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Synergistic Insulinotropic Action of Succinate, Acetate, and Glucose Esters in Islets from Normal and Diabetic Rats

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Succinic acid esters are currently under investigation as possible insulinotropic tools in the treatment of noninsulin-dependent diabetes mellitus. The present article introduces three novel nutrient esters and aims mainly to explore, in both normal and GK rats, the secretory response to such esters when tested alone or in combination. It documents that in pancreatic islets from normal rats, methyl acetate (10 m*M*), which fails to augment basal insulin output, potentiates the secretory response to succinate dimethyl ester (also 10 m*M*). It also reveals that α-D-glucose pentaacetate (αGPA) (1.7 m*M*) stimulates insulin release in the absence of any other exogenous nutrient and even more so in the presence of succinate methyl ester. Moreover, the methyl esters of succinic acid (10 m*M*), when used together with either methyl acetate or αGPA, provoked insulin secretion in islets from diabetic GK rats incubated in the absence of D-glucose, although no significant secretory response of such islets could be detected when each of these agents was tested separately. These findings thus draw attention to the insulinotropic potential in type 2 diabetes of selected combinations of nutrient esters, including a D-glucose ester presumably able to enter into islet cells without requiring the intervention of a hexose carrier.

Key Words: Nutrient esters; pancreatic islets; insulin release; GK rats.

Introduction

A few years ago, the proposal was first formulated that selected esters of carboxylic metabolites, which are intermediates of the Krebs cycle or their precursors, may represent useful insulinotropic tools for the treatment of noninsulin-dependent diabetes mellitus *(1,2)*. This novel therapeutic approach offers the advantages to allow stimulation of both proinsulin biosynthesis and insulin release, and to bypass site-specific defects of D-glucose transport and metabolism in the diseased B-cell *(3,4)*.

Several potential limitations of such an approach, enumerated elsewhere *(5)*, were recently circumvented. First, some of the esters were found to stimulate insulin release even when administered enterally or intraduodenally *(6–8)*. Second, several esters of succinic acid that are not susceptible to lead to the undesirable generation of methanol by intracellular hydrolysis displayed an insulinotropic potential comparable to that of the methyl esters used in our pilot experiments *(9–12)*. Third, in our most recent work, esters were developed that stimulate insulin release when tested in the micromolar range *(13)*, thus avoiding the need to administer a large amount of these exogenous nutrients. Last, since the latter esters increase plasma insulin concentration in vivo when given in amounts not exceeding 1 μmol/g body wt, their possible use as gluconeogenic precursor in the liver *(14)* becomes a quite negligible issue.

The aims of the present article are to introduce novel esters of D-glucose and acetic acid that could act as insulinotropic agents in synergism with the esters of succinic acid, and to compare the secretory response to such associations of esters in islets from normal and diabetic rats. The selection of the new esters used in this work was motivated by the following considerations. The pentaacetate ester of D-glucose was recently proposed as a tool to supply the sugar to cells by a process that does apparently not involve the carrier-mediated transport of the hexose across the plasma membrane *(15)*. The methyl and ethyl esters of acetate were used as controls to assess the possible role of the acetyl moiety of α-D-glucose pentaacetate in its insulinotropic action.

Results

Metabolic and Hormonal Status of Control and GK Rats

As indicated in Table 1, the GK rats were slightly heavier ($P < 0.001$) than the control animals, with a mean GK/control ratio of 116.9 ± 4.4% (degree of freedom [df] = 36). The plasma glucose and insulin concentrations were also higher in GK than control rats, with mean GK/control ratios of 167.4 ± 9.0% (df = 34; $P < 0.001$) and 149.2 ± 21.9% (df = 34; $P < 0.05$),

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Table 1
Metabolic and Hormonal Status of Control and GK Rats

Experiments	First series		Second series	
Rats	Control	GK	Control	GK
Body weight (g)	205 ± 3 (16)	240 ± 11 (16)	208 ± 5 (4)	244 ± 1 (4)
Plasma glucose (m*M*)	8.99 ± 0.22 (15)	15.33 ± 0.96 (15)	8.34 ± 0.16 (4)	12.88 ± 0.82 (4)
Plasma insulin (μU/mL)	16.1 ± 2.9 (15)	25.1 ± 3.3 (15)	22.4 ± 2.3 (4)	27.3 ± 1.7 (4)
Plasma insulin/glucose ratio (U/mol)	1.87 ± 0.35 (15)	1.59 ± 0.14 (15)	2.67 ± 0.23 (4)	2.15 ± 0.22 (4)

Table 2
Effects of Nutrient Secretagogs on Insulin Output in Islets from Control and GK Rats

Expt., nr.	Line, nr.	Nutrients, m*M*	Insulin output, μU/islet/90 min		*p*
			Control rats	GK rats	
1	1	Nil	21.8 ± 2.4 (42)	28.3 ± 3.4 (38)	> 0.1
	2	D-Glucose (8.3)	65.5 ± 2.5 (28)	40.0 ± 2.9 (8)	< 0.001
	3	D-Glucose (16.7)	160.0 ± 9.3 (40)	67.5 ± 8.6 (40)	< 0.001
	4	SAM (10.0)	70.5 ± 1.9 (53)	27.0 ± 2.8 (39)	< 0.001
	5	α-D-glucose pentaacetate (1.7)	51.2 ± 2.7 (52)	34.5 ± 4.4 (36)	< 0.001
	6	SAM (10.0) + α-D-glucose pentaacetate (1.7)	111.1 ± 3.4 (53)	48.2 ± 4.9 (39)	< 0.001
2	7	Nil	10.3 ± 1.1 (27)	20.8 ± 4.8 (8)	< 0.005
	8	SAD (10.0)	26.5 ± 2.0 (28)	22.8 ± 2.8 (8)	> 0.3
	9	Acetate methyl ester (10.0)	11.4 ± 3.5 (17)	24.1 ± 3.3 (7)	< 0.05
	10	Acetate ethyl ester (10.0)	13.3 ± 2.4 (16)	23.9 ± 4.6 (8)	< 0.05
	11	SAD (10.0) + acetate methyl ester (10.0)	46.8 ± 3.5 (18)	32.3 ± 3.0 (8)	< 0.02
	12	SAD (10.0) + acetate ethyl ester (10.0)	29.8 ± 1.6 (18)	21.7 ± 1.5 (8)	< 0.005

respectively. The paired plasma insulin/glucose ratio was somewhat lower, although not significantly so ($P > 0.3$), in GK rats than in control animals, the GK/control ratio averaging 84.1 ± 16.3% (df = 34).

In the first series of experiments, the insulin content of the islets averaged 1.58 ± 0.07 mU/islet ($n = 25$) in control rats, as distinct ($P < 0.01$) from 1.23 ± 0.08 μU/islet ($n = 23$) in GK rats. The islet insulin content was not measured in the second experimental series.

Secretory Data

The basal release of insulin measured in the absence of exogenous nutrient was significantly higher in islets from GK rats than in those prepared from control animals. Pooling all available data, it averaged 17.3 ± 1.6 ($n = 69$) and 27.0 ± 3.0 ($n = 46$) μU/islet/90 min ($P < 0.005$) in control and GK rats, respectively. When considering only those measurements made within the same experiment(s), the difference in basal insulin release between GK and control rats amounted to 8.5 ± 3.4 μU/islet/90 min (df = 63; $P < 0.02$).

D-Glucose, tested at concentrations of 8.3 and 16.7 m*M*, augmented ($P < 0.005$ or less) insulin release above basal value. The secretory rates recorded in the presence of the hexose were lower in GK rats than in control animals (Table 2, lines 2 and 3). As judged from measurements made within each individual experiment, the glucose-induced increment in insulin output averaged, at 8.3 and 16.7 m*M*, respectively, 54.8 ± 2.3 (df = 49) and 134.2 ± 8.2 (df = 70) μU/islet/90 min in control rats, as distinct ($P < 0.001$) from 19.2 ± 4.9 (df = 14) and 39.6 ± 8.1 (df = 70) μU/islet/90 min in GK rats. The secretory response to the hexose in GK rats thus represented no more than 31.2 ± 4.7 % (df = 203; $P < 0.001$) of that found in control animals.

Further experiments were designed to compare the effects of several esters on insulin release in the normal and diabetic animals.

In normal rats, all esters or combination of esters augmented insulin release above basal value ($P < 0.001$ in all cases), except the methyl and ethyl esters of acetic acid, which, when tested alone at a concentration of 10 m*M*, failed to do so (Table 2, lines 4–6 and 8–12).

The increase in secretory rate attributable to α-D-glucose pentaacetate was higher ($P < 0.02$) in the presence of succinic acid monomethl ester (SAM) (40.7 ± 3.0 μU/islet/90 min; df = 96) than in its absence (30.0 ± 2.9 μU/islet/90 min; df = 93). Likewise, the methyl ester of acetic acid significantly augmented insulin release ($P < 0.001$) in the presence of SAD, but failed to do so ($P > 0.6$) in the absence of the succinate ester (Fig. 1). Ethyl acetate, however, did not affect insulin release significantly ($P > 0.1$ or more), whether in the absence or presence of succinic acid dimethyl ester (SAD).

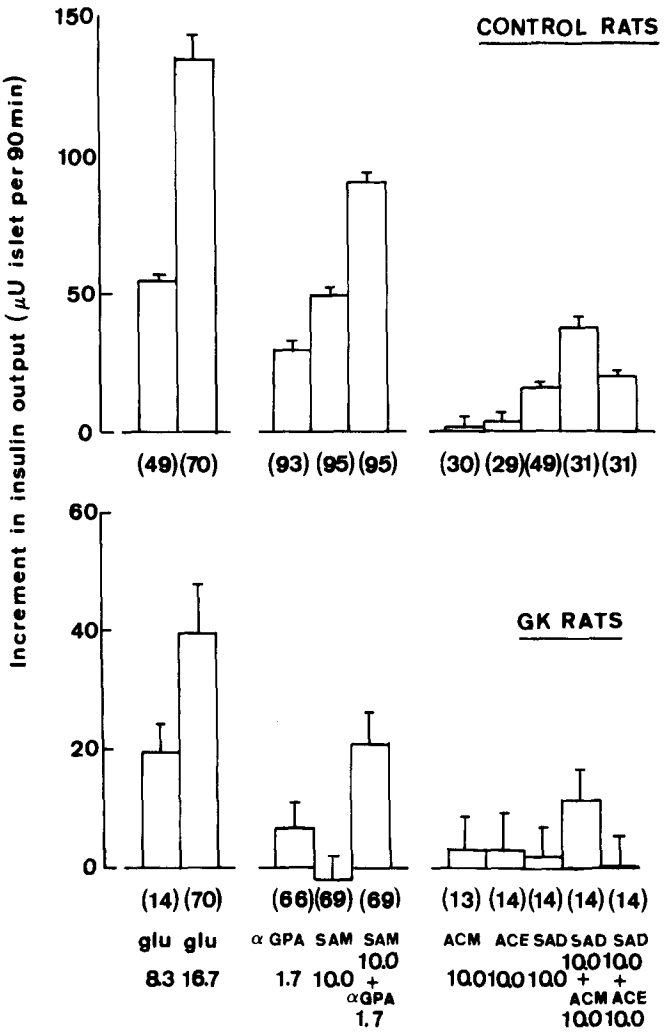

Fig. 1. Increment in insulin output above basal value evoked by D-glucose (glu), SAM or SAD, αGPA, and acetic acid methyl ester (ACM) or ethyl ester (ACE) in islets from control animals (upper panel) and GK rats (lower panel). Mean values (±SEM) refer to the degree of freedom indicated in parentheses below each column. Note the difference in scale of the ordinates in the two panels. The concentration of each nutrient is indicated as m*M*.

A somewhat different situation prevailed in islets from GK rats. When tested separately, neither the monomethyl and dimethyl esters of succinic acid, nor the methyl and ethyl esters of acetic acid were able to augment significantly insulin release above basal value (Table 2, lines 4 and 8–10). Even α-D-glucose pentaacetate failed to increase insulin release significantly, the mean increment in insulin output attributable to the hexose ester not exceeding 6.8 ± 4.5 μU/islet/90 min (df = 66; $P > 0.1$). A significant positive secretory response was only recorded in the simultaneous presence of α-D-glucose pentaacetate and SAM or methyl acetate and SAD, in which cases the increment in insulin output averaged, respectively, 20.9 ± 5.0 (df = 69; $P < 0.001$) and 11.5 ± 5.0 (df = 14; $P < 0.04$) μU/islet/90 min. The latter two values remained significantly lower, however, than the corresponding increments observed in islets from control rats (Fig. 2).

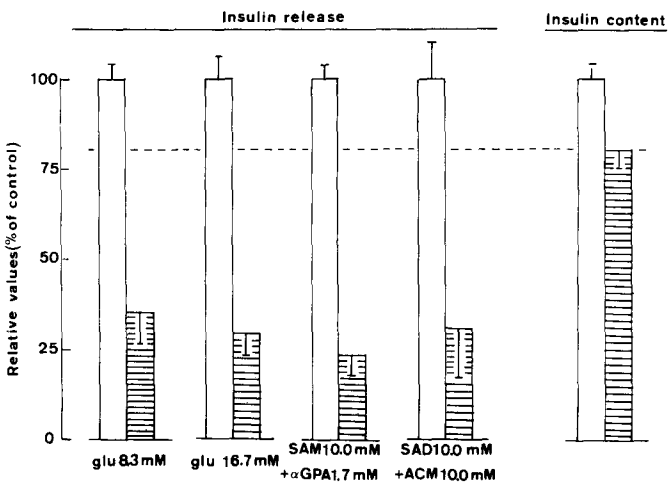

Fig. 2. Mean values (±SEM) for the increment in insulin output above basal value evoked by glu, SAM or SAD, αGPA, and ACM, and for the insulin content of pancreatic islets in control (open columns) and GK (shaded columns) rats are expressed in percent of the mean corresponding control value. The horizontal dashed line refers to the mean insulin content of islets from GK rats, and documents the lack of proportionality between insulin release and content in such animals.

In the GK rats, like in control animals, the increment in insulin output provoked by α-D-glucose pentaacetate was greater ($P < 0.02$) in the presence of SAM (22.0 ± 5.1 μU/islet/90 min; df = 70) than in its absence (6.8 ± 4.5 μU/islet/90 min; df = 66). Likewise, methyl acetate, but not ethyl acetate, augmented significantly ($P < 0.05$) the release of insulin in islets from GK rats exposed to SAD while failing to affect ($P > 0.5$) basal insulin output.

It should be stressed that in the present series of experiments, the increment in insulin output evoked by nutrient secretagogs in islets from GK rats, when expressed relative to that found in control animals, was always lower than the GK/control ratio for the insulin content of the islets (80.6 ± 6.3%; df = 46), suggesting that the impairment of the secretory response could not be attributed solely to a decrease in hormonal stores (Fig. 2).

Discussion

The present study introduces α-D-glucose pentaacetate as a stimulus for insulin release in islets from both normal and diabetic rats. The ester was here tested at a concentration of 1.7 m*M* for two reasons. First, this concentration is close to the limit of solubility of the ester. Second, at lower concentrations (0.9 m*M* or less), α-D-glucose pentaacetate fails to stimulate insulin release in islets from normal rats incubated in the absence of any other exogenous nutrient. The experiments dealing with the insulinotropic action of α-D-glucose pentaacetate and/or other esters were indeed purposely conducted in the absence of D-glucose in order to avoid interference of the modulating action of the hexose on the secretory response to these esters *(1)*. Such a modu-

lating role is likely to be different in normal and GK rats owing to the altered responsiveness of the B-cell to D-glucose in the latter animals. By avoiding the above-mentioned interference, the insulinotropic efficiency of the esters themselves could be adequately assessed and compared in control and diabetic rats.

Our results suggest that the insulinotropic action of α-D-glucose pentaacetate at the concentration of 1.7 m*M* used in the present study is attributable, in part at least, to the D-glucose moiety of the ester. Indeed, when either methyl acetate or ethyl acetate was tested at a concentration (10 m*M*) in excess of that of the acetate moiety of the hexose ester (8.5 m*M*), no stimulation of insulin release could be detected, at least in the absence of another exogenous nutrient. However, methyl acetate augmented significantly SAD-stimulated insulin secretion, suggesting that this acetate ester is able to enter into islet cells, and undergo intracellular hydrolysis and further metabolism. The concept that the intracellular generation of D-glucose participates in the secretory response to α-D-glucose pentaacetate is further supported by the recent finding that β-D-galactose pentaacetate does not stimulate insulin release *(15)*.

In both normal and diabetic rats, the increment in insulin output attributable to α-D-glucose pentaacetate was more marked in the presence of SAM than in its absence. This situation could merely reflect the well-known threshold phenomenon for nutrient-stimulated insulin release as a function of O_2 uptake and ATP generation *(16)*.

Likewise, the absence of a sizable secretory response to methyl acetate, when tested alone, and its efficient enhancing action on SAD-induced insulin secretion could conceivably indicate that the increase in O_2 consumption attributable to the catabolism of the acetate ester is not sufficient to reach the threshold value required for stimulation of insulin release. However, in the simultaneous presence of methyl acetate and SAD, the stimulation of insulin release may also involve a reciprocal action of the two nutrients on their respective catabolism.

Whatever the intimate mode of action of these esters in islet cells, the present findings indicate that they may act synergistically on insulin secretion. For instance, in islets of GK rats, none of the esters used in the present study increased insulin output above basal value to any significant extent, whereas the association of α-D-glucose pentaacetate and SAM or that of methyl acetate and SAD both caused obvious stimulation of insulin release.

In conclusion, therefore, the present study draws attention both to the potential use of novel nutrient esters, other than the esters of succinic acid, as possible tools for stimulation of insulin release in type 2 diabetes and, in the same perspective, to the advantage that could result from the administration of selected combinations of these insulinotropic agents.

Materials and Methods

The monomethyl ester of succinic acid ($CH_3OCOCH_2CH_2CO_2H$; SAM), its dimethyl ester ($CH_3OCOCH_2CH_2COOCH_3$; SAD), and 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (α-D-glucose pentaacetate) were obtained from Sigma (St. Louis, MO). The methyl ester of acetic acid (CH_3OCOCH_3) and its ethyl ester ($CH_3CH_2OCOCH_3$) were synthetized by a method adapted from that proposed by Vogel *(17)*.

Twenty male and female GK rats, 28.9 ± 1.7-wk-old, from our colony, were given free access to food up to the time of killing. Normal Wistar rats of grossly comparable body weight were used as control animals.

The animals were weighed and then decapitated. Blood was collected in heparinized tubes for the measurement of plasma glucose by the glucose oxidase method *(18)* and plasma insulin by radioimmunoassay *(19)*.

In each experiment, pancreatic islets were isolated by the collagenase method *(20)* from three to four rats in the same group. The methods used to measure the insulin content of the islets and the secretion of insulin over 90 min of incubation at 37°C were identical to those described elsewhere *(20)*.

Two separate series of experiments were conducted dealing, respectively, with the islet secretory response to D-glucose, SAM, and α-D-glucose pentaacetate (first series), and to SAD, methyl acetate, and ethyl acetate (second series).

The experimental procedure used in this study was approved by the commission éthique du bien-être animal of our faculty.

All results are expressed as mean values (±SEM) together with either the number of individual observations *(n)* or degree of freedom (df). The statistical significance of differences between mean values was assessed by use of Student's *t*-test.

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References

1. Malaisse, W. J., Rasschaert, J., Villanueva-Peñacarrillo, M. L., and Valverde, I. (1993). *Am. J. Physiol.* **264,** E428–E433.
2. Malaisse, W. J. and Sener, A. (1993). *Am. J. Physiol.* **264,** E434–E440.
3. Malaisse, W. J. (1993). *Endocrinologia* **40,** 309–313.
4. Malaisse, W. J. (1994). *Diabetologia* **37(Suppl. 2),** S36–S42.
5. Malaisse, W. J. (1995). *Gen. Pharmacol.* **26,** 1133–1141.
6. Garcia-Martinez, J. A., Villanueva-Peñacarrillo, M. L., Valverde, I., and Malaisse, W. J. (1995). *Arch. Int. Pharmacodyn.* **330,** 116–124.

7. Malaisse-Lagae, F., Bakkali Nadi, A., and Malaisse, W. J. (1994). *Arch. Int. Pharmacodynamie* **328,** 235–242.
8. Malaisse-Lagae, F., Zhang, T.-M., Bakkali Nadi, A., and Malaisse, W. J. (1994). *Med. Sci. Res.* **22,** 365–367.
9. Björkling, F., Malaisse-Lagae, F., and Malaisse, W. J. (1996). *Pharmacol. Res.* **33,** 273–275.
10. Garcia-Martinez, J. A., Zhang, T.-M., Villanueva-Peñacarrillo, M. L., Valverde, I., Björkling, F., and Malaisse, W. J. (1996). *Diabetologia* **39(Suppl. 1),** A125 (abstract).
11. Malaisse, W. J., Blaehr, L., and Björkling, F. (1995). *Med. Sci. Res.* **23,** 9,10.
12. Zhang, T.-M., Björkling, F., and Malaisse, W. J. (1995). *Horm. Metab. Res.* **27,** 251–252.
13. Malaisse, W. J., Ladrière, L., Garcia-Martinez, J. A., Viñambres, C., Villanueva-Peñacarrillo, M. L., Valverde, I., and Björkling, F. (1997). *Diabetologia* **40(Suppl. 1),** AIII (abstract).
14. Zhang, T.-M., Sener, A., and Malaisse, W. J. (1994). *Arch. Biochem. Biophys.* **314,** 186–192.
15. Malaisse, W. J., Jijakli, H., Kadiata, M., Sener, A., and Kirk, O. (1997). *Biochem. Biophys. Res. Commun.* **231,** 435,436.
16. Hutton, J. C. and Malaisse, W. J. (1980). *Diabetologia* **18,** 395–405.
17. Vogel, A. I. (1956). *A Textbook of Practical Organic Chemistry,* 3rd ed. Langmans, Green: London, p. 383.
18. Bergmeyer, H. U. and Berndt, E. (1974). In: *Methods of Enzymatic Analysis.* Bergmeyer, H. U. (ed.). Academic: New York, pp. 1205–1215.
19. Leclercq-Meyer, V., Marchand, J., Woussen-Colle, M.-C., Giroix, M.-H., and Malaisse, W. J. (1985). *Endocrinology* **116,** 1168–1174.
20. Malaisse-Lagae, F. and Malaisse, W. J. (1984). *Methods in Diabetes Research,* vol. 1. Larner, J. and Pohl, S. L. (eds.). Wiley: New York, pp. 147–152.