Title

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Journal

Metabolism, 43(7)

ISSN

0026-0495

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Publication Date

1994-07-01

DOI

10.1016/0026-0495(94)90267-4

Peer reviewed

Redundant Parasympathetic and Sympathoadrenal Mediation of Increased Glucagon Secretion During Insulin-Induced Hypoglycemia in Conscious Rats

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Both the parasympathetic and sympathoadrenal inputs to the pancreas can stimulate glucagon release and are activated during hypoglycemia. However, blockade of only one branch of the autonomic nervous system may not reduce hypoglycemia-induced glucagon secretion, because the unblocked neural input is sufficient to mediate the glucagon response, ie, the neural inputs are redundant. Therefore, to determine if parasympathetic and sympathoadrenal activation redundantly mediate increased glucagon secretion during hypoglycemia, insulin was administered to conscious rats pretreated with a muscarinic antagonist (methylatropine, n = 7), combined α- and β-adrenergic receptor blockade (tolazoline + propranolol, n = 5), or adrenergic blockade + methylatropine (n = 7). Insulin administration produced similar hypoglycemia in control and antagonist-treated rats (25 to 32 mg/dL). In control rats (n = 9), plasma immunoreactive glucagon (IRG) increased from a baseline level of 125 ± 11 to 1,102 ± 102 pg/mL during hypoglycemia (ΔIRG = +977 ± 98 pg/mL, $P < .0005$). The plasma IRG response was not significantly altered either by methylatropine (ΔIRG = +677 ± 141 pg/mL) or by adrenergic blockade (ΔIRG = +1,374 ± 314 pg/mL). However, the IRG response to hypoglycemia was reduced to 25% of the control value by the combination of adrenergic blockade + methylatropine (ΔIRG = +250 ± 83 pg/mL, $P < .01$ v control rats). These results suggest that the plasma glucagon response to hypoglycemia in conscious rats is predominately the result of autonomic neural activation, and is redundantly mediated by the parasympathetic and sympathoadrenal divisions of the autonomic nervous system.

IT HAS BEEN WELL ESTABLISHED that activation of three different autonomic inputs to the pancreas can stimulate glucagon secretion. For example, activation of the parasympathetic input to the pancreas by electrical stimulation of the vagal nerve trunks increases pancreatic glucagon release in a number of species.[1,2] In addition, simulating the activation of the adrenal medulla by epinephrine (EPI) infusion stimulates glucagon secretion.[3,4] Lastly, activation of the local sympathetic innervation of the pancreas by electrical stimulation also increases pancreatic glucagon secretion.[5,6] The activation of each of these autonomic inputs to the pancreas has been demonstrated in response to hypoglycemia. Thus, activation of pancreatic parasympathetic nerves has been demonstrated during hypoglycemia by measuring increased secretion of the vagally sensitive islet hormone, pancreatic polypeptide (PP).[7,8] Activation of sympathetic nerves innervating the adrenal medulla results in an increase of circulating EPI.[9,10] Activation of the direct sympathetic neural input to the pancreas during hypoglycemia has been assessed by the increased spillover of norepinephrine (NE) from sympathetic nerves into the pancreatic venous effluent.[11,12]

Although autonomic activation has been demonstrated to contribute to increased glucagon secretion in response to a number of physiological stressors,[13,14] it has been more difficult to demonstrate a definitive role for the autonomic nervous system in mediating glucagon responses during hypoglycemia, because hypoglycemia can also directly increase glucagon secretion in the absence of autonomic neural activation. For example, decreasing the perfusate glucagon concentration can increase glucagon release from isolated islets[15] or perfused pancreas preparations.[16] In addition, a number of studies, mostly conducted in human subjects, found no significant effect of various pharmacological or physical disruptions of autonomic activation on glucagon responses to insulin-induced hypoglycemia.[17-20] Therefore, the prevailing view has been that a low plasma glucose concentration at the level of the islet, not autonomic nervous system activation, is the predominant stimulus for increased glucagon secretion during hypoglycemia.

However, other studies designed to investigate the contribution of the autonomic nervous system, versus the direct effects of decreased plasma glucose concentrations, have demonstrated a significant autonomic component to hypoglycemia-induced glucagon secretion in calves,[21] dogs,[22] and mice.[23] These studies are characterized by the use of pharmacological or surgical approaches that interrupt all three autonomic inputs to the A cell. Thus, it was proposed that during hypoglycemia, the parasympathetic and sympathoadrenal inputs to the pancreas may function in a redundant manner such that blockade or ablation of one or a portion of one autonomic subdivision is not sufficient to attenuate the glucagon response.[24] Accordingly, atropine administration or surgical vagotomy,[17] administration of adrenergic antagonists,[18] adrenalectomy,[19] or sympathectomy[20] alone do not reduce glucagon responses to insulin-induced hypoglycemia. However, if the neural control is redundant, then simultaneous blockade or ablation of the

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Submitted May 6, 1993; accepted August 13, 1993.

Supported by National Institutes of Health Grants No. DK-12829, DK-18899, T32-DK-07355, DK-35747, DK-20579, and 5-R29-DK-4073202 and the Research Service of the Veterans Administration. P.J.H. was supported in part by the Northern California Chapter of the Achievement Rewards for College Scientists (ARCS Inc).

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0026-0495/94/4307-0012$03.00/0

parasympathetic, adrenal medullary, and direct sympathetic inputs to the pancreas would be required to reveal the full autonomic contribution to the glucagon response to hypoglycemia.

Therefore, to determine if parasympathetic and sympathoadrenal mechanisms can redundantly mediate increased glucagon secretion during hypoglycemia, we examined glucagon responses to insulin-induced hypoglycemia in conscious rats treated with the muscarinic receptor antagonist methylatropine, with a combination of α- and β-adrenergic receptor blockers, or with both muscarinic and combined adrenergic blockade together. These responses were compared with those measured in control, saline-pretreated rats. Plasma PP and plasma catecholamine responses to hypoglycemia were measured as indices of parasympathetic and sympathoadrenal activation, respectively.

MATERIALS AND METHODS

Animals and Cannula Implantation

Adult male Sprague–Dawley rats (390 to 510 g) were used for these studies. Rats were individually housed in polycarbonate cages and fed a stock diet (Ralston Purina, St Louis, MO) and water ad libitum. The light-dark cycle was 12 hours on and 12 hours off, with lights on at 6 AM. To access mixed venous blood, each animal had a silicon jugular catheter (ID, 0.3 mm, OD 0.6 mm, American Scientific, McGraw Park, IL) implanted in the right atrium via the right jugular vein under pentobarbital anesthesia (65 to 80 mg/kg intraperitoneally), as previously described.[8] Catheters were flushed with heparinized saline (50 U/mL) and capped when not in use. Following catheter implantation, a minimum of 72 hours was allowed for recovery before experiments were conducted. The animal preparation and experimental protocols were approved by the institutional animal use and care committee, and were conducted in accordance with the National Institutes of Health Guide for the Use and Care of Laboratory Animals.

Hypoglycemia Protocol

All animals were fasted overnight before the experiments, which were conducted between 11 AM and 4 PM. In the control rats, saline (1 mL/kg) was administered intravenously. A second group of rats received atropine methyl bromide 10 mg/kg (Sigma Chemical, St Louis, MO) intravenously. Atropine methyl bromide was used rather than atropine sulfate because atropine methyl bromide does not readily cross the blood-brain barrier[25] and thus should not impair central nervous system muscarinic, cholinergic neurotransmission. A third group received a combination of a β-adrenergic blocker, propranolol HCl (1 mg/kg, Inderal, Ayerst Laboratories, New York, NY), and an α-adrenergic blocker, tolazoline HCl (10 mg/kg, Priscoline, Ciba-Geigy, Summit, NJ). The dose of propranolol exceeds that which has been demonstrated to significantly impair cardiovascular reflexes in rats.[26] Tolazoline was administered at a dose five to 10 times the dose (per kg) reported to produce significant α-adrenergic blockade in humans.[27] Lastly, both muscarinic blockade (methylatropine) and combined adrenergic blockade (propranolol + tolazoline) were induced in a fourth group of rats. Fifteen minutes after administration of saline or autonomic antagonists, baseline samples were drawn from the jugular cannula. Then 1.0 to 2.0 U/kg regular porcine insulin (Eli Lilly and Co, Indianapolis, IN) was administered intravenously as a bolus. A second blood sample was drawn 30 minutes following the insulin injection. After each blood sample was drawn, an equal volume of heparinized blood (20 U/mL) collected from donor rats was replaced via the jugular cannula.

Assays and Data Analysis

Blood samples for glucose and PP determination were placed in tubes containing EDTA. Blood samples for catecholamine determination were placed in tubes containing EGTA and reduced glutathione. Blood samples for determination of immunoreactive glucagon (IRG) were placed in tubes containing heparin and 50 μL aprotinin (24 TIU/mL). All samples were kept on ice until centrifugation (2,500 rpm for 20 minutes at 4°C). The plasma was then decanted and frozen at −20°C until assayed.

Plasma glucose was assayed by the glucose oxidase method with a glucose analyzer (Beckman Instruments, Fullerton, CA). The plasma immunoreactive PP level was measured radioimmunologically in unextracted plasma with a guinea pig–derived antisera, as previously described.[28] Plasma NE and EPI levels were measured in duplicate with a sensitive and specific radioenzymatic assay.[29] The plasma IRG level was measured according to the method of Faloona and Unger by radioimmunoassay with an antibody that has a high degree of specificity for the COOH-terminal portion of the glucagon molecule.[30] The range of the standard curve is 0 to 2,000 pg/mL. Intraassay and interassay coefficients of variation for the glucagon assay in this laboratory are 11% and 12%, respectively.

The changes of plasma glucose, PP, NE, EPI, and IRG in Tables 1 through 5 were calculated by subtracting the 0-minute baseline value from the 30-minute value following the injection of insulin. The percentage data in Figs 1 through 4 were calculated by dividing the change of IRG in individual rats by the mean change of IRG in the control animals × 100. Data are expressed as the mean ± SE. Statistical comparisons of means within a group were made with a paired *t* test. For statistical comparisons of means of different groups, ANOVA was performed with a Dunnett's post-test.

RESULTS

Plasma Glucose

Baseline plasma glucose averaged 100 ± 3 mg/dL in control rats, and decreased by 71 ± 2 mg/dL to a mean of 29 ± 2 mg/dL. Baseline plasma glucose levels, the glucose level 30 minutes after insulin administration, and the plasma glucose decrement did not differ significantly from control values in any of the groups of animals treated with autonomic antagonists (Table 1).

Plasma PP

Plasma PP increased more than sevenfold during insulin-induced hypoglycemia (Table 2). Plasma PP was also significantly increased during hypoglycemia in atropinized

Table 1. Baseline Plasma Glucose Concentrations and Glucose Levels During IIH

Treatment	Plasma Glucose (mg/dL)		
	Baseline	IIH 30 Minutes	Δ 30 Minutes
Control (n = 9)	100 ± 3	29 ± 2	−71 ± 2
Methylatropine (n = 7)	105 ± 4	32 ± 2	−73 ± 5
Adrenergic blockade (n = 5)	90 ± 6	29 ± 3	−61 ± 5
Methylatropine + adrenergic blockade (n = 7)	91 ± 4	25 ± 2	−66 ± 4

NOTE. Values are the mean ± SEM.

Abbreviation: IIH, insulin-induced hypoglycemia.

Table 2. Baseline PP Concentrations and PP Responses During IIH

Treatment	PP (pg/mL) Baseline	IIH 30 Minutes	Δ 30 Minutes
Control (n = 9)	29 ± 3	276 ± 46	+247 ± 46†
Methylatropine (n = 7)	27 ± 2	112 ± 24	+85 ± 24*†
Adrenergic blockade (n = 5)	48 ± 9	228 ± 36	+179 ± 36†
Methylatropine + adrenergic blockade (n = 7)	39 ± 6	66 ± 7	+26 ± 7*†

NOTE. Values are the mean ± SE.
Abbreviation: IIH, insulin-induced hypoglycemia.
*$P < .01$ *v* control.
†$P < .01$ *v* baseline.

rats, but this response was reduced by approximately 65% and was significantly smaller than the response in control rats ($P < .01$). The increase of plasma PP during hypoglycemia in rats treated with both α- and β-adrenergic antagonists was not significantly different from the response in control rats. In rats that received both methylatropine and combined adrenergic blockade, the response was significantly reduced compared with that in control rats ($P < .01$; Fig 1, Table 2).

Plasma NE and EPI

Baseline NE was increased in rats that received adrenergic antagonists or methylatropine and adrenergic antagonists together. Plasma NE increased during hypoglycemia in all four groups of rats, but the increase of NE was significantly larger in animals that received adrenergic blockers alone than in the control animals (Fig 2, Table 3). Baseline EPI levels were also increased in rats that received adrenergic blockade or methylatropine plus adrenergic blockade; however, the increase of EPI during hypoglycemia was similar in all four groups of animals (Fig 3, Table 4).

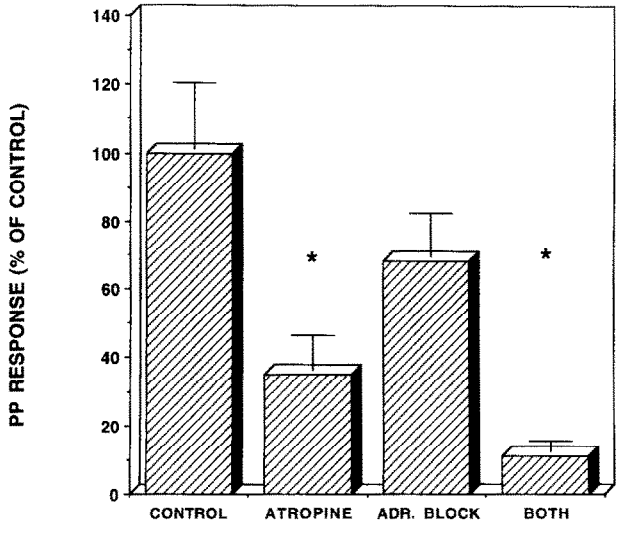

Fig 1. Plasma PP responses 30 minutes after insulin injection as a percentage of the mean control response to insulin-induced hypoglycemia in conscious rats. Animals received either saline (CONTROL, n = 9), methylatropine 10 mg/kg (ATROPINE, n = 7), tolazoline 10 mg/kg + propranolol 1 mg/kg (ADR. BLOCK, n = 5), or methylatropine + adrenergic antagonists (BOTH, n = 7). *$P < .01$ *v* CONTROL. Absolute plasma PP levels are provided in Table 2.

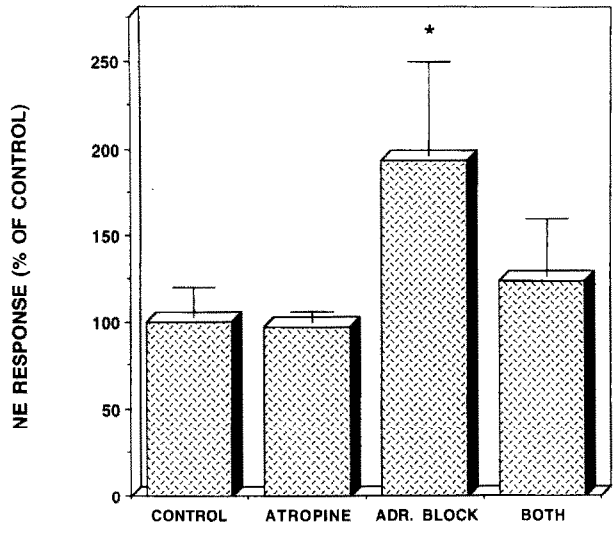

Fig 2. Plasma NE responses 30 minutes after insulin injection as a percentage of the mean control response to insulin-induced hypoglycemia in conscious rats. Animals received either saline (CONTROL, n = 9), methylatropine 10 mg/kg (ATROPINE, n = 7), tolazoline 10 mg/kg + propranolol 1 mg/kg (ADR. BLOCK, n = 5), or methylatropine + adrenergic antagonists (BOTH, n = 7). *$P < .01$ *v* CONTROL. Absolute plasma NE levels are provided in Table 3.

Plasma IRG

In control rats, plasma IRG was increased by nearly eightfold during insulin-induced hypoglycemia ($P < .0005$; Table 5). The increases of plasma IRG in rats pretreated with methylatropine or the α- and β-adrenergic antagonists were not significantly different from the response in control animals. The plasma IRG response in rats that received both methylatropine and adrenergic antagonists was reduced to 25% of that in the control rats ($P < .01$; Fig 4, Table 5).

DISCUSSION

Activation of both parasympathetic and sympathoadrenal inputs to the pancreas during hypoglycemia has been

Table 3. Baseline NE Concentrations and NE Responses During IIH

Treatment	NE (pg/mL) Baseline	IIH 30 Minutes	Δ 30 Minutes
Control (n = 9)	210 ± 30	1,560 ± 230	+1,350 ± 230†
Methylatropine (n = 7)	350 ± 30	1,650 ± 80	+1,300 ± 80†
Adrenergic blockade (n = 5)	1,430 ± 410*	4,040 ± 1,050	+2,610 ± 710*†
Methylatropine + adrenergic blockade (n = 7)	1,210 ± 250*	2,870 ± 420	+1,660 ± 450†

NOTE. Values are the mean ± SE.
Abbreviation: IIH, insulin-induced hypoglycemia.
*$P < .01$ *v* control.
†$P < .05$ *v* baseline.

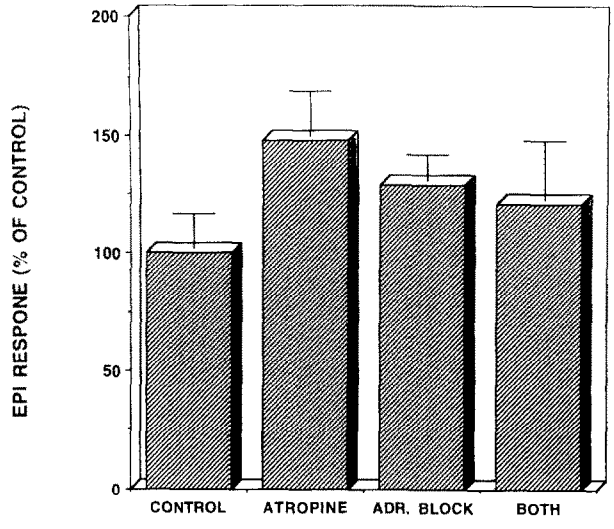

Fig 3. Plasma EPI responses 30 minutes after insulin injection as a percentage of the mean control response to insulin-induced hypoglycemia in conscious rats. Animals received either saline (CONTROL, n = 9), methylatropine 10 mg/kg (ATROPINE, n = 7), tolazoline 10 mg/kg + propranolol 1 mg/kg (ADR. BLOCK, n = 5), or methylatropine + adrenergic antagonists (BOTH, n = 7). Absolute plasma EPI levels are provided in Table 4.

demonstrated. This study was designed to determine if the parasympathetic and sympathoadrenal divisions of the autonomic nervous system redundantly contribute to increased pancreatic glucagon secretion during hypoglycemia in conscious rats. Although the effects of parasympathetic and sympathoadrenal activation on numerous physiological parameters are often antagonistic, during hypoglycemia the activation of either major autonomic subdivision has a similar result—increased glucagon secretion. However, for redundancy between these systems to exist, selective blockade of either the parasympathetic or sympathoadrenal inputs to the pancreas should not significantly reduce the glucagon response to insulin-induced hypoglycemia, but blockade of both autonomic divisions together must result in a substantial reduction of the glucagon response. Therefore, in the present study, hypoglycemia was induced by insulin injection in four groups of chronically cannulated

Table 4. Baseline EPI Concentrations and EPI Responses During IIH

	EPI (pg/mL)		
Treatment	Baseline	IIH 30 Minutes	Δ 30 Minutes
Control (n = 9)	130 ± 40	7,570 ± 1,170	+7,440 ± 1,150†
Methylatropine (n = 7)	200 ± 30	11,220 ± 1,430	+11,020 ± 1,420†
Adrenergic blockade (n = 5)	590 ± 140*	10,190 ± 760	+9,600 ± 820†
Methylatropine + adrenergic blockade (n = 7)	970 ± 200*	9,940 ± 1,990	+8,970 ± 1,890†

NOTE. Values are the mean ± SE.
Abbreviation: IIH, insulin-induced hypoglycemia.
*$P < .025$ *v* control.
†$P < .01$ *v* baseline.

Table 5. Baseline IRG Concentrations and IRG Responses During IIH

	IRG (pg/mL)		
Treatment	Baseline	IIH 30 Minutes	Δ 30 Minutes
Control (n = 9)	125 ± 11	1,102 ± 102	+977 ± 98†
Methylatropine (n = 7)	183 ± 30	861 ± 139	+677 ± 141†
Adrenergic blockade (n = 5)	153 ± 8	1,260 ± 209	+1,107 ± 202†
Methylatropine + adrenergic blockade (n = 7)	262 ± 55*	512 ± 55	+250 ± 83*†

NOTE. Values are the mean ± SE.
Abbreviation: IIH, insulin-induced hypoglycemia.
*$P < .01$ *v* control.
†$P < .01$ *v* baseline.

conscious rats, ie, control saline-treated rats and rats pretreated either with the muscarinic antagonist methylatropine to impair the effects of parasympathetic neural activation, with a combination of α- and β-adrenergic receptor antagonists to impair the effects of adrenal medullary and sympathetic neuronal activation, or with both methylatropine and adrenergic antagonists together.

Insulin administration produced similar hypoglycemia and similar decrements of plasma glucose in all four groups of animals. Thus, the glucopenic stimulus at the brain and at the islet was comparable between treatments. As a result of this central neuroglucopenia, plasma PP levels were markedly increased both in the control rats and in the rats pretreated with adrenergic antagonists, demonstrating an increase of the parasympathetic vagal input to the islet in these animals. Plasma levels of PP have been used as an indirect index of activation of the parasympathetic, cholin-

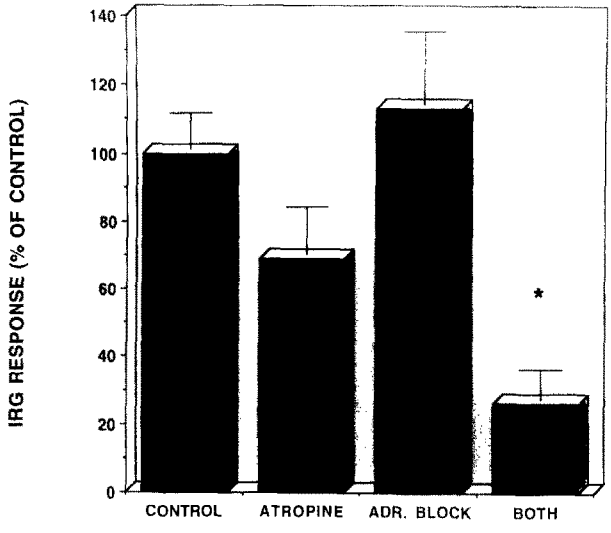

Fig 4. Plasma IRG responses 30 minutes after insulin injection as a percentage of the mean control response to insulin-induced hypoglycemia in conscious rats. Animals received either saline (CONTROL, n = 9), methylatropine 10 mg/kg (ATROPINE, n = 7), tolazoline 10 mg/kg + propranolol 1 mg/kg (ADR. BLOCK, n = 5), or methylatropine + adrenergic antagonists (BOTH, n = 7). *$P < .01$ *v* CONTROL. Absolute plasma IRG levels are provided in Table 5.

ergic input to the islet.[7,8] Accordingly, plasma PP responses were reduced by 65% to 90% in rats that received methylatropine or methylatropine plus adrenergic antagonists, suggesting that pancreatic cholinergic receptor activation was substantially reduced in these animals.

Plasma EPI levels were increased during hypoglycemia in all rats, reflecting increased adrenal medullary secretion of this sympathetic neurohormone. The increase of plasma NE during insulin-induced hypoglycemia in rats is primarily the result of neuronal NE release rather than of NE secretion from the adrenal medulla,[31] and therefore, increased plasma NE levels indicate increased spillover from sympathetic nerve terminals. Plasma catecholamine responses to hypoglycemia would not be expected to be reduced by adrenergic blockade, as the PP response is by atropine, because adrenergic antagonists block the action and not the release of EPI and NE. In fact, pretreatment of rats with the adrenergic antagonists propranolol and tolazoline increased baseline plasma levels of both NE and EPI. Plasma NE, but not EPI responses to insulin-induced hypoglycemia were also increased by adrenergic blockade. Increased baseline and hypoglycemia-stimulated plasma NE levels may result from an action of the α-adrenergic antagonist to block presynaptic α_2-receptors that inhibit neurotransmitter release from sympathetic nerves.[32] Increased baseline EPI may be due to blockade of β-adrenergic receptors that are involved in the clearance of plasma EPI.[33] Alternatively, adrenergic receptor blockade could result in increased basal or hypoglycemia-stimulated plasma catecholamines if the blockade produced significant hypotension. Since blood pressure was not measured in this study, it is not known what degree of hypotension may have resulted from administration of the adrenergic blockers.

Pharmacological blockade of peripheral parasympathetic, muscarinic receptors with methylatropine in conscious rats did not significantly reduce the increase of plasma glucagon during insulin-induced hypoglycemia compared with the response observed in control animals. Although it is possible that a statistically significant effect of methylatropine to reduce the glucagon response to hypoglycemia would have been found if larger numbers of animals were used, this effect would likely be small because at least 70% of the glucagon response was intact in these animals. This result conflicts with a previous study conducted in anesthetized rats in which atropine partially reduced the glucagon response to insulin-induced hypoglycemia.[34] However, in that experiment, atropine rather than methylatropine was used. Since atropine more readily crosses the blood-brain barrier,[25] it is uncertain whether the activation of central cholinergic receptors is also impaired after atropine administration. We have found that atropine sulfate does significantly reduce the glucagon response to insulin-induced hypoglycemia in conscious rats (unpublished observations), in contrast to the lack of effect of methylatropine reported in the present study. In human duodenal-ulcer patients with truncal vagotomy, the glucagon response to insulin-induced hypoglycemia was significantly reduced compared with the response in control subjects.[35] However, other studies in human subjects found that neither atropine administration nor vagotomy reduced the glucagon response to insulin-induced hypoglycemia when compared with responses observed in control subjects,[17] suggesting that selective interruption of the parasympathetic input to the islet alone is not always sufficient to impair glucagon responses to hypoglycemia.

Similar to methylatropine-treated animals, the glucagon response to hypoglycemia in rats treated with the α- and β-adrenergic antagonists tolazoline and propranolol was not significantly different from the response in control rats. In a previous study, it was observed that combined adrenergic blockade did not impair the glucagon response to insulin-induced hypoglycemia in anesthetized rats.[36] Another study showed no reduction of glucagon responses to hypoglycemia in adrenodemedullated rats that were also chemically sympathectomized with reserpine.[37] A similar lack of effect of combined adrenergic blockade[18] or functional sympathoadrenalectomy[20] on hypoglycemia-induced glucagon responses has been reported in human subjects. Functional adrenalectomy, produced by diverting adrenal venous blood from the systemic circulation, did result in an impaired glucagon response to insulin-induced hypoglycemia in pentobarbital-anesthetized dogs.[38] However, pentobarbital anesthesia abolishes the activation of cholinergic input to the islet during central neuroglucopenia in dogs.[39] Therefore, both parasympathetic and adrenal medullary inputs to the A cell would be expected to be diminished under these experimental conditions. Thus, selective impairment of sympathoadrenal activation or its effects does not usually result in decreased glucagon responses to hypoglycemia.

Administration of both methylatropine and adrenergic antagonists together before insulin injection reduced the glucagon response to hypoglycemia to 25% of that observed in control animals. Therefore, simultaneous blockade of both parasympathetic, muscarinic and sympathoadrenal adrenergic receptors reveals that autonomic neural activation makes a major contribution to hypoglycemia-induced glucagon secretion in conscious rats. This result is compatible with results from previous experiments in which the administration of ganglionic blocking agents, which impair both parasympathetic and sympathoadrenal neural activation, eliminates the majority of the glucagon response to insulin-induced hypoglycemia in dogs[22] and mice.[23] Similarly, the combination of vagotomy and surgical sympathoadrenalectomy markedly reduces the glucagon response to hypoglycemia in dogs.[22] In addition, the selective infusion of glucose into the carotid and vertebral arteries supplying the brain in order to prevent central neuroglucopenia in conscious dogs reduces both parasympathetic and sympathoadrenal activation and eliminates the glucagon response to insulin-induced hypoglycemia.[40]

In the present study, methylatropine and combined adrenergic blockade together prevented the majority of the glucagon response to hypoglycemia; however, a significant portion (25%) of the response persisted despite the blockade. This portion of the glucagon response could be a direct result of low plasma glucose concentrations at the level of the islet.[13,14] In addition to a possible direct effect, low

plasma glucose levels may potentiate neurally mediated glucagon secretion, since it is likely that in the absence of hypoglycemia autonomic activation would be a less potent stimulus for glucagon release. Thus, both low islet glucose concentrations and autonomic activation may be required for full expression of the glucagon response. Alternatively, it is possible that parasympathetic neuropeptides such as vasoactive intestinal polypeptide or sympathetic neuropeptides such as galanin contribute to the portion of the glucagon response that was not blocked by atropine and combined adrenergic blockade. Both vasoactive intestinal polypeptide and galanin stimulate glucagon secretion[41,42] and are released from the pancreas during electrical stimulation of parasympathetic or sympathetic nerves, respectively.[43,44] In addition, it has recently been shown that galanin is co-released with NE from pancreatic sympathetic nerves during insulin-induced hypoglycemia in dogs.[12] The actions of these neuropeptides would not be expected to be blocked by the classic muscarinic and adrenergic receptor antagonists.

In human subjects, administration of a ganglionic blocking agent, which would impair both parasympathetic and sympathoadrenal activation, significantly reduced glucagon responses to insulin-induced hypoglycemia.[45] However, in another study, administration of atropine and adrenergic antagonists together did not alter the glucagon response to hypoglycemia,[46] suggesting that autonomic activation may not contribute to hypoglycemia-induced glucagon secretion in humans. Alternatively, noncholinergic, nonadrenergic mechanisms (peptides) could contribute to glucagon responses to hypoglycemia in human subjects after administration of classic muscarinic and adrenergic blockers.

Together, the results of this study provide evidence to support the hypothesis that parasympathetic and sympathoadrenal autonomic activation mediate glucagon responses to hypoglycemia in a redundant fashion, such that interfering with either autonomic subdivision alone will not impair the glucagon response in rats. There is some experimental evidence from other species that supports this idea. For example, in anesthetized dogs, it was found that neither bilateral vagotomy nor surgical sympathectomy alone affected plasma glucose recovery after insulin administration, but that both procedures together produced a marked impairment of plasma glucose recovery.[47] Unfortunately, plasma glucagon levels were not measured in this study; however, since increased glucagon secretion is the primary mechanism for recovery of plasma glucose from insulin-induced hypoglycemia,[48,49] this study provides indirect support for the redundancy hypothesis. More direct evidence is provided by the observation that atropine or splanchnic-nerve transection delayed or modestly reduced glucagon responses to hypoglycemia in conscious calves, whereas atropine and splanchnic-nerve transection together markedly impaired the glucagon response.[21] Therefore, redundancy between the major autonomic subdivisions, if unrecognized, could lead to underestimation of the autonomic contribution to hypoglycemia-induced glucagon secretion.

In summary, neither methylatropine nor combined α- and β-adrenergic blockade alone alter the glucagon response to insulin-induced hypoglycemia in conscious rats, but methylatropine and adrenergic blockade together eliminate 75% of this response. Thus, activation of the autonomic nervous system is responsible for the majority of the glucagon response to hypoglycemia in rats, and this response can be redundantly mediated by either parasympathetic or sympathoadrenal neural activation.

ACKNOWLEDGMENT

The authors would like to acknowledge the expert technical assistance of Marcia Beverly, Julie Lee, David Flatness, Richard Chan, and Hong Nguyen, and Dr Roger Unger for the generous gift of the 04A glucagon antisera. None of the plasma NE, EPI, or the glucagon data have been previously published. The PP data have been published in a report on the neural control of PP secretion in rats[8] and are included as an index of activation of the parasympathetic input to the islet during hypoglycemia and to document the effect of methylatropine to impair this activation.

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