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Effects of Galanin on Hormone Secretion from the *in Situ* Perfused Rat Pancreas and on Glucose Production in Rat Hepatocytes *in Vitro**

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ABSTRACT. Galanin is a novel peptide, widely distributed throughout the central and peripheral nervous system, including nerve endings surrounding the pancreatic islets. In dogs, galanin infusion has been reported to induce hyperglycemia along with a reduction of circulating insulin. In this work, we have studied the effect of galanin (a 200 ng bolus followed by constant infusion at a concentration of 16.8 ng/ml for 22-24 min) on insulin, glucagon, and somatostatin secretion in the perfused rat pancreas. In addition, we have investigated the effect of galanin (10 and 100 nM) on glycogenolysis and gluconeogenesis in isolated rat hepatocytes.

In the rat pancreas, galanin infusion marked inhibited unstimulated insulin release as well as the insulin responses to glucose (11 mM), tolbutamide (100 mg/liter) and arginine (5 mM). Galanin failed to alter the glucagon and somatostatin responses to glucose, tolbutamide, and arginine.

In isolated rat hepatocytes, galanin did not influence glycogenolysis or glucagon phosphorylase *a* activity. Gluconeogenesis and the hepatocyte concentration of fructose 2,6-bisphosphate were also unaffected by galanin.

In conclusion: 1) in the perfused rat pancreas, galanin inhibited insulin secretion without modifying glucagon and somatostatin output, thus pointing to a direct effect of galanin on the B cell; and 2) in rat hepatocytes, galanin did not affect glycogenolysis or gluconeogenesis; hence, the reported hyperglycemia induced by exogenous galanin does not seem to be accounted for by a direct effect of this peptide on hepatic glucose production. (*Endocrinology* **121:** 378–383, 1987)

GALANIN is a 29 amino acid straight chain peptide, recently isolated and characterized by Tatemoto *et al.* (1) from porcine intestinal extracts. Galanin immunoreactivity has since been shown to be widely distributed throughout the central and peripheral nervous system, both in cell bodies and nerve fibers, and is now considered to be a neuropeptide (1–5). Although galanin presents partial structural similarities to physalaemin, substance P, and gonadoliberin, as well as to propiomelanocortin, its unique amino acid sequence suggests it belongs to a new family of peptides (1).

As for the biological activity of galanin, the original study by Tatemoto and associates (1) showed that besides contracting smooth muscle preparations of the rat, when infused iv in conscious dogs, this peptide induced a mild, sustained elevation of fasting glycemia. Subsequent work by McDonald *et al.* (6) confirmed the hyperglycemic effect of exogenous galanin in the dog, both in the fasted state and during parenteral glucose administration; this was accompanied by a reduction of plasma insulin levels.

To further explore the influence of galanin on pancreatic hormone secretion, we have investigated the effect of this peptide on insulin, glucagon and somatostatin release in the perfused rat pancreas. In view of the hyperglycemic effect of galanin, we have also studied its possible influence on glucose production, glycogenolysis and gluconeogenesis, in isolated rat hepatocytes.

Materials and Methods

Perfused rat pancreas system and hormone determination

Fed male Wistar rats (200–250 g BW) from our inbred colony were used as donors. After anesthesia of the rat with pentobarbital sodium (50 mg/kg, ip), the pancreas was dissected and perfused *in situ* according to the procedure of Leclercq-Meyer *et al.* (7); all adjacent organs, including duodenum, were excluded. The pancreas was perfused through the celiac and

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superior mesenteric arteries, via a cannula inserted into the aorta. Effluent samples were collected from the portal vein, without recycling, at 2-min intervals (flow rate, 2 ml/min) in tubes containing 2000 kallikrein inhibiting units Trasylol (Bayer AG, Leverkusen, West Germany), and frozen at −20 C until the time of assay.

The perfusion medium consisted of a Krebs-Ringer bicarbonate buffer (gas phase 95:5, 0_2-CO_2; pH 7.4) supplemented with 4% (wt/vol) dextran T-70, 0.5% (wt/vol) bovine albumin (Cohn fraction V), and glucose (5.5 mM). After an equilibration period of 35 min, baseline samples were collected for 12 min. At zero time, synthetic porcine galanin (Peninsula Laboratories, Inc., Belmont, CA) were infused through a sidearm cannula as a priming dose (200 ng in 15 sec), followed by constant infusion at a rate of 33.6 ng/min (16.8 ng/ml) for 22 or 24 min. As secretagogues of the endocrine pancreas, glucose, L-arginine hydrochloride (Sigma Chemical Co., St. Louis, MO) and tolbutamide (Boehringer Mannheim GmbH, Mannheim, West Germany) were employed. Addition of these substances to the perfusate was performed as described in the corresponding figures. In control experiments, an identical volume of perfusion medium was infused.

RIA was employed to measure insulin (8), glucagon (9), and somatostatin (10). Antiglucagon serum (30 K) and antisomatostatin serum (80 C) were kindly donated by Dr. R. H. Unger (University of Texas Health Science Center at Dallas, TX). Samples for each given series of experiments were analyzed in the same run.

Incubation of rat hepatocytes and measurement of glycogenolysis and gluconeogenesis

Hepatocytes were isolated from fed male Wistar rats (200–250 g BW) by perfusion of the liver with collagenase (Boehringer Mannheim GmbH) (11), and subsequently incubated in Krebs-Ringer bicarbonate buffer as described elsewhere (12). Five minutes after galanin addition (10 and 100 nM), aliquots of cell suspension (0.1 ml) were taken and immediately frozen in liquid N_2 until the time of glycogen phosphorylase *a* activity and fructose 2,6-bisphosphate assays. In control incubations, a suboptimal concentration of glucagon (0.1 nM) (Novo Industri A/S, Copenhagen, Denmark) was similarly tested.

Glycogenolysis was estimated by the rate of net glucose production (13). In these experiments, neither glucose nor gluconeogenic precursor were added to the incubation medium. Gluconeogenesis was measured by the rate of radioactive glucose formation (12) using 4 mM L-(U-^{14}C)lactate (0.5 μCi/ml) (Amersham International, Amersham, UK) as gluconeogenic precursor. Glycogen phosphorylase *a* activity was measured at 37 C, as described by Hue *et al.* (11). Fructose 2,6-bisphosphate was assayed by its ability to activate the enzyme pyrophosphate fructose 6-phosphate 1-phosphotransferase from potato tuber (Sigma Chemical Co.), as previously described (14). All the above measurements are expressed per gram (wet weight) of hepatocytes; 1 g packed hepatocytes corresponds to 220 ± 5 mg protein (n = 12). Protein was measured by the method of Bradford (Bio-Rad Protein Assay, Bio-Rad Laboratories, München, West Germany) using BSA as standard (15).

Expression of results

Results are presented as the mean ± SEM. Hormone response, from zero min until the end of infusion, was calculated as the integrated area of the curve above or below the mean preinfusion level (average of all baseline levels), using the trapezoidal method. Differences between values were tested for significance by Student's paired or unpaired *t* test.

Results

Effect of galanin on unstimulated insulin release by the perfused rat pancreas (Fig. 1)

Baseline values from control and galanin experiments overlapped. As expected, in control perfusions, insulin output did not vary significantly throughout the experimental period. Incorporation of galanin into the perfusate was followed by a depression of insulin release (preinfusion value: 1 ± 0.1 ng/4 ml·2 min; 4-min value: 0.45 ± 0.1 ng/4 ml·2 min, $P < 0.01$) which remained statistically significant until the 20-min point. Furthermore, during galanin infusion, insulin secretion, as calculated by the integrated area under the response curve, was reduced as compared to control perfusions (13.1 ± 1.9 ng/4 ml·24 min *vs.* 20.9 ± 0.9 ng/4 ml·24 min, $P < 0.01$).

Effect of galanin on the insulin, glucagon, and somatostatin responses to glucose (11 mM) by the perfused rat pancreas (Fig. 2)

In these experiments, galanin markedly blocked the insulin secretion evoked by glucose (incremental response: 82.8 ± 32.6 ng/4 ml·22 min *vs.* 325 ± 61.4 ng/4 ml·22 min in control experiments; $P < 0.01$). Galanin did not significantly affect the suppressor effect of glucose on glucagon secretion. Glucose, at the concentration employed in these perfusions, did not stimulate somatostatin release; when galanin was simultaneously infused, no change in the secretion of somatostatin was observed.

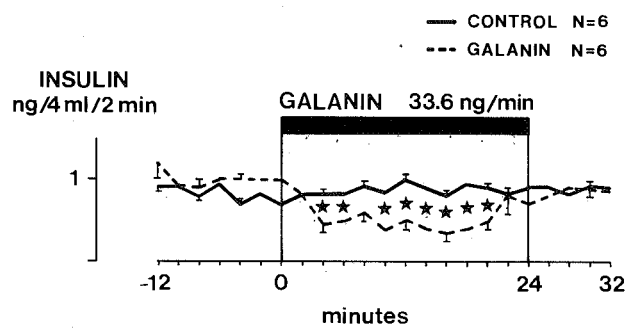

FIG. 1. Effect of galanin (a priming dose of 200 ng, followed by constant infusion at a rate of 33.6 ng/min) on unstimulated insulin release by the perfused rat pancreas. *Solid and broken lines* correspond to control and galanin experiments, respectively (means ± SEM). ★, Statistically significant differences ($P < 0.05$) between galanin and control perfusions at a given time.

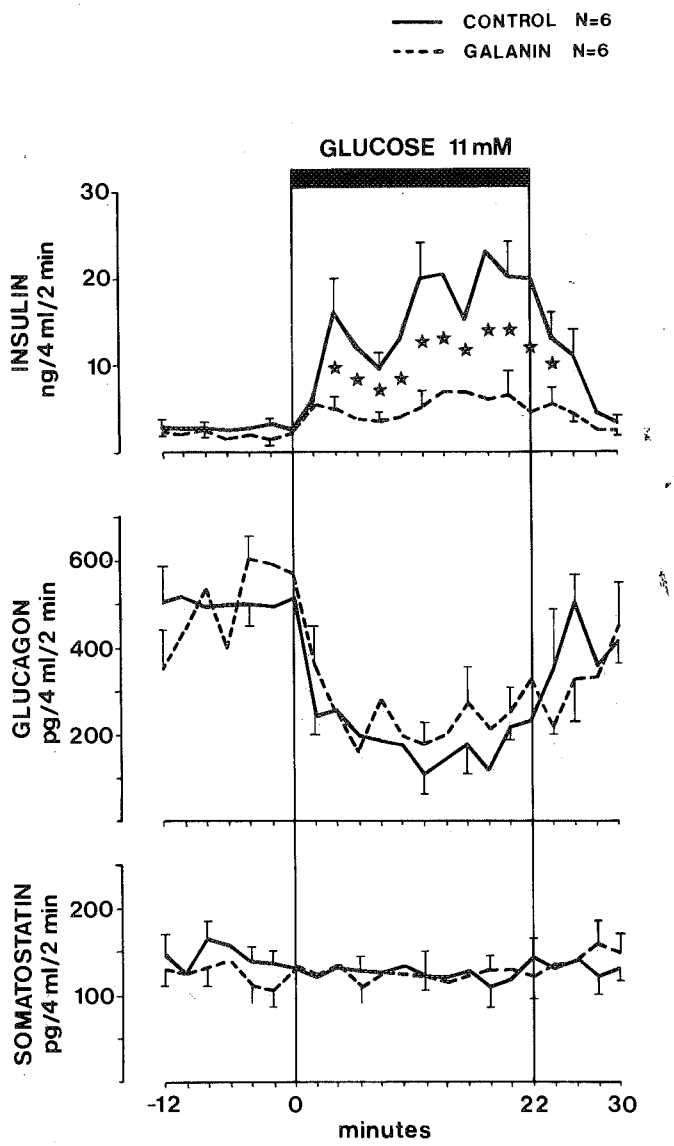

FIG. 2. Effect of galanin (a priming dose of 200 ng, followed by constant infusion at a rate of 33.6 ng/min from 0 to 22 min) on the insulin, glucagon, and somatostatin responses to glucose (11 mM) by the perfused rat pancreas. *Solid lines* represent glucose experiments. *Broken lines* represent glucose plus galanin experiments (means ± SEM). ★, Statistically significant differences ($P < 0.05$) between galanin and control perfusions at a given time.

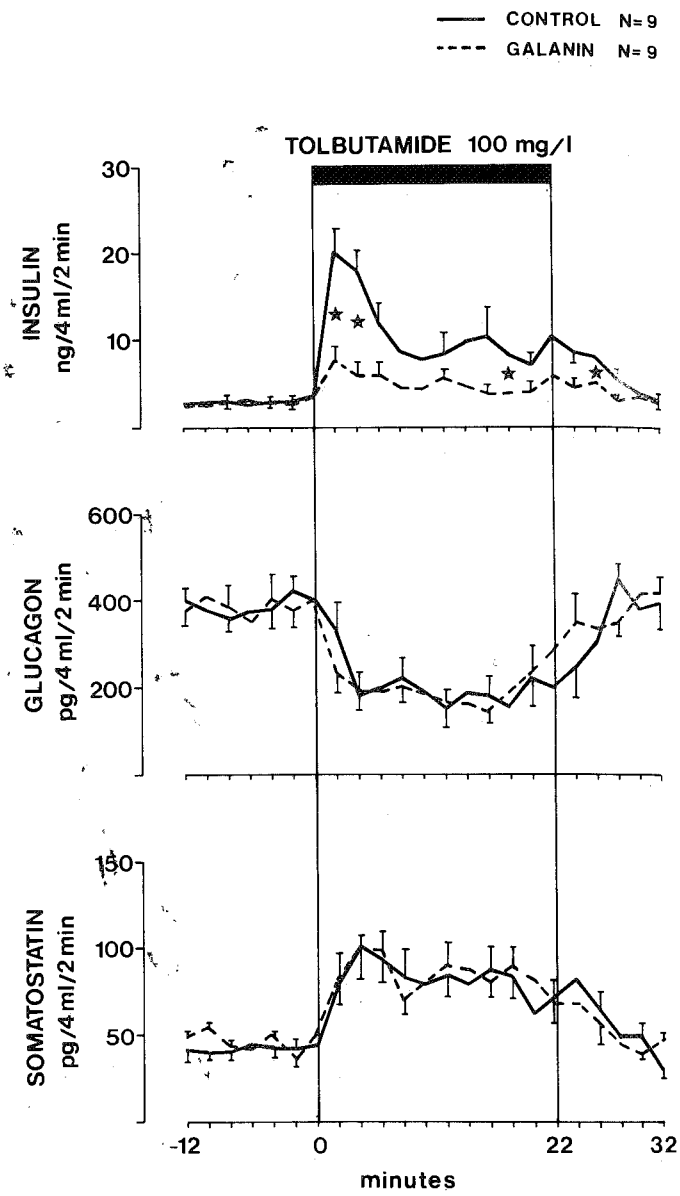

FIG. 3. Effect of galanin (a priming dose of 200 ng, followed by constant infusion at a rate of 33.6 ng/min from 0 to 22 min) on the insulin, glucagon, and somatostatin responses to tolbutamide (100 mg/liter) by the perfused rat pancreas. *Solid lines* represent tolbutamide experiments. *Broken lines* represent tolbutamide plus galanin experiments (means ± SEM). ★, Statistically significant differences ($P < 0.05$) between galanin and control perfusions at a given time.

Effect of galanin on the insulin, glucagon, and somatostatin responses to tolbutamide (100 mg/liter) by the perfused rat pancreas (Fig. 3)

The release of insulin induced by tolbutamide (incremental response: 164 ± 41 ng/4 ml·22 min) was intensely blocked when galanin was added to the perfusate (incremental response: 51 ± 17 ng/4 ml·22 min, $P < 0.025$). The reduction of glucagon output observed during tolbutamide infusion (decremental response: 3991 ± 473 pg/4 ml·22 min) was not significantly affected by galanin (decremental response: 4036 ± 540 pg/4 ml·22 min). Nor was tolbutamide-induced somatostatin release (incremental response: 877 ± 148 pg/4 ml·22 min) significantly modified by galanin (incremental response: 795 ± 195 pg/4 ml·22 min).

Effect of galanin on the insulin, glucagon, and somatostatin responses to two different concentrations of arginine, 10 mM (Fig. 4) and 5 mM (Fig. 5)

As shown in Fig. 4, galanin failed to suppress the insulin secretion elicited by 10 mM arginine (incremental

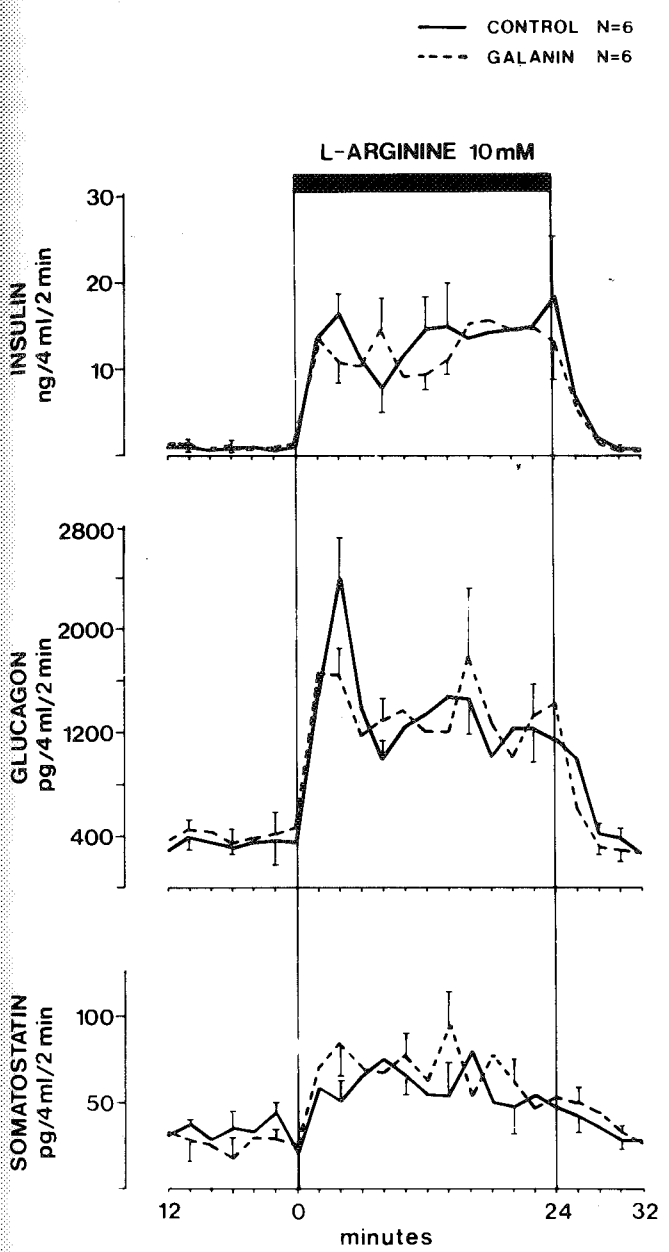

FIG. 4. Effect of galanin (a priming dose of 200 ng, followed by constant infusion at a rate of 33.6 ng/min from 0 to 24 min) on the insulin, glucagon, and somatostatin responses to arginine (10 mM) by the perfused rat pancreas. *Solid lines* represent arginine experiments. *Broken lines* represent arginine plus galanin experiments (mean ± SEM). There were no statistically significant differences between galanin and control perfusions at any given time.

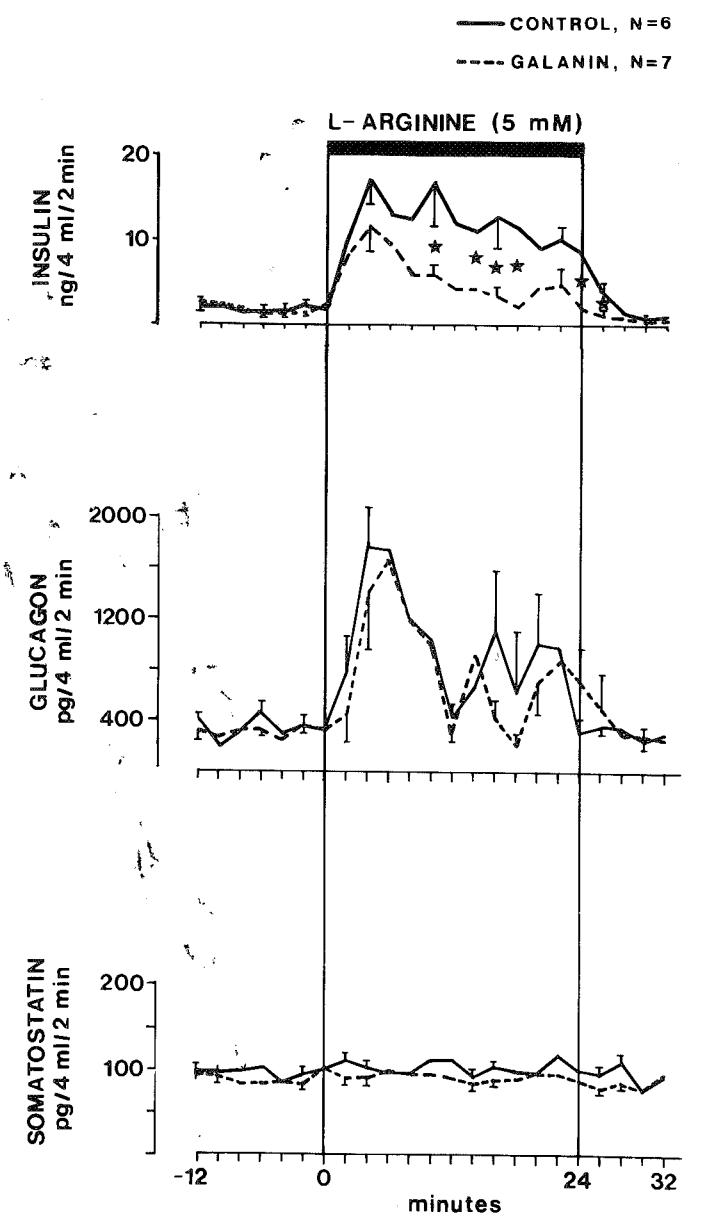

FIG. 5. Effect of galanin (a priming dose of 200 ng, followed by constant infusion at a rate of 33.6 ng/min from 0 to 24 min) on the insulin, glucagon, and somatostatin responses to arginine (5 mM) by the perfused rat pancreas. *Solid lines* represent arginine experiments. *Broken lines* represent arginine plus galanin experiments (means ± SEM). ★, Statistically significant differences ($P < 0.05$) between galanin and control perfusions at a given time.

response: 271 ± 38 ng/4 ml·24 min *vs.* 251 ± 74 ng/4 ml·24 min in control perfusions). The glucagon responses to arginine with or without galanin were comparable (24,111 ± 4,833 and 22,016 ± 6,745 pg/4 ml·24 min, respectively). Galanin failed as well to significantly affect arginine-induced somatostatin release (incremental response: 941 ± 137 pg/4 ml·24 min *vs.* 572 ± 219 pg/4 ml·24 min in control experiments; $P = 0.21$).

In view of the lack of effect of galanin on the insulin secretion evoked by 10 mM arginine, we tested the effect of galanin on the insulin response to a lower concentration of arginine, 5 mM.

As shown in Fig. 5, in these experiments, galanin significantly blocked arginine-induced insulin output (incremental response: 89 ± 19 ng/4 ml·24 min *vs.* 238 ± 53 ng/4 ml·24 min in control experiments; $P < 0.025$). The glucagon secretion elicited by 5 mM arginine (incre-

mental response: 15,249 ± 4,148 pg/4 ml·24 min) was not significantly modified by galanin (incremental response: 12,075 ± 2,443 pg/4 ml·24 min; $P = 0.5$). Finally, arginine, at 5 mM, did not stimulate somatostatin release; when galanin was simultaneously infused, no change in the secretion of somatostatin was observed.

Effect of galanin on glycogenolysis and gluconeogenesis in isolated rat hepatocytes (Table 1)

Galanin (10 and 100 nM) did not significantly modify glycogenolysis or gluconeogenesis in isolated rat hepatocytes. Glycogen phosphorylase *a* activity and the concentration of fructose 2,6-bisphosphate in these cells were also unaffected by the addition of galanin to the incubation medium. In parallel control incubations, glucagon (0.1 nM) showed its well known stimulatory effect on glycogenolysis, gluconeogenesis, and glycogen phosphorylase *a* activity, and reduced the hepatocyte concentration of fructose 2,6-bisphosphate.

Discussion

The foregoing results show that in the perfused rat pancreas, galanin infusion reversibly inhibits unstimulated insulin release as well as the insulin response to glucose. These findings are in agreement with those of McDonald *et al.* (6) who first observed, in the conscious dog, the suppressor effect of galanin on both fasting and glucose-stimulated plasma insulin levels. Our study also demonstrates that galanin inhibits insulin secretion as induced by tolbutamide and by arginine.

As of this writing, two animal studies have been published confirming the inhibitory effect of galanin on insulin secretion. Manabe *et al.* (16) have reported that, in the unanesthetized dog, iv galanin infusion reduces fasting insulin concentrations in peripheral plasma. Dunning *et al.* (17) have described that in the pentobarbital-anesthetized dog, iv administration of galanin reduces insulin output without modifying arterial and pancreatic venous catecholamine levels; moreover, they observed that injection of a peripherally ineffective dose of galanin into the pancreatic artery also blocks insulin secretion.

In our preparation of rat pancreas, galanin did not modify the glucagon responses to either stimulatory (arginine) or inhibitory (glucose and tolbutamide) agents. Accordingly, McDonald *et al.* (6) and Manabe *et al.* (16) found no significant changes in the plasma glucagon levels of dogs subjected to galanin infusion. Conversely, Dunning *et al.* (17) observed that exogenous galanin stimulates pancreatic glucagon output, this effect not being reflected in arterial plasma.

Galanin also failed to significantly alter the somatostatin responses to either tolbutamide or arginine in the perfused rat pancreas. Similarly, it did not modify the release of somatostatin observed during glucose (11 mM) infusion. It should be commented that in our pancreas model, the secretagogue effect of glucose on somatostatin secretion becomes manifest at a higher glucose concentration (16.6 mM) than that employed in the present study (18). Again, these findings are in contrast with the blocking effect of galanin on somatostatin output found by Dunning *et al.* (17) in their preparation of dog pancreas. The reason for the discrepancies between our data and those of Dunning *et al.* (17) is not clear and might represent a species difference.

In summary, our observations show that, in the rat pancreas, galanin inhibits insulin secretion without modifying glucagon and somatostatin release, thus suggesting a direct effect of galanin on the B cell. The presence of galanin-like immunoreactivity in nerve endings surrounding the islets of dog pancreas (17) favors the concept of galanin as a regulatory peptide implicated in the control of insulin release.

Finally, our studies in isolated rat hepatocytes show that galanin does not affect glycogenolysis or glycogen phosphorylase *a* activity. Gluconeogenesis and the hepatocyte concentration of fructose 2,6-bisphosphate, a

TABLE 1. Effect of galanin on glycogenolysis, gluoneogenesis, glycogen phosphorylase activity, and fructose 2,6-bisphosphate concentration in hepatocytes isolated from fed rats

Additions	Glyogenolysis (μmol glucose/g cells × 20 min) (n = 6)	L-(U-^{14}C)Lactate converted to glucose (μmol/g cells × 20 min) (n = 9)	Glycogen phosphorylase *a* (U/g cells) (n = 9)	Fructose 2,6-bisphosphate (nmol/g cells) (n = 6)
Saline	21.9 ± 1.24	2.47 ± 0.20	4.62 ± 0.40	12.2 ± 0.72
Galanin (10 nM)	18.7 ± 1.62 (NS)	2.53 ± 0.17 (NS)	5.15 ± 0.33 (NS)	12.2 ± 0.60 (NS)
Galanin (100 nM)	20.8 ± 1.15 (NS)	2.52 ± 0.24 (NS)	4.50 ± 0.41 (NS)	12.3 ± 0.76 (NS)
Glucagon (0.1 nM)	44.6 ± 4.1[a]	12.60 ± 0.40[a]	12.22 ± 0.71[a]	2.74 ± 0.12[a]

Hepatocytes were incubated for 30 min in Krebs-Henseleit medium with 10 mM glucose. Galanin, glucagon, or saline, and the gluconeogenic precursor were then added to the incubation medium; 5 min later, aliquots of the cell suspension were taken for the assays of glycogen phosphorylase activity and fructose 2,6-bisphosphate. To measure glycogenolysis, hepatocytes were incubated for 20 min without adding glucose to the medium. Values are means ± SEM of six or nine incubations.

[a] P values *vs.* saline incubation of less than 0.01; NS, nonsignificant.

regulatory metabolite that plays an important role in the control of this process (19), were also unaffected by galanin, Thus, the reported hyperglycemia induced by exogenous galanin does not seem to be accounted for by a direct effect of galanin on hepatic glucose production.

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