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Suppression by Exendin(9-39)amide of Glucagon-Like Peptide-1 Insulinotropic Action in Rats Infused with Dimethyl Ester of Succinic Acid

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Glucagon-like peptide-1 (GLP-1) acts as a nutrient-dependent insulin-releasing agent, and its insulinotropic action is enhanced by nutrient secretagogues, such as the dimethyl ester of succinic acid (SAD). In the present study, a primed constant infusion of SAD (0.5 µmol followed by 0.25 µmol/min both per g of body wt) was found to increase plasma insulin concentration in fed anesthetized rats, to potentiate the B-cell secretory response to GLP-1 (0.5 pmol/g of body wt), and to unmask the hypoglycemic potential of the gastrointestinal hormone. In the SAD-infused rats, the infusion of exendin(9-39)amide (5.0 pmol/min per g of body wt), 1 min before and 3 min after GLP-1 injection, decreased plasma insulin concentration before GLP-1 injection, suppressed the B-cell secretory response to GLP-1, and both delayed and minimized its hypoglycemic action. It is proposed, therefore, that exendin (9-39)amide could represent a tool in the treatment of alimentary or reactive hypoglycemia.

Key Words: Exendin(9-39)amide; glucagon-like peptide-1; succinic acid dimethyl ester; insulin secretion.

Introduction

Glucagon-like peptide-1 (GLP-1) is often considered a glucose-dependent insulinotropic agent. However, it actually acts as a nutrient-dependent insulin secretagogue. Indeed, in the isolated perfused pancreas from either normal rats or Goto-Kakizaki rats, GLP-1 fails to stimulate insulin release in the absence of any exogenous nutrient but markedly enhances insulin output in the presence of the dimethyl ester of succinic acid (SAD) *(1,2)*.

Likewise, we have recently documented that in vivo, SAD potentiates the insulinotropic action of GLP-1 in both normal rats and adult rats that were injected with streptozotocin during the neonatal period, with the latter animals currently used as another animal model of type 2 diabetes *(3,4)*.

The aim of the present study was to explore to which extent exendin(9-39)amide (Ex[9-39]), a truncated form of exendin-4 (a peptide structurally related to GLP-1 and isolated from *Heloderma suspectum* venom) acting as an antagonist of GLP-1, may oppose the insulinotropic action of GLP-1 in rats infused with SAD.

Results

The results of control experiments conducted under experimental conditions identical to those used in the present study but including the primed-constant infusion of saline (instead of SAD) and the injection of saline (instead of GLP-1) at the min 5 of the test were previously reported *(5)* and are therefore not duplicated in the present report. In these control experiments, the plasma D-glucose and insulin concentrations were affected little during the experiments, with mean paired differences (min 15 vs min 0) of –0.64 ± 0.63 m*M* and –0.90 ± 1.04 ng/mL, respectively ($n = 4$ in both cases).

Likewise, in the animals infused with saline but injected with GLP-1, the plasma D-glucose concentration remained fairly constant (Fig. 1, left). Such was also the case up to min 10 in the animals infused with SAD and then receiving both Ex(9-39) and GLP-1 (Fig. 1, right). In the rats infused with SAD in the absence of Ex(9-39), however, the injection of GLP-1 caused a progressive decrease in plasma D-glucose concentration. The paired difference between the measurements made at min 5 (just before GLP-1 injection) and at later times averaged in these rats 0.55 ± 0.12 m*M* at min 7, 1.25 ± 0.30 m*M* at min 10, and 2.32 ± 0.37 m*M* at min 15 ($n = 7$ and $p < 0.01$ or less in all cases). In the rats receiving Ex(9-39), a modest but not significant ($p > 0.1$) fall in plasma D-glucose concentration was also recorded during the last 5 min of the test, i.e., after cessation of SAD infusion. In these rats, the paired difference in plasma D-glucose concentration at min 5 and 15 corresponded to a mean decrease of 1.07 ± 0.54 m*M* ($n = 4$; $p > 0.1$), as compared ($p < 0.075$) to 2.32 ± 0.37 m*M* in the absence of Ex(9-39).

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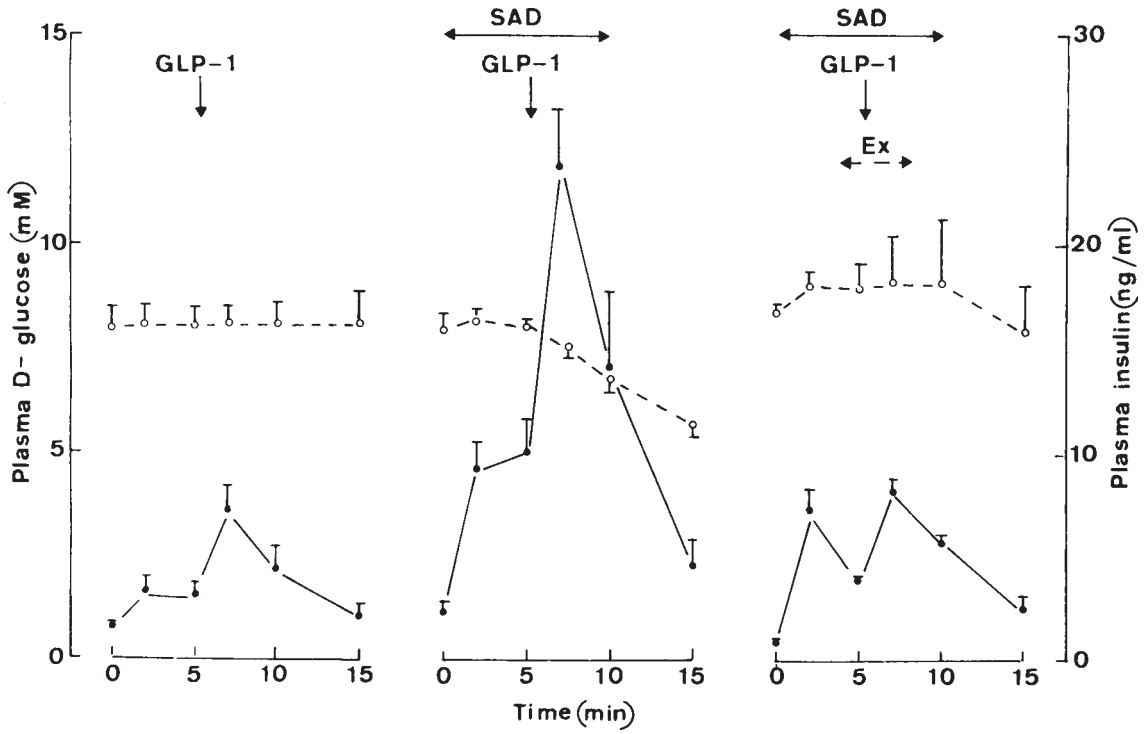

Fig. 1. Plasma D-glucose (– –○– –) and insulin (—●—) concentrations in rats receiving a primed constant infusion of SAD; an injection of GLP-1; and, when required, an infusion of Ex(9-39). In the control experiments (**left**), saline was administered according to the same protocol. From left to right, mean values (±SEM) refer to six, seven, and five individual experiments.

The primed-constant infusion of SAD resulted, within 2 min, in a 6.62 ± 0.90 ng/mL ($n = 12$; $p < 0.001$) increase in plasma insulin concentration above the paired basal value, as distinct ($p < 0.005$) from a mean change of 1.70 ± 0.78 ng/mL ($n = 6$; $p > 0.05$) in the saline-infused animals. In the absence of Ex(9-39), the plasma insulin concentration remained elevated between min 2 and 5 after initiating the administration of SAD (paired change: +0.93 ± 1.49 ng/mL; $n = 7$; $p > 0.5$). However, in the presence of Ex(9-39), which was administered for 4 min (from s 241to 480 of the test), the plasma insulin concentration decreased by 3.22 ± 0.99 ng/mL ($n = 5$; $p < 0.05$) between min 2 and 5 during the administration of SAD.

The integrated increment in plasma insulin concentration over the 5 min following the injection of GLP-1 and above the paired value recorded just before such an injection (min 5) averaged 11.93 ± 2.73 (ng·min)/mL ($n = 6$) in the rats infused with saline, 40.16 ± 6.60 (ng·min)/mL ($n = 7$) in the animals infused with SAD, and 13.26 ± 2.03 (ng·min)/mL ($n = 5$) when Ex(9-39) was administered to the rats infused with SAD. Thus, despite the higher plasma insulin concentration at min 5 in the SAD-infused rats than in the saline-infused rats (9.97 ± 1.65 vs 3.05 ± 0.70 ng/mL; $n = 5$–7; $p < 0.01$), the increment in insulin concentration caused by GLP-1 was much higher ($p < 0.005$) in the former than latter animals. Ex(9-39) severely decreased ($p < 0.01$) the B-cell secretory response to GLP-1 in the SAD-infused rats. The integrated increment in plasma insulin concentration evoked by GLP-1 in the presence of both SAD and Ex(9-39) was not significantly different ($p > 0.7$) from that recorded in the saline-infused rats in the absence of Ex(9-39).

During the last 5 min of the experiments, the plasma insulin concentration decreased by 2.12 ± 1.17 ng/mL ($n = 6$) in the saline-infused rats. Over the same period, the fall in plasma insulin concentration in the rats infused with SAD (in the absence of Ex[9-39]) averaged 9.40 ± 3.61 ng/mL ($n = 7$). In this case, it coincided, however, with the cessation of SAD infusion. In the rats infused with SAD and Ex(9-39), the decrease in plasma insulin concentration between min 10 and 15 averaged 3.26 ± 1.02 ng/mL ($n = 5$).

Discussion

The present results extend to a low dose of GLP-1 (0.5 pmol/g of body wt) the knowledge recently documented with a 10 times higher dose of GLP-1 (5.0 pmol/g of body wt) that SAD given as a primed-constant infusion markedly potentiates the B-cell secretory response to the gastrointestinal hormone *(3)*. They also confirm that SAD provokes a sustained increase in plasma insulin concentration when administered as a primed-constant infusion. Last, they indicate that, even at the low dose used in the present study, GLP-1 lowers the plasma D-glucose concentration in the SAD-infused rats. This may be owing, in part at least, to its insulinotropic action. The lowering of glycemia by GLP-1

may also entail its extrapancreatic and insulinomimetic effect *(6)*. At variance with the metabolic response to GLP-1 and prior to its administration, the increase in plasma insulin concentration caused by the infusion of SAD did not result in any fall in glycemia, probably because the ester also acts as a gluconeogenic precursor *(7)*.

The experiments conducted in the presence of Ex(9-39) provide two novel pieces of information. First, they reveal that this GLP-1 antagonist opposes the insulinotropic action of SAD. Whether this effect reflects inhibition of endogenous GLP-1 insulinotropic action in the fed rats used in our experiments or results from a direct effect of Ex(9-39) on the B-cell secretory response to SAD, independently of any suppression of the insulin-releasing action of endogenous GLP-1, cannot be answered on the sole basis of the present experiments. Second, the present results indicate that Ex(9-39) severely decreases the B-cell secretory response to GLP-1 in the SAD-infused rats. This coincides with the fact that the hypoglycemic action of GLP-1 is both delayed and minimized by Ex(9-39).

The implications of these findings should not be overlooked. The present results justify further work, to be conducted both in vitro and in vivo, to investigate the direct effect of Ex(9-39) on the secretory response of the B-cell to SAD and other nutrient secretagogues, in the absence of any significant contribution of exogenous or endogenous GLP-1 in the control of insulin release. Furthermore, in light of the present study, Ex(9-39) could be considered a possible tool to investigate to which extent syndromes of alimentary or reactive hypoglycemia entail an excessive stimulation of GLP-1 secretion *(8–12)*.

Materials and Methods

Animal housing and protocols were approved by the Animal Use Committee of the Fundación Jiménez Diaz (Madrid, Spain).

Eighteen male Wistar rats (241 ± 8 g of body wt) were obtained from a colony maintained at the Fundación Jiménez Diaz and kept on a standard pellet diet (UAR; Panlab, Barcelona, Spain). They were anesthetized with pentobarbital administered intraperitoneally (60 µg/g of body wt) (Pentothal; Abbot, Madrid, Spain). At time zero, SAD (Sigma, St. Louis, MO) in saline was given intravenously for 10 min as a primed-constant infusion (0.5 µmol of SAD in 2.5 µL of saline followed by 0.25 µmol of SAD in 0.5 µL of saline per min, both indicated per g of body wt). Five minutes later, GLP-1 (Bachem, Bubendorf, Switzerland) placed in saline (0.2 µ*M*) containing 10 g/L of human serum albumin was injected over 30 s in an amount of 0.5 pmol/g of body wt. In control experiments, the same volume of saline was administered intravenously instead of SAD and/or GLP-1. In one set of SAD-infused rats, Ex(9-39) (a gift from Dr. J. Eng [IAMC, New York]) solubilized (3.9 µ*M*) in saline also containing 10 g/L of human serum albumin was infused (1.3 µL or 5.0 pmol per min and per g of body wt) intravenously for 4 min (s 241–480).

Blood samples (0.5 mL) were collected from a catheter inserted in a carotid artery for the measurement of plasma D-glucose *(13)* and insulin *(14)* concentrations by methods described in the cited references. Immediately after the test, the rats were killed by iv injection of a high dose of pentobarbital.

Statistical Analyses

All results including those already mentioned are presented as mean values (±SEM). The integrated changes in metabolic variables above or below a suitable paired reference value were calculated by planimetry. The statistical significance of differences between mean values was assessed by use of the student's *t*-test.

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References

1. Leclercq-Meyer, V. and Malaisse, W. J. (1996). *Life Sci.* **58,** 1195–1199.
2. Leclercq-Meyer, V. and Malaisse, W. J. (1996). *Biochem. Mol. Med.* **59,** 87–90.
3. García-Martínez, J., Cancelas, J., Villanueva-Peñacarrillo, M.L., Valverde, I., and Malaisse, W. J. (2000). *Horm. Metab. Res.* **32,** 306–309.
4. García-Martínez, J., Cancelas, J., Villanueva-Peñacarrillo, M. L., Valverde, I., and Malaisse, W. J. (2000). *Int. J. Mol. Med.* **6,** 319–321.
5. Cancelas, J., Villanueva-Peñacarrillo, M. L., Valverde, I., and Malaisse, W. J. (2001). *Int. J. Mol. Med.*, in press.
6. Alcántara, A. I., Morales, M., Delgado, E., López-Delgado, M. I., Clemente, F., Luque, M. A., Malaisse, W. J., Valverde, I., and Villanueva-Peñacarrillo, M. L. (1997). *Arch. Biochem. Biophys.* **341,** 1–7.
7. Zhang, T.-M., Sener, A., and Malaisse, W. J. (1994). *Arch. Biochem. Biophys.* **314,** 186–192.
8. Miholic, J., Ørskov, C., Holst, J. J., Kotzerke, J., and Meyer, H. J. (1991). *Digest. Dis. Sci.* **36,** 1361–1370.
9. Miholic, J., Ørskov, C., Holst, J. J., Kotzerke, J., and Pichlmayr, R. (1993). *Digestion* **54,** 73–78.
10. Andreasen, J. J., Ørskov, C., and Holst, J. J. (1994). *Digestion* **55,** 221–228.
11. Owada, K., Wasada, T., Miyazono, Y., Yoshino, H., Hasumi, S., Kuroki, H., Yano, K., Maruyama, A., Kawai, K., and Omori, Y. (1995). *Endocr. J.* **42,** 147–151.
12. Toft-Nielsen, M., Madsbad, S., and Holst, J. J. (1998). *Diabetologia* **41,** 1180–1186.
13. Bergmeyer, H. U. and Berndt, E. (1974). In: *Methods of enzymatic analysis*. Bergmeyer, H. U. (ed.). Academic: New York.
14. Valverde, I., Barreto, M., and Malaisse, W. J. (1988). *Endocrinology* **122,** 1443–1448.