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Autonomic Mediation of Glucagon Secretion During Insulin-Induced Hypoglycemia in Rhesus Monkeys

Peter J. Havel and Celia Valverde

Autonomic activation mediates the majority of the increase of glucagon secretion during insulin-induced hypoglycemia in several species including dogs, mice, and rats. However, the role of the autonomic nervous system to increase glucagon during hypoglycemia in humans remains controversial, and investigations in nonhuman primates have not been previously conducted. The autonomic contribution to glucagon secretion during hypoglycemia in a nonhuman primate was examined by two independent pharmacological approaches. Glucagon responses to clamped insulin-induced hypoglycemia were compared in conscious rhesus monkeys in the presence or absence of ganglionic blockade with trimethaphan, or during combined muscarinic and adrenergic receptor blockade with atropine, propranolol, and tolazoline. Insulin-induced hypoglycemia (plasma glucose = 1.9 ± 0.1 mmol/l) activated parasympathetic nerves to the pancreas as assessed by increased plasma pancreatic polypeptide (PP) levels (Δ = 135.0 ± 36.8 pmol/l, $P < 0.01$), produced sympathoadrenal activation as assessed by elevations of plasma epinephrine (EPI) (Δ = 22.3 ± 2.95 nmol/l, $P < 0.0005$) and norepinephrine (NE) (Δ = 3.72 ± 0.77 nmol/l, $P < 0.0025$) and increased plasma immunoreactive glucagon (IRG) (Δ = 920 ± 294 ng/l, $P < 0.025$). Nicotinic ganglionic blockade with trimethaphan prevented parasympathetic (ΔPP = 16.5 ± 16.3 pmol/l, $P <$ 0.01 vs. control) and sympathoadrenal (ΔEPI = 1.52 ± 0.98 nmol/l; ΔNE = −0.62 ± 0.24 nmol/l, both $P < 0.0025$ vs. control) activation during hypoglycemia and inhibited the IRG response by 70% (Δ = 278 ± 67 ng/l, $P < 0.025$ vs. control). Combined muscarinic and adrenergic receptor blockade reduced parasympathetic activation (ΔPP = 48.3 ± 16.3 pmol/l, $P < 0.01$ vs. control) and inhibited the IRG response by a similar degree to ganglionic blockade (ΔIRG = 284 ± 60 ng/l, $P < 0.025$ vs. control). These results demonstrate by two independent pharmacological approaches that autonomic activation makes a substantial contribution to increased glucagon secretion during hypoglycemia of ~2.0 mmol/l in a species of nonhuman primate. ***Diabetes*** **45:960–966, 1996**

Increased glucagon secretion, in concert with adrenergic mechanisms, is a primary counterregulatory response for the recovery of plasma glucose levels during hypoglycemia (1,2). However, the question of which mechanisms are responsible for stimulating glucagon secretion during hypoglycemia remains controversial. Hypoglycemia activates three autonomic inputs to the pancreas, which include pancreatic parasympathetic nerves (3,4), the direct sympathetic innervation of the pancreas (5), and epinephrine from the adrenal medulla (6). Although each of these autonomic inputs to the pancreas is capable of stimulating glucagon secretion (7–10), hypoglycemia per se may contribute to increased glucagon secretion as lowered glucose concentrations can increase glucagon secretion in vitro (11,12).

A number of early studies in human subjects found that selective impairment of parasympathetic or sympathoadrenal inputs to the pancreas did not reduce the glucagon response to hypoglycemia (13–16). These results contrast with those from studies conducted in several species of animals in which autonomic activation was demonstrated to mediate the majority of the glucagon response to hypoglycemia (17–22). The animal studies are generally characterized by the use of interventions that impair activation of both the parasympathetic and the sympathoadrenal inputs to the pancreas.

The failure of the early human studies to find a significant autonomic contribution to the glucagon response to hypoglycemia could have been due to unrecognized redundancy among the autonomic inputs. In accord with this hypothesis, the glucagon response to hypoglycemia in rats is redundantly mediated such that simultaneous blockade of both the parasympathetic and sympathoadrenal inputs to the pancreas is required to reduce the response (21). However, more recent studies have found that the glucagon response to hypoglycemia was not reduced by the combination of classical muscarinic and adrenergic receptor blockade in humans (23,24). It is possible that the release of neuropeptides such as vasoactive intestinal polypeptide from pancreatic autonomic nerves could contribute to glucagon secretion during hypoglycemia (25). The actions of neuropeptides would not be expected to be blocked by classical receptor antagonists. In contrast, pharmacological blockade of nicotinic receptors in autonomic ganglia would be expected to impair autonomic activation whether classical or peptidergic. One limited study in humans found that administration of a ganglionic blocking agent significantly reduced the glucagon response to hypoglycemia (26): however, addi-

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CRPRC, California Regional Primate Research Center; EPI, epinephrine; IRG, immunoreactive glucagon; IRI, immunoreactive insulin; NE, norepinephrine; PP, pancreatic polypeptide; sBP, systolic blood pressure.

tional experiments are needed to determine the autonomic contribution in humans.

In the present study, we sought to determine the autonomic contribution to the glucagon response to hypoglycemia in a species more closely related to humans than other laboratory animal species. Plasma glucagon responses to insulin-induced hypoglycemia were measured in conscious rhesus monkeys with chronically implanted arterial catheters and in the same animals during ganglionic blockade with trimethaphan. As a second independent approach to this question, an additional experiment was conducted with atropine, tolazoline, and propranolol to block the autonomic inputs to classical muscarinic and adrenergic receptors. Plasma pancreatic polypeptide (PP) responses to hypoglycemia were measured in all three studies as an index of the activation of pancreatic parasympathetic nerves during hypoglycemia, and the effectiveness of ganglionic blockade or atropine to impair parasympathetic activation. Plasma catecholamine responses to hypoglycemia were measured in the control and trimethaphan studies as an index of the effectiveness of ganglionic blockade to impair sympathoadrenal activation.

RESEARCH DESIGN AND METHODS

Animals and catheter implantation. Six adult male rhesus monkeys (*Macaca mulatta*) (weight = 8.4–14.4 kg, mean ± SE = 10.9 ± 0.9 kg) were used for these studies. Before selection for the study, a physical examination, complete blood count, and serum biochemistry panel were performed on each animal. The animals had previously been acclimated to several hours of chair restraint with a minimum of 10 training sessions (27). One week before the first experiment, an iliac or femoral artery was catheterized under ketamine/isoflurane anesthesia with a polyurethane catheter connected to a subcutaneous vascular-access-port (Access Technologies, Skokie, IL) as previously described (28). Animals were housed in the American Association for the Accreditation of Laboratory Animal Care accredited facilities of the California Regional Primate Research Center (CRPRC) in accordance with standards established by the U.S. Animal Welfare Act and the Institute of Laboratory Animal Resources. The experimental protocols were approved by the Institutional Animal Use and Care Committee at the University of California, Davis and the CRPRC and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Hypoglycemia protocol. The animals were fasted overnight and placed in restraint chairs at least 1 h before the experiment. A cephalic vein was catheterized for infusion of saline, autonomic blockers, and insulin. Two baseline arterial blood samples (3 ml) for plasma glucose, PP, catecholamine, and glucagon determination were drawn 10 min apart, then regular human insulin was infused intravenously via a cephalic vein catheter at a rate of 3.5 mU · kg^{-1} · min^{-1}. Small (0.2 ml) blood samples were drawn frequently (every 5–15 min) from the arterial catheter for on-line determination of plasma glucose levels with a Beckman glucose analyzer (Beckman, Fullerton, CA). The arterial plasma glucose was maintained at ~2.0 mmol/l by administering small boluses of glucose (5–10 mg/kg) and infusing glucose at a variable rate (1–5 mg · kg^{-1} · min^{-1}). A plasma glucose level of 2.0 mmol/l is a moderate degree of hypoglycemia in adult male rhesus monkeys since the normal fasting serum glucose (~4.0 mmol/l) is lower in rhesus monkeys than in many other species (29). The plasma glucose reference range in adult male rhesus monkeys at the CRPRC was 3.1–4.9 mmol/l (mean ± 1 SD = 4.0 ± 0.9 mmol/l) (n = 35). The animals showed no adverse effects during hypoglycemia other than slight drowsiness. Blood samples for hormone measurement were drawn every 15 min during the hypoglycemic period (90 min), and then the glucose infusion rate was increased (125 mg/kg + 12.5 mg · kg^{-1} · min^{-1}) to reverse the hypoglycemia, and additional samples were drawn at 15, 30, and 60 min.

Pharmacological autonomic blockade. To induce ganglionic autonomic blockade and impair parasympathetic and sympathoadrenal activation during hypoglycemia (30), trimethaphan camsylate (Arfonad, Roche, Nutley, NJ) (0.1–0.5 mg/min i.v.) was infused for 40 min before the induction of hypoglycemia and throughout the study. Arterial blood pressure was monitored continuously with a digital blood pressure analyzer (DigiMed, Louisville, KY). The infusion rate was adjusted to decrease systolic blood pressure (sBP) by ~40 mmHg without lowering sBP below 80 mmHg. To produce pharmacological muscarinic and adrenergic blockade, in a third experiment the same animals received atropine sulfate (Elkins-Sinn, Cherry Hill, NJ) (0.1 mg/kg + 0.001 mg · kg^{-1} · min^{-1}), plus combined α- and β-adrenergic blockade with tolazoline HCl (Priscoline, Ciba-Geigy, Summit, NJ) (2 mg/kg + 0.033 mg · kg^{-1} · min^{-1}) and propranolol HCl (Solopak, Elk Grove Village, IL) (0.3 mg/kg + 0.003 mg · kg^{-1} · min^{-1}), intravenously for 30 min before the induction of hypoglycemia and throughout the experiment. The control and trimethaphan studies were performed in random order 7 days apart. The muscarinic and adrenergic blockade studies were conducted 3 months later.

TABLE 1
Heart rate in rhesus monkeys before and during insulin-induced hypoglycemia in the control study, and before and after ganglionic blockage with trimethaphan or muscarinic plus α- and β-adrenergic receptor blockade

	Control study	Ganglion block	Receptor block
Baseline heart rate	194 ± 8	190 ± 13	168 ± 12
After blockers	—	171 ± 6	153 ± 8
Δheart rate	—	−19 ± 8*	−15 ± 16
During insulin-induced hypoglycemia	180 ± 7	158 ± 5	128 ± 8
Δheart rate	−14 ± 7	−13 ± 3†	−24 ± 5†

Data are means ± SE. *$P < 0.05$ vs. baseline. †$P < 0.005$ vs. baseline.

Assays and data analysis. Blood samples for plasma glucose determination were drawn and placed in tubes containing heparin. Blood samples for glucagon and PP determination were placed in tubes containing EDTA and aprotinin (Sigma, St. Louis, MO). Blood samples for catecholamine determination were placed in tubes containing EGTA and glutathione. All samples were kept on ice until centrifugation (20 min at 4°C). The plasma was decanted and frozen at −20°C until assayed.

Plasma glucose was assayed by the glucose oxidase method with a Beckman glucose analyzer (Beckman). Plasma PP was measured with a radioimmunoassay (31). Plasma norepinephrine (NE) and epinephrine (EPI) were measured in duplicate with a highly sensitive and specific radioenzymatic assay (32). The intra- and interassay coefficients of variation for the plasma catecholamine assay are 6 and 12%, respectively. Plasma immunoreactive glucagon (IRG) and insulin (IRI) were measured radioimmunologically in unextracted plasma with reagents supplied by Linco (St. Louis, MO). The intra- and interassay coefficients of variation for the plasma insulin assay are <10 and 14%, respectively. The antibody used for the plasma glucagon assay has high specificity for the COOH-terminal portion of the glucagon molecule. The intra- and interassay coefficients of variation for the plasma glucagon assay are <10 and 11%, respectively.

Calculations and data analysis. All values are means ± SE. The changes in blood pressure, heart rate, arterial plasma glucose, PP, EPI and NE, and glucagon were calculated by subtracting the mean of the −10- and 0-min baseline values from the mean of the 30-, 45-, 60-, 75-, and 90-min values after the infusion of insulin. Statistical comparison of means within a treatment group were made with a paired t test. For comparison of means of various treatment groups, analysis of variance with a Dunnett's post-test was performed.

RESULTS

Heart rate and blood pressure. Baseline heart rate was similar in all three studies. Trimethaphan, but not muscarinic plus adrenergic blockade, significantly reduced heart rate ($P < 0.05$). Heart rate was lower or tended to be lower during hypoglycemia than in the prehypoglycemic period in all three studies (Table 1).

Baseline systolic and mean arterial blood pressure was similar in all three studies. As required by the experimental design, trimethaphan infusion significantly reduced both

TABLE 2
Systolic arterial pressure and mean arterial pressure in rhesus monkeys before and during insulin-induced hypoglycemia in the control study and before and after ganglionic blockade with trimethaphan or muscarinic plus α- and β-adrenergic receptor blockade

	Control study	Ganglionic block	Receptor block
Systolic pressure (mmHg)			
Baseline systolic pressure	130 ± 7	136 ± 5	133 ± 4
After blockers	—	97 ± 3	116 ± 8
Δsystolic arterial pressure	—	−39 ± 4‡	−16 ± 6†
During insulin-induced hypoglycemia	117 ± 7	84 ± 6	107 ± 7
Δsystolic arterial pressure	−13 ± 5†	−13 ± 4†	−9 ± 5
Mean pressure (mmHg)			
Baseline mean pressure	109 ± 6	117 ± 15	112 ± 4
After blockers	—	80 ± 3	99 ± 7
Δmean arterial pressure	—	−37 ± 3‡	−13 ± 5†
During insulin-induced hypoglycemia	97 ± 4	71 ± 5	90 ± 7
Δmean arterial pressure	−12 ± 3‡	−9 ± 5	−9 ± 4*

Data are means ± SE. *$P < 0.05$ vs. baseline. †$P < 0.025$ vs. baseline. ‡$P < 0.01$ vs. baseline.

systolic and mean arterial blood pressure (both $P < 0.0005$ vs. initial values); however, marked hypotension was not produced by these rates of trimethaphan infusion. Muscarinic plus adrenergic blockade produced a modest reduction of systolic and mean arterial pressure (both $P < 0.05$ vs. initial values). Systolic and mean arterial pressure were lower or tended to be lower during hypoglycemia than before hypoglycemia in all three studies (Table 2).

Plasma glucose and plasma IRI. The initial fasting plasma glucose in the control study averaged 4.9 ± 0.4 mmol/l. The initial plasma glucose before the blockers were administered was similar in the trimethaphan and atropine plus combined adrenergic blockade studies, averaging 4.7 ± 0.4 and 4.3 ± 0.2 mmol/l, respectively. Atropine and combined adrenergic blockade, but not trimethaphan, significantly reduced plasma glucose levels to 3.7 ± 0.1 mmol/l (Δ = −0.6 ± 0.1 mmol/l, $P < 0.0025$ vs. initial). In the control study, arterial plasma glucose during the hypoglycemic period was 1.9 ± 0.1 mmol/l. Plasma glucose during the hypoglycemic period was slightly lower in the trimethaphan study (1.8 ± 0.1 mmol/l, $P < 0.025$ vs. control), but not during the atropine plus combined adrenergic blockade study (1.9 ± 0.1 mmol/l) (Fig. 1).

Initial plasma IRI levels were similar in all three studies. Plasma IRI was modestly decreased by atropine plus adrenergic blockade, but not by trimethaphan. Plasma IRI levels during the hypoglycemic clamp were comparable in all three studies (Table 3).

Plasma PP. In the control study, baseline arterial plasma PP was 69.1 ± 19.8 pmol/l and increased to 204.1 ± 51.9 pmol/l during hypoglycemia (Δ = 135.0 ± 36.8 pmol/l, $P < 0.01$). The initial plasma PP level was not significantly different in the trimethaphan or atropine plus combined adrenergic blockade studies; however, trimethaphan eliminated the increase of plasma PP during hypoglycemia (Δ = 16.5 ± 16.3 pmol/l, NS vs. prehypoglycemia, $P < 0.01$ vs. control). Atropine plus combined adrenergic blockade significantly reduced but did not abolish the PP response (Δ = 48.3 ± 16.3 pmol/l, $P < 0.02$ vs. prehypoglycemia, $P < 0.01$ vs. control) (Fig. 2).

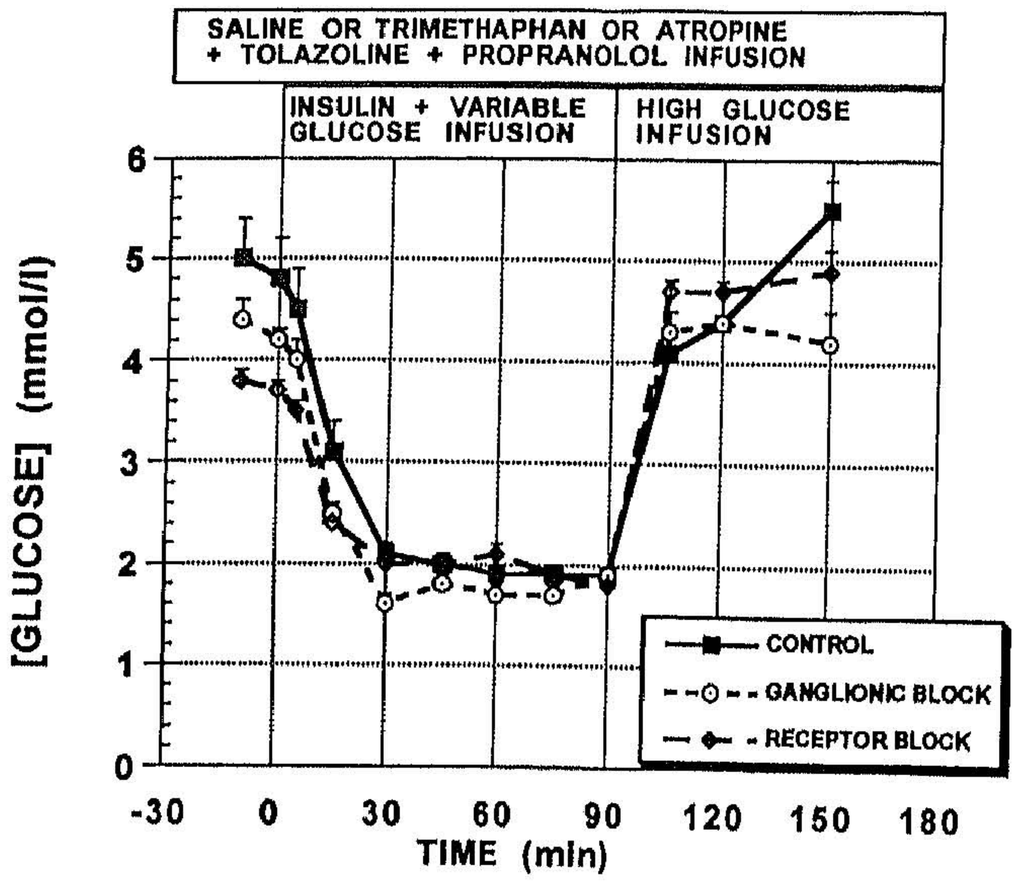

FIG. 1. Arterial plasma glucose before and after insulin plus variable rate glucose infusion, and during posthypoglycemia glucose infusion in six saline-infused rhesus monkeys (control) and in the same monkeys during ganglionic block with trimethaphan or muscarinic plus combined α- and β-adrenergic receptor block with atropine, tolazoline, and propranolol.

Plasma catecholamines: EPI and NE. In the control study, baseline arterial plasma EPI averaged 5.51 ± 0.66 nmol/l and increased to 27.78 ± 3.44 pg/ml (Δ = 22.21 ± 2.95 nmol/l, $P < 0.0005$). The initial plasma EPI level in trimethaphan study (6.66 ± 0.76 pg/ml) was not different from the control study. Trimethaphan administration lowered plasma EPI to 2.89 ± 0.44 nmol/l (Δ = −3.77 ± 0.76 nmol/l, $P < 0.0025$ vs. baseline) and prevented plasma EPI from increasing significantly during hypoglycemia (Δ = 1.53 ± 0.98 nmol/l, NS vs. prehypoglycemia, $P < 0.001$ vs. control) (Fig. 3).

Baseline arterial plasma NE in the control study was 8.87 ± 1.06 nmol/l and increased to 12.58 ± 1.77 nmol/l during hypoglycemia (Δ = 3.72 ± 0.77 nmol/l, $P < 0.0025$). The initial plasma NE in the trimethaphan study before trimethaphan infusion was similar to that in the control study (10.22 ± 1.36 nmol/l); however, trimethaphan lowered the NE level before hypoglycemia to 4.49 ± 0.61 nmol/l (Δ = −5.73 ± 1.12 nmol/l, $P < 0.0025$ vs. initial NE). Arterial plasma NE did not increase, and in fact decreased slightly, during hypoglycemia in the trimethaphan study (Δ = −0.65 ± 0.23 nmol/l, $P < 0.02$ vs. prehypoglycemia, $P < 0.01$ vs. control) (Fig. 4). Plasma catecholamines were not measured in the study with combined adrenergic blockade because adrenergic antagonists block the action and not the

TABLE 3
Plasma IRI levels in rhesus monkeys before and during insulin-induced hypoglycemia in the control study and before and after ganglionic blockade with trimethaphan or muscarinic plus α- and β-adrenergic receptor blockade

	Control study	Ganglion block	Receptor block
Baseline IRI	145 ± 47	115 ± 35	108 ± 14
After blockers	—	107 ± 25	82 ± 10
ΔIRI	—	−8 ± 22	−26 ± 11*
During insulin-induced hypoglycemia	2,352 ± 320	2,569 ± 280	2,620 ± 440

Data are means ± SE (pmol/l). *$P < 0.05$ vs. baseline.

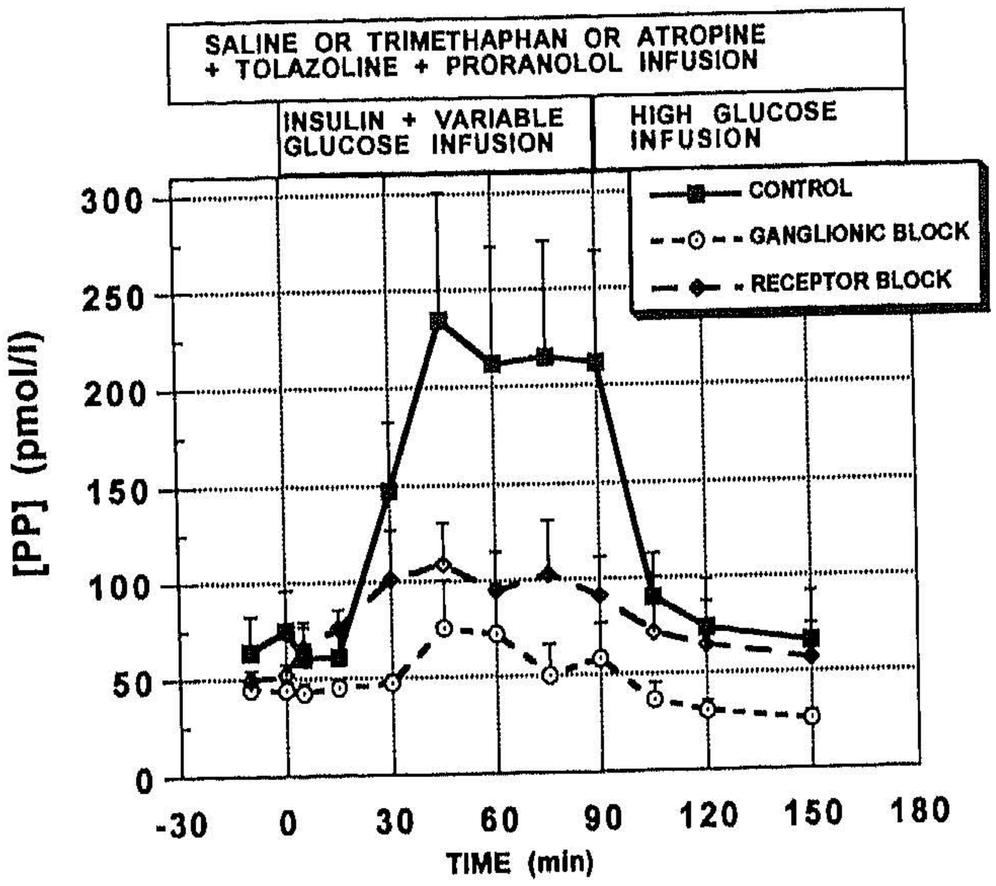

FIG. 2. Arterial plasma PP before and after insulin plus variable-rate glucose infusion and during posthypoglycemia glucose infusion in six rhesus monkeys (control) and in the same monkeys during ganglionic block with trimethaphan or muscarinic plus combined α- and β-adrenergic receptor block with atropine, tolazoline, and propranolol.

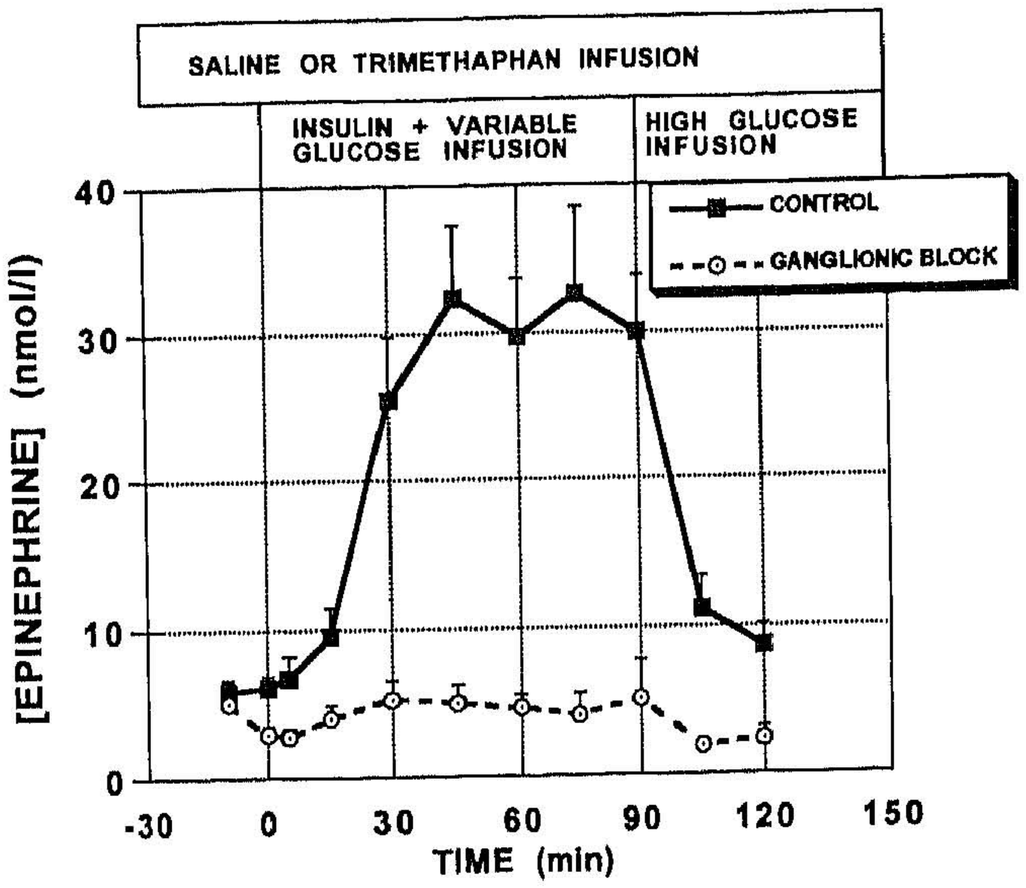

FIG. 3. Arterial plasma epinephrine before and after insulin plus variable rate glucose infusion, and during posthypoglycemia glucose infusion in six saline-infused rhesus monkeys (control) and in the same monkeys during ganglionic block with trimethaphan.

release of NE and EPI. Therefore, circulating plasma catecholamine levels are not a relevant index of sympathoadrenal input to the islet during adrenergic receptor blockade.

Plasma IRG. Baseline arterial plasma IRG in the control study averaged 414 ± 24 ng/l and increased to 1,334 ± 297 ng/l during hypoglycemia (Δ = 920 ± 294 ng/l, $P < 0.02$). The initial plasma IRG level was not significantly different in the trimethaphan and the atropine plus combined adrenergic blockade studies, averaging 458 ± 80 and 454 ± 46 ng/l, respectively. Plasma IRG was significantly reduced by muscarinic plus combined adrenergic blockade ($\Delta = -122 \pm 27$ ng/l, $P < 0.005$), but not trimethaphan ($\Delta = -61 \pm 46$ ng/l, NS vs. initial IRG). The increases of plasma glucagon during insulin-induced hypoglycemia were reduced, but not abolished by trimethaphan (Δ = 278 ± 67 ng/l, $P < 0.005$ vs. baseline, $P < 0.025$ vs. control) or atropine plus combined adrenergic blockade (Δ = 284 ± 60 ng/l, $P < 0.005$ vs. baseline, $P < 0.025$ vs. control) (Fig. 5).

Glucose infusion rates. The mean glucose infusion rate required to maintain the plasma glucose level at 1.9 ± 0.1 mmol/l during the insulin infusion in the control study was 1.1 ± 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$. Higher glucose infusion rates were required to maintain plasma glucose levels at 1.8 ± 0.1 and 1.9 ± 0.1 mmol/l, respectively, in the trimethaphan (2.6 ± 0.7 $mg \cdot kg^{-1} \cdot min^{-1}$, $P < 0.025$ vs. control) and atropine plus combined adrenergic blockade studies (3.4 ± 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$, $P < 0.005$ vs. control) (Fig. 6).

DISCUSSION

The major finding of the studies described in this report is that either pharmacological impairment of autonomic neural activation or autonomic receptor blockade reduces the increase of plasma glucagon during insulin-induced hypoglycemia by 70% in rhesus monkeys. Thus, the autonomic contribution was defined by two separate but complementary pharmacological approaches, demonstrating a prominent role for the autonomic nervous system to contribute to increased glucagon secretion during hypoglycemia in a nonhuman primate. It is unlikely that the autonomic antagonists by themselves directly impaired glucagon secretion since these agents do not directly suppress glucagon secretion in vitro from isolated perfused pancreas preparations (33–37).

The effectiveness of the ganglionic blockade produced by trimethaphan infusion was demonstrated by the lack of both parasympathetic (plasma PP) and sympathoadrenal (EPI and NE) responses to hypoglycemia, all of which increased in the control study. Plasma PP levels are useful as an index of the activation of the parasympathetic nerves to the pancreas during hypoglycemia (3,4,22,38). Plasma PP responses to hypoglycemia were abolished by ganglionic blockade, but only reduced by 65% during muscarinic plus combined adrenergic blockade, suggesting that noncholinergic, nonadrenergic (peptidergic) mechanisms could contribute increased PP secretion during hypoglycemia in rhesus monkeys.

It should be noted that while effective ganglionic blockade

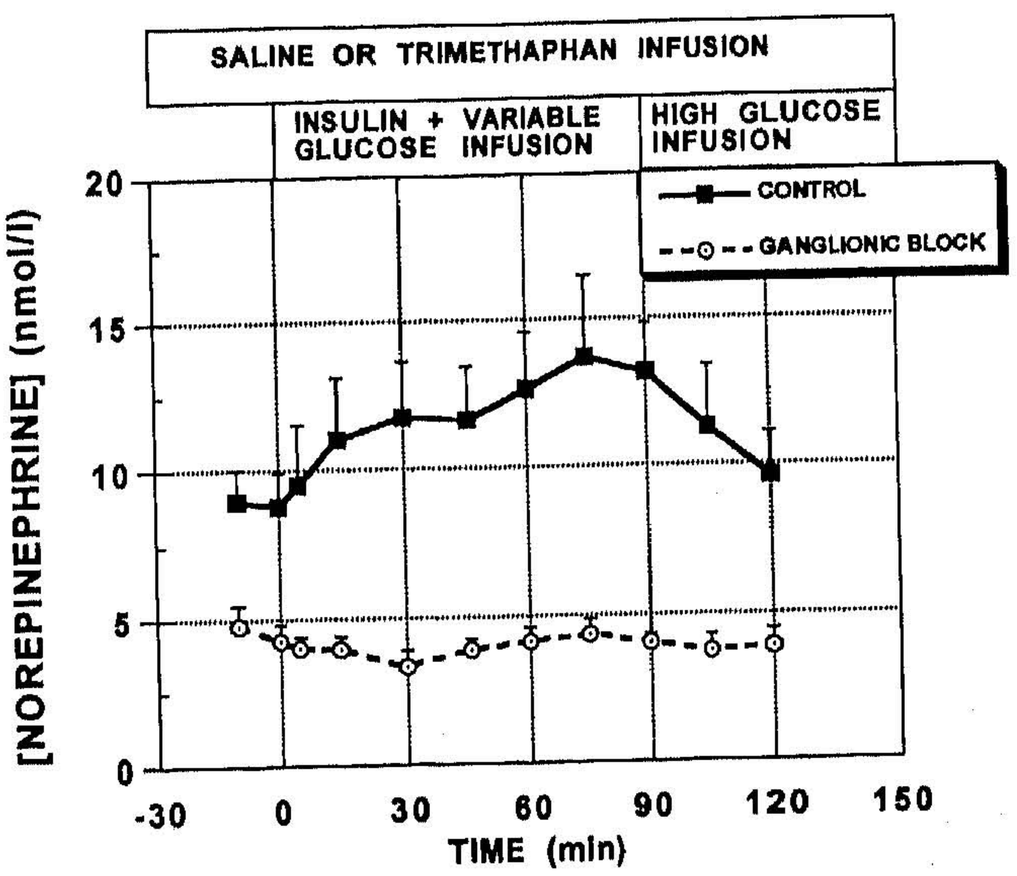

FIG. 4. Arterial plasma NE before and after insulin plus variable rate glucose infusion and during posthypoglycemia glucose infusion in six rhesus monkeys (control) and in the same monkeys during ganglionic block with trimethaphan.

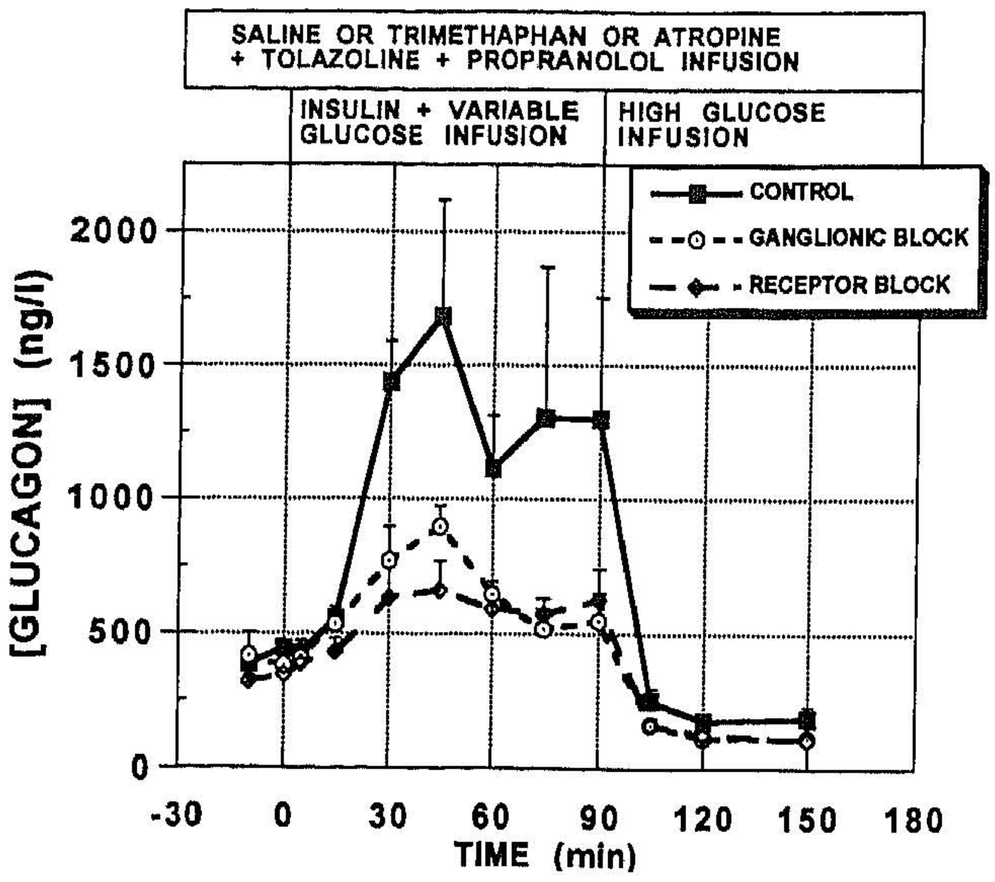

FIG. 5. Arterial plasma IRG before and after insulin plus variable rate glucose infusion, and during posthypoglycemia glucose infusion in six rhesus monkeys (control) and in the same monkeys during ganglionic block with trimethaphan or muscarinic plus combined α- and β-adrenergic receptor block with atropine, tolazoline, and propranolol.

or large doses of autonomic antagonists eliminated the majority (70%) of the increase of glucagon secretion, a significant portion of the glucagon response to hypoglycemia persisted despite the pharmacological interventions. Thus, autonomic activation is not likely to be the sole mediator of the glucagon response in rhesus monkeys. Other potential mechanisms include effects at the level of the islet. These islet effects could either be due to a direct action of low glucose levels on the A-cell itself or indirect via release of the A-cell from tonic paracrine inhibition by insulin (39), since intraislet insulin levels are directly suppressed by hypoglycemia per se and are likely to be reduced by exogenous insulin (40).

The role of the autonomic nervous system in mediating increased glucagon secretion during hypoglycemia was previously examined in a number of animal species, including dogs (17,18), rats (20,21), and mice (22), but not in nonhuman primates. One related study in baboons showed a contribution of sympathoadrenal activation to glucagon secretion during central neuroglucopenia, in the absence of hypoglycemia, because α- or β-adrenergic blockers alone, or together, reduced plasma glucagon responses after 2-deoxy-D-glucose administration (41). In rats, the glucagon response to hypoglycemia is redundantly mediated by parasympathetic, cholinergic, and sympathoadrenal adrenergic activation, such that input from both autonomic subdivisions must be simultaneously blocked to significantly impair the glucagon response (21). In dogs, there is recent evidence that the direct sympathetic innervation of the pancreas can contribute to increased glucagon secretion during hypoglycemia, independently from the vagal and adrenal medullary inputs (42). Thus, autonomic inputs to the pancreas have been shown to make an important contribution to increased glucagon secretion during hypoglycemia in several species (25) and in the present study in rhesus monkeys.

In contrast, the role of the autonomic nervous system in mediating increased glucagon secretion during hypoglycemia in humans is more controversial. Results of early studies in human subjects in which one or two, but not all three, of the autonomic inputs to the pancreas were blocked usually did not support a significant contribution of the autonomic nervous system (10). However, in a more recent short report, ganglionic blockade, which would impair activation of all three autonomic inputs, significantly reduced the glucagon response to hypoglycemia in human subjects (26). Other recent studies of acute hypoglycemia-associated autonomic failure in humans also support the case for autonomic mediation. In these experiments, a prior episode of hypoglycemia markedly reduces both parasympathetic activation (PP responses) and adrenal medullary activation (EPI responses) during subsequent hypoglycemia, 24 h later (43). Since prior hypoglycemia also markedly reduced the glucagon response and the degree of hypoglycemia in the two episodes was closely matched, it is unlikely that a direct effect of hypoglycemia on the islet, either to directly stimulate the A-cell or to release the A-cell from tonic paracrine restraint by high levels of intraislet insulin (39), is solely responsible for mediating the glucagon response to hypoglycemia in humans. Thus, it is possible that the glucagon response to hypoglycemia in humans is mediated, at least in part, by activation of the autonomic nervous system.

There are, however, other data in humans that seem to argue against an autonomic contribution. Classical cholinergic and adrenergic receptor antagonists did not reduce the glucagon response to hypoglycemia in two studies in human subjects (23,24), in contrast with the marked effect of these agents in rhesus monkeys. However, in the human studies much lower doses of autonomic antagonists were used than in the present study, suggesting that higher doses might be necessary to abolish the effects of hypoglycemia-induced autonomic activation on A-cell receptors. In accordance with this idea, it has been shown that the low doses of atropine often used in human studies do not provide sufficient muscarinic blockade to suppress cardiovascular parasympathetic reflexes (44). Alternatively, it is possible that neuro-

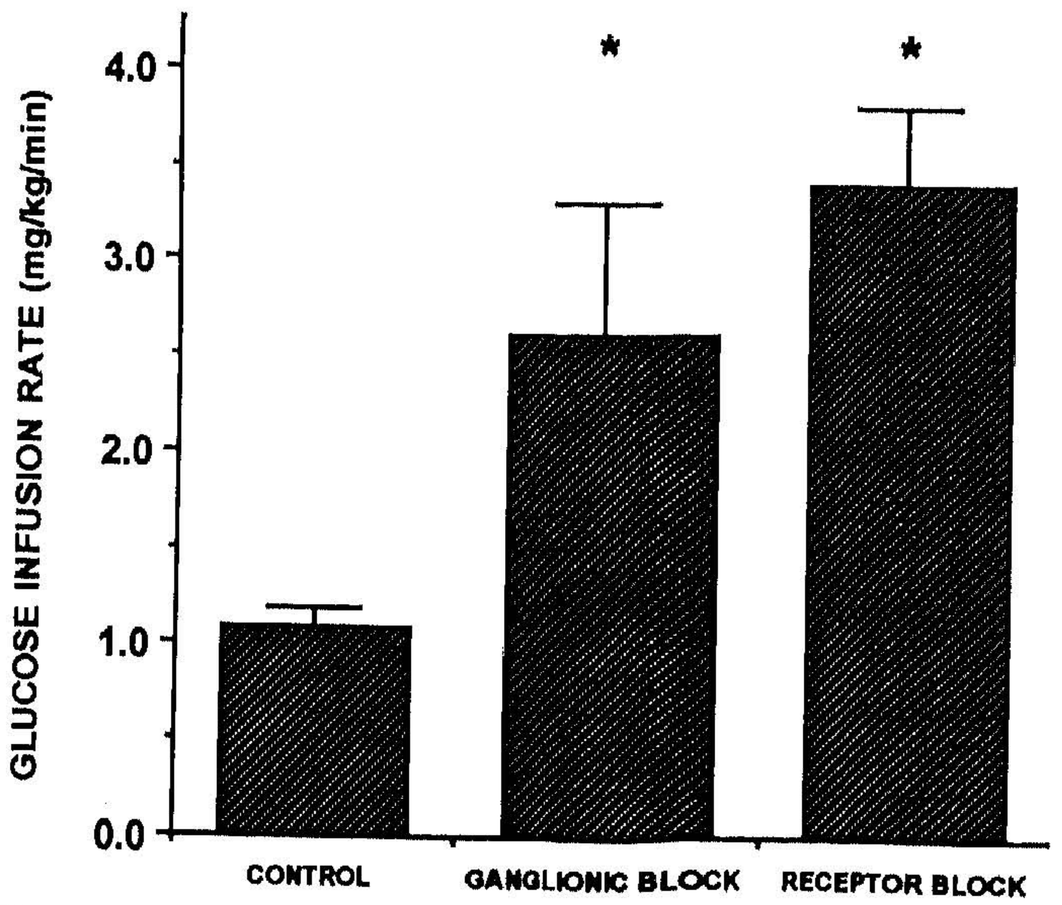

FIG. 6. The mean glucose infusion rate required to maintain the plasma glucose level at ~2.0 mmol/l during insulin plus variable rate glucose infusion in six rhesus monkeys and in the same monkeys during ganglionic block with trimethaphan or muscarinic plus adrenergic receptor block with atropine, tolazoline, and propranolol. *$P < 0.025$ vs. control.

peptides, such as vasoactive intestinal polypeptide, could be involved in the autonomic stimulation of glucagon secretion during hypoglycemia in humans (25). The actions of neuropeptides would not be likely to be blocked by classical muscarinic and adrenergic receptor antagonists. Another explanation for the discrepancies between the human and animal studies is the level of hypoglycemia examined. The present study and most animal studies only examined one level of hypoglycemia (~2.0 mmol/l). It is possible that the autonomic contribution may differ at other degrees of hypoglycemia and that autonomic mechanisms are relatively unimportant for stimulating glucagon secretion during the moderate hypoglycemia usually used in human studies, whereas during more marked hypoglycemia studied in animals, autonomic activation makes a substantial contribution. Clearly, further experiments examining other degrees of hypoglycemia and lower doses of autonomic blockers will be required to address these issues.

In summary, the present study demonstrates that activation of the autonomic nervous system mediates a substantial portion of increased plasma glucagon during hypoglycemia in rhesus monkeys. These results extend observations made in several other animal species to a species of nonhuman primate. However, defining a role for the autonomic nervous system in mediating glucagon responses to hypoglycemia in humans will require new studies with ganglionic blockers and/or peptidergic antagonists.

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