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Interrelationship among insulin, glucagon and somatostatin secretory responses to exendin-4 in the perfused rat pancreas

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Abstract

We have investigated the effect of exendin-4 on insulin, glucagon and somatostatin output in the perfused rat pancreas. At 9 mM glucose, exendin-4 potentiated the insulin and somatostatin responses to arginine and reduced the glucagon response to this amino acid. Thus, this reduction might be thought to be paracrine-mediated through the concomitant increase in insulin and somatostatin concentrations. At 3.2 mM glucose, exendin-4 did not affect insulin secretion, reduced glucagon release and stimulated somatostatin output. Furthermore, exendin-4 reduced glucagon secretion as induced by a glucose decline (from 11 to 3.2 mM) without affecting insulin or somatostatin responses. In summary, exendin-4 stimulated insulin and somatostatin secretion and reduced glucagon release. The glucagonostatic effect of exendin-4 was observed under conditions in which insulin and somatostatin were not affected, thus indicating that exendin-4, per se, inhibits A-cell secretion. Indeed, an additional glucagonostatic effect of exendin-4, mediated by its stimulation of insulin and/or somatostatin secretion, cannot be ruled out.

Keywords: Exendin-4; Insulin; Glucagon; Somatostatin

1. Introduction

Exendin-4 is a molecule isolated from the salivary secretions of the lizard *Heloderma suspectum* whose sequence is ~ 50% identical to that of the intestinal insulinotropic peptide GLP-1 (Eng et al., 1992). Interestingly, exendin-4 and GLP-1 represent related yet distinct peptides encoded by different genes in the lizard (Chen and Drucker, 1997). Exendin-4, interacts with the GLP-1 receptor on human pancreatic islets (Thorens et al., 1993) and on rat insulinoma-derived cells (Göke et al., 1993). These analogies have led to the investigation of the influence of exendin-4 on insulin secretion and its potential antidiabetic effect. The insulin secretory activity of exendin-4, first demonstrated by Göke et al. (1993), has been amply documented (Chepurny et al., 2002; Creutzfeldt, 2001; Egan et al., 2002; Greig et al., 1999; Parkes et al., 2001a; Rodríguez-Gallardo et al., 2000; Tourrel et al., 2002). Preliminary studies have shown that exendin-4 inhibits glucagon secretion both in vivo (Baron et al., 2000; Gedulin et al., 1999; Kolterman et al., 2000; Parkes et al., 2001b) and in vitro (Rodríguez-Gallardo et al., 2002). There is no information, however, on the effect of this peptide on pancreatic somatostatin secretion, although a stimulatory effect of exendin-4 on gastric somatostatin secretion has been described (Eissele et al., 1994).

To gain further insight into the influence of exendin-4 on islet cell secretion, in this work, we have investigated the interrelationship among somatostatin, insulin and glucagon output in the perfused rat pancreas.

2. Materials and methods

Male Wistar rats (200–225 g body weight) from our inbred colony, fed ad libitum, were used as donors. Animals were maintained in accordance with the guidelines established by the European Union (86/609). After anesthesia of the rat with pentobarbital sodium (50 mg/kg, i.p.), the pancreas was dissected and perfused in situ according to the procedure of Leclercq-Meyer et al. (1976) as adapted in our laboratory (Silvestre et al., 1986). Effluent samples were collected from the portal vein, without recycling, at 1-min

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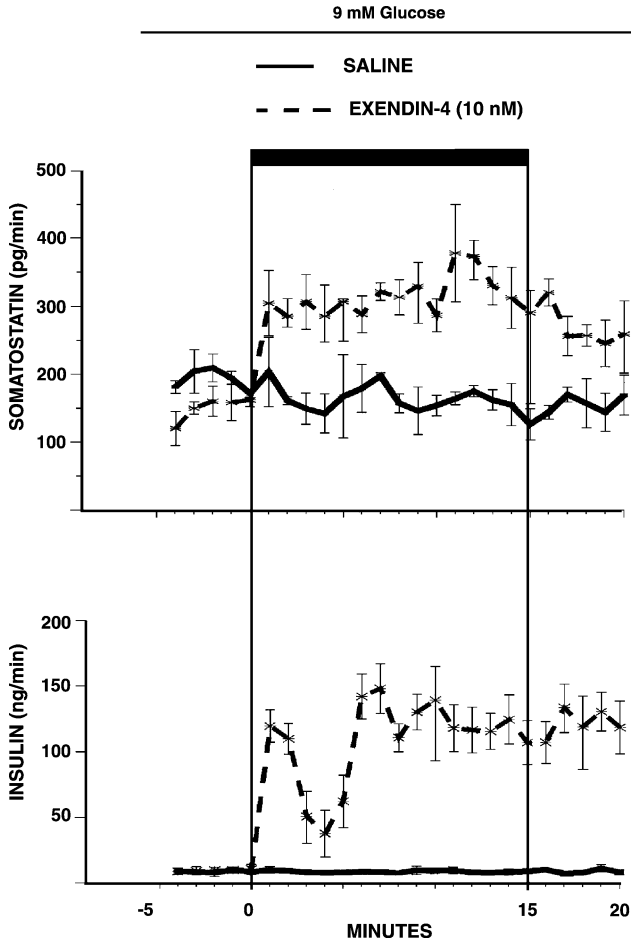

Fig. 1. Effect of 10 nM exendin-4 on somatostatin and insulin secretion in the rat pancreas perfused at constant glucose concentration (9 mM). Solid lines correspond to control perfusions: from 0- to 15-min saline infusion ($N=4$). Broken lines correspond to exendin-4 perfusions: from 0- to 15-min exendin-4 infusion ($N=4$). Means ± S.E.M.

intervals (flow rate, 2 ml/min) and frozen at −20 °C until the time of assay. The perfusion medium consisted of a Krebs–Henseleit buffer: 115 mM NaCl, 4.7 mM KCl, 2.6 mM $CaCl_2$, 1.19 mM H_2KPO_4, 1.19 mM $MgSO_4 \cdot 7H_2O$ and 24.9 mM $HNaCO_3$ (gas phase 95:5, O_2/CO_2; pH 7.4), supplemented with 4% (w/v) dextran T-70 (Pharmacia LKB Biotechnology, Uppsala, Sweden), 0.5% (w/v) Cohn Fraction V bovine albumin (Sigma) and glucose (3.2, 9 or 11 mM; Sigma). Exendin-4 (kindly donated by Amylin Pharmaceuticals, San Diego, CA, USA) was dissolved in 0.9% NaCl, containing 0.1% bovine albumin (Cohn Fraction V). This solution was prepared daily, immediately before experiments. When added to the perfusate, the final exendin-4 concentration was 1 or 10 nM. After a 35-min equilibration period, baseline samples were collected for 5 min and, at zero time, saline with or without exendin-4 was infused through a sidearm cannula. Glucose and L-arginine hydrochloride (Sigma) were used as hormone secretagogues. Insulin, glucagon and somatostatin were analyzed by radioimmunoassay (Faloona and Unger, 1974; Harris et al., 1978; Herbert et al., 1965; Yalow and Bergson, 1960). Anti-pig insulin serum (I8510, Sigma) and rat insulin standards (Novo, Nordisk, Denmark) were employed. Anti-glucagon serum (O4A) and anti-somatostatin serum (80C) were kindly donated by R.H. Unger (University of Texas, Health Sciences Center, Dallas, TX, USA). All samples for each series of experiments were analyzed within the same assay. Results are expressed as the means ± S.E.M. Hormone response was calculated as the integrated area of the curve above or below the mean preinfusion level (average of all the baseline levels for each perfusion) using the trapezoidal method. The normal distribution of our data was demon-

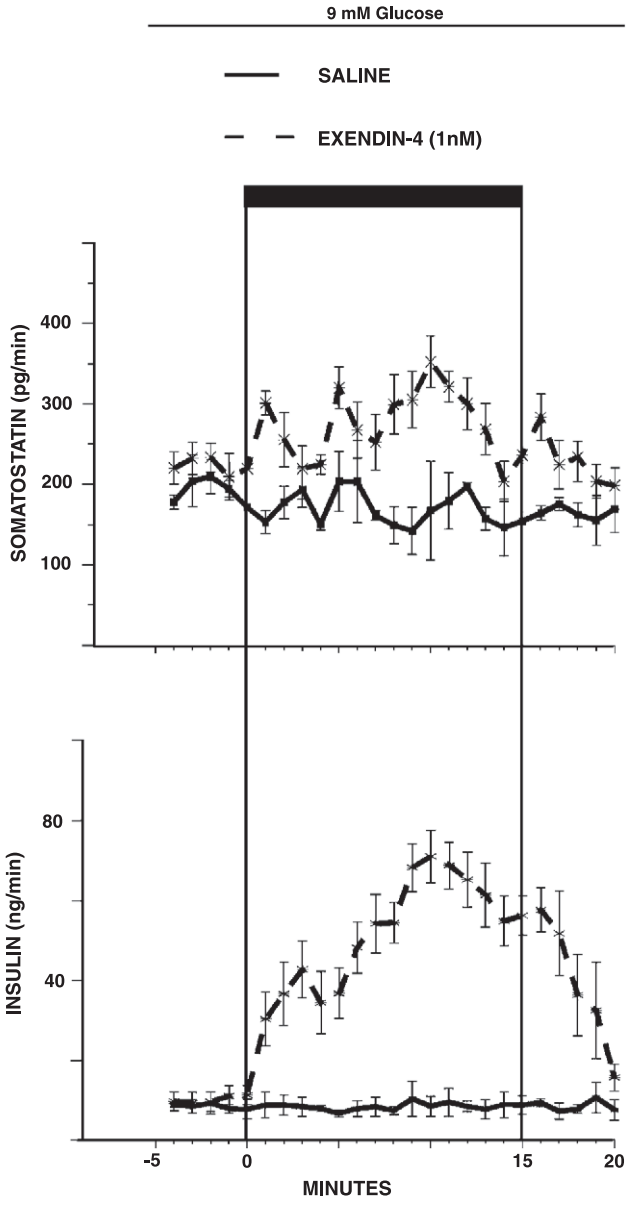

Fig. 2. Effect of 1 nM exendin-4 on somatostatin and insulin secretion in the rat pancreas perfused at constant glucose concentration (9 mM). Solid lines correspond to control perfusions: from 0- to 15-min saline infusion ($N=3$). Broken lines correspond to exendin-4 perfusions: from 0- to 15-min exendin-4 infusion ($N=7$). Means ± S.E.M.

strated by the Kolmogorov–Smirnov test (Siegel, 1978). Differences between values were tested for significance by analysis of variance and by the Student's *t*-test for unpaired samples.

3. Results

In a first series of experiments, we tested the effect of a high (10 nM) exendin-4 concentration on unstimulated somatostatin output. To verify the biological activity of our exendin-4 preparation, insulin was also measured. As shown in Fig. 1, infusion of 10 nM exendin-4, at 9 mM glucose, clearly stimulated somatostatin release (incremental area: 2365 ± 438 pg/15 min, $F(15,45) = 4.48$, $P < 0.01$) and, as expected, insulin output was markedly augmented (incremental area: 1440 ± 163 ng/15 min, $F(15,45) = 14.8$, $P < 0.01$).

Fig. 2 demonstrates that 1-nM exendin-4 also induced an increase in somatostatin secretion (incremental area: 763 ± 200 pg/15 min, $F(15,90) = 2.68$, $P < 0.01$), as well as in insulin secretion (incremental area: 608 ± 62 ng/15

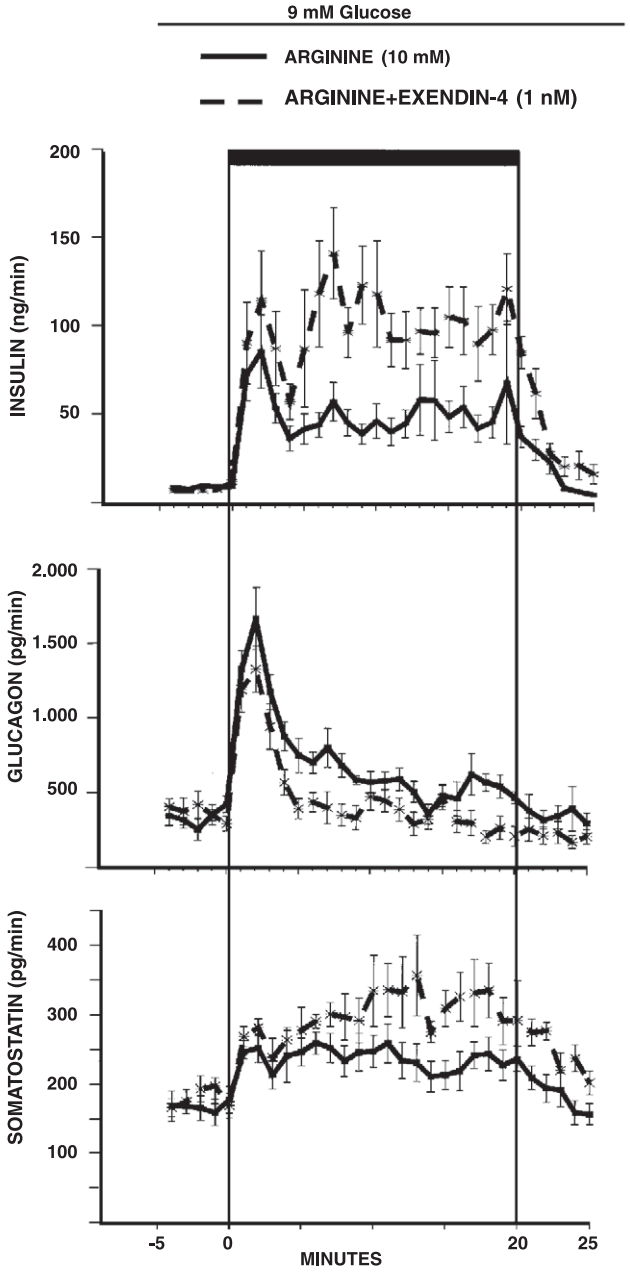

Fig. 3. Effect of 1 nM exendin-4 on the insulin, glucagon and somatostatin responses to 10 mM arginine in the perfused rat pancreas. Solid lines correspond to control experiments: from 0- to 20-min arginine infusion ($N = 9$). Broken lines correspond to exendin-4 experiments: from 0- to 20-min arginine + exendin-4 infusion ($N = 8$). Means ± S.E.M.

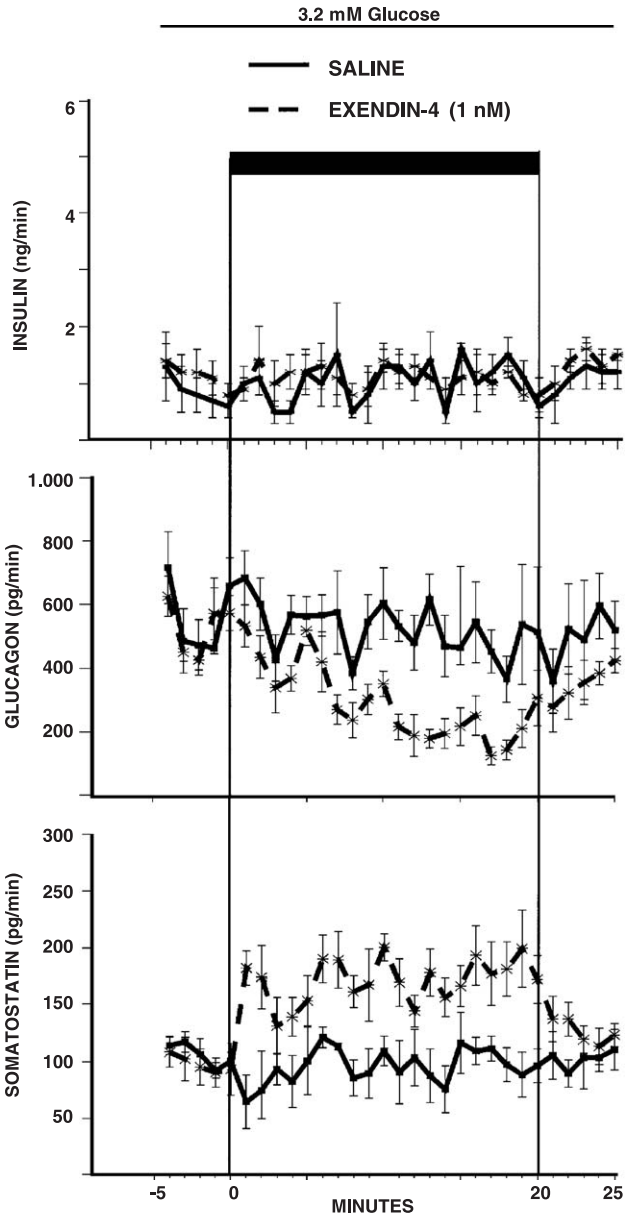

Fig. 4. Effect of 1 nM exendin-4 on the insulin, glucagon and somatostatin secretion in the rat pancreas perfused at constant glucose concentration (3.2 mM). Solid lines correspond to control experiments: from 0- to 20-min saline infusion ($N = 5$). Broken lines correspond to exendin-4 experiments: from 0- to 20-min saline + exendin-4 infusion ($N = 6$). Means ± S.E.M.

min, $F(15,90) = 14.58$, $P < 0.01$). Given that exendin-4 was biologically active at 1 nM, in subsequent experiments, it was tested at this concentration.

Fig. 3 shows the effect of 1 nM exendin-4 on pancreatic hormone responses to arginine, a common secretagogue of the B, A and D cells. Infusion of 10 mM arginine at a constant 9 mM glucose concentration stimulated the secretion of these three cells (incremental area: insulin response, 830 ± 175 ng/20 min; glucagon response, 7510 ± 1089 pg/20 min; somatostatin response, 1447 ± 182 pg/20 min). Co-infusion of exendin-4 (1 nM) potentiated both the insulin and the somatostatin secretion induced by arginine (1826 ± 284 ng/20 min, $P < 0.01$, and 2471 ± 358 pg/20 min, $P < 0.025$ vs. controls, respectively), while it inhibited the glucagon response to this amino acid (2370 ± 714 pg/20 min, $P < 0.01$ vs. controls).

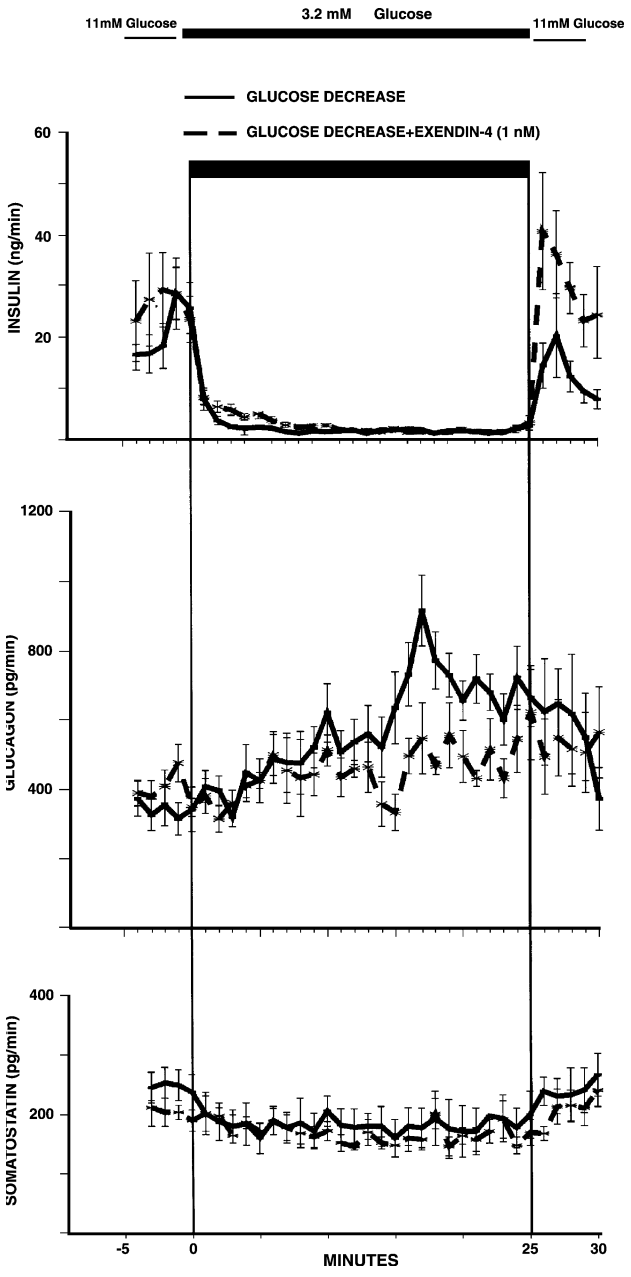

Fig. 5. Effect of 1 nM exendin-4 on the insulin, glucagon and somatostatin responses to an abrupt decline in perfusate glucose concentration (from 11 to 3.2 mM) in the perfused rat pancreas. Solid lines correspond to control experiments: from 0- to 20-min 3.2 mM glucose infusion ($N = 6$). Broken lines correspond to exendin-4 experiments: from 0- to 20-min 3.2 mM glucose + exendin-4 infusion ($N = 5$). Means ± S.E.M.

Fig. 4 shows the effect of exendin-4 (1 nM) at a constant low glucose concentration (3.2 mM). Under this condition, exendin-4 was without effect on the insulin secretory rate, reduced glucagon secretion (decremental area: 4657 ± 788 pg/20 min, $F(20,100) = 5.27$, $P < 0.01$) and stimulated somatostatin secretion (incremental area: 1430 ± 65 pg/20 min, $F(20,100) = 1.80$, $P < 0.05$).

Fig. 5 demonstrates the insulin, glucagon and somatostatin responses to an abrupt reduction of the perfusate glucose level (from 11 to 3.2 mM). As expected, the decline in glucose was accompanied by a prompt, dramatic reduction of insulin (output basal value: 24 ± 6 ng/min; nadir at 23 min: 0.6 ± 0.2 ng/min). This inhibitory response was not modified by infusion of 1 nM exendin-4. The progressive increase in glucagon secretion observed in control experiments (incremental area: 6436 ± 801 pg/25 min) was significantly reduced by exendin-4 (incremental area: 1263 ± 919 pg/25 min, $P < 0.01$). In both control and exendin-4 experiments, reduction in perfusate glucose levels was accompanied by a trend toward lower somatostatin values which did not attain the 5% level of statistical significance ($F(26,125) = 1.43$, and $F(25,100) = 1.56$, respectively). Somatostatin secretion curves in control and exendin-4 perfusions overlapped.

4. Discussion

Our results first demonstrate that, in the rat pancreas perfused at a glucose concentration within the type 2 diabetic range (9 mM), infusion of exendin-4 stimulates somatostatin secretion in a dose-dependent manner. The well-known insulinotropic effect of exendin-4 was also observed in these experiments. Given that exendin-4 has also been shown to increase gastric somatostatin secretion (Eissele et al., 1994), it is tempting to speculate that this peptide might behave as a general stimulator of D-cell function in other tissues, a point worthy of further investigation.

Co-infusion of exendin-4 (1 nM) potentiated both the somatostatin and the insulin responses to arginine, while it reduced the glucagon response to this amino acid. Although the potential interactions within islet cells have not been conclusively elucidated (Matschinsky et al., 1980; Silvestre et al., 1986), there is evidence for an inhibitory paracrine effect of both insulin and somatostatin on A-cell secretion (Pipeleers et al., 1985; Samols et al., 1983; Taborsky, 1983). Thus, the glucagonostatic effect of exendin-4 could be thought to be mediated by its induction of an increase in

intraislet insulin and/or somatostatin. Likewise, the inhibitory effect of GLP-1 on glucagon secretion observed in vitro (Kawai et al., 1989; Komatsu et al. 1989) has been proposed to be paracrine mediated by the concomitant stimulation of insulin and/or somatostatin output induced by GLP-1 (Orskov, 1992; Drucker, 1998; Weir et al. 1989; D'Alessio et al., 1989).

To investigate whether the glucagonostatic effect of exendin-4 is mediated by an increase in insulin output, we tested the effect of this peptide on insulin and glucagon output in pancreases perfused at a low glucose concentration (3.2 mM), a condition in which exendin-4 does not stimulate insulin release in vitro (Rodríguez-Gallardo et al., 2000). As expected, in these experiments, exendin-4 did not modify the insulin secretory rate, whilst it significantly reduced glucagon output (Fig. 4). Thus, it is apparent that exendin-4 can inhibit glucagon secretion in the absence of a concomitant increase in insulin secretion.

As already mentioned, somatostatin has also been proposed as a paracrine inhibitor of glucagon release. In our pancreas preparation, the inhibitory effect of exendin-4 both on unstimulated and on arginine-induced glucagon release was accompanied by an increase in somatostatin output which, in turn, could be responsible for inhibiting A-cell secretion. However, when glucagon secretion was stimulated by an abrupt decline of the perfusate glucose level, exendin-4 blocked said response without affecting somatostatin release (Fig. 5). Furthermore, exendin-4 did not alter the expected suppression of insulin output elicited by the decrease in glucose.

These findings indicate that exendin-4, per se, directly inhibits A-cell secretion. Indeed, the possibility of an additional glucagonostatic effect of exendin-4, mediated by its stimulation of insulin and/or somatostatin secretion, cannot be ruled out.

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