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Potentiation and Prolongation of the Insulinotropic Action of Glucagon-Like Peptide 1 by Methyl Pyruvate or Dimethyl Ester of L-Glutamic Acid in a Type 2 Diabetes Animal Model

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Methyl pyruvate and the dimethyl ester of L-glutamic acid were administered intravenously, as a primed constant infusion (1.0–2.0 µmol followed by 0.5–1.0 µmol/min, both expressed per gram of body wt), in adult rats that had been injected with streptozotocin during the neonatal period. Each ester augmented plasma insulin concentration and potentiated and/or prolonged the insulinotropic action of glucagon-like peptide 1 (GLP-1) injected intravenously (5 pmol/g of body wt) at min 5 of the test. It is proposed, therefore, that suitable nonglucidic nutrients, susceptible to bypassing the site-specific defects of D-glucose transport and metabolism responsible for the preferential impairment of the B-cell secretory response to D-glucose in non-insulin-dependent diabetes, could be used to optimize the insulinotropic action of GLP-1.

Key Words: Glucagon-like peptide 1; methyl pyruvate; L-glutamic acid; insulin secretion.

Introduction

Glucagon-like peptide 1 (GLP-1) is presently contemplated as a potential agent for the treatment of type 2 diabetes mellitus. The possible use of GLP-1 as an antidiabetic agent is inspired mainly by its well-known insulinotropic capacity *(1)*. It may also benefit from the insulinomimetic action of GLP-1 in extrapancreatic sites *(2,3)*. It is well established that the secretory response of the pancreatic islet B-cell to GLP-1 is, under normal conditions, modulated by the extracellular concentration of D-glucose, being virtually suppressed at low concentrations of the hexose. In type 2 diabetes, however, the recognition of D-glucose by the B-cell is often preferentially impaired relative to that of other insulin secretagogues *(4)*. Hence, this apparent blindness of the diseased B-cell to the hexose could well impede GLP-1 to exert its full insulinotropic action in noninsulin-dependent diabetes. It was recently demonstrated, however, that GLP-1 is not truly a glucose-dependent, but rather a fuel-dependent, insulin secretagogue. Indeed, in the isolated perfused rat pancreas of either normal or GK rats and in the absence of any other exogenous nutrient, nonglucidic substrates such as the dimethyl ester of succinic acid restore the insulinotropic action of GLP-1 *(5,6)*. Likewise, it was recently reported that, in normal rats, nonglucidic nutrients such as the dimethyl esters of succinic acid and L-glutamic acid or the monomethyl ester of pyruvic acid potentiate in vivo the B-cell secretory response to GLP-1 *(7–9)*. The major aim of the present study was to investigate whether two of these nonglucidic nutrients, methyl pyruvate and the dimethyl ester of L-glutamic acid (DMG), also potentiate the insulinotropic action of GLP-1 in an animal model of type 2 diabetes, namely in adult rats that were injected with the β-cytotoxic agent streptozotocin during the neonatal period *(10)*.

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Results

Metabolic Status

The 32 streptozotocin-injected rats used had a mean body wt of 250 ± 6 g. They were selected on the basis of a D-glucose assimilation coefficient (K) below 2.5×10^{-2}/min. Actually, the mean K in these rats did not exceed $1.94 \pm 0.09 \times 10^{-2}$/min ($n = 32$). At time zero of the test, their plasma D-glucose and insulin concentrations averaged, respectively, 10.24 ± 0.37 mM and 1.05 ± 0.13 ng/mL ($n = 32$ in both cases).

Plasma D-Glucose

In the saline-infused rats, whether injected with GLP-1 or not, no significant change in plasma D-glucose concentration was observed (Table 1). The mean integrated plasma D-glucose concentration (min 0–20 inclusive) was 0.47 ± 0.30 mM higher ($n = 12$; $p > 0.1$) than the paired basal value.

Whether in the rats injected with GLP-1 or not, the infusion of methyl pyruvate caused a progressive increase in

Table 1
Plasma D-Glucose
Concentration in Streptozotocin-Injected Rats

Infused nutrient (identity)	GLP-1 (pmol/g body wt)	Basal value (m*M*)	Min 0–20 integrated increment (m*M*)
Nil	Nil	9.03 ± 1.00 (6)	+0.69 ± 0.50 (6)
Nil	5.0	10.49 ± 0.83 (6)	+0.25 ± 0.36 (6)
Methyl pyruvate	Nil	9.84 ± 0.34 (5)	+1.37 ± 0.40 (5)
Methyl pyruvate	5.0	9.80 ± 0.74 (5)	+1.67 ± 0.42 (5)
DMG	Nil	10.56 ± 0.78 (5)	+0.83 ± 0.40 (5)
DMG	5.0	11.91 ± 1.78 (5)	+0.26 ± 0.47 (5)

plasma D-glucose concentration. It averaged 9.82 ± 0.39 m*M* at time zero, 10.47 ± 0.39 m*M* at min 5, 11.26 ± 0.31 m*M* at min 10, and 13.39 ± 0.26 m*M* at min 20. The mean integrated plasma D-glucose concentration (min 0–20 inclusive) was, in the methyl pyruvate-infused rats, 1.56 ± 0.29 m*M* higher ($n = 10$; $p < 0.001$) than the paired basal value.

There was a trend toward an increase in plasma D-glucose concentration only in the DMG-infused rats, the mean integrated value (min 0–20 inclusive) being 0.83 ± 0.40 m*M* higher ($n = 5$; $p > 0.1$) than the paired basal value. This trend was no longer observed when the DMG-infused rats were injected with GLP-1, the mean integrated value now only 0.26 ± 0.47 m*M* higher ($n = 5$; $p > 0.6$) than the paired basal measurement.

Plasma Insulin

At time zero, the plasma insulin concentration averaged 1.05 ± 0.13 ng/mL ($n = 32$). In the control rats infused and injected with saline, the plasma insulin concentration remained fairly stable throughout the experiment (Fig. 1). In the saline-infused rats, the iv injection of GLP-1 provoked a peak-shaped monophasic increase in plasma insulin concentration. Such an increment averaged between min 5 and 15 of the test, 1.72 ± 0.40 ng/mL ($n = 6$; $p < 0.01$).

Over the first 5 min of the test, the infusion of methyl pyruvate increased the plasma insulin concentration by 1.44 ± 0.25 ng/mL ($n = 10$; $p < 0.001$). During infusion of methyl pyruvate, and in the absence of GLP-1, the plasma insulin concentration decreased by 2.24 ± 0.53 ng/mL ($n = 5$; $p < 0.02$) between min 2 and 15. When the infusion of methyl pyruvate was halted at min 15, a further decrease in the plasma insulin concentration averaging 1.44 ± 0.56 ng/mL ($n = 10$; $p < 0.05$) was observed. In the methyl pyruvate-infused rats, the injection of GLP-1 again caused a monophasic increase in plasma insulin concentration, the peak value being reached within 2 min after administration of the hormone. Between min 5 and 15, the GLP-1-induced increment in plasma insulin concentration averaged 6.49 ± 1.01 ng/mL ($n = 5$), a value about four times higher ($p < 0.002$) than that recorded in the control saline-infused rats (Table 2). In these calculations, the reference value (min 5) was also higher ($p < 0.05$) in the methyl pyruvate-infused rats (2.58 ± 0.69 ng/mL; $n = 5$) than in the saline-infused animals (0.95 ± 0.19 ng/mL; $n = 6$).

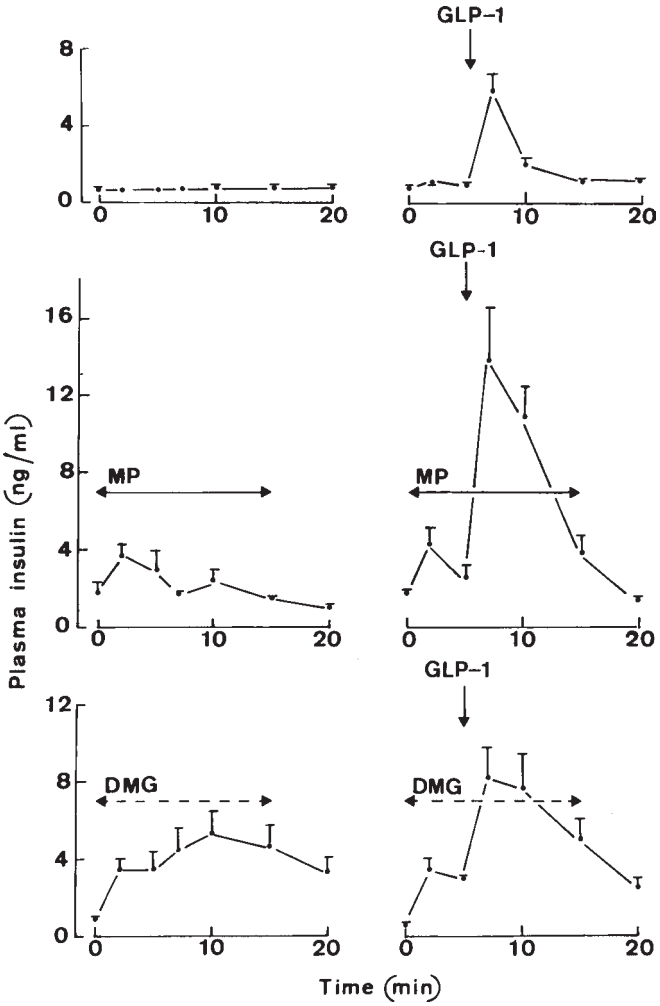

Fig. 1. Time course for changes in plasma insulin concentration caused by infusion of methyl pyruvate (MP) or DMG (double-headed solid and dashed horizontal arrows, respectively) and injection of GLP-1 (vertical arrows) in streptozotocin-injected rats. Mean values (±SEM) refer to five (middle and lower panels) or six (upper panels) individual experiments.

The potentiation by methyl pyruvate of GLP-1's insulinotropic action could not be blamed on the increase in glycemia provoked by the pyruvate ester. Indeed, between min 5 and 15 and after administration of GLP-1, the integrated mean insulinogenic index (i.e., the paired ratio between plasma insulin and D-glucose concentration) was also higher ($p < 0.001$) in the methyl pyruvate-infused rats (8.06 ± 1.21 mg/mol; $n = 5$) than in the saline-infused animals (2.43 ± 0.29 mg/mol; $n = 6$).

Table 2
Plasma Insulin Concentration
in Streptozotocin-Treated Rats Injected with GLP-1

Infused nutrient (identity)	Min (ng/mL)	Min 7 peak increment (ng/mL)	Min 5–15 integrated increment (ng/mL)
Nil	0.95 ± 0.19 (6)	+4.87 ± 0.96 (6)	+1.72 ± 0.40 (6)
Methyl pyruvate	2.58 ± 0.69 (5)	+11.32 ± 2.46 (5)	+6.49 ± 1.01 (5)
DMG	2.96 ± 0.18 (5)	+5.12 ± 1.62 (5)	+3.62 ± 0.15 (5)

Over the first 5 min of the test, the infusion of DMG increased the plasma insulin concentration by 2.06 ± 0.33 ng/mL ($n = 10$; $p < 0.01$). Such an increment was not significantly higher than that evoked by methyl pyruvate. In the DMG-infused rats, the plasma insulin concentration remained significantly higher than basal value (time zero) throughout the test, with the highest value usually recorded at min 10. During the last 5 min, after halting the infusion of DMG, the plasma insulin concentration decreased by 1.78 ± 0.74 ng/mL ($n = 10$; $p < 0.05$). In the animals infused with DMG, the mean GLP-1-induced increment in plasma insulin concentration between min 5 and 15 was twice as high (+3.62 ± 1.15 ng/mL; $n = 5$) as in the saline-infused rats (+1.72 ± 0.40 ng/mL; $n = 6$), despite the fact that the min-5 reference value was much higher ($p < 0.001$) in the DMG-infused animals (2.96 ± 0.18 ng/mL; $n = 5$) than in the saline-infused rats (0.95 ± 0.19 ng/mL; $n = 6$). In terms of the GLP-1-induced increment in plasma insulin concentration, however, the difference between saline- and DMG-infused rats achieved virtual statistical significance ($p < 0.06$) only after exclusion of one abnormally low individual value in each group (individual value less than the mean minus $t_{0.05} \times$ SEM). Nevertheless, the mean absolute plasma insulin concentration after the administration of GLP-1 (min 5–15) was much higher ($p < 0.02$) in the DMG-infused rats (6.62 ± 1.30 ng/mL; $n = 5$) than in the saline-infused animals (2.66 ± 0.44 ng/mL; $n = 6$).

The data illustrated in Fig. 1 (right) indicate that the enhancing action of methyl pyruvate and DMG on the B-cell response to GLP-1 was attributable, at least in part, to a more sustained insulinotropic action of the gastrointestinal hormone in the presence than in the absence of these nutrient esters. Thus, in the rats injected with GLP-1, the paired min-10/min-7 ratio in plasma insulin concentration averaged 82.6 ± 7.3% ($n = 5$) during infusion of MP and 94.7 ± 14.2% ($n = 5$) during infusion of DMG, as distinct ($p < 0.005$ or less) from only 39.0 ± 5.5% ($n = 6$) in the saline-infused rats. In other terms, the plasma insulin concentration decreased significantly ($p < 0.001$) between min 2 and 5 after GLP-1 injection to saline-infused rats, whereas such was not the case ($p > 0.05$ or more) in the methyl pyruvate- or DMG-infused rats.

Discussion

Streptozotocin-injected rats, selected on the basis of an abnormally low D-glucose assimilation coefficient *(11)*, displayed lower than normal basal insulinemia *(12)*, despite higher than normal plasma D-glucose concentration *(12)*. Our study reveals that, in these diabetic animals, methyl pyruvate and DMG both stimulated insulin secretion, as judged from the increase in plasma insulin concentration recorded at min 2 and 5 during administration of these nutrients as a primed constant infusion. The magnitude of such an early secretory response to methyl pyruvate and DMG was again lower ($p < 0.001$) in the streptozotocin-injected rats than that recorded in prior experiments conducted in normal rats under otherwise identical experimental conditions *(8,9)*. Indeed, the values recorded in the streptozotocin-injected rats averaged 40.7 ± 5.8% ($n = 20$) of the mean corresponding control values (100.0 ± 12.9%; $n = 30$) found in normal animals.

Our findings also extend to an animal model of type 2 diabetes the knowledge, recently documented in normal rats *(8,9)*, that both methyl pyruvate and DMG enhance in vivo the pancreatic islet B-cell secretory response to GLP-1. This enhancing action included a more sustained insulinotropic action of the gastrointestinal hormone. It could not be attributed solely to the modest increase in glycemia caused by methyl pyruvate. Moreover, in the streptozotocin-injected rats, DMG also potentiated the insulinotropic action of GLP-1, while failing to affect significantly the plasma D-glucose concentration.

Our results are reminiscent of those recently collected in streptozotocin-injected rats infused with the dimethyl ester of succinic acid *(12)*. In such a case, the ester was also found to prolong the insulinotropic action of GLP-1.

Taken as a whole, therefore, these findings indicate that suitable nutrients, susceptible to bypassing the site-specific defects responsible for the preferential impairment of the B-cell secretory response to D-glucose in noninsulin-dependent diabetes mellitus *(4)*, could be used to potentiate and/or prolong the insulinotropic action of such antidiabetic agents as GLP-1. In this perspective, it could be objected that, under the present experimental conditions, GLP-1 failed to display any obvious effect on plasma D-glucose concentration. However, the hypoglycemic potential of GLP-1 was already well documented in prior studies conducted in both normal subjects and those with type 2 diabetes *(13)*.

Materials and Methods

Nutrients

Methyl pyruvate and DMG were both obtained from Sigma (St. Louis, MO).

Procedure

The experiments were approved by the Animal Use Committee of the Fundación Jiménez Diaz (Madrid, Spain).

Thirty-two rats injected with streptozotocin during the neonatal period *(10)* were selected on the basis of a D-glucose assimilation coefficient (K) below 2.5×10^{-2}/min during an iv glucose tolerance test *(11)*. These rats were maintained at the Fundación Jiménez Diaz (Madrid, Spain) and kept on a standard pellet diet (UAR; Panlab, Barcelona, Spain). Close to an equal number of male and female animals were used either in the experiments conducted in saline-infused rats or in those including the infusion of methyl pyruvate and DMG.

The rats were anesthetized with pentobarbital administered intraperitoneally (60 µg/g of body wt) (Penthothal; Abbot, Madrid, Spain). At time zero, methyl pyruvate or DMG was given intravenously (left femoral vein catheter) for 15 min as a primed constant infusion (2 µmol of methyl pyruvate or 1 µmol of DMG in 2.5 µL of saline followed by 1 µmol of methyl pyruvate or 0.5 µmol of DMG in 0.5 µL of saline/min, all values indicated per gram of body wt). Five minutes later, GLP-1 (Bachem, Bubendorf, Switzerland) placed in saline (2.0 µM) containing 10 mg/mL of human serum albumin was injected intravenously (right femoral vein catheter) over 30 s in an amount of 5 pmol/g of body wt. In control experiments, the same volumes of saline were administered intravenously instead of methyl pyruvate (or DMG) and/or GLP-1.

Blood samples (0.5 mL) were collected from a catheter inserted in a carotid artery for the measurement of plasma D-glucose *(14)* and insulin *(15)* concentrations by methods described in the cited references.

Statistical Analyses

All results are presented as mean values (± SEM), together with the number of individual observations (n). 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 Student's t-test.

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