

# Adrenergic Mediation of Hypoglycemia-Associated Autonomic Failure

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**OBJECTIVE**—We tested the hypothesis that adrenergic activation, cholinergic activation, or both, mediate the effect of recent antecedent hypoglycemia to reduce the sympathoadrenal response to subsequent hypoglycemia, the key feature of hypoglycemia-associated autonomic failure in diabetes, in humans.

**RESEARCH DESIGN AND METHODS**—Seventeen healthy adults were studied on 2 consecutive days on three occasions. Day 1 involved hyperinsulinemic euglycemic ( $90 \text{ mg/dL} \times 1 \text{ h}$ ), then hypoglycemic ( $54 \text{ mg/dL} \times 2 \text{ h}$ ) clamps, in the morning and afternoon on all three occasions with 1) saline infusion, 2) adrenergic blockade with the nonselective  $\alpha$ -adrenergic and  $\beta$ -adrenergic antagonists phentolamine and propranolol, or 3) adrenergic blockade plus cholinergic blockade with the muscarinic cholinergic antagonist atropine in random sequence. Day 2 involved similar morning euglycemic and hypoglycemic clamps, with saline infusion, on all three occasions.

**RESULTS**—Compared with the responses to hypoglycemia during saline infusion on day 1, the plasma epinephrine and norepinephrine responses to hypoglycemia were reduced on day 2 ( $351 \pm 13$  vs.  $214 \pm 22 \text{ pg/mL}$  for epinephrine and  $252 \pm 4$  vs.  $226 \pm 7 \text{ pg/mL}$  for norepinephrine during the last hour; both  $P < 0.0001$ ). However, the plasma epinephrine and norepinephrine responses to hypoglycemia were not reduced on day 2 when adrenergic or adrenergic plus cholinergic blockade was produced during hypoglycemia on day 1.

**CONCLUSIONS**—Adrenergic blockade prevents the effect of hypoglycemia to reduce the plasma catecholamine responses to subsequent hypoglycemia. Thus, adrenergic activation mediates the effect of recent antecedent hypoglycemia to reduce the sympathoadrenal response to subsequent hypoglycemia, the key feature of hypoglycemia-associated autonomic failure in diabetes, in humans. *Diabetes* 60:602–606, 2011

**I**atrogenic hypoglycemia is the limiting factor in the glycemic management of diabetes (1). It causes recurrent morbidity in most people with type 1 diabetes and in many with type 2 diabetes, and is sometimes fatal, impairs physiologic and behavioral defenses against subsequent hypoglycemia, and generally precludes maintenance of euglycemia over a lifetime of diabetes. The concept of hypoglycemia-associated autonomic failure (HAAF) in diabetes posits that recent antecedent hypoglycemia, as well as prior exercise or sleep,

causes both the syndrome of defective glucose counterregulation (by reducing the adrenomedullary epinephrine response to subsequent hypoglycemia in the setting of absent decrements in insulin and absent increments in glucagon) and of hypoglycemia unawareness (by reducing the sympathoadrenal and resulting neurogenic symptom responses to subsequent hypoglycemia) (1–4). These two components of HAAF are both associated with a substantially increased incidence of hypoglycemia during intensive therapy for diabetes (1). Perhaps the most compelling evidence of the clinical effect of HAAF is the finding, originally in three independent laboratories (5–8), that as little as 2 to 3 weeks of scrupulous avoidance of hypoglycemia reverses hypoglycemia unawareness and improves the attenuated epinephrine component of defective glucose counterregulation in most affected patients.

The mechanisms of the attenuated sympathoadrenal response to hypoglycemia, the key feature of the pathogenesis of HAAF in diabetes (1–8), are unknown (1). Although much of the neuroscience research into this issue has focused on the hypothalamus (9), recent translational research has raised the possibility that a complex cerebral network normally regulates the hypothalamic (and thus the systemic sympathoadrenal) response to falling plasma glucose concentrations (10–12) and that an inhibitory signal mediated through the thalamus might be involved in the pathogenesis of HAAF (12).

Hypoglycemia activates the sympathoadrenal system (1,13,14). This includes the release of catecholamines that interact with  $\alpha$ -adrenergic and  $\beta$ -adrenergic receptors—norepinephrine from sympathetic postganglionic neurons and epinephrine and norepinephrine from the adrenal medullae—and acetylcholine that interacts with muscarinic cholinergic receptors—from sympathetic postganglionic neurons. Hypoglycemia also activates central nervous system circuits, including those that involve adrenergic and cholinergic neurotransmission. There are, of course, an array of other neurotransmitters released in the peripheral and the central nervous systems.

We used the original model of HAAF (2) and the nonselective  $\alpha$ -adrenergic and  $\beta$ -adrenergic antagonists phentolamine and propranolol as well as the muscarinic cholinergic antagonist atropine in doses shown previously to be both safe and effective (15) to test the hypothesis that adrenergic activation, cholinergic activation, or both, mediate the effect of recent antecedent hypoglycemia to reduce the sympathoadrenal response to subsequent hypoglycemia, the key feature of HAAF in diabetes (1–8), in humans.

## RESEARCH DESIGN AND METHODS

This study was approved by the Washington University Human Research Protection Office and was conducted at the Washington University Clinical Research Unit (CRU).

**Participants.** Study participants comprised 17 adults (7 women, 10 men), with a mean ( $\pm$  SD) age of  $29 \pm 5$  years and a BMI of  $26.5 \pm 4.6 \text{ kg/m}^2$ , who gave

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their written consent. They were in good health as determined by medical history, physical examination, and fasting plasma glucose and creatinine concentrations, hematocrits, and electrocardiograms that were within normal reference ranges.

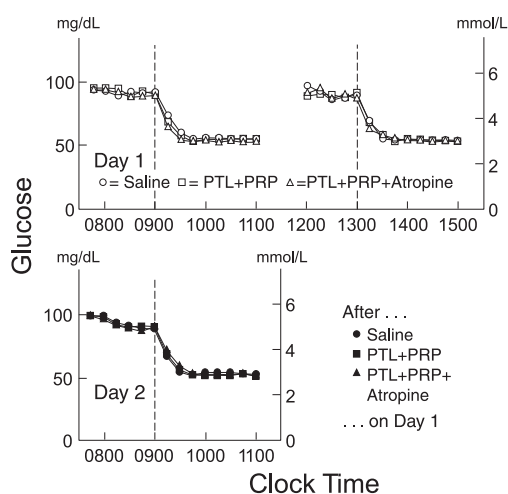
**Experimental design.** Participants were studied on 2 consecutive days on three occasions, separated by at least 2 weeks, after overnight fasts. Intravenous catheters were inserted into a hand vein, with that hand kept in a  $\sim 55^{\circ}\text{C}$  plexiglas box for arterialized venous blood sampling, and into a contralateral antecubital vein for insulin, glucose, and drug infusions and injections.

Day 1 involved hyperinsulinemic (2.0 mU/kg/min), euglycemic (90 mg/dL [5.0 mmol/L]  $\times$  1 h), and then hypoglycemic (54 mg/dL [3.0 mmol/L]  $\times$  2 h) clamps in the morning and again in the afternoon on all three occasions. Intravenous infusions during these day 1 glucose clamps were 1) saline, 2) the nonselective  $\alpha$ -adrenergic receptor antagonist phentolamine mesylate (70  $\mu\text{g}/\text{kg}$ , followed by 7.0  $\mu\text{g}/\text{kg}/\text{min}$ ) and the nonselective  $\beta$ -adrenergic antagonist propranolol hydrochloride (14  $\mu\text{g}/\text{kg}$ , followed by 1.4  $\mu\text{g}/\text{kg}/\text{min}$ ), or 3) phentolamine plus propranolol and the muscarinic cholinergic antagonist atropine (1.0 mg injected intravenously at the end of each euglycemic clamp and 60 min into each hypoglycemic clamp) in random sequence. This experimental design is illustrated, with the actual plasma glucose concentration data, in Fig. 1.

Plasma glucose concentrations were measured every 5 min at the bedside (YSI Glucose Analyzer, Yellow Springs Instruments, Yellow Springs, OH) to guide 20% glucose infusions. Heart rates and blood pressures (GE Dash 3000, Fairfield, CT) were recorded at 15-min intervals, and the electrocardiogram was monitored throughout. Arterialized venous samples for the additional analytes detailed below were drawn at 15-min intervals during the morning euglycemic and hypoglycemic clamps on both days. This model of morning and afternoon hypoglycemia reduces the sympathoadrenal response to hypoglycemia the following day (1–4), and these doses of phentolamine, propranolol, and atropine are safe and hemodynamically, metabolically, and symptomatically effective (15).

Day 2 involved identical hyperinsulinemic euglycemic and then hypoglycemic clamps, with saline infusion, in the morning on all three occasions. Arterialized venous samples were again used to measure plasma glucose concentrations every 5 min and were drawn for the analytes detailed subsequently every 15 min.

**Analytic methods.** Plasma glucose concentrations were measured with a glucose oxidase method (YSI Glucose Analyzer). Plasma insulin, C-peptide, growth hormone, and cortisol concentrations were measured with two site chemiluminescent assays (Immuline 1000, Siemens Corp., Los Angeles, CA). Plasma glucagon and pancreatic polypeptide concentrations were measured with Linco radioimmunoassays (Millipore, Temecula, CA). Plasma epinephrine and norepinephrine concentrations were measured with a single isotope-derivative (radioenzymatic) method (16).



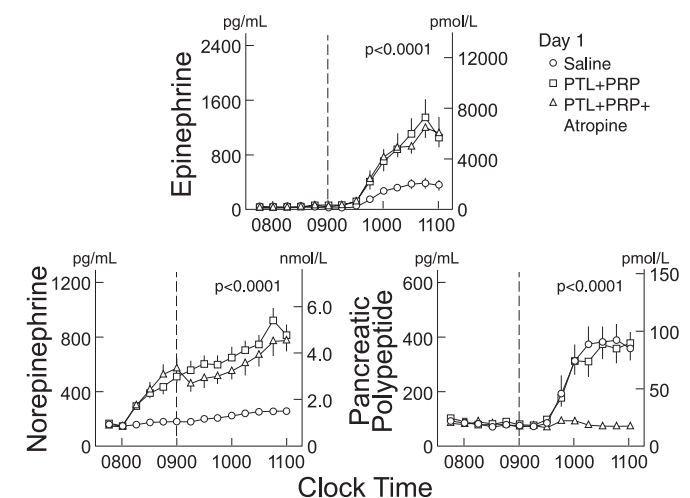
**FIG. 1.** Mean (SEs within the symbols) plasma glucose concentrations are shown during morning and afternoon hyperinsulinemic euglycemic and hypoglycemic clamps with intravenous infusions of saline ( $\circ$ ), phentolamine plus propranolol (PTL + PRP,  $\square$ ) or phentolamine plus propranolol with injection of atropine (PTL + PRP + Atropine,  $\triangle$ ) on day 1 and during morning hyperinsulinemic euglycemic and hypoglycemic clamps with intravenous infusion of saline on day 2 after saline ( $\bullet$ ), PTL + PRP ( $\blacksquare$ ) or PTL + PRP + Atropine ( $\blacktriangle$ ) on day 1 on three separate occasions.

**Statistical methods.** Time and condition related variables were analyzed by repeated measures mixed model ANOVA. Values of  $P < 0.05$  were considered to indicate statistically significant differences. Data are expressed as the mean  $\pm$  SE, except where the SD is indicated.

## RESULTS

**Plasma glucose concentrations, day 1 and day 2.** Plasma glucose concentrations were clamped at euglycemic ( $\sim 90$  mg/dL [5.0 mmol/L]) and then hypoglycemic ( $\sim 54$  mg/dL [3.0 mmol/L]) levels in the morning and again in the afternoon—with intravenous infusions of saline, phentolamine plus propranolol (adrenergic blockade), or phentolamine plus propranolol with injection of atropine (adrenergic plus cholinergic blockade)—on day 1 and on the morning of day 2—with intravenous infusion of saline—after saline, phentolamine plus propranolol, or phentolamine plus propranolol with atropine on day 1 on three separate occasions (Fig. 1). The glucose infusion rates required to maintain the clamps were similar on all occasions (data not shown).

**Responses on day 1.** Compared with those during saline, increments in plasma epinephrine and norepinephrine concentrations during hypoglycemia were increased about threefold during adrenergic blockade and during adrenergic plus cholinergic blockade (both  $P < 0.0001$ ; Fig. 2). The final epinephrine values were  $352 \pm 46$  pg/mL ( $1,920 \pm 251$  pmol/L),  $1,040 \pm 160$  pg/mL ( $5,680 \pm 873$  pmol/L), and  $1,130 \pm 193$  pg/mL ( $6,170 \pm 1,050$  pmol/L), respectively. The final norepinephrine values were  $257 \pm 19$  pg/mL ( $1.52 \pm 0.11$  nmol/L),  $809 \pm 84$  pg/mL ( $4.78 \pm 0.50$  nmol/L), and  $786 \pm 100$  pg/mL ( $4.65 \pm 0.59$  nmol/L), respectively. Increments in plasma pancreatic polypeptide concentrations during hypoglycemia were similar during saline and during adrenergic blockade but were prevented during adrenergic plus cholinergic blockade. The final pancreatic polypeptide values were  $356 \pm 45$  pg/mL ( $85 \pm 11$  pmol/L),  $374 \pm 42$  pg/mL ( $89 \pm 10$  pmol/L), and  $75 \pm 9$  pg/mL ( $18 \pm 2$  pmol/L), respectively.



**FIG. 2.** Mean ( $\pm$  SE) plasma epinephrine, norepinephrine, and pancreatic polypeptide concentrations are shown during morning hyperinsulinemic euglycemic and hypoglycemic clamps with intravenous infusions of saline ( $\circ$ ), phentolamine plus propranolol (PTL + PRP,  $\square$ ), or phentolamine plus propranolol with atropine ( $\triangle$ ) on day 1. The  $P$  values represent comparisons with the values during saline infusion (both  $P < 0.0001$ ) for epinephrine and norepinephrine and comparisons with values during phentolamine plus propranolol with atropine ( $P < 0.0001$ ) for pancreatic polypeptide.

Plasma concentrations of infused insulin stabilized at  $\sim 95 \mu\text{U/mL}$  ( $570 \text{ pmol/L}$ ) during infusion of saline but rose to  $\sim 120 \mu\text{U/mL}$  ( $720 \text{ pmol/L}$ ) during adrenergic blockade and during adrenergic plus cholinergic blockade (both  $P < 0.0001$ ; Fig. 3). The final insulin values were  $96 \pm 8 \mu\text{U/mL}$  ( $576 \pm 48 \text{ pmol/L}$ ),  $122 \pm 12 \mu\text{U/mL}$  ( $732 \pm 72 \text{ pmol/L}$ ), and  $123 \pm 9 \mu\text{U/mL}$  ( $738 \pm 54 \text{ pmol/L}$ ), respectively. Increments in plasma glucagon concentrations during hypoglycemia were similar on all three occasions (Fig. 3). The final glucagon values were  $98 \pm 11 \text{ pg/mL}$  ( $28 \pm 3 \text{ pmol/L}$ ),  $110 \pm 10 \text{ pg/mL}$  ( $32 \pm 3 \text{ pmol/L}$ ), and  $104 \pm 7 \text{ pg/mL}$  ( $30 \pm 2 \text{ pmol/L}$ ), respectively.

Compared with those during saline, increments in plasma growth hormone and cortisol concentrations during hypoglycemia were increased during adrenergic blockade and adrenergic plus cholinergic blockade ( $P < 0.0001$  under both conditions for both hormones; Fig. 3). The final growth hormone values were  $13.3 \pm 2.2 \text{ ng/mL}$  ( $587 \pm 97 \text{ pmol/L}$ ),  $25.2 \pm 3.2 \text{ ng/mL}$  ( $1,110 \pm 141 \text{ pmol/L}$ ), and  $20.7 \pm 2.8 \text{ ng/mL}$  ( $914 \pm 124 \text{ pmol/L}$ ), respectively. The final cortisol values were  $18.4 \pm 1.5 \mu\text{g/dL}$  ( $508 \pm 41 \text{ nmol/L}$ ),  $22.1 \pm 1.4 \mu\text{g/dL}$  ( $610 \pm 39 \text{ nmol/L}$ ), and  $23.7 \pm 1.6 \mu\text{g/dL}$  ( $654 \pm 44 \text{ nmol/L}$ ), respectively.

Heart rate responses to hypoglycemia were decreased during adrenergic blockade and increased during adrenergic plus cholinergic blockade compared with those during saline (both  $P < 0.0001$ ; Table 1). Systolic and diastolic blood pressures were reduced during adrenergic blockade and during adrenergic plus cholinergic blockade (both  $P < 0.0001$ ; Table 1).

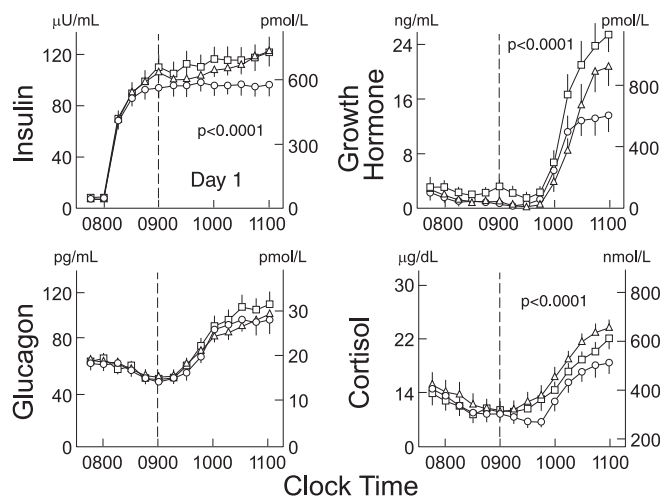
**Responses on day 2 compared with those during saline on day 1.** Compared with that during saline on day 1, the increment in the plasma epinephrine concentration during hypoglycemia was attenuated by  $\sim 50\%$  on day 2 ( $P < 0.0001$ ; Fig. 4). The epinephrine values during the last hour of hypoglycemia were  $351 \pm 13 \text{ pg/mL}$  ( $1920 \pm 71 \text{ pmol/L}$ ) and  $214 \pm 22 \text{ pg/mL}$  ( $1170 \pm 120 \text{ pmol/L}$ ), respectively. In contrast, the increments in plasma epinephrine during hypoglycemia were not reduced on day 2 after adrenergic blockade or adrenergic plus cholinergic

blockade on day 1 (Fig. 4). The epinephrine values during the last hour of hypoglycemia were  $359 \pm 26 \text{ pg/mL}$  ( $1,960 \pm 142 \text{ pmol/L}$ ) and  $375 \pm 11 \text{ pg/mL}$  ( $2,050 \pm 60 \text{ pmol/L}$ ), respectively.

Similarly, the increment in the plasma norepinephrine concentration during hypoglycemia was attenuated on day 2 compared with that during saline on day 1 ( $P < 0.0001$ ; Fig. 4). The norepinephrine values during the last hour of hypoglycemia were  $252 \pm 4 \text{ pg/mL}$  ( $1.49 \pm 0.02 \text{ nmol/L}$ ) and  $226 \pm 7 \text{ pg/mL}$  ( $1.34 \pm 0.04 \text{ nmol/L}$ ), respectively. The increments in plasma norepinephrine during hypoglycemia were not reduced on day 2 after adrenergic blockade or adrenergic plus cholinergic blockade on day 1 (Fig. 4). The norepinephrine values during the last hour of hypoglycemia were  $284 \pm 11 \text{ pg/mL}$  ( $1.68 \pm 0.06 \text{ nmol/L}$ ) and  $256 \pm 11 \text{ pg/mL}$  ( $1.51 \pm 0.06 \text{ nmol/L}$ ), respectively. Compared with that during saline on day 1, the increment in the plasma pancreatic polypeptide concentration during hypoglycemia was attenuated by  $\sim 50\%$  on day 2 under all three study conditions ( $P < 0.0001$ ; Fig. 4). The pancreatic polypeptide values during the last hour of hypoglycemia were  $373 \pm 6 \text{ pg/mL}$  ( $89 \pm 1 \text{ pmol/L}$ ) on day 1 and  $216 \pm 7 \text{ pg/mL}$  ( $52 \pm 2 \text{ pmol/L}$ ),  $214 \pm 17 \text{ pg/mL}$  ( $51 \pm 4 \text{ pmol/L}$ ), and  $239 \pm 10 \text{ pg/mL}$  ( $57 \pm 2 \text{ pmol/L}$ ), respectively, on day 2.

Plasma concentrations of infused insulin were stable and comparable to those during saline on day 1 during all three hyperinsulinemic euglycemic and hypoglycemic clamps on day 2 (Fig. 5). Compared with that during saline on day 1, the increments in the plasma glucagon concentrations during hypoglycemia were reduced under all three conditions on day 2 ( $P = 0.0263$ ,  $0.0008$  and  $<0.0001$ , respectively; Fig. 5). The glucagon values during the last hour of hypoglycemia during saline infusion on day 1 were  $99 \pm 1 \text{ pg/mL}$  ( $28 \pm 0 \text{ pmol/L}$ ). The glucagon values during the last hour of hypoglycemia on day 2 were  $89 \pm 3 \text{ pg/mL}$  ( $26 \pm 1 \text{ pmol/L}$ ),  $83 \pm 2 \text{ pg/mL}$  ( $24 \pm 1 \text{ pmol/L}$ ), and  $86 \pm 2 \text{ pg/mL}$  ( $25 \pm 1 \text{ pmol/L}$ ) after saline, adrenergic blockade, and adrenergic plus cholinergic blockade on day 1, respectively.

This was also the case for increments in the plasma cortisol concentrations during hypoglycemia on day 2 ( $P = 0.0001$ ,  $<0.0001$  and  $0.0030$ ) (Fig. 5). The cortisol values during the last hour of hypoglycemia during saline infusion on day 1 were  $17.3 \pm 0.7 \mu\text{g/dL}$  ( $480 \pm 19 \text{ nmol/L}$ ). Cortisol values during the last hour of hypoglycemia on day 2 were  $14.2 \pm 0.6 \mu\text{g/dL}$  ( $390 \pm 17 \text{ nmol/L}$ ),  $13.5 \pm 1.0 \mu\text{g/dL}$  ( $370 \pm 28 \text{ nmol/L}$ ), and  $15.0 \pm 1.1 \mu\text{g/dL}$  ( $410 \pm 30 \text{ nmol/L}$ ) after saline, adrenergic blockade, and adrenergic plus cholinergic blockade on day 1, respectively. Compared with that during saline on day 1, the increment in the plasma growth hormone concentration during hypoglycemia was reduced on day 2 ( $P = 0.0028$ ; Fig. 5). The growth hormone values during the last hour of hypoglycemia were  $12.6 \pm 0.5 \text{ ng/mL}$  ( $560 \pm 22 \text{ pmol/L}$ ) on day 1 and  $7.7 \pm 0.2 \text{ ng/mL}$  ( $340 \pm 9 \text{ pmol/L}$ ) on day 2. In contrast, growth hormone values during the last hour of hypoglycemia on day 2 were not reduced after adrenergic blockade or adrenergic plus cholinergic blockade on day 1— $10.8 \pm 0.8 \text{ ng/mL}$  ( $480 \pm 35 \text{ pmol/L}$ ) and  $14.0 \pm 1.4 \text{ ng/mL}$  ( $620 \pm 62 \text{ pmol/L}$ ), respectively.



**FIG. 3.** Mean ( $\pm$  SE) plasma insulin, glucagon, growth hormone, and cortisol concentrations are shown during morning hyperinsulinemic euglycemic and hypoglycemic clamps with intravenous infusions of saline ( $\circ$ ), phentolamine plus propranolol ( $\square$ ), or phentolamine plus propranolol with atropine ( $\triangle$ ) on day 1. The  $P$  values represent comparisons with the values during saline, both  $P < 0.0001$  for insulin, growth hormone, and cortisol.

## DISCUSSION

We used a well-documented model of HAAF in diabetes (hypoglycemia attenuates the sympathoadrenal response to hypoglycemia the following day) (1–4) and the classic adrenergic and cholinergic antagonists phentolamine,

TABLE 1

Heart rate and blood pressure responses on day 1 during clamped hyperinsulinemic euglycemia (0800–0900 h) and hypoglycemia (0900–1100 h)

Clock time	Saline			PTL + PRP			PTL + PRP + Atropine		
	HR (bpm)	sBP (mmHg)	dBP (mmHg)	HR (bpm)	sBP (mmHg)	dBP (mmHg)	HR (bpm)	sBP (mmHg)	dBP (mmHg)
0745 h	69 ± 3	125 ± 3	72 ± 2	69 ± 2	120 ± 3	68 ± 2	67 ± 3	119 ± 3	67 ± 2
0800 h	68 ± 2	125 ± 3	71 ± 2	70 ± 2	118 ± 4	66 ± 2	69 ± 3	118 ± 3	68 ± 2
0815 h	70 ± 2	124 ± 3	70 ± 3	71 ± 2	117 ± 3	63 ± 2	71 ± 3	114 ± 3	64 ± 2
0830 h	71 ± 3	125 ± 3	70 ± 2	72 ± 2	115 ± 3	60 ± 1	69 ± 3	112 ± 3	64 ± 2
0845 h	70 ± 2	124 ± 3	70 ± 2	69 ± 2	112 ± 3	60 ± 2	69 ± 2	111 ± 3	60 ± 2
0900 h	71 ± 2	124 ± 3	68 ± 2	68 ± 2	113 ± 3	58 ± 1	77 ± 4	112 ± 3	59 ± 2
0915 h	71 ± 2	125 ± 4	69 ± 3	69 ± 2	111 ± 3	57 ± 2	90 ± 3	111 ± 3	62 ± 2
0930 h	72 ± 3	124 ± 4	68 ± 3	67 ± 2	110 ± 3	57 ± 2	86 ± 3	113 ± 3	60 ± 2
0945 h	74 ± 3	123 ± 4	65 ± 3	67 ± 2	110 ± 3	55 ± 2	82 ± 3	110 ± 3	58 ± 2
1000 h	76 ± 3	126 ± 4	64 ± 2	66 ± 2	108 ± 2	53 ± 1	82 ± 2	109 ± 3	57 ± 2
1015 h	73 ± 2	125 ± 3	63 ± 3	66 ± 2	109 ± 3	52 ± 2	86 ± 2	109 ± 3	55 ± 2
1030 h	73 ± 2	122 ± 4	61 ± 3	63 ± 2	105 ± 3	52 ± 1	84 ± 2	106 ± 3	53 ± 2
1045 h	72 ± 3	122 ± 4	60 ± 2	60 ± 2	106 ± 3	51 ± 1	80 ± 2	106 ± 3	51 ± 2
1145 h	73 ± 3	119 ± 4	60 ± 2	60 ± 2	106 ± 3	51 ± 1	77 ± 2	105 ± 3	51 ± 2
<i>P</i> vs. saline	—	—	—	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Values are mean ± SE. DBP, diastolic blood pressure; HR, heart rate; sBP, systolic blood pressure; PTL, phentolamine; PRP, propranolol.

propranolol and atropine (13–15) to test the hypothesis that adrenergic activation, cholinergic activation, or both, mediate the effect of recent antecedent hypoglycemia to reduce the sympathoadrenal response to subsequent hypoglycemia, the key feature of HAAF in diabetes (1–8), in humans.

The data indicate that adrenergic mechanisms mediate the effect of recent antecedent hypoglycemia to reduce the sympathoadrenal response to subsequent hypoglycemia. Morning and afternoon hypoglycemia with saline infusion on day 1 led to attenuated increments in plasma epinephrine and norepinephrine concentrations, markers of the

sympathoadrenal, primarily adrenomedullary, response to hypoglycemia (17), during hypoglycemia on day 2. Thus, the phenomenon that we sought to explore mechanistically—recent antecedent hypoglycemia reduces the sympathoadrenal response to subsequent hypoglycemia (1–8)—was reproduced.

However, when adrenergic blockade (without or with cholinergic blockade) was produced during hypoglycemia on day 1, the increments in plasma epinephrine and norepinephrine concentrations during hypoglycemia on day 2 were not attenuated. Thus, combined adrenergic blockade with the nonselective  $\alpha$ -adrenergic antagonist phentolamine and the nonselective  $\beta$ -adrenergic antagonist propranolol prevented the effect of hypoglycemia to reduce the

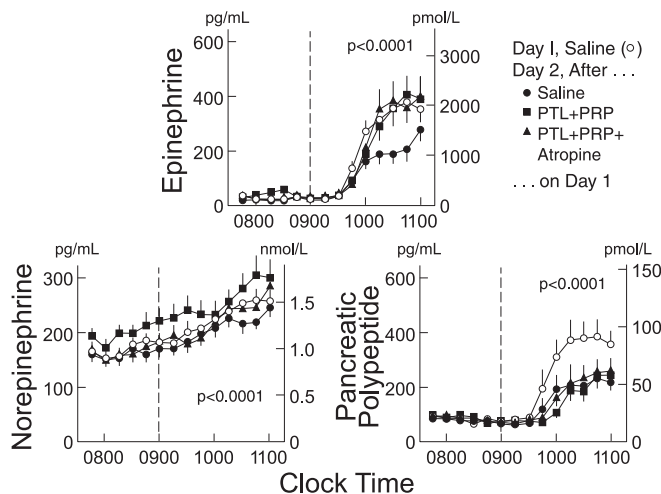


FIG. 4. Mean ( $\pm$  SE) plasma epinephrine, norepinephrine, and pancreatic polypeptide concentrations are shown during morning hyperinsulinemic euglycemic and hypoglycemic clamps on day 1 with saline infusion ( $\circ$ ) and on day 2 after saline ( $\bullet$ ), phentolamine plus propranolol (PTL + PRP,  $\blacksquare$ ), or phentolamine plus propranolol with atropine (PTL + PRP + Atropine,  $\blacktriangle$ ) during the clamps on day 1. The *P* values represent comparisons with the values during saline infusion on day 1 ( $\circ$ ), *P* < 0.0001 for epinephrine and norepinephrine on day 2 ( $\bullet$ ), and *P* < 0.0001 for pancreatic polypeptide under all three conditions on day 2 ( $\bullet$ ,  $\blacksquare$ , and  $\blacktriangle$ ).

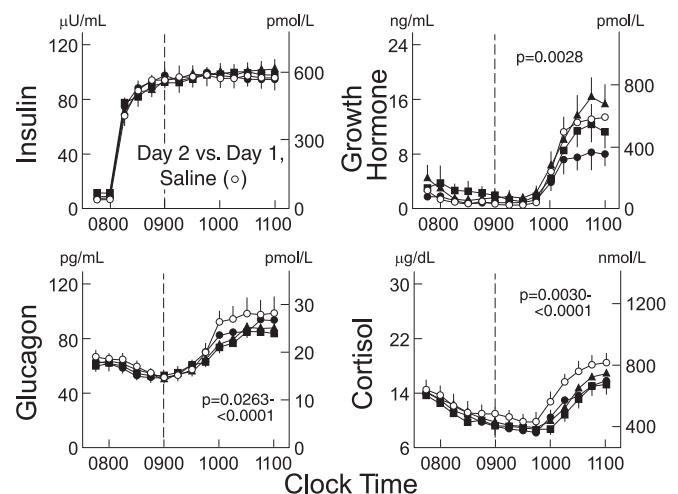


FIG. 5. Mean ( $\pm$  SE) plasma insulin, glucagon, growth hormone, and cortisol concentrations are shown during morning hyperinsulinemic euglycemic and hypoglycemic clamps on day 1 with saline infusion ( $\circ$ ) and on day 2 after saline ( $\bullet$ ), phentolamine plus propranolol ( $\blacksquare$ ), or phentolamine plus propranolol with atropine ( $\blacktriangle$ ) during the clamps on day 1. The *P* values represent comparisons with the values during saline on day 1 ( $\circ$ ); see text for details.



sympathoadrenal response to subsequent hypoglycemia. The latter is the key feature of HAAF in diabetes (1–8).

The data do not disclose—but do not categorically exclude—an additional cholinergic mechanism. The sympathoadrenal response to hypoglycemia on day 2 was similar whether the muscarinic cholinergic antagonist atropine was or was not administered with phentolamine and propranolol during hypoglycemia on day 1.

Morning and afternoon hypoglycemia with saline infusion on day 1 also led to attenuated increments in the plasma pancreatic polypeptide concentration, a marker of the parasympathetic response to hypoglycemia (18), during hypoglycemia on day 2. In contrast to the effect on the sympathoadrenal response, adrenergic blockade (without or with cholinergic blockade) during hypoglycemia on day 1 did not prevent the attenuated pancreatic polypeptide response to hypoglycemia on day 2. An altered parasympathetic response is not known to be involved in the pathogenesis of HAAF in diabetes (1).

To the extent the drugs used enter the central nervous system (propranolol and atropine are known to do so), the data do not distinguish between central and peripheral (autonomic) nervous system mediation of the effect of antecedent hypoglycemia to reduce the sympathoadrenal response to subsequent hypoglycemia. However, because the adrenergic antagonists block the heart rate and pressor responses to hypoglycemia, which are normally mediated by the autonomic response, one might speculate that it was blockade of adrenergic sympathoadrenal actions during hypoglycemia on day 1 that prevented an attenuated plasma catecholamine response to hypoglycemia on day 2.

The data confirm substantially higher plasma epinephrine and norepinephrine concentrations in response to hypoglycemia during adrenergic blockade on day 1 (15). That is expected because both catecholamines are cleared through  $\beta$ -adrenergic mechanisms in humans (19). (Given the short half-times of the antagonists and the catecholamines, a carry-over effect to the next day is unlikely.) The current findings of higher plasma concentrations of infused insulin and of endogenous growth hormone and cortisol during hypoglycemia during adrenergic blockade suggest that adrenergic mechanisms are also involved in the clearance of these hormones. The growth hormone responses to hypoglycemia, like the epinephrine and norepinephrine responses, were reduced after hypoglycemia with saline infusion but not after hypoglycemia with adrenergic blockade (without or with cholinergic blockade). An interesting finding was that the pancreatic polypeptide, glucagon, and cortisol responses to hypoglycemia were reduced on day 2 regardless of the day 1 conditions. Among these, a reduced glucagon response is a feature of HAAF (1). Thus, the data further support the view that the mechanisms of the reduced glucagon and epinephrine responses to hypoglycemia in HAAF are different (1).

In conclusion, the data indicate that adrenergic mechanisms mediate the effect of recent antecedent hypoglycemia to reduce the sympathoadrenal response to subsequent hypoglycemia, the key feature of HAAF in diabetes, in humans.

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R.R. and P.E.C. designed the study, R.R. conducted it, and R.R. and P.E.C. analyzed the data and wrote the manuscript.

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