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European Journal of Pharmacology 325 (1997) 65–68

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Potentiation of the insulinotropic and hypoglycemic action of gliquidone by succinic acid esters

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Received 18 November 1996; revised 27 January 1997; accepted 4 February 1997

Abstract

The monoethyl, monopropyl and monoisopropyl esters of succinic acid, administered intravenously at the dose of 2 μmol/g body weight, were found to increase the insulinotropic action of gliquidone (0.2 nmol/g body weight) in anaesthetized rats. The monoisopropyl ester of succinic acid also doubled the hypoglycemic action of gliquidone. These findings indicate that it is possible to design esters of succinic acid that are devoid of the risk of generating methanol by intracellular hydrolysis, and yet susceptible to increase both the insulinotropic and hypoglycemic responses to antidiabetic agents.

Keywords: Succinic acid monoethyl ester; Succinic acid monopropyl ester; Succinic acid monoisopropyl ester; Gliquidone; Insulin secretion

1. Introduction

The esters of selected carboxylic metabolites, that are intermediates of the Krebs cycle or their precursors, such as pyruvic acid, succinic acid and glutamic acid, are currently under investigation as potential insulinotropic tools in the treatment of non-insulin-dependent diabetes mellitus (Malaisse, 1995). This approach offers the advantage of bypassing site-specific defects of D-glucose metabolism in the diseased B-cell and stimulating both proinsulin biosynthesis and insulin release (Malaisse, 1994, 1995). Moreover, the esters so far tested for such a purpose remain able to enhance insulin output in experimental models of cell glucotoxicity, even when the pancreas is exposed to a high concentration of glucose (Leclercq-Meyer and Malaisse, 1994).

Potentially unfavourable aspects of this novel therapeutical approach should not be ignored, however. They include the undesirable generation of methanol by intracellular hydrolysis of the methyl esters of these carboxylic metabolites and the extrapancreatic nutritional value of the esters, e.g., as hepatic gluconeogenic precursors (Malaisse, 1995; Zhang et al., 1994).

In recent reports, we have indicated that the first of these two objections can be overcome, since several esters of succinic acid not susceptible to generate methanol by deesterification remain potent insulinotropic agents (Björkling et al., 1996; Garcia-Martinez et al., 1997; Malaisse et al., 1995; Zhang et al., 1995). The present study now reveals that some of these novel esters are able to potentiate both the insulinotropic and hypoglycemic actions of the hypoglycemic sulfonylurea gliquidone.

2. Materials and methods

2.1. Materials

The esters used in this study were synthesized, and their purity assessed, by methods similar to those described elsewhere (Malaisse et al., 1994, 1995).

The succinic acid esters (monoethyl ester, monopropyl ester and monoisopropyl ester) were dissolved in saline (pH 7.0–7.5) at an 0.8 M concentration. Gliquidone (80

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PII S0014-2999(97)00099-X

μM) was dissolved in a bicarbonate-buffered medium containing dimethyl sulfoxide (0.6%, v/v).

2.2. Animals

Fed male Wistar rats (FJD inbred, Madrid, Spain) were anaesthetized with pentobarbital administered intraperitoneally (60 μg/g body weight; Pentothal, Abbott, Madrid, Spain). The body weight of the rats averaged 201 ± 5 g ($n = 23$). At time zero, the rats were injected through a catheter placed in a femoral vein over 30 s with a solution of gliquidone alone or in combination with one of the succinic acid esters. The dose administered corresponded to 2 μmol/g body weight for succinic acid esters and 0.2 nmol/g body weight for gliquidone.

Blood samples (0.5 ml) were collected, from a catheter placed in a carotid artery, before and at time intervals after administration of the agents, and were replaced by an equal volume of 155 mM NaCl through the catheter placed in a femoral vein.

The plasma concentrations of glucose (Bergmeyer and Berndt, 1974) and insulin (Valverde et al., 1988) were measured as described in the cited references.

2.3. Statistical analysis

All results are given as means ± S.E.M. together with the number of individual observations (n). The statistical significance of differences between mean values was assessed by Student's t-test.

3. Results

The effect of gliquidone, administered in increasing amounts (0.2–2.0 nmol/g body weight), was first examined. In all cases, the sulfonylurea caused stimulation of insulin release and lowering of glycemia (Fig. 1). The initial increment in plasma insulin concentration, observed at the 2nd min after gliquidone administration, displayed a comparable magnitude with distinct amounts of sulfonylurea (Fig. 1, upper panels). However, at later times, the insulinemia was obviously related to the dose of gliquidone, as documented by the dose–action relationship for the integrated incremental insulin response from min 0 to 30 (Fig. 2, left panel). Likewise, the dependency of the hypoglycemic action of gliquidone to the amount of sulfonylurea injected intravenously (Fig. 2, right panel) reflected differences in plasma glucose concentration during the late (min 10 to 30) rather than early (min 0 to 5) period after drug administration (Fig. 1, lower panels).

The three succinic acid esters tested in this study significantly increased the secretory response to gliquidone (Fig. 3). For instance, at the 2nd min of the test, the increment in plasma insulin concentration above paired basal value averaged 17.5 ± 1.3, 13.3 ± 1.6 and 16.8 ± 2.0 ng/ml

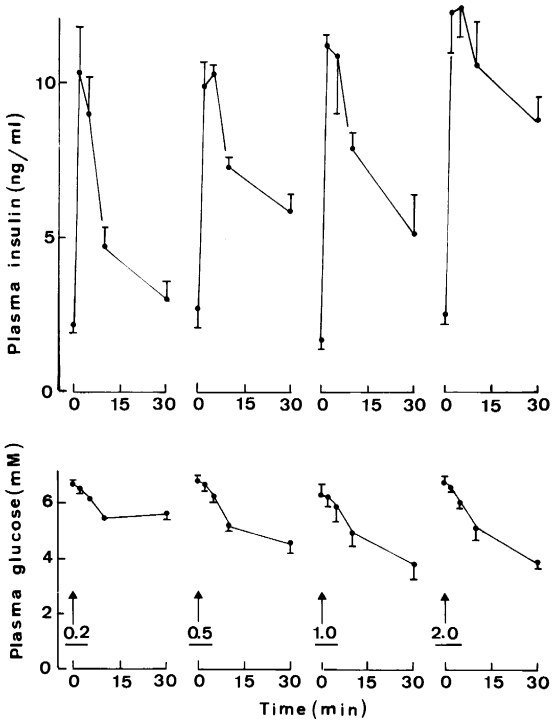

Fig. 1. Time-course for the changes in plasma insulin and glucose concentrations in response to the intravenous administration of increasing amounts of gliquidone (from left to right: 0.2, 0.5, 1.0 and 2.0 nmol/g body weight). Mean values (±S.E.M.) refer to 3–5 individual experiments.

($n = 6$ in all cases) when gliquidone was administered together with the monopropyl, monoethyl and monoisopropyl ester, respectively, as distinct ($P < 0.05$ or less) from only 8.2 ± 1.2 ng/ml ($n = 5$) after injection of gliquidone alone. In a parallel series of experiments, the increments recorded after injection of the esters alone averaged 8.0 ± 0.5 ($n = 6$), 4.6 ± 0.3 ($n = 10$) and 4.7 ± 0.5 ($n = 6$) ng/ml in the case of the monopropyl, mo-

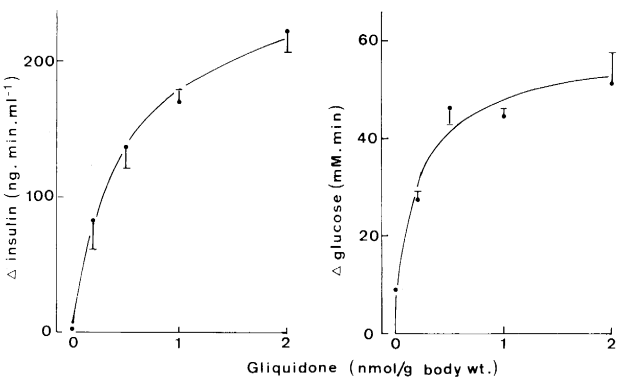

Fig. 2. Dose–action relationship for the insulinotropic and hypoglycemic actions of gliquidone. Mean values (±S.E.M.) for the integrated increment in plasma insulin concentration and decrement in plasma glucose concentration over 30 min of observation refer to 3–5 individual experiments. The data for the zero abscissa refer to the administration of the gliquidone solvent in the absence of the hypoglycemic sulfonylurea.

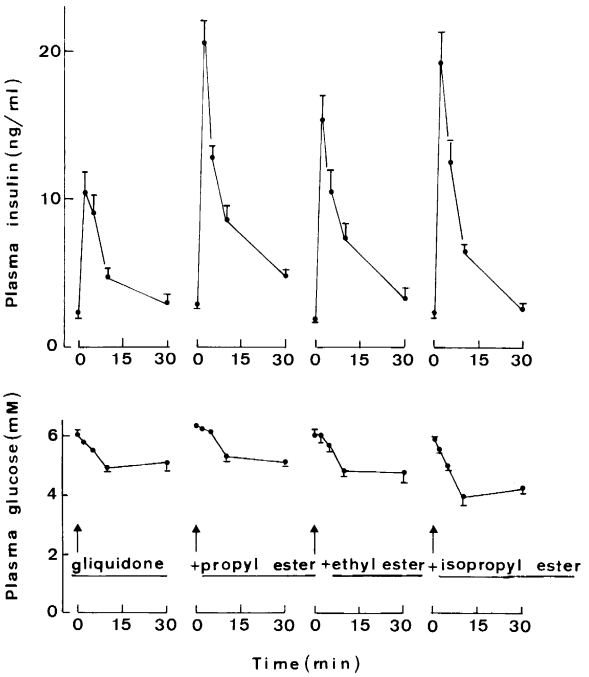

Fig. 3. Time-course for the changes in plasma insulin and glucose concentrations in response to the intravenous administration of gliquidone (0.2 nmol/g body weight) alone or together with the monopropyl ester, monoethyl ester or monoisopropyl ester of succinic acid (2.0 μmol/g body weight). Mean values (±S.E.M.) refer to 5–6 individual experiments.

noethyl and monoisopropyl ester, respectively. The comparison of the data recorded in the presence and absence of gliquidone thus indicates that the succinic acid esters and the hypoglycemic sulfonylurea acted in an additive manner upon insulin secretion. Indeed, the mean increments in plasma insulin concentration caused by gliquidone and the esters, when injected together, were not significantly different ($P > 0.09$ or more) from the sum of the increments attributable to the separate administration of each of these secretagogues.

The hypoglycemic action of gliquidone was not significantly affected by either the monopropyl or monoethyl ester of succinic acid, but almost doubled ($P < 0.005$) by the monoisopropyl ester (Table 1). Although there was no significant difference in the secretory response evoked by gliquidone, when administered together with either the monopropyl or monoisopropyl ester of succinic acid, the decremental glucose response was significantly more pronounced ($P < 0.005$) with the latter, rather than former, ester. When the esters of succinic acid were injected without gliquidone, no significant fall in plasma glucose concentration was observed when comparing the measurements made between the 2nd and 30th min of the test to the paired initial value, except in the case of the monoisopropyl ester, which transiently decreased the glucose concentration by 0.99 ± 0.14 mM ($n = 6$; $P < 0.001$) at the 10th min of the test.

Table 1
Effect of succinic acid esters upon the incremental (min 0 to 30) plasma insulin and glucose response to gliquidone (0.2 nmol/g body weight)

Succinic acid ester (2.0 μmol/g)	ΔInsulin (ng·min/ml)	ΔGlucose (mM·min)
Nil	81.4 ± 20.3 (5)	27.4 ± 1.7 (5)
Monopropyl	168.6 ± 14.1 (6) [b]	27.0 ± 3.5 (6)
Monoethyl	150.6 ± 21.6 (6) [a]	31.1 ± 7.5 (6)
Monoisopropyl	134.2 ± 13.6 (6)	50.6 ± 4.3 (6) [c]

[a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.005$, as compared to the response to gliquidone alone (first line).

4. Discussion

The present study affords three pieces of information. First, it indicates that, after intravenous injection, the dose dependency for the insulinotropic and hypoglycemic actions of gliquidone concerns the changes in plasma insulin and glucose concentration observed beyond the 5th min after administration of the sulfonylurea, rather than the early secretory and metabolic responses.

Second, it extends to three novel esters of succinic acid, that are devoid of the risk of generating methanol by intracellular hydrolysis, the knowledge that these non-glucidic nutrients potentiate the cell secretory response to antidiabetic agents such as hypoglycemic sulfonylureas (Leclercq-Meyer and Malaisse, 1994; Malaisse et al., 1993; Vicent et al., 1993, 1995), meglitinide analogs (Bakkali Nadi et al., 1994, 1996; Garcia-Martinez et al., 1995) or glucagon-like peptide 1 (Leclercq-Meyer and Malaisse, 1996a,b). The dose of 2 μmol/g body weight used for the esters tested in the present work was identical to that found, in a recent study (Garcia-Martinez et al., 1997), to cause stimulation of insulin release in the absence of any other exogenous insulinotropic agents. At variance with the situation found in response to increasing amounts of gliquidone, the enhancing action of succinic acid esters upon the insulinotropic effect of the hypoglycemic sulfonylurea was as or more pronounced during the early as/than late phase of B-cell stimulation.

These findings reinforce the idea that the potential use of non-glucidic nutrients as insulinotropic agents in the treatment of non-insulin-dependent diabetes should be considered in the light of their capacity to improve the insulinotropic potential of hypoglycemic agents in the diseased B-cell, rather than in the sole perspective of the endocrine pancreatic response to the nutrient itself, when administered alone.

Last, and most importantly, the present work reveals that distinct esters, that exert a comparable potentiating effect upon the insulinotropic action of hypoglycemic sulfonylurea, may affect to a vastly different extent the hypoglycemic efficiency of the antidiabetic agent. In a previous study (Vicent et al., 1993), we were unable to

detect any significant effect of the monomethyl and dimethyl esters of succinic acid upon plasma glucagon concentration. It seems most likely, therefore, that the hypoglycemic action of the monoisopropyl ester observed when injected alone or together with gliquidone reflects a lesser stimulation of hepatic gluconeogenesis than that presumably caused by the monopropyl or monoethyl ester of succinic acid.

In conclusion, our results reveal that it is possible to design esters of succinic acid which potentiate the hypoglycemic action of antidiabetic agents. Thus, the extrapancreatic metabolism of succinic acid esters, e.g., their role as gluconeogenic precursor in the liver, is not an universal obstacle to their use in a therapeutic perspective.

Acknowledgements

This work was supported by grants from the Fondo de Investigaciones Sanitarias (FIS, 96/1383), the Dirección General de Investigaciones Científica y Técnica (DGICYT, 95/0048), a Concerted Research Action of the French Community of Belgium (94/99-183), and the Belgian Foundation for Scientific Medical Research (3.4513.94). We are grateful to M. Valverde for technical assistance, and to C. Demesmaeker for secretarial help.

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