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Mechanism of 3-phenylpyruvate-induced insulin release

Secretory, ionic and oxidative aspects

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1. 3-Phenylpyruvate caused a dose-related stimulation of insulin release from rat pancreatic islets deprived of exogenous nutrient or incubated in the presence of 5.6 or 8.3 mM-D-glucose. 2. 3-Phenylpyruvate inhibited insulin release evoked by high concentrations of D-glucose (16.7 or 27.8 mM) or 4-methyl-2-oxopentanoate (10.0 mM). This inhibitory effect appeared to be attributable to impairment of 2-oxo-acid transport into the mitochondria, with resulting inhibition of D-glucose, pyruvate or 4-methyl-2-oxopentanoate oxidation. 3. 3-Phenylpyruvate failed to affect the oxidation of, and secretory response to, L-leucine, and did not augment insulin release evoked by a non-metabolized analogue of the latter amino acid. 4. L-Glutamine augmented 3-phenylpyruvate-induced insulin release. The release of insulin evoked by the combination of 3-phenylpyruvate and L-glutamine represented a sustained phenomenon, abolished in the absence of extracellular Ca^{2+} or the presence of menadione and potentiated by theophylline. 5. Whether in the presence or in the absence of L-glutamine, the secretory response to 3-phenylpyruvate coincided with an increase in O_2 uptake, a decrease in K^+ conductance, a stimulation of both Ca^{2+} inflow and $^{45}Ca^{2+}$ net uptake and an increase in cyclic AMP content. 6. It is concluded that the release of insulin induced by 3-phenylpyruvate displays features classically encountered when the B-cell is stimulated by nutrient secretagogues, and is indeed attributable to an increase in nutrient catabolism.

Panten & Langer (1981) recently reported that 3-phenylpyruvate evokes insulin release from mouse pancreatic islets. Being unable to reveal a mechanism by which 3-phenylpyruvate could increase catabolic fluxes in islet cells, they proposed that a specific B-cell membrane receptor acts as mediator of the insulin-releasing capacity of 3-phenylpyruvate.

A simple metabolic explanation for the insulin-releasing capacity of 3-phenylpyruvate could, however, be visualized. Thus, 3-phenylpyruvate could be converted into phenylalanine by transamination with endogenous amino acids. The 2-oxo-acids derived from the endogenous amino acids would then be further utilized so that the release of insulin evoked by 3-phenylpyruvate would indeed coincide with an increase in catabolic fluxes in islet cells. This hypothesis is supported by a number of experimental data in the present and the following paper (Malaisse *et al.*, 1983). The results of the present study indicate that the secretory, ionic and oxidative responses of rat pancreatic islets to 3-phenylpyruvate are indeed compatible with the view that this 2-oxo acid stimulates islet metabolism. The nature of the metabolic events induced by 3-phenylpyruvate in islet cells are analysed in the following paper (Malaisse *et al.*, 1983).

Abbreviation used: BCH, 2-*endo*-aminonorbornane-2-carboxylic acid.

Materials and methods

All experiments were performed with islets isolated by collagenase digestion of pancreases removed from fed rats (Lacy & Kostianovsky, 1967). The methods used to measure insulin release from incubated (Malaisse *et al.*, 1970) or perifused

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(Herchuelz & Malaisse, 1978) islets, the fractional outflow rate of $^{45}Ca^{2+}$ (Herchuelz *et al.*, 1980*b*) and $^{86}Rb^{+}$ (Carpinelli & Malaisse, 1980) from prelabelled and perifused islets, the net uptake of $^{45}Ca^{2+}$ by the islets (Malaisse-Lagae & Malaisse, 1971), the cyclic AMP content of the islets and incubation media (Valverde *et al.*, 1983) and the oxidation of exogenous nutrients (Carpinelli *et al.*, 1980) have all been described previously in the cited publications.

For measuring O_2 uptake, rat islets were cultured overnight in Parker's culture medium TC 199 containing 5.6mM-glucose and then placed in Cartesian divers in a Krebs–Ringer solution (Na^+, 143.5mM; K^+, 5.9mM; Mg^{2+}, 1.2mM; Ca^{2+}, 2.5mM; Cl^-, 144.3mM; SO_4^{2-}, 1.2mM; PO_4^{3-}, 1.2mM) containing 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (10mM; pH 7.4) and equilibrated against ambient air. The respiratory rate was recorded over two periods of 60min each before and after addition of 3-phenylpyruvate from a side drop, the medium used throughout incubation being either deprived of exogenous nutrient or containing 10mM-L-glutamine (Hellerström, 1967).

All results are expressed as means (±S.E.M.) together with the number of individual observations (given in parentheses). The statistical significance of differences between mean values was tested by using Student's *t* test.

Results

Secretory data

At a 10mM concentration, 3-phenylpyruvate significantly augmented insulin release from islets incubated for 90min in the absence of exogenous nutrient (Table 1). The secretory response to 3-phenylpyruvate was a rapid and reversible process (Fig. 1*c*). The increment in insulin output evoked by 3-phenylpyruvate was greater in the presence of D-glucose (5.6 or 8.3mM) or L-glutamine (2.0 or 10mM) than in the absence of exogenous nutrient (Tables 1 and 2; Fig. 1). In the presence of 5.6mM-D-glucose, the secretory response to 3-phenylpyruvate was dose-related in the 1.0–10mM range (Fig. 2*a*). In the presence of 10mM-L-glutamine, which itself failed to stimulate insulin release (Table 2), the stimulant action of 3-phenylpyruvate upon insulin release represented a sustained phenomenon whether judged from the dynamic pattern illustrated in Fig. 1 or the results of static experiments (Fig. 2*b*). In the presence of L-glutamine (10mM), the secretory response to 3-phenylpyruvate was abolished in the absence of extracellular Ca^{2+} (Table 2) or in the presence of menadione (25μM) and markedly augmented by theophylline (1.4mM), which itself fails to stimulate insulin release in the present system (Brisson *et al.*, 1972).

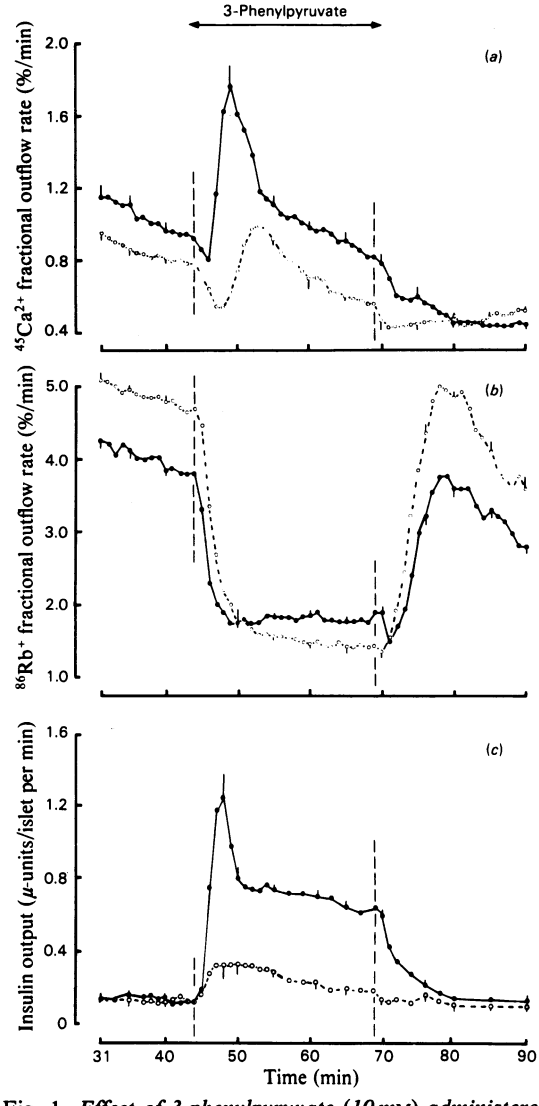

Fig. 1. *Effect of 3-phenylpyruvate (10mM) administered during the period indicated between the vertical broken lines upon $^{45}Ca^{2+}$ and $^{86}Rb^{+}$ fractional outflow rates and insulin output from perifused islets*

The perifusate circulated throughout the 90min was either deprived of exogenous nutrient (○) or contained 10mM-L-glutamine (●). Results are means ± S.E.M. and refer to four ($^{45}Ca^{2+}$ and $^{86}Rb^{+}$) or eight (insulin release) individual experiments.

In contrast with 3-phenylpyruvate, L-phenylalanine failed to stimulate insulin release in the absence of exogenous nutrient or in the presence of L-glutamine (Table 2). In good agreement with a prior observation (Sener *et al.*, 1981), L-phenylalanine augmented insulin output in the presence of 8.3mM-D-glucose, but this stimulatory effect faded out at a higher concentration of the sugar (Table 1).

Table 1. *Effect of 3-phenylpyruvate, L-phenylalanine and D-glucose upon insulin release*
Pancreatic islets were incubated for 90min in the sole presence of D-glucose or in the simultaneous presence of D-glucose and either 3-phenylpyruvate (10mM) or L-phenylalanine (10mM). Results are means ± S.E.M.

	Insulin output (μ-units/90min per islet)		
[D-Glucose] (mM)	D-Glucose alone	D-Glucose + 3-phenylpyruvate	D-Glucose + L-phenylalanine
Nil	10.8 ± 3.0 (45)	51.3 ± 3.8 (73)	14.3 ± 7.0 (9)
5.6	41.4 ± 10.0 (19)	153.9 ± 8.6 (19)	
8.3	126.2 ± 11.9 (18)	226.1 ± 16.0 (9)	190.0 ± 22.0 (9)
16.7	595.0 ± 26.3 (28)	267.2 ± 19.6 (19)	601.9 ± 29.3 (9)
27.8	668.8 ± 30.3 (10)	297.5 ± 14.3 (10)	

Table 2. *Effect of 3-phenylpyruvate, L-phenylalanine and L-glutamine upon insulin release*
Results are means ± S.E.M.

Nutrient(s)	Modifier	Insulin output (μ-units/90min per islet)
Nil		10.8 ± 3.0 (45)
L-Glutamine (10mM)		11.7 ± 2.0 (49)
3-Phenylpyruvate (10mM)		51.3 ± 3.8 (73)
L-Glutamine (2mM) + 3-phenylpyruvate (10mM)		116.8 ± 11.5 (17)
L-Glutamine (10mM) + 3-phenylpyruvate (10mM)		152.3 ± 7.4 (55)
L-Glutamine (10mM) + 3-phenylpyruvate (10mM)	No $CaCl_2$	12.1 ± 8.6 (10)
L-Glutamine (10mM) + 3-phenylpyruvate (10mM)	Menadione (25μM)	24.7 ± 9.4 (10)
L-Glutamine (10mM) + 3-phenylpyruvate (10mM)	Theophylline (1.4mM)	365.8 ± 23.5 (10)
L-Phenylalanine (10mM)		14.3 ± 7.0 (9)
L-Glutamine (10mM) + L-phenylalanine (10mM)		15.5 ± 8.3 (9)

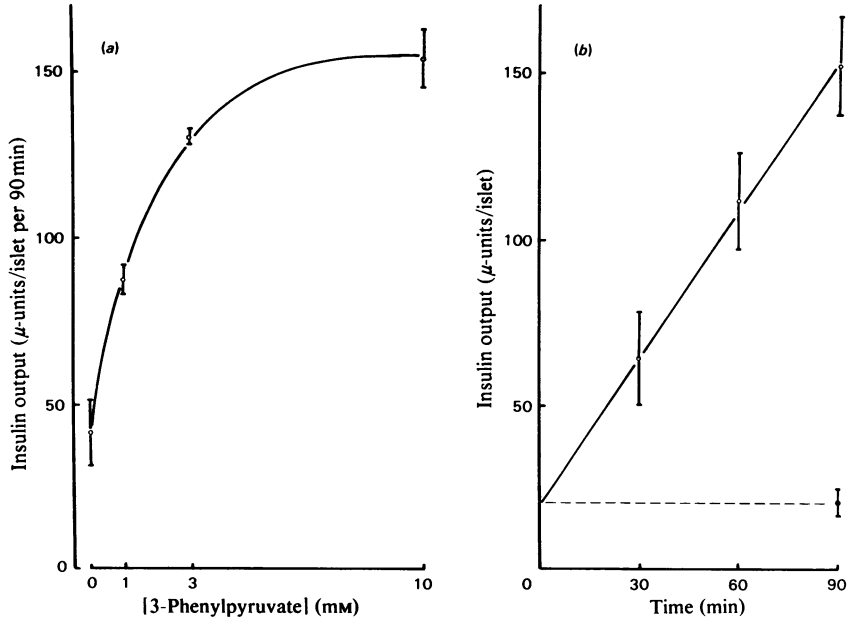

Fig. 2. *Effect of increasing concentrations of 3-phenylpyruvate upon insulin release in the presence of 5.6 mM-D-glucose (a) and time course for insulin release evoked by 3-phenylpyruvate (10 mM) in the presence of 10 mM-L-glutamine (b)*
In (*b*), the basal value for insulin release over 90min incubation is indicated by the horizontal broken line. Results are means ± S.E.M. indicated by the bars and refer to nine or ten individual observations.

Whereas 3-phenylpyruvate augmented insulin output in the presence of 5.6 or 8.3 mM-D-glucose (Table 1), it severely inhibited insulin release evoked by D-glucose in higher concentrations (16.7 and 27.8 mM). 3-Phenylpyruvate also inhibited insulin release evoked by 10 mM-4-methyl-2-oxopentanoate (Table 3). However, 3-phenylpyruvate failed to affect insulin release evoked by 10 mM-L-leucine or 20 mM-(±)-BCH (Table 3). In the absence of exogenous nutrient, the secretory response to 20 mM-(±)-BCH was significantly higher than that evoked by 10 mM-3-phenylpyruvate ($P<0.001$). Likewise, in the presence of L-glutamine (10 mM), the release of insulin evoked by 20 mM-(±)-BCH (312.8 ± 2.8 μ-units/90 min per islet; $n = 9$) largely exceeded that evoked by 10 mM-3-phenylpyruvate (152.3 ± 7.4 μ-units/90 min per islet; $n = 55$).

Ionic data

In static experiments, 3-phenylpyruvate significantly augmented $^{45}Ca^{2+}$ net uptake in the absence of exogenous nutrient and, even more so, in the presence of L-glutamine (Table 4). The relationship between $^{45}Ca^{2+}$ net uptake and insulin release displayed the same pattern as that repeatedly documented with other nutrient secretagogues and characterized by a threshold value for the stimulant action of Ca^{2+} upon insulin release (Malaisse-Lagae & Malaisse, 1971), there being a tight correlation between the mean values for $^{45}Ca^{2+}$ net uptake and insulin release respectively ($r = 0.9772$; $n = 4$; $P < 0.05$). 3-Phenylpyruvate augmented the cyclic AMP content of the islets and their surrounding incubation medium, as measured after 60 min incubation in the presence of 1.0 mM-3-isobutyl-1-methylxanthine (Table 4). The cyclic AMP content was further increased ($P<0.001$) in the simultaneous presence of 3-phenylpyruvate and L-glutamine. Incidentally, L-glutamine alone augmented the cyclic AMP content above the basal value. This confirms a prior observation (Valverde *et al.*, 1983) and is consistent with the knowledge that L-glutamine stimulates insulin release provided that the islets are incubated in the presence of a phosphodiesterase inhibitor (Sener & Malaisse, 1980).

In dynamic experiments, 3-phenylpyruvate caused an initial decrease and a later rise in the fractional outflow rate of $^{45}Ca^{2+}$ from prelabelled islets perifused in the absence or in the presence of L-glutamine (Fig. 1*a*). The mean value for $^{45}Ca^{2+}$ fractional outflow rate before 3-phenylpyruvate administration was slightly but not significantly higher in the presence than in the absence of L-glutamine. The 3-phenylpyruvate-induced initial deceleration in $^{45}Ca^{2+}$ fractional outflow rate was similar in both series of experiments, with a mean

Table 3. *Effect of 3-phenylpyruvate upon insulin release evoked by 4-methyl-2-oxopentanoate, L-leucine and (±)-BCH*
Pancreatic islets were incubated for 90 min in the sole presence of 4-methyl-2-oxopentanoate, L-leucine or (±)-BCH or in the simultaneous presence of one of these nutrients and 3-phenylpyruvate (10 mM). Results are means ± S.E.M. The statistical significance (P) of the 3-phenylpyruvate-induced changes in insulin output is indicated in each case.

	Insulin release (μ-units/90 min per islet)		
3-Phenylpyruvate ...	Nil	10 mM	P
4-Methyl-2-oxopentanoate (10 mM)	231.0 ± 12.7 (9)	120.2 ± 8.3 (9)	<0.001
L-Leucine (10 mM)	80.1 ± 12.0 (10)	75.9 ± 7.7 (10)	>0.7
(±)-BCH (20 mM)	83.7 ± 4.7 (18)	87.8 ± 7.0 (9)	>0.6

Table 4. *Effects of 3-phenylpyruvate and L-glutamine upon O_2 uptake, $^{45}Ca^{2+}$ net uptake and cyclic AMP content*
The statistical significance (*, $P<0.05$; †, $P<0.01$; ‡$P<0.001$) of the 3-phenylpyruvate-induced changes (second compared with first line and fourth compared with third line) in O_2 uptake was established by paired comparison. For $^{45}Ca^{2+}$ net uptake and cyclic AMP content, the statistical indices refer to mean differences from basal value (first line) as tested by group comparison. The cyclic AMP content of the islets and media was measured after 60 min incubation in the presence of 1.0 mM-3-isobutyl-1-methylxanthine.

	O_2 uptake (nl/60 min per islet)	$^{45}Ca^{2+}$ net uptake (pmol/90 min per islet)	Cyclic AMP (fmol/60 min per islet)
Nil	2.60 ± 0.48 (5)	0.84 ± 0.05 (12)	53.0 ± 4.1 (25)
3-Phenylpyruvate (10 mM)	3.15 ± 0.47 (5)*	2.12 ± 0.23 (12)‡	71.7 ± 5.5 (26)†
L-Glutamine (10 mM)	2.59 ± 0.16 (5)	1.15 ± 0.07 (12)†	88.1 ± 8.3 (26)‡
L-Glutamine (10 mM) + 3-phenylpyruvate (10 mM)	4.42 ± 0.42 (4)‡	3.43 ± 0.30 (12)‡	105.9 ± 7.6 (30)‡

Table 5. *Effects of 3-phenylpyruvate upon the oxidation of* D-*glucose, pyruvate, 4-methyl-2-oxopentanoate and* L-*leucine*
Pancreatic islets were incubated for 120min in the sole presence of the nutrients listed or in the simultaneous presence of one of these nutrients and 3-phenylpyruvate (10mM). Results are means ± S.E.M. The statistical significance (*P*) of the 3-phenylpyruvate-induced changes in oxidation rate is indicated in each case.

	Oxidation rate (pmol of nutrient oxidized/120min per islet)		
3-Phenylpyruvate concn. ...	Nil	10mM	*P*
D-[U-^{14}C]Glucose (5.6mM)	21.5 ± 1.6 (10)	20.4 ± 1.5 (10)	>0.6
D-[U-^{14}C]Glucose (8.3mM)	28.2 ± 1.3 (10)	21.7 ± 2.0 (10)	<0.02
D-[U-^{14}C]Glucose (16.7mM)	44.8 ± 1.3 (10)	29.8 ± 1.2 (10)	<0.001
[2-^{14}C]Pyruvate (0.5mM)	8.7 ± 1.2 (10)	5.6 ± 0.8 (9)	<0.06
[2-^{14}C]Pyruvate (5.0mM)	45.8 ± 5.0 (9)	32.5 ± 4.3 (9)	<0.07
[U-^{14}C]4-Methyl-2-oxopentanoate (10.0mM)	26.3 ± 4.3 (14)	13.3 ± 2.1 (14)	<0.02
L-[U-^{14}C]Leucine (10.0mM)	24.7 ± 2.1 (10)	27.7 ± 2.5 (10)	>0.3
L-[1-^{14}C]Leucine (10.0mM)	53.2 ± 5.0 (10)	56.6 ± 2.6 (10)	>0.5

value of 842 ± 139 per million/min^2 ($n = 8$), but was more rapidly masked by the secondary rise in $^{45}Ca^{2+}$ efflux in the presence of L-glutamine. The magnitude of such a secondary rise was also greater ($P < 0.05$) in the presence of L-glutamine (0.97 ± 0.17%/min) than in its absence (0.44 ± 0.02%/min).

3-Phenylpyruvate provoked a marked and rapidly reversible decrease in $^{86}Rb^+$ outflow from prelabelled islets (Fig. 1*b*). The initial value (at 40–44min) for $^{86}Rb^+$ fractional outflow rate was significantly lower ($P < 0.001$) in the presence of L-glutamine (3.84 ± 0.06%/min) than in its absence (4.73 ± 0.05%/min). The value reached within 6min exposure to 3-phenylpyruvate was similar in both series of experiments. Thereafter, however, the $^{86}Rb^+$ fractional outflow rate continued to slowly decrease in the absence of L-glutamine and slightly increased in the presence of the amino acid, so that the mean value reached between 64 and 68min of perifusion was higher ($P < 0.001$) in the presence (1.78 ± 0.03%/min) than in the absence of L-glutamine (1.46 ± 0.03%/min).

Oxidative data

In the absence of exogenous nutrient, 3-phenylpyruvate (10mM) augmented O_2 uptake by 23.2 ± 5.6% ($P < 0.05$) relative to the paired basal value (Table 4). In the presence of L-glutamine (10mM), the paired increment in O_2 uptake was greater, averaging 69.3 ± 7.5% ($P < 0.001$). All respiratory rates were linear with time. There were tight correlations between the mean values for O_2 uptake and the corresponding mean values for either $^{45}Ca^{2+}$ net uptake ($r = 0.9798$; $n = 4$; $P < 0.01$) or insulin release ($r = 0.9997$, $n = 4$, $P < 0.001$).

The finding that 3-phenylpyruvate inhibited insulin release evoked by high concentrations of D-glucose or 4-methyl-2-oxopentanoate led us to examine the effect of the 2-oxo acid on the oxidation of these and other nutrients. As shown in Table 5, 3-phenylpyruvate failed to affect significantly D-[U-^{14}C]glucose oxidation at a low concentration of the sugar (5.6mM), but inhibited oxidation at higher glucose concentrations (8.3 and 16.7mM). 3-Phenylpyruvate also inhibited the oxidation of [2-^{14}C]-pyruvate (0.5 or 5.0mM), the experimental values averaging 67.8 ± 6.6% ($n = 18$) of the mean control values (100.0 ± 8.7%, $n = 19$) found at the same concentration of pyruvate ($P < 0.01$). Likewise, 3-phenylpyruvate inhibited the oxidation of [U-^{14}C]4-methyl-2-oxopentanoate, but failed to affect the oxidation of L-[U-^{14}C]leucine or L-[1-^{14}C]leucine. There was a significant correlation between the mean control values for $^{14}CO_2$ output from islets exposed to labelled D-glucose, pyruvate or 4-methyl-2-oxopentanoate and the corresponding mean decreases in $^{14}CO_2$ output evoked by 3-phenylpyruvate ($r = 0.8260$; $n = 6$; $P < 0.05$). There was also a significant correlation ($r = 0.9033$; $n = 4$; $P < 0.05$) between the mean changes evoked by 3-phenylpyruvate in the output of $^{14}CO_2$ from islets exposed to D-[U-^{14}C]glucose (5.6, 8.3 or 16.7mM) or [U-^{14}C]4-methyl-2-oxopentanoate (10mM) and the corresponding mean changes evoked by 3-phenylpyruvate in the secretory response to these nutrient secretagogues.

Discussion

The present results confirm that 3-phenylpyruvate stimulates insulin release, as recently reported by Panten & Langer (1981). At variance with the view expressed by the latter authors, several findings in our study suggest that the insulin-releasing capacity of 3-phenylpyruvate coincides with, and

may be secondary to, an increase in the catabolism of nutrients in the islet cells.

First, 3-phenylpyruvate increased O_2 uptake by the islets. The increment in insulin release evoked by 3-phenylpyruvate, whether in the absence or in the presence of L-glutamine, was proportional to the increment in O_2 uptake, with a mean ratio of 51.5 μ-units/nl of O_2 or 1.2 μ-units/pmol of O_2.

Secondly, the secretory response to 3-phenylpyruvate coincided with a decrease in $^{86}Rb^+$ fractional outflow rate, a phenomenon that fails to occur in response to such non-nutrient secretagogues as L-arginine or the ionophore A23187 (Boschero & Malaisse, 1979; Henquin & Meissner, 1981). 3-Phenylpyruvate caused an initial decrease and a later rise in $^{45}Ca^{2+}$ efflux. This dual effect is observed in response to nutrient secretagogues such as D-glucose (Herchuelz & Malaisse, 1980), L-leucine (Malaisse *et al.*, 1980*a*) or 4-methyl-2-oxopentanoate (Hutton *et al.*, 1980), and is thought to reflect inhibition of Na^+/Ca^{2+} counter-transport (initial fall) and facilitation of $^{40}Ca^{2+}$ influx (late rise) respectively (Herchuelz *et al.*, 1980*a*,*b*). In good agreement with a recent report (Lenzen & Panten, 1981), 3-phenylpyruvate stimulated $^{45}Ca^{2+}$ net uptake. The 2-oxo-acid also provoked the accumulation of cyclic AMP in the islets and their surrounding incubation media. These findings suggest that, as is the case with other nutrient secretagogues, the functional response to 3-phenylpyruvate involves a decrease in K^+ conductance, the subsequent gating of voltage-sensitive Ca^{2+} channels, the accumulation of Ca^{2+} in the cytosol of islet cells and the resulting activation via Ca^{2+}–calmodulin of adenylate cyclase (Malaisse *et al.*, 1981*a*).

Thirdly, the relationship between changes in $^{86}Rb^+$ fractional outflow rate and insulin release in the present series of experiments was in good agreement with present knowledge on the interdependency of these two variables in response to activation of the B-cell by nutrient secretagogues. L-Glutamine alone decreased $^{86}Rb^+$ fractional outflow rate, but failed to stimulate insulin release. This finding, which confirms a prior observation (Malaisse *et al.*, 1980*b*), is identical with that observed at low concentrations of D-glucose (Carpinelli & Malaisse, 1981) and suggests that the cell membrane depolarization resulting from the decrease in K^+ conductance must reach a critical level in order to cause the gating of Ca^{2+} channels and subsequent stimulation of insulin release. The value of $^{86}Rb^+$ fractional outflow rate reached at 6 min after exposure to 3-phenylpyruvate was not lower in the presence of L-glutamine than in its absence, despite the higher rate of insulin release recorded in the presence of the amino acid. This is also similar to what is seen whenever D-glucose is used in the range of concentrations in excess of the threshold value required for stimulation of insulin release (Carpinelli & Malaisse, 1981). Moreover, the steady-state value for $^{86}Rb^+$ outflow reached after prolonged exposure to 3-phenylpyruvate was somewhat higher in the presence than in the absence of L-glutamine. This is again reminiscent of what is observed in response to D-glucose, in which case a secondary activation of a Ca^{2+}-sensitive modality of K^+ extrusion is held responsible for the higher value of $^{86}Rb^+$ fractional outflow observed at high than at intermediate glucose concentrations (Lebrun *et al.*, 1982).

Fourthly, the fact that 3-phenylpyruvate, unlike other nutrient secretagogues, did not merely shift to the left the sigmoidal curve relating insulin output to the ambient glucose concentration, does not detract but, on the contrary, reinforces the view that the secretory response to 3-phenylpyruvate depends closely on the regulation of oxidative events in the islet cells. Indeed, the inhibitory effect of 3-phenylpyruvate upon insulin release evoked by high concentrations of D-glucose or by 4-methyl-2-oxopentanoate coincided with a decrease in glucose or 4-methyl-2-oxopentanoate oxidation. Since 3-phenylpyruvate also inhibited pyruvate oxidation, it seems most likely that 3-phenylpyruvate interferes with the transport of 2-oxo-acids (e.g., pyruvate derived from either an exogenous source or endogenous glucose metabolism) into the mitochondria (Halestrap *et al.*, 1974). This interpretation is consistent with the observation that 3-phenylpyruvate failed to affect L-leucine oxidation, since the oxidation of this amino acid depends on the mitochondrial rather than extramitochondrial conversion of L-leucine into 4-methyl-2-oxopentanoate (Malaisse *et al.*, 1982*a*). Our interpretation is also supported by the existence of a significant correlation between the magnitude of the 3-phenylpyruvate-induced decrease in $^{14}CO_2$ output and the control value for such an output, suggesting that the inhibitory effect of 3-phenylpyruvate is most evident when the capacity of the mitochondrial transport system is close to saturation.

Fifthly, L-glutamine markedly enhanced the oxidative, ionic and secretory response to 3-phenylpyruvate. In previous studies, we have demonstrated that the capacity of L-glutamine to augment insulin release evoked by either activators of glutamate dehydrogenase (e.g., leucine of BCH) or 2-oxo-acids acting as 'partners' in the transamination of L-glutamate (derived from exogenous L-glutamine) to 2-oxoglutarate (e.g., 4-methyl-2-oxopentanoate) can be readily accounted for by the changes in oxidative fluxes evoked by L-glutamine in the islet cells (Malaisse *et al.*, 1981*b*, 1982*b*; Malaisse-Lagae *et al.*, 1982). The capacity of L-glutamine to augment 3-phenylpyruvate-stimulated insulin release is likely therefore to be attributable to a comparable process. Since 3-phenyl-

pyruvate does not activate glutamate dehydrogenase (Panten & Langer, 1981), interference by 3-phenylpyruvate of the transamination of L-glutamate would appear to be the most likely explanation for the synergistic effect of 3-phenylpyruvate and L-glutamine on insulin release. This interpretation is further supported by the finding that 3-phenylpyruvate failed to augment insulin release evoked by either L-leucine or its non-metabolized analogue BCH. These two amino acids are known to increase considerably the oxidative deamination and further catabolism of endogenous amino acids in the islet cells. If 3-phenylpyruvate were to act in a comparable but not identical manner, namely by facilitating the transamination and further catabolism of endogenous amino acids, it would seem reasonable to speculate that 3-phenylpyruvate would not augment the secretory response to L-leucine (or BCH), the availability of endogenous amino acids eventually representing a rate-limiting factor. The latter view is again supported by the knowledge that exogenous L-glutamine augments 3-phenylpyruvate-induced insulin release.

Lastly, the secretory response to the combination of 3-phenylpyruvate and L-glutamine was abolished by menadione, which inhibits nutrient-stimulated functional events in the islet cells by lowering their content in reduced nicotinamide nucleotides (Malaisse *et al.*, 1978). This finding again suggests that the response to 3-phenylpyruvate includes an increase in the catabolism of nutrients in the islet cells, with induction of a more reduced redox state.

The mechanism by which 3-phenylpyruvate indeed stimulates catabolic events in the islet cells is considered in the following paper (Malaisse *et al.*, 1983).

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