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Resistance of Succinic Acid Dimethyl Ester Insulinotropic Action to Exendin (9-39) Amide

Abstract

Exendin (9-39) amide (Ex [9-39]) was recently proposed for use in the treatment of alimentary or reactive hypoglycaemia. It was indeed found to antagonise the insulinotropic action of GLP-1 in rats infused with the dimethyl ester of succinic acid (SAD). We have now investigated whether, under comparable experimental conditions, Ex (9-39) also opposes the insulin-releasing action of SAD itself. Since this was not the case, Ex (9-39) could be safely used to abolish the incretin effect of GLP-1 without interfering with the control of insulin secretion by circulating nutrients.

Key words
Exendin (9-39) Amide · Succinic Acid Dimethyl Ester · Insulin Secretion · Anaesthetized Rats

Introduction

Exendin (9-39) amide (Ex [9-39]) is a truncated form of exendin-4 (a peptide structurally related to GLP-1 and isolated from *Meloderma suspectum* venom) that acts as an antagonist to glucagon-like peptide 1 (GLP-1). It was recently reported [1] to suppress GLP-1 insulinotropic action in anaesthetized rats infused with succinic acid dimethyl ester (SAD). The latter ester is currently used to support the insulinotropic action of GLP-1 *in vitro* or *in vivo* [1 – 5]. It was proposed, therefore, that Ex (9-39) might be of use in the treatment of alimentary or reactive hypoglycaemia [1]. With this possibility in mind, the major aim of the present study was to explore whether Ex (9-39), administered according to the same modality as that used in the above-mentioned experiments [1], also antagonized the insulinotropic action of SAD.

Materials and Methods

Eight male Wistar rats (264 ± 7 g body wt) given free access to food (UAR; Panlab, Barcelona, Spain) and six overnight fasted animals (225 ± 11 g) all obtained from a colony maintained at the Fundación Jiménez Diaz (Madrid, Spain) were anaesthetized with pentobarbital administered intraperitoneally (60 µg/g body wt; Pentothal, Abbot Laboratories, Madrid, Spain).

At time zero, SAD (Sigma Chemical Company, St. Louis, MO, USA) in saline was given intravenously for 10 – 15 min as a primed constant infusion (0.5 µmol SAD in 2.5 µl saline followed by 0.25 µmol SAD in 0.5 µl saline per min, both indicated per g body wt). Four min later, Ex (9-39), a gift from Dr. J. Eng, (VAMC, NY, USA) solubilised (3.9 µM) in saline containing 10 g/l human serum albumin, was infused (1.3 µl or 5.0 pmol per min and per g body wt) intravenously for 4 min (241st to 480th s).

Blood samples (0.5 ml) were collected from a catheter inserted in a carotid artery for measuring plasma D-glucose [6] and insulin [7] concentrations by methods described in the cited references.

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Bibliography
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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 using Student's *t*-test.

Results

The basal plasma D-glucose concentration averaged 7.24 ± 0.16 mM (n = 8) in fed rats, as compared to only 5.73 ± 0.25 mM (n = 6) in overnight fasted animals ($p < 0.001$). In the latter, the administration of SAD increased ($p < 0.05$) the plasma D-glucose concentration over the 10-min period of observation (min 1 to 10) to a mean level 0.44 ± 0.14 mM higher (n = 6) than the paired basal value (Fig. **1**). This was not the case in fed rats, in which the mean plasma D-glucose concentration over the same period was not significantly higher (+ 0.16 ± 0.19 mM; n = 8; $p > 0.4$) than paired basal value.

In both fed and starved rats, the mean value for the integrated SAD-induced increase in plasma D-glucose concentration was somewhat higher in the presence of Ex (9-39) than in its absence. However, this difference only achieved statistical significance ($p < 0.05$) when pooling the data collected in fed and starved rats (0.07 ± 0.14 mM; n = 8 in the absence of Ex (9-39) vs. 0.57 ± 0.18 mM; n = 6 in its presence).

In fed rats, the administration of SAD increased the plasma insulin concentration by 11.5 ± 0.9 ng/ml (n = 8; $p < 0.001$) above the paired basal value (1.9 ± 0.3 ng/ml; n = 8) within 2 min. Relative to the paired plasma insulin reached at the end of the second minute, the mean concentration of the hormone between the 5th and 10th min of the experiment averaged 94.3 ± 6.6% (n = 5) and 84.4 ± 13.3% (n = 3) in the absence and presence of Ex (9-39), respectively (Fig. **2**). These two percentages were not significantly different from one another ($p > 0.4$), suggesting that Ex (9-39) did not interfere with the insulinotropic action of SAD under the present experimental conditions.

Likewise, whether Ex (9-39) was absent (44.0 ± 6.0%; n = 3) or present (53.4 ± 4.7%; n = 3) did not seem to make a difference in the mean plasma insulin concentration reached between the 5th and 10th min of the test relative to the paired peak value recorded at min 2 in overnight fasted rats ($p > 0.25$). In these starved animals, plasma insulin concentrations were 6.8 ± 0.9 ng/ml higher (n = 6; $p < 0.001$) than the paired basal value (0.9 ± 0.2 ng/ml; n = 6) at the end of the second minute. Both of the latter two mean values were significantly lower ($p < 0.02$ or less) than those recorded in fed rats.

Even when the insulinogenic index (the paired ratio between plasma insulin and D-glucose concentration) was taken into consideration, there was no obvious evidence that Ex (9-39) affected SAD-stimulated insulin secretion, whether in fed or fasted rats. At the 2nd min of the experiments, the insulinogenic index aver-

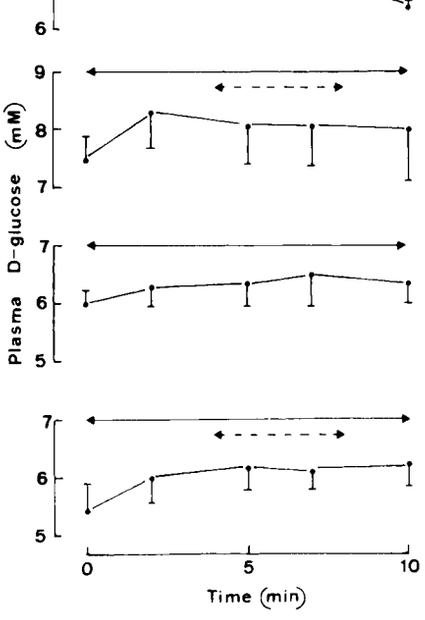

Fig. **1** Plasma D-glucose concentrations in either fed (upper panels) or starved (lower panels) rats infused with SAD (horizontal double-headed solid arrow) and, for 4 min, either saline or Ex (9-39) (horizontal double-headed dashed arrow). Mean values (± SEM) refer to 3 – 5 individual experiments.

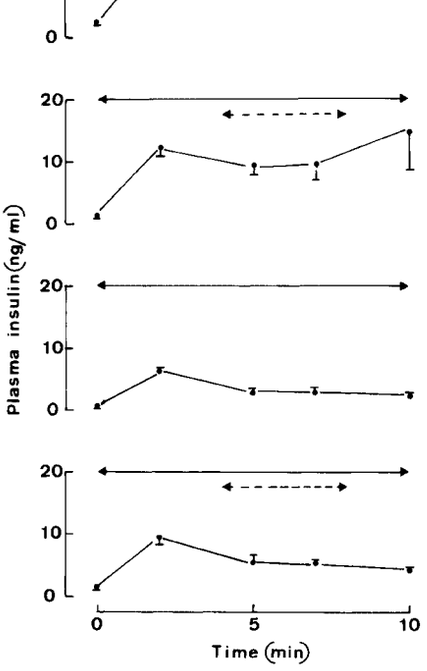

Fig. **2** Plasma insulin concentrations in either fed (upper panels) or starved (lower panels) rats infused with SAD (horizontal double-headed solid arrow) and, for 4 min, either saline or Ex (9-39) (horizontal double-headed dashed arrow). Mean values (± SEM) refer to 3 – 5 individual experiments.

aged 763 ± 126 % (n = 8) and 995 ± 143 % (n = 6) of paired basal value in fed and starved rats, respectively. Relative to these 2nd-min measurements, the mean insulinogenic index recorded in each animal from min 5 to 10 inclusive averaged, in the absence and presence of Ex (9-39) respectively, 102.6 ± 7.5 and 87.2 ± 12.9 % (n = 5 and 3; $p > 0.3$) in fed rats, and 43.1 ± 6.4 and 51.8 ± 3.0 % (n = 3 in both cases; $p > 0.2$) in starved animals.

Discussion

The present results convincingly document that Ex (9-39) infused for 4 min at the rate of 5.0 pmol/min per g body wt during administration of SAD to either fed or fasted anaesthetized rats, fails to cause any sizeable change in plasma insulin concentration. Since the secretory response of insulin-producing B-cells to SAD is modulated by the extracellular concentration of D-glucose [8], these findings suggest that, under the present experimental conditions, Ex (9-39) does not antagonise the insulinotropic action of circulating nutrients.

Yet, under the same conditions, Ex (9-39) suppresses the insulin-releasing effect of GLP-1 [1]. Hence, Ex (9-39) indeed appears to act on insulin-producing cells as a selective antagonist of GLP-1.

It could be argued that Ex (9-39) tended to augment the SAD-induced increase in plasma D-glucose concentration, as if the peptide might indeed oppose the insulinotropic action of SAD to some extent. It should be stressed, however, that the SAD-induced increase in plasma D-glucose concentration is mainly attributable to the gluconeogenic capacity of this succinic acid ester. It was indeed quite obvious in starved rats, but failed to achieve statistical significance in fed animals. It is conceivable, therefore, that Ex (9-39) might interfere with the metabolic response to SAD through some extrapancreatic effects that are independent of any change in insulin secretion.

In conclusion, the present work suggests that Ex (9-39) specifically antagonizes the insulinotropic action of GLP-1 whilst failing to exert any obvious effect upon the islet B-cell response to circulating nutrients. Such attributes should not be ignored when considering the use of Ex (9-39) in the treatment of alimentary or reactive hypoglycemia.

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References

[1] Cancelas J, Villanueva-Peñacarrillo ML, Valverde I, Malaisse WJ. Suppression by exendin (9-39) amide of glucagon-like peptide 1 insulinotropic action in rats infused with dimethyl ester of succinic acid. Endocrine 2001; 15: 283 – 285

[2] Leclercq-Meyer V, Malaisse WJ. Potentiation of glucagon-like peptide I insulinotropic action by succinic acid dimethyl ester. Life Sci 1996; 58: 1195 – 1199

[3] Leclercq-Meyer V, Malaisse WJ. Potentiation of GLP-1 insulinotropic action by a non-glucidic nutrient in the pancreas of diabetic GK rats. Biochem Mol Med 1996; 59: 87 – 90

[4] García-Martínez JA, Cancelas J, Villanueva-Peñacarrillo ML, Valverde I, Malaisse WJ. Potentiation of the insulinotropic action of GLP-1 by succinic acid dimethyl ester in fed anaesthetised rats. Horm Metab Res 2000; 32: 306 – 309

[5] García-Martínez JA, Cancelas J, Villanueva-Peñacarrillo ML, Valverde I, Malaisse WJ. Prolongation of the insulinotropic action of glucagon-like peptide 1 by the dimethyl ester of succinic acid in an animal model of type-2 diabetes. Int J Mol Med 2000; 6: 319 – 321

[6] Bergmeyer HU, Berndt E. Glucose determination with glucose oxidase and peroxidase. In: Bergmeyer HU (ed). In: Methods of enzymatic analysis. New York: Academic Press, 1974: 1205 – 1215

[7] Valverde I, Barreto M, Malaisse WJ. Stimulation by D-glucose of protein biosynthesis in tumoral insulin-producing cells (RINm5F line). Endocrinology 1988; 122: 1443 – 1448

[8] Malaisse WJ, Rasschaert J, Villanueva-Peñacarrillo ML, Valverde I. Respiratory, ionic and functional effects of succinate esters in pancreatic islets. Am J Physiol 1993; 264: E428 – E433