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Autonomic control of pancreatic polypeptide and glucagon secretion during neuroglucopenia and hypoglycemia in mice

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Havel, Peter J., Jones O. Akpan, Donald L. Curry, Judith S. Stern, Ronald L. Gingerich, and Bo Ahren. Autonomic control of pancreatic polypeptide and glucagon secretion during neuroglucopenia and hypoglycemia in mice. *Am. J. Physiol.* 265 (*Regulatory Integrative Comp. Physiol.* 34): R246-R254, 1993.—Neural control of pancreatic polypeptide (PP) release has not been previously investigated in the mouse. In addition, it is not known to what extent increased glucagon secretion during hypoglycemia in mice is neurally mediated vs. an effect of hypoglycemia to directly stimulate glucagon secretion at the level of the islet. Feeding or the cholinergic agonist carbachol increased plasma PP levels in conscious mice (+74 ± 18 pg/ml vs. fasted mice and +141 ± 17 pg/ml vs. control, respectively). Neuroglucopenia induced by 2-deoxy-D-glucose or insulin-induced hypoglycemia also increased plasma PP (+79 ± 18 and +89 ± 11 pg/ml vs. control, respectively). These increases were abolished by hexamethonium and reduced by atropine methylnitrate (atropine). Hypoglycemia-induced hyperglucagonemia (+1,243 ± 275 pg/ml) was reduced to 31 ± 7% of control by atropine (+382 ± 85 pg/ml), to 48 ± 9% of control by combined adrenergic blockade (+601 ± 112 pg/ml), and nearly abolished by atropine plus combined blockade (+143 ± 41 pg/ml; 11 ± 3% of control) or hexamethonium (+151 ± 38 pg/ml; 12 ± 3% of control). We conclude the following in the mouse. *1*) Feeding or cholinergic agonists increase plasma PP. *2*) During neuroglucopenia or hypoglycemia, plasma PP is increased via nicotinic and muscarinic mechanisms. *3*) The glucagon response to hypoglycemia is predominantly the result of autonomic activation and is mediated by both muscarinic and adrenergic mechanisms.

carbachol; 2-deoxy-D-glucose; insulin-induced hypoglycemia; atropine; hexamethonium; propranolol; phentolamine; parasympathetic nervous system; sympathetic nervous system

THE EXPERIMENTS reported in this paper were conducted to address two fundamental questions. The first was to investigate the neural regulation of the islet hormone, pancreatic polypeptide (PP), to determine whether plasma PP levels reflect cholinergic, parasympathetic input to the pancreas in the mouse and might therefore be useful to assess the activation of parasympathetic input to the pancreas during neuroglucopenia in experiments performed on mice.

The secretion of PP in several species is predominantly controlled by the vagal input to the pancreas and involves both nicotinic and muscarinic receptors (for review, see Refs. 24 and 48). For example, electrical stimulation of the vagus nerves or the administration of cholinergic agonists markedly increases PP secretion via an atropine-sensitive mechanism (5, 50). Cholinergic control of PP release is also mediated by autonomic neurotransmission between pre- and postganglionic parasympathetic nerves because nicotinic receptor antagonists that block ganglionic transmission abolish the PP response to vagal nerve stimulation (5). Another stimulus for PP release is neuroglucopenic stress produced by either insulin-induced hypoglycemia or by administration of 2-deoxy-D-glucose (2-DG). Neuroglucopenia-induced PP secretion has been demonstrated to be mediated by vagal, muscarinic activation in several species (1, 28, 47, 50). Thus measurements of plasma PP and PP responses to neuroglucopenia have been used as an index of the parasympathetic input to both the endocrine and exocrine pancreas (8, 9, 21, 35).

However, this potentially useful index of parasympathetic input to the endocrine pancreas has not been widely used for studies in rodents because of a scarcity of radioimmunoassays for measuring plasma PP in rodents. The primary structure of PP in rodents differs from that of several other mammalian species by eight amino acids (36). Therefore, radioimmunoassays used to measure plasma PP in other species are not always effective for measuring plasma PP in rodents. A new radioimmunoassay that is both sensitive and specific has recently been developed and validated for the measurement of PP in rat and mouse plasma (7). A recent study of autonomic control of PP secretion in rats, which used this new assay, found that the majority of the PP response to insulin-induced hypoglycemia in this species is mediated by cholinergic, muscarinic activation (28). However, this study also suggested that adrenergic activation may make a minor contribution to this response. In the present study, we examined plasma PP levels after feeding or administration of a cholinergic agonist and during 2-DG-induced neuroglucopenia or insulin-induced hypoglycemia and evaluated the role of nicotinic neurotransmission and muscarinic, cholinergic receptors in the regulation of PP release in the mouse.

The second major question addressed in these experiments was to determine the contribution of autonomic nervous system activation vs. the role of low glucose levels acting at the level of the islet in mediating increases in plasma glucagon during insulin-induced hypoglycemia in the mouse. It is well-known that glucagon secretion increases during insulin-induced hypoglycemia and that this increased secretion is the primary factor responsible for the recovery of plasma glucose levels from acute hypoglycemia (20), as well as a major determinant of the glucose nadir after insulin administration (25). However, hypoglycemia is also known to activate the autonomic nervous system, increasing the level of circulating epinephrine (12, 18) and activating the parasympathetic nerves to the islet, as reflected by

increased secretion of PP (28, 47). In addition, it has been demonstrated that the direct sympathetic innervation to the pancreas can be activated during glucopenic stress (23, 29). Activation of each of these autonomic inputs to the pancreas has the potential to increase glucagon secretion (4, 6, 19) and therefore to mediate the glucagon response to hypoglycemia. However, a number of experiments performed to test this question have shown that interfering with one portion of the autonomic activation, or blocking its effects with classical receptor antagonists, has little or no influence on the glucagon response to hypoglycemia (17, 39, 40, 41, 44, 45, 54). These results, and the demonstration that lowering the perfusate glucose level can stimulate glucagon secretion from isolated islets (38) and from the isolated, perfused pancreas, (56) have led to the view that the increase in glucagon secretion during insulin-induced hypoglycemia is due to a direct effect of low glucose at the level of the islet. However, recent studies have demonstrated that the autonomic nervous system can make a major contribution to increased pancreatic glucagon secretion during hypoglycemia in dogs (9, 26), and there is additional evidence from studies in other species (10, 27, 42).

Therefore, to determine the contribution of autonomic neural activation to the glucagon response to insulin-induced hypoglycemia in mice, we administered insulin to conscious mice pretreated with either saline or the ganglionic blocking agent hexamethonium, which impairs both parasympathetic and sympathoadrenal activation during hypoglycemia (51), and compared plasma glucagon levels during insulin-induced hypoglycemia. To determine the relative contributions of cholinergic vs. adrenergic mechanisms to the glucagon response, hypoglycemia was induced in mice pretreated with either saline, atropine methylnitrate (atropine), α- and β-adrenergic antagonists, or the combination of atropine and adrenergic antagonists.

MATERIALS AND METHODS

Animals. Female mice of the NMRI strain (ALAB, Stockholm, Sweden) weighing 25-30 g were used. The animals were fed a standard pellet diet (Astra-Ewos, Sodertalje, Sweden) and tap water ad libitum. Animals used for the carbachol experiments had access to food before and throughout the experiments. Animals used for the 2-DG and insulin hypoglycemia experiments were fasted overnight before the experiments. All experiments were conducted in conscious mice.

Protocols. Plasma glucose and pancreatic hormone levels were compared between fed and fasted mice (Table 1). For each pharmacological experiment animals were randomly divided into four groups with 8-16 animals per group. In *experiment 1* (carbachol ± atropine; Table 2) one group received two intraperitoneal injections of saline and served as controls for the groups that were administered drugs. The other groups received either atropine (2.5 mg/kg ip) or saline and then, 15 min later, received an intraperitoneal injection of an equal volume of saline or carbachol (750 μg/kg). Atropine methylnitrate was chosen rather than atropine sulfate because atropine methylnitrate does not readily cross the blood-brain barrier (55) and therefore should not impair muscarinic neurotransmission in the central nervous system.

Experiments 2 and *3* (2-DG ± atropine or hexamethonium; Table 3) were similar to *experiment 1*, except that the animals received intraperitoneal injections of saline, atropine (2.5 mg/kg), or hexamethonium (30 mg/kg) 15 min before the administration of saline or 2-DG (500 mg/kg ip). In *experiment 4* (insulin ± hexamethonium; Table 4), saline or insulin (1-2 U/kg ip) was administered 15 min after pretreatment with hexamethonium (30 mg/kg). Less insulin (1 U/kg) was administered to the hexamethonium-treated mice because we had previously observed that smaller doses of insulin were necessary to produce equal or lower glucose nadirs in mice treated with hexamethonium vs. saline-treated control mice.

In *experiment 5* (insulin ± atropine, adrenergic blockade, or atropine + adrenergic blockade; Table 5) mice were divided into eight groups of 15 or 16 animals per group. These mice received either saline or insulin after pretreatment with atropine (2.5 mg/kg), combined α- and β-adrenergic blockade with phentolamine (10 mg/kg ip) and propranolol (2.5 mg/kg ip), or both atropine and phentolamine + propranolol. To obtain plasma for hormone assay, a single blood sample was obtained from the intraorbital sinus with a heparinized glass pipette, 2 min after carbachol or saline injection (*experiment 1*), 15 min after 2-DG or saline injection (*experiments 2* and *3*), and 45 min after insulin or saline injection (*experiments 4* and *5*). Tables 1-5 outline the protocols for the specific treatments and the number of animals used in each experiment. Plasma was separated and frozen at −20°C until the assays were performed. The drug dosages and sampling times were selected based on previous studies of the regulation of insulin and glucagon secretion in mice (2, 3).

Assays. Plasma glucose was determined by the glucose oxidase method (11). Plasma samples for PP determination were lyophilized and shipped from Lund, Sweden, to St. Louis, MO. The samples were stored at −20°C until they were reconstituted to the original volume with distilled H_2O. PP concentration was determined with a new radioimmunoassay (Linco Research, St. Louis, MO) that is able to detect rodent PP but does not cross-react with neuropeptide Y or peptide YY (7). Plasma levels of immunoreactive insulin were determined by radioimmunoassay

Table 1. *Comparison of plasma glucose and pancreatic hormone levels in fed vs. overnight-fasted mice*

Treatment	n	Concentration			
		Glucose, mg/dl	PP, pg/ml	IRG, pg/ml	IRI, μU/ml
Fasted	18	78±2*	117±9*	338±20*	3±1*
Fed	24	97±4†	191±18†	276±24†	46±6†

Values are means ± SE. PP, pancreatic polypeptide; IRG, immunoreactive glucagon; IRI, immunoreactive insulin; *n*, no. of animals. Values in same column with different superscripts are significantly different by at least $P < 0.05$.

Table 2. *Experiment 1*

Treatment	Concentration			
	Glucose, mg/dl	PP, pg/ml	IRG, pg/ml	IRI, μU/ml
Saline/saline	102±4*	221±28*	281±29*	38±13*
Saline/carbachol	78±4†	362±17†	2,044±275†	100±6†
Atropine/saline	91±5*	191±40*	232±22*	34±11*
Atropine/carbachol	109±6*	184±35*	248±21*	48±8*

Values are means ± SE indicating plasma glucose and pancreatic hormone levels in fed mice treated with saline or atropine methylnitrate (atropine; 2.5 mg/kg) 2 min after carbachol (750 μg/kg) or saline. Values in same column with different superscripts are significantly different by at least $P < 0.05$; $n = 8$ in each group.

Table 3. *Experiments 2 and 3*

Treatment	n	Concentration		
		Glucose, mg/dl	PP, pg/ml	IRG, pg/ml
		Experiment 2		
Saline/saline	16	71±3*	106±4*	298±14*
Saline/2-DG	16	151±8‡	153±10†	1,095±141†
Hex/saline	16	52±2†	108±5*	247±21*
Hex/2-DG	16	92±4§	84±4‡	344±26*
		Experiment 3		
Saline/saline	10	81±3*	105±8*	295±23*
Saline/2-DG	10	141±3†	184±18†	986±125†
Atropine/saline	10	79±3*	108±9*	258±16*
Atropine/2-DG	10	108±6*	108±6*	512±68*

Values are means ± SE indicating plasma glucose, PP, and IRG levels in fasted mice treated with saline, hexamethonium (Hex; 30 mg/kg), or atropine (2.5 mg/kg), 15 min after 2-deoxy-D-glucose (2-DG; 500 mg/kg) or saline. Experiment 2, $n = 16$; Experiment 3, $n = 10$ animals. Values in same column with different superscripts are significantly different by at least $P < 0.05$.

Table 4. *Experiment 4*

Treatment	n	Concentration		
		Glucose, mg/dl	PP, pg/ml	IRG, pg/ml
Saline/saline	12	80±6*	91±4*	359±27*
Saline/insulin	15	30±1†	180±11†	1,856±202†
Hex/saline	15	51±2‡	102±3*	373±31*
Hex/insulin	14	18±2§	103±4*	524±27*

Values are means ± SE indicating plasma glucose, PP, and IRG levels in fasted mice treated with saline or Hex (30 mg/kg), 45 min after insulin (1-2 U/kg) or saline; n, no. of animals. Values in same column with different superscripts are significantly different by at least $P < 0.05$.

Table 5. *Experiment 5*

Treatment	n	Concentration		
		Glucose, mg/dl	PP, pg/ml	IRG, pg/ml
Saline/saline	16	88±4*	67±7*	507±48*
Saline/insulin	15	34±3†	118±5†	1,750±275†
Atropine/saline	16	87±3*	71±6*	397±38*
Atropine/insulin	15	23±2†	86±7*	779±85*
Adr Bl/saline	15	63±3‡	104±9†	673±87*
Adr Bl/insulin	15	33±2†	130±11†	1,274±112‡
Both/saline	16	58±3‡	70±5*	428±52*
Both/insulin	16	26±2†	85±6*	571±41*

Values are means ± SE indicating plasma glucose, PP, and IRG levels in fasted mice treated with saline, atropine (2.5 mg/kg), phentolamine (2.5 mg/kg) + propranolol (10 mg/kg) [adrenergic blockade (Adr Bl)], or atropine + combined adrenergic blockade (Both) 45 min after insulin (2 U/kg) or saline. Values in same column with different superscripts are significantly different by at least $P < 0.05$.

(30). Plasma immunoreactive glucagon was determined in unextracted plasma with a radioimmunoassay that is specific for pancreatic glucagon (31).

Sources. Hexamethonium bromide and 2-DG were obtained from Sigma Chemical, St. Louis, MO. Atropine methylnitrate was obtained from Vitrum, Stockholm, Sweden. Carbachol was obtained from British Drug Houses, Poole, UK, and regular porcine insulin was obtained from Novo, Bagsvaerd, Denmark. Phentolamine methanesulfonate was from Ciba-Geigy, Basel, Switzerland, and L-propranolol was from ICI, Macclesfield, UK.

Data analysis. Data are presented as means ± SE. Statistical comparisons of means of two groups were made with a two-sample t test. Statistical comparisons of means from more than two groups were made by analysis of variance with a Dunnett's post-test. The data in Figs. 1-4 are calculated as PP, glucagon, or insulin concentrations in each individual animal divided by the mean hormone concentration in the control (saline/saline) group. The glucagon responses in Fig. 5 are calculated as the concentration of glucagon in each animal in the experimental groups (saline/insulin, hexamethonium/insulin, atropine/insulin, adrenergic blockade/insulin, or atropine + adrenergic blockade/insulin) minus the mean level in their respective control groups (saline/saline, hexamethonium/saline, atropine/saline, adrenergic blockade/saline, or atropine + adrenergic blockade/saline) divided by the mean response in control animals (saline/insulin − saline/saline). A P value ≤0.05 was considered statistically significant.

RESULTS

Comparison of plasma glucose and pancreatic hormone levels in fasted vs. fed mice. Plasma glucose (+19 mg/dl), PP (+74 pg/ml), and immunoreactive insulin (+43 μU/ml) were all significantly higher in fed mice compared with mice that were fasted overnight. Plasma immunoreactive glucagon was modestly, but significantly, lower (−62 pg/ml) in fed mice compared with overnight-fasted mice (Table 1).

Experiment 1: plasma glucose and pancreatic hormones in fed mice 2 min after carbachol with or without atropine pretreatment. Plasma glucose was significantly decreased in mice that received carbachol (−24 mg/dl) (Table 2). Plasma PP (+141 pg/ml), glucagon (+1,763 pg/ml), and insulin (+62 μU/ml) were all significantly increased by carbachol administration. These increases were prevented by the prior administration of atropine (Fig. 1 *A*, *B*, and *C*; Table 2).

Experiments 2 and 3: plasma glucose, PP, and glucagon levels in fasted mice 15 min after 2-DG with or without hexamethonium or atropine pretreatment. Plasma glucose was significantly decreased by hexamethonium administration (−19 mg/dl) (Table 3). Measured plasma glucose was approximately doubled in mice that received 2-DG (+60-80 mg/dl). The increases in plasma glucose were partially reduced by hexamethonium (−40 mg/dl) or atropine (−29 mg/dl) pretreatment, with the effect of hexamethonium being somewhat more pronounced (Table 3). Plasma PP levels were significantly increased by 2-DG (+47-79 pg/ml), and these increases were absent in mice treated with hexamethonium (−24 pg/ml) or atropine (0 pg/ml) pretreatment (Fig. 2, *A* and *B*; Table 3). Similarly, 2-DG administration increased plasma glucagon levels (+691-797 pg/ml), and this increase was largely prevented by pretreatment with hexamethonium (+97 pg/ml) or atropine (+254 pg/ml), with the effect of hexamethonium again being more pronounced (Fig. 3, *A* and *B*; Table 3).

Experiment 4: plasma glucose, PP, and glucagon in fasted mice 45 min after insulin with or without hexamethonium. In this experiment, hexamethonium administration alone also significantly lowered plasma glucose levels (−29 mg/dl) (Table 4). Insulin injection lowered plasma glucose in both saline- (−50 mg/dl) and hexame-

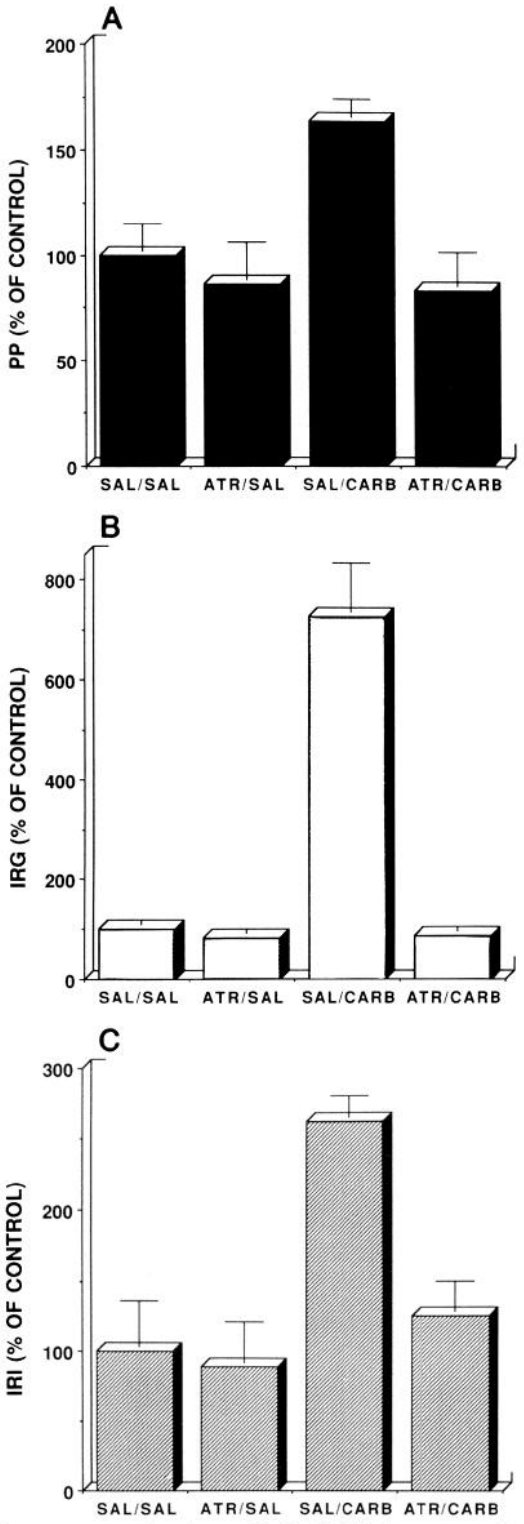

Fig. 1. Plasma pancreatic polypeptide (PP) (*A*), immunoreactive glucagon (IRG) (*B*), and immunoreactive insulin (IRI) (*C*) levels as % of mean control levels [saline/saline (sal/sal)] in fed mice 2 min after injection of saline or carbachol. Atr/sal, atropine methylnitrate (atropine)/saline; sal/carb, saline/carbachol; atr/carb, atropine/carbachol; $n = 8$ animals/group. Absolute plasma PP, IRG, and IRI concentrations are provided in Table 2.

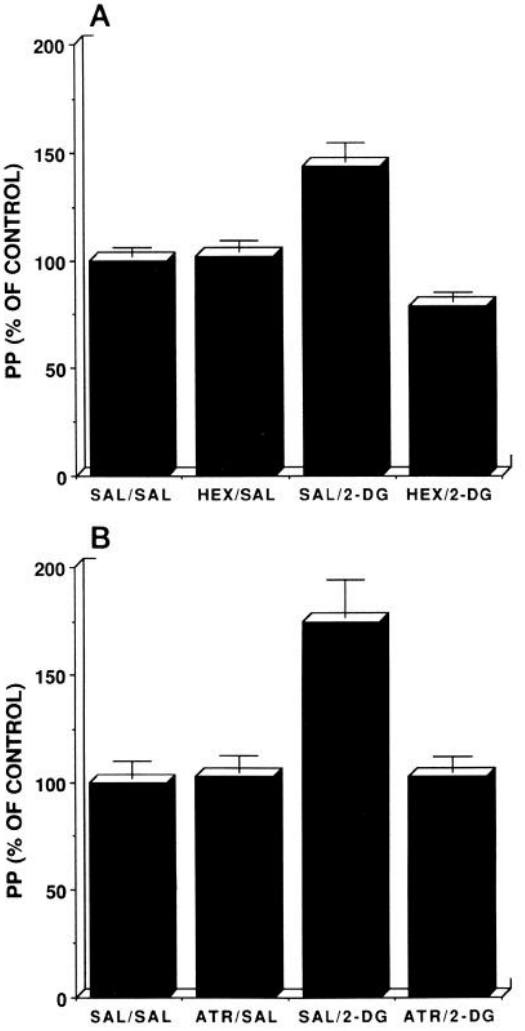

Fig. 2. Plasma PP levels as % of mean control levels (sal/sal) in fasted mice 15 min after injection of saline or 2-deoxy-D-glucose (2-DG). *A*: $n = 16$ animals/group; hex, hexamethonium. *B*: $n = 10$ animals/group. Absolute plasma PP concentrations are provided in Table 3.

thonium-treated mice (−33 mg/dl). The plasma glucose level after insulin was lower in hexamethonium-treated than in saline-treated mice (18 ± 2 vs. 30 ± 1 mg/dl) (Table 4). Plasma PP levels were increased (+89 pg/ml) during insulin-induced hypoglycemia, and the increase was prevented by hexamethonium (+1 pg/ml) (Fig. 4*A*; Table 4). Likewise, insulin-induced hypoglycemia markedly increased plasma glucagon levels (+1,497 pg/ml), and this increase was largely (90%) prevented by pretreatment with hexamethonium (+151 pg/ml) (Fig. 5; Table 4).

Experiment 5: plasma glucose, PP, and glucagon in fasted mice 45 min after insulin pretreatment with or without atropine, adrenergic blockade, or atropine + adrenergic blockade. Baseline plasma glucose levels were lower in mice after adrenergic blockade (−25 mg/dl) or adrenergic blockade + atropine (−30 mg/dl) than in saline-treated control mice. Insulin administration produced significant hypoglycemia in all groups of mice (Table 5). Plasma PP was increased by hypoglycemia in saline-pretreated mice (+51 pg/ml), and this increase was significantly reduced by atropine (+15 pg/ml) (Fig. 4*B*; Table 5) or atropine + adrenergic blockade (+15 pg/ml) (Table 5). Plasma PP was elevated in mice with lowered plasma glucose levels resulting from administration of

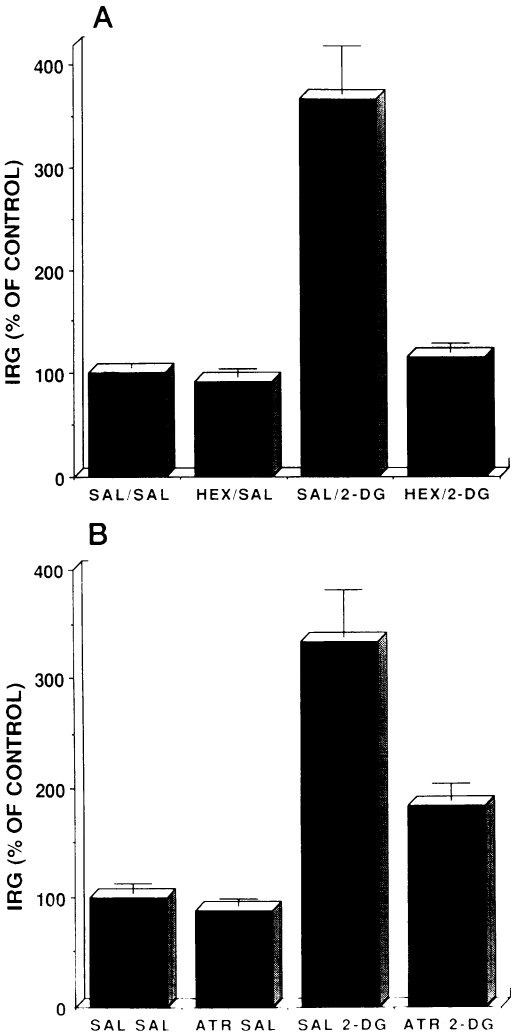

Fig. 3. Plasma IRG levels as % of mean control levels (sal/sal) in fasted mice 15 min after injection of saline or 2-DG. *A*: $n = 16$ animals/group. *B*: $n = 10$ animals/group. Absolute plasma IRG concentrations are provided in Table 3.

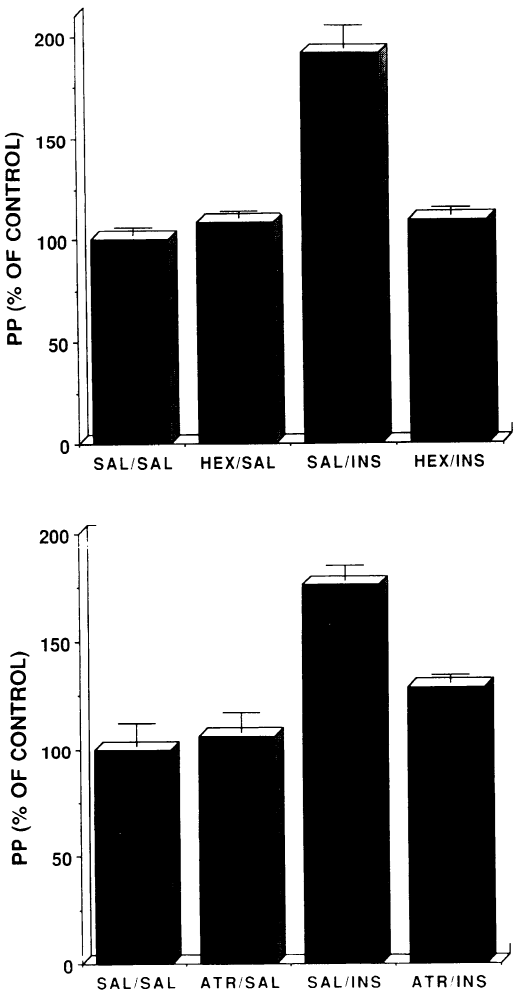

Fig. 4. Plasma PP levels as % of mean control levels (sal/sal; $n = 12$) in fasted mice 45 min after injection of saline or insulin. Hex/sal, sal/ins, atr/sal, and atr/ins groups: $n = 15$ animals/group. Hex/ins, $n = 14$. Sal/ins, $n = 16$. Absolute plasma PP concentrations are provided in Tables 4 and 5.

adrenergic antagonists (+37 pg/ml) but not in mice treated with atropine + adrenergic antagonists (+3 pg/ml) (Table 5). Plasma glucagon was markedly increased by hypoglycemia in control mice (+1,243 pg/ml). The increase in plasma glucagon during insulin-induced hypoglycemia was significantly smaller in mice pretreated with atropine (+382 pg/ml, 31 ± 7% of control), adrenergic antagonists (+601 pg/ml, 48 ± 9% of control), or atropine + adrenergic blockers together (+143 pg/ml, 12 ± 3% of control) (Fig. 6; Table 5).

DISCUSSION

The purposes of the experiments conducted in this study were twofold: *1*) to investigate the neural regulation of PP secretion in the mouse and *2*) to determine the relative contribution of autonomic neural activation vs. direct islet effects of lowered plasma glucose concentrations in mediating increased glucagon secretion during insulin-induced hypoglycemia in mice.

A new radioimmunoassay for measuring plasma PP in rodents has recently been developed and validated (7). This assay has now been employed to examine neural control of PP secretion in rats (28). As in other species, PP secretion during hypoglycemia in the rat was found to be largely mediated by parasympathetic, muscarinic neural activation. However, unlike other species, the results from this study suggested an additional minor adrenergic contribution to the PP response to hypoglycemia in this species. Investigations of neural regulation of PP release have not been previously reported in mice.

In the present study, plasma PP levels were higher in fed vs. fasted mice, suggesting that nutrient absorption (46, 52) and/or neural activation associated with feeding (43, 53) stimulate islet PP release. In addition, plasma PP levels were increased in mice that received the parasympathomimetic acetylcholine analogue carbachol, and this increase was prevented by atropine pretreatment. Carbachol also increased plasma insulin and glucagon levels by an atropine-sensitive mechanism. These results are similar to those observed in other species (21, 50) and suggest that, in the mouse, increased plasma levels of PP can reflect increased cholinergic input to the islet and are associated with the secretion of insulin and glucagon.

The increases in plasma PP during neuroglucopenia produced by 2-DG or insulin-induced hypoglycemia were

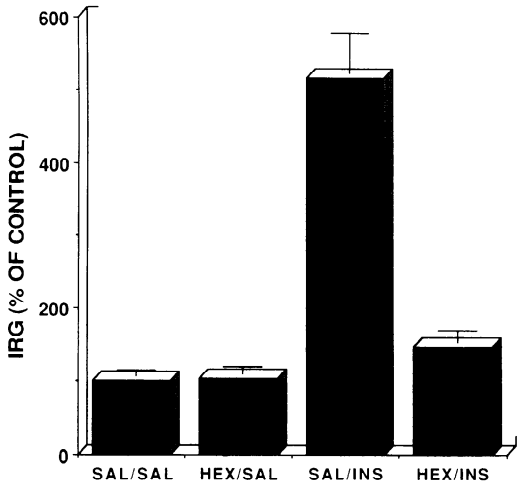

Fig. 5. Plasma IRG levels as % of mean control levels (sal/sal; $n = 12$) in mice 45 min after injection of saline or insulin. Hex/sal and sal/ins groups, $n = 15$ animals/group; hex/ins, $n = 14$. Absolute plasma IRG concentrations are provided in Table 4.

eliminated by pretreatment with the ganglionic blocker hexamethonium, suggesting that, as in other species (28), the PP response to this stimulus in mice is neurally mediated via nicotinic transmission. Furthermore, the PP responses to 2-DG or hypoglycemia were markedly reduced by atropine, suggesting major muscarinic, cholinergic mediation of the PP response to central neuroglucopenia. There was no evidence for an adrenergic contribution to the PP response to insulin-induced hypoglycemia in mice, as was suggested by the results of a previous study in rats (28); no further decrease in plasma PP levels was observed in mice treated with both atropine and adrenergic blockers vs. mice treated with atropine alone. These results suggest that under carefully controlled conditions plasma PP levels may be useful as an index of parasympathetic, cholinergic input to the pancreatic islets in mice.

The second major goal of these studies was to investigate the role of the autonomic nervous system in mediating increased glucagon secretion during insulin-induced hypoglycemia. Central neuroglucopenia produced by hypoglycemia can activate three different autonomic inputs to the pancreas. These inputs include parasympathetic neural activation (28, 47), adrenal medullary catecholamine release (12, 18, 22), and activation of the direct sympathetic innervation of the pancreas (23, 29), all of which could potentially stimulate glucagon secretion (4, 6, 19). Results from recent studies conducted in dogs (9, 26) and other experiments in rats (27, 42) and calves (10) suggest that activation of these autonomic inputs can make a major contribution to glucagon responses to hypoglycemia in these species.

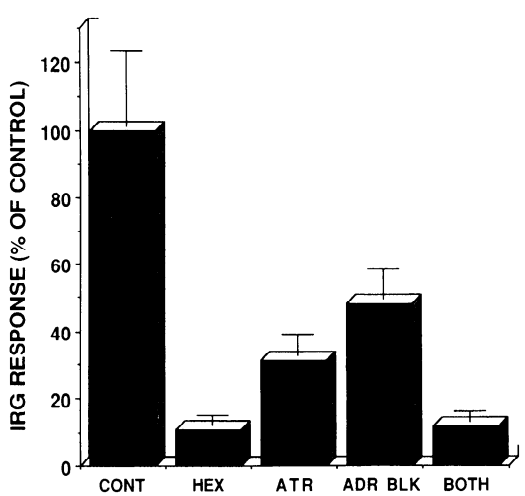

Fig. 6. Plasma IRG responses to insulin-induced hypoglycemia as % of mean control responses (sal/ins − sal/sal) in mice pretreated with saline [control (cont); $n = 16$]; hex, $n = 14$; atr, $n = 15$; phentolamine + propranolol [adrenergic blockade (adr blk); $n = 15$], or atropine and adrenergic blockade together (both; $n = 16$). Absolute plasma IRG concentrations are provided in Table 5.

In contrast, a number of other studies conducted primarily in human subjects, in which portions of the autonomic response to hypoglycemia were blocked or ablated, have not found a significant autonomic component to the glucagon response (17, 39, 40, 44, 54). Furthermore, results from in vitro studies suggest that low glucose levels can stimulate glucagon secretion in the absence of neural activation (38, 56). Together, these results have led to the view that the low plasma glucose level, rather than autonomic neural activation, is the major determinant of glucagon responses to hypoglycemia in vivo. In a recent review article (24), it was hypothesized that the glucagon response to hypoglycemia may be redundantly mediated by parasympathetic and sympathoadrenal activation and therefore under certain conditions, i.e., partial blockade or ablation, the autonomic contribution to this counterregulatory response could be obscured by the redundantly functioning unblocked or unablated autonomic neural input to the pancreas. Thus it would be necessary to ablate or block all of the autonomic inputs to the pancreas to reveal the full contribution of the autonomic nervous system to hypoglycemia-induced glucagon release. In accordance with this hypothesis, one recent study in human subjects demonstrated a significant reduction in the glucagon response to insulin-induced hypoglycemia after the administration of a ganglionic blocking agent (14), which would be expected to impair the activation of all autonomic input to the pancreatic A-cell (24).

To our knowledge, the autonomic contribution to hypoglycemia-induced glucagon secretion has not been previously investigated in the mouse. Therefore, in the present study, we examined the contribution of autonomic activation to the glucagon response by examining the effect of the ganglionic blocker, hexamethonium, on glucagon responses to insulin-induced hypoglycemia in conscious mice. Hexamethonium impairs the activation of all three autonomic inputs to the pancreas because all require nicotinic neurotransmission across autonomic ganglia (51).

To determine the relative contributions of cholinergic, muscarinic, and adrenergic mechanisms and their potential for redundancy in mediating hypoglycemia-induced glucagon responses, atropine, α- and β-adrenergic antagonists, or atropine and adrenergic antagonists together were administered to mice before the induction of hypoglycemia. As expected, insulin-induced hypoglycemia resulted in a large (3.5- to 5-fold) increase in immunoreactive plasma glucagon levels in saline-treated mice.

Hexamethonium pretreatment reduced the increase in plasma glucagon during hypoglycemia by ~90%, revealing a major neural contribution to this response. Pretreatment with atropine reduced the glucagon response by ~70%. In both hexamethonium- and atropine-pretreated animals, the PP response to hypoglycemia was also markedly reduced by atropine, suggesting that a significant level of nicotinic or muscarinic blockade was present. Pretreatment with combined α- and β-adrenergic blockers reduced the glucagon response by ~50%.

Atropine and combined adrenergic blockade together reduced the glucagon response by ~90%. Thus there is ~20–30% overlap, i.e., potential for redundancy, between cholinergic and adrenergic mechanisms in the autonomic mediation of the glucagon response to this degree of hypoglycemia in the mouse.

The 10% of the glucagon response that was not eliminated by hexamethonium or atropine and combined adrenergic blockade together could be due to either inadequate levels of the autonomic antagonists necessary to produce a complete blockade or to a direct effect of low plasma glucose levels on the islet. In addition, peptidergic neurotransmitters such as vasoactive intestinal polypeptide and galanin have been demonstrated to stimulate glucagon secretion (15, 16, 37) and to be released during electrical stimulation of autonomic nerves (15, 16, 32) or insulin-induced hypoglycemia (29). The effects of these neuropeptides on glucagon release would be unlikely to be blocked by classical autonomic antagonists. However, there appears to be little potential role for these peptides in mediating the glucagon response to hypoglycemia in mice, because 90% of the response was eliminated by the pretreatment with classical cholinergic and adrenergic antagonists.

The plasma glucose responses to 2-DG provide some insight into the mechanism of 2-DG-induced hyperglycemia. For example, hexamethonium abolished the glucagon response to intraperitoneal 2-DG administration in fasted mice and reduced the measured increase of plasma glucose by >60%. Atropine was slightly less effective in inhibiting the glucagon response to 2-DG but inhibited 2-DG-induced hyperglycemia by 50%. These results with intraperitoneal administration of 2-DG in fasted mice confirm those of previous studies, which demonstrated that hexamethonium or atropine inhibits the glucagon and hyperglycemic response to intravenous 2-DG in fed mice (33, 34), and further suggest that a significant portion of 2-DG-induced hyperglycemia is the result of vagally mediated increases in glucagon secretion.

The plasma glucose levels in mice pretreated with autonomic blockers alone reveal potential for an autonomic contribution to glucose homeostasis during prolonged fasting in mice. In the two experiments in which hexamethonium was employed, hexamethonium injection by itself produced significant hypoglycemia in fasted mice, suggesting that plasma glucose may be supported by autonomic outflow in long-term fasted mice. Hexamethonium treatment alone was not associated with significant decreases in baseline plasma glucagon levels. In addition, combined adrenergic blockade or adrenergic blockade and atropine together both produced similar hypoglycemia. In fact, the degree of hypoglycemia produced by combined adrenergic blockade was sufficient to induce a modest, yet significant, rise in baseline plasma PP, which was prevented by the addition of atropine. These data suggest that sympathoadrenal outflow may support plasma glucose in fasting mice via adrenergic receptor activation and are consistent with the known effects of catecholamines to stimulate hepatic gluconeogenesis (13) and increase hepatic glucose output (49). However, it should be noted that a 24-h fast in mice is likely to be equivalent to a much longer period of food deprivation in larger animals. Thus these results do not provide evidence for autonomic support of plasma glucose during fasting of less prolonged duration, and this interpretation should not be extended to other species without additional experiments.

In summary, we have demonstrated that plasma PP levels are increased by a cholinergic, muscarinic mechanism in the mouse. Therefore, under controlled conditions, plasma PP levels may be useful as an index of parasympathetic input to the pancreas in this species. We have also found that the large majority of the plasma glucagon response to insulin-induced hypoglycemia in mice is the consequence of autonomic neural activation, rather than a direct effect of low plasma glucose at the level of the islet, and that this response is mediated partly by parasympathetic, muscarinic receptor activation and partly by sympathoadrenal, adrenergic receptor activation.

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