine 1 mg/kg, s.c., as bolus alone (30 min); or combination of yohimbine, 1 mg/kg, and propranolol, 5 mg/kg, s.c., as bolus alone (30 min).

kept in the refrigerator at 4°C until centrifuged. Plasma glucose levels were determined. Immediately after that, either one of the following adrenergic blocking drugs or a combination was injected as follows: (1) phentolamine, 0.2 mg/kg, i.p., as bolus and 0.05 mg/kg/h infused i.v. for 1.5 h; or (2) propranolol, 5 mg/kg, i.p., as bolus and 0.5 mg/kg/h infused i.v. for 1.5 h; or (3) combination of phentolamine and propranolol infused i.v. for 1.5 h; or (4) yohimbine, 1 mg/kg, s.c., as bolus alone (30 min); or (5) combination of yohimbine, 1 mg/kg, and propranolol, 5 mg/kg, s.c., as bolus alone (30 min).

Ninety minutes after each adrenergic blocker (except 30 min after yohimbine and yohimbine with propranolol), insulin was injected i.v. according to the following formula: 2.0 + (PG − 100) × 0.02 U/kg (PG = plasma glucose). Immediately after a bolus insulin injection via jugular vein, insulin (5 U/kg/h) and chloral hydrate (25 mg/kg/ml) were infused at the rate of 2.3–2.6 ml/h using an infusion pump. No adrenergic blocker was infused along with insulin injection. Heart rates or blood pressure were not monitored to document the adequacy of adrenergic blockade due to lack of facilities to undertake such studies.

Approximately 70 μl of blood was collected periodically (maximum 10–15 times) to determine plasma glucose levels to monitor hypoglycemia. The second 2.0-ml blood samples were collected when the plasma glucose level dropped to 30–40 mg/dl. As mentioned before, blood samples were transferred to tubes for glucagon estimations. All blood samples were centrifuged at 1500 × *g* at 4°C for 20 min. Plasma samples were separated and stored at −20°C for glucagon determinations at a later date.

To determine the effects of adrenergic blockers on basal plasma glucose and plasma glucagon levels, the following experiments were carried out. Fasting blood samples were collected from anesthetized rats as described earlier. The second blood samples were collected at the above specified times after each adrenergic blocking agent. Plasma glucose and plasma glucagon levels were determined.

Both young and old rats were used to study the role of aging in the adrenergic mechanism of the secretion of glucagon. Fasting blood samples were collected from anesthetized rats as described earlier. Epinephrine bitartrate was infused in a dose of 0.2 μg/kg/min via jugular vein catheter for 20 min. The second blood samples were collected at that time. Plasma glucose and plasma glucagon levels were measured.

Analytic techniques. Plasma glucose was determined by the glucose-oxidase method, using a glucose analyzer (Beckman Instruments, Fullerton, California). Plasma glucagon was measured by radioimmunoassay, using crystalline porcine pancreatic glucagon as standard (Eli Lilly and Company, Indianapolis, Indiana).[28] To eliminate spurious increments in glucagon values caused by the unidentified "big plasma glucagon," both standards and samples were extracted with ice-cold acetone as described by Von Schenck and Nilsson.[29] Statistical significance was computed by either Student's *t*-test or Duncan's multiple range test. Differences between means were considered statistically significant when $P < 0.05$.

RESULTS

Table 1 depicts plasma glucose and plasma glucagon before and 20 min after epinephrine infusion in both young and old rats. Fasting plasma glucose levels in both young and

TABLE 4
Plasma glucose and glucagon levels before and after adrenergic blocking drugs in diabetic rats (10–15 days post-STZ injection)

Drugs	Plasma glucose (mg/dl)		Plasma glucagon (pg/ml)	
	Basal	After drugs	Basal	After drugs
Phentolamine (6)	494 ± 76	550 ± 65	68 ± 34	63 ± 10
Propranolol (8)	449 ± 76	436 ± 65	94 ± 46	91 ± 44
Yohimbine (8)	316 ± 56	310 ± 46	89 ± 46	66 ± 27
Yohimbine + propranolol (8)	289 ± 62	283 ± 58	59 ± 30	41 ± 21

All results are expressed as mean ± SD. Numbers of observations are in parentheses.
Phentolamine 0.2 mg/kg, i.p., as bolus, and 0.05 mg/kg/h infused i.v. for 1.5 h; or propranolol 5 mg/kg, i.p., as bolus, and 0.5 mg/kg/h infused i.v. for 1.5 h; or yohimbine 1 mg/kg, s.c., as bolus alone (30 min); or combination of yohimbine, 1 mg/kg, and propranolol, 5 mg/kg, s.c., as bolus alone (30 min).

TABLE 5
Δ Glucagon over fasting (plasma) levels in response to insulin hypoglycemia in control and diabetic (10–15 days post-STZ injection) male rats

Test drugs	Δ Glucagon (pg/ml)	
	Control rats	Diabetic rats
No blocker	(8) 841 ± 226	(7) 723 ± 182
α-Blocker (phentolamine)	(6) 889 ± 277	(7) 987 ± 110
α_2-Blocker (yohimbine)	(8) 1180 ± 415	(7) 681 ± 402
β-Blocker (propranolol)	(10) 1405 ± 370*	(5) 796 ± 290
α + β-Blocker (phentolamine + propranolol)	(6) 1167 ± 182†	(6) 513 ± 120
α_2 + β-Blocker (yohimbine + propranolol)	(8) 856 ± 598	(7) 547 ± 398

All results are expressed as mean ± SD. Numbers of observations are in parentheses.
Phentolamine 0.2 mg/kg, i.p., as bolus, and 0.05 mg/kg/h infused i.v. for 1.5 h; or propranolol 5 mg/kg, i.p., as bolus, and 0.5 mg/kg/h infused i.v. for 1.5 h; or combination of phentolamine and propranolol infused i.v. for 1.5 h; or yohimbine 1 mg/kg, s.c., as bolus alone (30 min); or combination of yohimbine 1 mg/kg, and propranolol, 5 mg/kg, s.c., as bolus alone (30 min).
*$P < 0.01$ compared with respective values of a group without blocker.
†$P < 0.05$ compared with respective values of a group without blocker.

old rats were essentially identical. However, fasting plasma glucagon levels in older rats were significantly higher compared with those in young rats (85 ± 21 and 93 ± 8 pg/ml versus 47 ± 12 and 43 ± 5 pg/ml, $P < 0.05$). There was also an identical rise in plasma glucagon levels due to epinephrine infusion in both young and old rats, and this rise in plasma glucagon levels was blocked by yohimbine pretreatment.

Table 2 shows plasma glucose in initial and hypoglycemic states and total insulin units required to obtain hypoglycemia in both normal and diabetic (10–15 days post-STZ injection) male rats with and without adrenergic blockers. Plasma glucose levels in initial (fasting) states were significantly higher in diabetic rats compared with those in normal rats; however, plasma glucose nadirs after insulin infusion were identical in both diabetic and control rats. The total amount of insulin required to achieve a comparable hypoglycemic state was of higher magnitude in the diabetic rats compared with that in the control rats.

Table 3 depicts plasma glucose and plasma glucagon levels before and after adrenergic blockers in normal male rats. Neither α- (phentolamine), α_2- (yohimbine), nor β- (propranolol) blockers alone nor a combination of α_2- and β-adrenergic blockers elicited any effect on basal plasma glucose or basal plasma glucagon values in normal adult male rats. It has previously been observed in this laboratory (unpublished data) that older male rats have significantly higher basal (fasting) plasma glucagon levels compared with those in young adult male rats. Rats in yohimbine- and yohimbine-with-propranolol–treated groups were much older compared with rats in phentolamine- or propranolol-alone–treated groups, which probably accounts for the higher basal values in those groups.

Effects of adrenergic blockers, alone or in combination, on basal plasma glucose and basal plasma glucagon concentration in diabetic (10–15 days post-STZ injection) male rats are shown in Table 4. Adrenergic blockers (α, α_2, β, or combination of α_2 + β) did not affect either basal plasma glucose or basal plasma glucagon levels in diabetic male rats.

The results in Table 5 are expressed as the changes in values (Δ) for plasma glucagon in response to insulin hypoglycemia in diabetic and age-matched control male rats and were computed and analyzed with Duncan's multiple range test. It was observed that Δ glucagon values in response to insulin hypoglycemia in control (841 ± 226 pg/ml) and in diabetic (723 ± 182 pg/ml) male rats, in the absence of adrenergic blockers, were essentially identical. The β-blocker alone or in combination with an α-blocker significantly increased the glucagon response compared with no blocker in control rats. However, the glucagon responses, with either α-blocker, α_2-blocker alone, or a combination of α_2- and β-blockers, were not different from the control values in normal male rats. Similarly, in diabetic rats, none of the tested adrenergic blockers altered the glucagon response from control levels except that α- (phentolamine) adrenergic blockade significantly increased the glucagon response over that in the control group. In the diabetic rats, as well as in the controls, blocking the α- or β-sites of adrenergic mechanisms did not diminish the glucagon response to insulin hypoglycemia.

DISCUSSION

The amount of insulin required to achieve almost identical nadir plasma glucose values in short-term diabetic rats was significantly higher than that for age-matched control rats. However, glucagon responses to insulin hypoglycemia in both short-term diabetic and control rats were essentially identical; therefore, inhibitory effects of insulin on glucagon release are irrelevant for the present study.

Several studies involving adrenergic agonists and antagonists[30–34] and neural stimulation[35,36] have strongly suggested that the sympathoadrenal system may modulate glucagon secretion. In addition, many states characterized by catecholamine excess are associated with hyperglucagonemia.[37–41] However, a cause-effect relationship between sympathetic nervous system discharge or epinephrine release from the adrenal, and stimulation of glucagon secretion in response to insulin hypoglycemia has not been identified. This study has examined the effects of both α- and β-adrenergic blockers separately or in combination on glucagon secretion in response to insulin hypoglycemia in normal as well as in short-term diabetic rats (10–15 days post-STZ injection).

Several studies indicated that phentolamine (an α-adrenergic blocking agent) increased glucagon secretion in various experimental conditions.[30] Luyckx and Lefebvre[32] reported that, in rats, i.p. injection of 25 mg/kg phentolamine led to a slight increase in plasma glucagon. However, a 5-mg/kg dose had no effect on basal plasma glucagon levels. The present results concur with these findings that 0.2 mg/kg phentolamine had no effect on basal glucagon levels in either normal or diabetic rats. In this study, a lower dose of phentolamine was used because even 1 mg/kg of phentolamine turned out to be toxic in diabetic rats. Most of the

diabetic rats died in less than 1 h after phentolamine (1 mg/kg) injection. The cause of mortality was not evaluated. None of the diabetic rats died after 0.2 mg/kg of phentolamine. Sasaki et al.[42] infused phentolamine (20 μg/kg/min) i.v. into mature, castrate, male sheep and showed that, in a cold environment, insulin secretion was significantly increased. During α-adrenergic blockade, the increased β-adrenergic stimulation in the cold became apparent, resulting in an increase in the insulin concentration. This suggests that the dose of phentolamine used in this study is adequate to block α-adrenergic receptors on islet cells. Phentolamine also had no effect on glucagon secretion in response to insulin hypoglycemia, which is in agreement with the findings of Luyckx and Lefebvre[32] in exercise-induced glucagon release. Walter et al.[21] infused phentolamine (5 mg as bolus and 0.5 mg/min for 150 min) during insulin hypoglycemia in normal subjects. They also observed that phentolamine did not affect plasma glucagon levels, nor did it block the rise in plasma glucagon values due to insulin hypoglycemia. In diabetic rats, phentolamine pretreatment significantly increased the rise in plasma glucagon levels in response to insulin hypoglycemia. It seems quite evident from these findings that α-adrenergic receptors do not play any inhibitory role in the glucagon release in response to insulin hypoglycemia in diabetic rats.

Recently, Cherksey et al.[27] demonstrated that α-adrenergic receptors on islets of rats are of the α_2-subtype. Therefore, yohimbine, a specific α_2-adrenergic blocker, was used to study the role of α-adrenergic receptors in the glucagon secretion in response to insulin hypoglycemia. Yohimbine, like phentolamine, had no effect on basal glucagon levels and also had no effect on the rise in glucagon values in response to insulin hypoglycemia in both normal and diabetic rats. However, yohimbine blocked the glucagon response to epinephrine infusion in control rats. This implies that the blockade of specific adrenergic receptors (α_2-subtype) on rat pancreatic islets does not affect the glucagon response to insulin hypoglycemia.

Harvey et al.[43] reported that propranolol potentiated glucagon rise in rats submitted to very intensive exercise, which was indeed associated with a prolonged hypoglycemia. Luyckx and Lefebvre[32] reported that, in rats, the exercise-induced rise in glucagon secretion was completely abolished by previous i.p. administration of propranolol at a dose of 5 mg/kg, whereas a relatively specific β_1-blocker, practalol (12.5 mg/kg), was devoid of effect. They concluded that β_2-adrenoreceptors of the α-cells were involved in glucagon secretion under these conditions. However, they also demonstrated that, in rats, propranolol did not prevent hypoglycemia-induced glucagon secretion that was, on the contrary, exaggerated, probably because of the marked decrease in blood glucose. Recently, DeFeo et al.[44] also demonstrated that in both diabetic (with normal glucagon response to insulin hypoglycemia) and normal subjects, plasma glucose concentrations were unaffected by propranolol. The rise in plasma glucagon was unaffected in the presence of propranolol. However, the present findings show significantly higher Δ glucagon in response to insulin hypoglycemia in the presence of propranolol. This could perhaps be due to species difference.

In most of the studies α- or β-adrenergic blockers were used separately. It has been suggested that both α_2- and β_2-adrenoreceptors are involved in the glucagon response. This laboratory has used a combination of α + β- and α_2 + β-adrenergic blockers to investigate their combined effect on the glucagon response to insulin hypoglycemia in both normal and diabetic rats. Neither yohimbine nor propranolol affected basal plasma glucose or plasma glucagon concentrations in both normal and diabetic rats. Similarly, yohimbine and propranolol pretreatment also had no effect on Δ glucagon response to insulin hypoglycemia. Phentolamine and propranolol significantly increased Δ glucagon in response to insulin hypoglycemia in normal rats. On the other hand, a combination of phentolamine and propranolol induced no change in Δ glucagon in response to insulin hypoglycemia in diabetic rats.

In conclusion, neither α- nor α_2- nor β- nor a combination of α + β- nor α_2 + β-blocker affects basal plasma glucose or plasma glucagon levels in normal and diabetic rats. Similarly, none of the adrenergic blockers studied blocked the glucagon response to insulin hypoglycemia in either normal or short-term diabetic rats (10–15 days post-STZ injection). In long-term diabetic rats (50 or more days post-STZ injection), both glucagon and epinephrine responses to insulin hypoglycemia are altered simultaneously.[9] However, when diabetic rats were maintained in prolonged euglycemic state with insulin mini-osmotic pump therapy, this laboratory was able to normalize the lack of epinephrine response, but not the lack of glucagon response, to insulin hypoglycemia.[26] Similar results were also obtained by Bolli et al.[45] when they maintained diabetic subjects in euglycemic state with insulin pump therapy. Therefore, it is improbable that the lack of glucagon response in long-term diabetic rats and diabetic subjects was dependent on a simultaneous decrease in epinephrine response. These abnormalities may, therefore, be independent events.

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