Mechanism of 3-phenylpyruvate-induced insulin release

Metabolic aspects

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1. The metabolism and metabolic effects of 3-phenylpyruvate were examined in rat pancreatic islets. 2. Islet homogenates catalysed transamination reactions between 3-phenylpyruvate and L-glutamate, L-leucine, L-norleucine or L-valine. 3-Phenylpyruvate failed to activate glutamate dehydrogenase. 3. 3-Phenylpyruvate rapidly entered into islet cells, was extensively converted into phenylalanine but slowly oxidized. 4. The conversion of phenylpyruvate into phenylalanine coincided with a fall in the content of several amino acids (especially glutamate and aspartate) in the islets and incubation medium, the accumulation of 2-oxoglutarate and a modest fall in the NH_4^+ production rate. 5. 3-Phenylpyruvate failed to affect $^{14}\text{CO}_2$ output from islets prelabelled with $[\text{U}-^{14}\text{C}]$ palmitate, but augmented $^{14}\text{CO}_2$ output from islets prelabelled with L- $[\text{U}-^{14}\text{C}]$ glutamine. 6. In the presence of L-glutamine, 3-phenylpyruvate augmented the ATP/ADP ratio and NAD(P)H islet content, and caused a rapid and sustained decrease in the outflow of radioactivity from islets prelabelled with $[2\text{-}^3\text{H}]$ adenosine. 7. These data support the view that the insulin-releasing capacity of 3-phenylpyruvate coincides with an increase in the catabolism of endogenous amino acids acting as 'partners' in transamination reactions leading to the conversion of 3-phenylpyruvate into phenylalanine.

3-Phenylpyruvate stimulates insulin release in mouse or rat pancreatic islets (Panten & Langer, 1981; Lenzen & Panten, 1981; Sener *et al.*, 1983). This coincides with an increase in O_2 consumption by the islets (Sener *et al.*, 1983). The present report aims at identifying the metabolic events responsible for the increase in O_2 uptake in islets stimulated by 3-phenylpyruvate.

Materials and methods

$[\text{U}-^{14}\text{C}]$ 3-Phenylpyruvate and 3-phenyl $[1\text{-}^{14}\text{C}]$ pyruvate were prepared from L- $[\text{U}-^{14}\text{C}]$ phenylalanine and L- $[1\text{-}^{14}\text{C}]$ phenylalanine respectively, by enzymic oxidation by the method of Meister (1952) and purified by gel filtration and ion-exchange chromatography, as described previously (Hutton *et al.*, 1979).

All experiments were performed with islets isolated by collagenase digestion of pancreases removed from fed rats (Lacy & Kostianovsky, 1967).

The methods used to measure the activity of glutamate dehydrogenase (Sener & Malaisse, 1980), lactate dehydrogenase (Malaisse *et al.*, 1979a) and transaminase (Sener *et al.*, 1982) in islet homogenates, the oxidation of exogenous nutrients by intact islets (Carpinelli *et al.*, 1980), the release of $^{14}\text{CO}_2$ from islets prelabelled with either $[\text{U}-^{14}\text{C}]$ palmitate (Sener *et al.*, 1978; Malaisse *et al.*, 1979a) or L- $[\text{U}-^{14}\text{C}]$ glutamine (Malaisse *et al.*, 1981), the islet content and/or output of 2-oxoglutarate (Malaisse *et al.*, 1980) and NH_4^+ (Malaisse *et al.*, 1981), the ATP/ADP ratio and total islet content in reduced nicotinamide nucleotides (Malaisse *et al.*, 1978) were as described in the cited publications.

Several of the above methods depend on the measurement of NAD^+ , which is converted into a fluorescent product by alkaline treatment. 3-Phenylpyruvate was found to interfere with the fluorescence of the latter product. To correct for such an interference, appropriate standards were always prepared in media containing 3-phenylpyruvate.

For measuring the uptake of $[\text{U}-^{14}\text{C}]$ 3-phenyl-

pyruvate, groups of 10 islets each were incubated for 5 or 10 min in 50 μ l of the usual bicarbonate-buffered incubation medium containing [6,6'- 3 H]sucrose (1 mM; 2.5 μ Ci/ml) and [U- 14 C]3-phenylpyruvate (10 mM; 0.5 μ Ci/ml) and layered on 150 μ l silicone oil (Versilube F-50; General Electric Co., Waterford, NY, U.S.A.). After incubation, the tubes were centrifuged for 1 min (Beckman Microfuge, model 152; Beckman Instruments, Palo Alto, CA, U.S.A.). The tip of the tube containing the pellet of islets was removed with a scalpel and examined for its radioactive content. In this series of experiments, the [3 H]sucrose space of distribution averaged 0.36 ± 0.03 nl/islet (mean $\pm$ S.E.M.; $n = 27$).

To measure the conversion of [U- 14 C]3-phenylpyruvate into its amination product, groups of 20 islets each were incubated in 150 μ l of the usual medium containing [U- 14 C]3-phenylpyruvate (10 mM; 1.0 μ Ci/ml). After 60 min incubation, the medium was separated from the islets, the latter being resuspended in 200 μ l of non-radioactive incubation medium, placed in liquid N_2 and disrupted by mechanical vibration (Malaisse *et al.*, 1978). The separation of [14 C]oxo-acids and amino acids was achieved as described previously (Hutton *et al.*, 1979).

For measuring the amino acid content of the islets and incubation medium, groups of 80 islets each were incubated for 120 min in 0.1 ml of the usual bicarbonate-buffered medium, in the absence or in the presence of 3-phenylpyruvate (10 mM). After addition of 0.1 ml of a solution of trichloroacetic acid (10%, w/v), the tubes containing the islets were placed in liquid N_2 and the islets were disrupted by mechanical vibration (Malaisse *et al.*, 1978). After thawing, the homogenate was centrifuged for 3 min in a microcentrifuge and a portion of the supernatant solution was examined for its amino acid content by chromatography in a Beckman Multi-chrom B amino acid analyser. In one out of the four batches of islets incubated in the absence of 3-phenylpyruvate and in one out of the four batches of islets incubated in the presence of 3-phenylpyruvate, the impression was gained that proteolysis may have been simulated during the 120 min incubation in the nutrient-free medium. This was suggested, amongst other things, by an elevated threonine/taurine ratio amounting to 1.93 and 2.93 as distinct from 0.72 ± 0.17 in the six other batches of islets ($P < 0.01$). To correct for such a proteolytic phenomenon, the amount of each individual amino acid recovered in the islets and medium was expressed either as a percentage of the total amino acid content, or relative to the paired value for threonine. The validity of this procedure was assessed by the observation that our estimation of the 3-phenylpyruvate-induced increment in phenylalanine production was not vastly different, whether

judged from the data below (Table 2) yielding a mean value of 43.9 ± 10.3 pmol/120 min per islet or estimated by comparison of the paired differences in phenylalanine and tyrosine content of control islets (0.8 ± 0.6 pmol/120 min per islet) and 3-phenylpyruvate-stimulated islets (49.5 ± 11.8 pmol/120 min per islet) respectively. In the latter comparison, any artefactual increase in phenylalanine content due to proteolysis is corrected for by subtracting the concomitantly increased tyrosine value. Indeed, the threonine/tyrosine ratio was fairly stable in all batches of islets, with a mean value of 1.12 ± 0.09 ($n = 8$).

The method used to measure the efflux of radioactive nucleotides from islets prelabelled with [3 H]adenosine has been described in detail by Welsh (1982). Briefly, groups of 60 islets each were cultured overnight in Parker's culture medium TC 199 containing 5.6 mM-glucose and then incubated at 45 min at 37°C in Krebs-Ringer medium containing 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (10 mM; pH 7.4) and [3 H]adenosine (0.1 mM; sp. radioactivity 10 Ci/mmol) and equilibrated against CO_2 /air (1:19). After prelabelling, the islets were washed four times at 4°C in the same medium (except for the absence of labelled adenosine) and transferred to the perfusion chamber. The perfusate was delivered at a rate of 1 ml/min. The efflux of radioactivity was expressed as a fractional outflow rate, taking into account the radioactivity recovered in the filter and islets at the end of each experiment.

All results are expressed as means ($\pm$ S.E.M.), together with the number of individual observations (given in parentheses).

Results

Enzymic data

Neither 3-phenylpyruvate nor L-phenylalanine affected the activity of glutamate dehydrogenase in islet homogenates. The control value and those collected in the presence of either 3-phenylpyruvate (10 mM) or L-phenylalanine (10 mM) averaged respectively 36 ± 3 , 34 ± 5 and 42 ± 4 pmol/min per islet in the absence of ADP and 208 ± 8 , 224 ± 22 and 210 ± 5 pmol/min per islet in the presence of 1.0 mM-ADP ($n \geq 3$ in each case). In the same series of experiments, ($\pm$)-BCH (2-*endo*-aminonorbomane-2-carboxylic acid) (20 mM) increased 4–5-fold the activity of glutamate dehydrogenase when measured in the absence of ADP. Incidentally, Panten & Langer (1981) observed that 3-phenylpyruvate (10 mM) causes a close to 50% increase in the activity of glutamate dehydrogenase in mouse islet homogenates.

Neither 3-phenylpyruvate (10 mM) nor L-phenylalanine (10 mM) affected the activity of lactate dehydrogenase (242 pmol/min per islet) in islet

Table 1. *Transamination of 3-phenylpyruvate in islet homogenates*

The transamination rate of 3-phenylpyruvate was judged from either the conversion of [U- 14 C]3-phenylpyruvate into [14 C]phenylalanine (a) or that of L-[U- 14 C]amino acids into the corresponding [14 C]2-oxo-acid (b). All substrates were used at a 10mM concentration. Results are means $\pm$ S.E.M. or individual deviation from mean values when $n = 2$. Abbreviation: N.D., not determined.

Amino acid	Transamination rate (pmol/min per islet)	
	(a)	(b)
Nil	0.27 $\pm$ 0.15 (3)	$\leq$ 0.25 $\pm$ 0.08 (2)
L-Glutamate	2.04 $\pm$ 0.37 (3)	2.23 $\pm$ 0.37 (9)
L-Leucine	1.84 $\pm$ 0.14 (2)	1.73 $\pm$ 0.47 (9)
L-Norleucine	1.15 $\pm$ 0.25 (3)	N.D.
L-Valine	N.D.	0.96 $\pm$ 0.25 (9)

homogenates incubated for 10–20 min at 30°C in the presence of 4 mM-pyruvate.

In the presence of L-glutamate, L-leucine, L-norleucine or L-valine, the islet homogenate catalysed the conversion of 3-phenylpyruvate into L-phenylalanine. The reaction velocity was virtually identical, whether judged from the amination of [U- 14 C]3-phenylpyruvate incubated in the presence of an unlabelled amino acid or from the deamination of L-[U- 14 C]amino acids incubated in the presence of unlabelled 3-phenylpyruvate (Table 1). No significant transamination activity was detected in the presence of 3-phenylpyruvate and L-aspartate. Control experiments performed with the same L-[U- 14 C]amino acids and unlabelled 2-oxoglutarate (all 10mM) yielded reaction velocities comparable with those recently reported by Sener *et al.* (1982). Such velocities were 1–2 orders of magnitude higher than those recorded in the presence of 3-phenylpyruvate. Indeed, in the presence of 2-oxoglutarate, the rate of transamination ranged from 19 pmol/min per islet (in the presence of L-[U- 14 C]valine) to 58 pmol/min per islet (in the presence of L-[U- 14 C]aspartate).

Fate of [14 C]3-phenylpyruvate

[U- 14 C]3-Phenylpyruvate (10mM) was rapidly taken up by the islets. After 5 and 10 min incubation, the radioactive content of the islets corresponded to an apparent uptake of 11.4 $\pm$ 1.9 and 11.0 $\pm$ 1.3 pmol of 3-phenylpyruvate/islet respectively ($n = 10$ in each case).

[U- 14 C]3-Phenylpyruvate (10mM) was converted into a radioactive amino acid, presumably L-[U- 14 C]phenylalanine, in the islets. After 60 min incubation, the amount of L-[U- 14 C]phenylalanine recovered in the islets and incubation media aver-

aged 11.2 $\pm$ 3.0 and 14.5 $\pm$ 4.6 pmol/islet respectively. The islet content in L-[U- 14 C]phenylalanine was fairly constant between the 30 and 120 min of incubation.

The oxidation of 3-phenyl[1- 14 C]pyruvate (10mM) and that of L-[1- 14 C]phenylalanine (10mM) occurred at almost the same rate, averaging 12.0 $\pm$ 2.1 and 10.0 $\pm$ 0.7 pmol/120 min per islet respectively. The oxidation of L-[1- 14 C]phenylalanine was time-related (1.83 $\pm$ 0.55 pmol/30 min, 4.16 $\pm$ 0.54 pmol/60 min and 10.04 $\pm$ 0.72 pmol/90 min per islet; $n = 11$ in all cases), and was decreased by L-glutamine (10mM) to 51.0 $\pm$ 7.2% of its control value. Over 120 min incubation, the oxidation of 3-phenylpyruvate, however, was not affected by L-glutamine (10mM) averaging 100.8 $\pm$ 19.5% ($n = 7$) of the control value found in the absence of the amino acid.

Effect of 3-phenylpyruvate on amino acid metabolism

Unlabelled amino acids were measured in the islets and their surrounding incubation medium after 120 min incubation in the absence or presence of 3-phenylpyruvate (10.0mM). The islet content and/or output of taurine and phosphoethanolamine was similar in each batch of islets averaging 25.6 $\pm$ 1.0 and 25.4 $\pm$ 1.7 pmol/islet (taurine) and 16.0 $\pm$ 0.3 and 16.0 $\pm$ 1.6 pmol/islet (phosphoethanolamine) in control and 3-phenylpyruvate-stimulated islets respectively ($n = 4$ in each case). The total islet content and/or output of other amino acids (Table 2) was not significantly different ($P > 0.5$) in control and stimulated islets and, expressed relative to the threonine value, averaged 20.59 $\pm$ 1.41 and 17.17 $\pm$ 2.73 in control and stimulated islets respectively ($n = 4$ in both cases; $P < 0.3$). In absolute terms, this corresponded to an overall mean value of 261.4 $\pm$ 36.4 pmol/islet ($n = 6$), after exclusion of one abnormally high reading in each group (see the Materials and methods section). 3-Phenylpyruvate caused a 4-fold increase in phenylalanine production whether judged from the relative data illustrated in Table 3 or the absolute values for phenylalanine content and/or output. In absolute terms, the 3-phenylpyruvate-induced increment in phenylalanine production averaged 75.3 $\pm$ 23.8 pmol/islet per 120 min (primary data) or, after correction for apparent proteolysis, 43.9 $\pm$ 10.3 pmol/120 min per islet ($P < 0.02$ or less). 3-Phenylpyruvate failed to affect significantly the islet content and/or output of tyrosine, and increased the phenylalanine/tyrosine ratio from a basal value of 1.03 $\pm$ 0.05 to 4.21 $\pm$ 0.77 ($n = 4$ in each case; $P < 0.01$).

The increase in phenylalanine production coincided with an obvious and highly significant decrease in the aspartate and glutamate content of

Table 2. *Effect of 3-phenylpyruvate upon the amino acid content of the islets and medium*

Results are means $\pm$ S.E.M. and are expressed either as a percentage of the total amount of amino acids or relative to the paired value for threonine. Also shown is the statistical significance (*, $P < 0.05$; †, $P < 0.02$; ‡, $P < 0.01$; §, $P < 0.005$; ||, $P < 0.001$) of the changes in mean values attributable to 3-phenylpyruvate (10 mM).

Amino acid	Amino acid content			
	(% of total)		(relative to threonine)	
	Control	3-Phenylpyruvate	Control	3-Phenylpyruvate
Threonine	4.9 $\pm$ 0.3	6.6 $\pm$ 1.0	1.00	1.00
Serine	7.7 $\pm$ 0.6	5.9 $\pm$ 0.2*	1.59 $\pm$ 0.21	0.98 $\pm$ 0.19
Glycine	14.1 $\pm$ 2.6	10.6 $\pm$ 2.3	2.95 $\pm$ 0.61	1.93 $\pm$ 0.76
Asparagine	2.9 $\pm$ 0.3	2.8 $\pm$ 0.2	0.58 $\pm$ 0.04	0.45 $\pm$ 0.04*
Aspartate	9.6 $\pm$ 1.4	4.3 $\pm$ 0.3	2.00 $\pm$ 0.33	0.72 $\pm$ 0.17†
Glutamine	3.0 $\pm$ 0.4	3.4 $\pm$ 0.5	0.60 $\pm$ 0.07	0.52 $\pm$ 0.02
Glutamate	12.2 $\pm$ 0.1	8.5 $\pm$ 0.5	2.50 $\pm$ 0.18	1.37 $\pm$ 0.20‡
Alanine	10.5 $\pm$ 0.6	10.2 $\pm$ 0.8	2.14 $\pm$ 0.10	1.61 $\pm$ 0.16*
Leucine	11.6 $\pm$ 1.7	10.9 $\pm$ 1.2	2.37 $\pm$ 0.35	1.80 $\pm$ 0.37
Isoleucine	4.3 $\pm$ 0.3	2.9 $\pm$ 0.9	0.87 $\pm$ 0.04	0.40 $\pm$ 0.09§
Valine	8.7 $\pm$ 1.4	6.6 $\pm$ 1.5	1.77 $\pm$ 0.30	0.98 $\pm$ 0.18
Tyrosine	5.3 $\pm$ 0.6	5.1 $\pm$ 0.7	1.08 $\pm$ 0.13	0.79 $\pm$ 0.06
Phenylalanine	5.4 $\pm$ 0.7	22.2 $\pm$ 3.1§	1.11 $\pm$ 0.14	3.89 $\pm$ 0.82*

the islets and incubation media (Table 2). In the 3-phenylpyruvate-stimulated islets, there was also a trend towards a decrease in the islet content and/or output of serine, glycine, leucine, isoleucine and valine. Taken as a whole, the relative amount of these five amino acids was one-fifth lower ($P < 0.001$) in 3-phenylpyruvate-stimulated islets ($37.3 \pm 0.7\%$; $n = 4$) than in control islets ($46.2 \pm 1.0\%$; $n = 4$).

The 3-phenylpyruvate-induced fall in the islet content of glutamate was associated with a time-related accumulation of 2-oxoglutarate in the islets (Table 3). This coincided with a decrease in the rate of NH_4^+ production (Table 3). Thus, taking into account the initial NH_4^+ content of the islets and incubation media as measured at the onset of incubation (18.0 ± 3.9 pmol/islet; $n = 12$), the rate of NH_4^+ production was decreased by 3-phenylpyruvate from a basal value of 579 ± 34 to 426 ± 42 fmol/min per islet ($n = 45-46$; $P < 0.01$).

3-Phenylpyruvate augmented the output of $^{14}\text{CO}_2$ from islets prelabelled with L-[U- ^{14}C]glutamine (Table 4). As judged from the $^{14}\text{CO}_2$ output/ ^{14}C content ratio, the stimulant action of 3-phenylpyruvate (10 mM) was less pronounced ($P < 0.005$) than that of ($\pm$)-BCH (20 mM). It corresponded to a doubling of the oxidation rate of endogenous L-glutamate (derived from exogenous L-glutamine), after correction for the initial islet content in $^{14}\text{CO}_2$ at the onset of incubation (as measured in antimycin A-treated islets).

3-Phenylpyruvate (10 mM) also augmented the oxidation rate of exogenous L-[U- ^{14}C]glutamine (10 mM) when both nutrients were present together in the incubation medium. Thus, over a 120 min

Table 3. *Effect of 3-phenylpyruvate upon the 2-oxoglutarate and NH_4^+ content of the islets and medium*

Results are means $\pm$ S.E.M. and refer to measurements performed after 45 or 120 min incubation in the absence (control) or presence of 3-phenylpyruvate (10 mM).

Metabolite	Time (min)	Content (pmol/islet)	
		Control	3-Phenylpyruvate
2-Oxoglutarate	45	1.72 $\pm$ 0.22 (10)	3.82 $\pm$ 0.61 (11)
	120	2.62 $\pm$ 0.38 (12)	14.06 $\pm$ 1.55 (12)
NH_4^+	45	42.4 $\pm$ 3.8 (11)	35.2 $\pm$ 2.4 (11)
	120	88.9 $\pm$ 4.3 (34)	70.9 $\pm$ 6.4 (35)

Table 4. *Effects of 3-phenylpyruvate and ($\pm$)-BCH upon $^{14}\text{CO}_2$ output from islets prelabelled with L-[U- ^{14}C]glutamine*

Results are means $\pm$ S.E.M. for eight individual observations in each case.

Agent	$^{14}\text{CO}_2$ output (pmol/30 min per islet)	Final ^{14}C content (pmol/islet)	^{14}C output/ ^{14}C total content (%)
Nil	2.20 $\pm$ 0.27	11.55 $\pm$ 1.03	15.7 $\pm$ 0.7
3-Phenylpyruvate (10 mM)	2.69 $\pm$ 0.29	9.14 $\pm$ 0.86	22.6 $\pm$ 0.8
($\pm$)-BCH (20 mM)	3.49 $\pm$ 0.18	9.02 $\pm$ 0.64	28.2 $\pm$ 1.3
Antimycin A (0.01 mM)	1.18 $\pm$ 0.13	12.25 $\pm$ 0.89	8.6 $\pm$ 0.4

incubation, the oxidation of L-[U- ^{14}C]glutamine averaged 65.7 ± 3.4 and 85.2 ± 4.8 pmol/120 min per islet ($n = 24-25$) in the absence and in the presence of 3-phenylpyruvate respectively ($P < 0.005$).

Table 5. *Effects of 3-phenylpyruvate and L-phenylalanine upon $^{14}\text{CO}_2$ output from islets prelabelled with $[\text{U-}^{14}\text{C}]$ -palmitate*Results are means $\pm$ S.E.M. and are shown together with the number of individual observations (*n*).

Agent	$^{14}\text{CO}_2$ output (fmol/ 120 min per islet)	Final ^{14}C content (pmol/islet)	^{14}C output/ ^{14}C total content (%)	<i>n</i>
Nil	144 $\pm$ 11	3.43 $\pm$ 0.44	4.22 $\pm$ 0.30	9
3-Phenylpyruvate (10 mM)	149 $\pm$ 13	3.41 $\pm$ 0.41	4.40 $\pm$ 0.27	10
L-Phenylalanine (10 mM)	134 $\pm$ 9	2.95 $\pm$ 0.20	4.41 $\pm$ 0.22	10
Antimycin A (0.01 mM), rotenone (0.01 mM) and oligomycin (0.01 mM)	31 $\pm$ 2	3.42 $\pm$ 0.33	0.94 $\pm$ 0.08	10

Table 6. *Effect of 3-phenylpyruvate, L-glutamine and D-glucose upon the islet content of NADH and NADPH and ATP/ADP ratio*All measurements were performed after 30 min incubation. Results are means $\pm$ S.E.M. Statistical significance (*, $P < 0.05$; †, $P < 0.02$; ‡, $P < 0.01$) of differences from the values shown for glutamine alone are given.

Agent	NADH + NADPH (fmol/islet)	ATP (pmol/islet)	ADP (pmol/islet)	ATP/ADP ratio
L-Glutamine (10 mM)	253 $\pm$ 11 (44)	8.32 $\pm$ 0.40 (29)	4.29 $\pm$ 0.27 (29)	2.14 $\pm$ 0.13 (29)
L-Glutamine (10 mM)	299 $\pm$ 18 (44)*	8.84 $\pm$ 0.51 (30)	3.84 $\pm$ 0.36 (30)	3.01 $\pm$ 0.31 (30)†
+ 3-phenylpyruvate (10 mM)				
D-Glucose (16.7 mM)	337 $\pm$ 21 (34)‡	10.36 $\pm$ 0.57 (27)†	3.03 $\pm$ 0.35 (27)†	4.93 $\pm$ 0.66 (27)‡

Fuel utilization in 3-phenylpyruvate-stimulated islets

3-Phenylpyruvate, as well as L-phenylalanine, failed to affect significantly the production of $^{14}\text{CO}_2$ from islets prelabelled with $[\text{U-}^{14}\text{C}]$ palmitate (Table 5).

The catabolism of 3-phenylpyruvate, as judged from its rate of oxidation, may account for a modest increase in O_2 consumption (2–3% of the basal respiratory rate in freshly isolated islets incubated in a bicarbonate-buffered medium; see Hutton & Malaisse, 1980), 3 mol of O_2 being utilized/mol of 3-phenylpyruvate converted into acetoacetate and fumarate (McGilvery, 1970). However, such a conversion does not entail any gain in the production of reducing equivalents and ATP. Moreover, acetoacetate is not further oxidized in the islets (Hutton *et al.*, 1979).

The results so far presented nevertheless indicate that 3-phenylpyruvate indeed augments the generation rate of reducing equivalents. Indeed, after correction for basal values, the amount of phenylalanine accumulating in the islets and medium (43.9 ± 10.3 pmol/120 min per islet) and further oxidized (12.0 ± 2.1 pmol/120 min per islet) largely exceeded the fall in NH_4^+ production rate (18.4 ± 6.5 pmol/120 min per islet) evoked by 3-phenylpyruvate. The mean difference between these two sets of values (i.e. 37.5 pmol/120 min per islet), which may be taken as representative of the 3-phenylpyruvate-induced increase in the rate of

transamination of endogenous amino acids to their corresponding 2-oxo acids, is quantitatively compatible with the 3-phenylpyruvate-induced increase in O_2 consumption rate (Sener *et al.*, 1983). Indeed, this increase in transamination rate, when expressed relative to the basal NH_4^+ production rate (69.5 ± 4.1 pmol/120 min per islet), would represent a 54% increase in the catabolism of endogenous amino acids. The latter catabolism accounts for approx. 60% of the basal respiratory rate (Malaisse *et al.*, 1981, 1982). The 3-phenylpyruvate-induced increase in the catabolism of endogenous amino acids would thus correspond to a 32% ($54\% \times 60\%$) increase in basal respiratory rate. The latter value is in good agreement with the $23 \pm 6\%$ increase in O_2 consumption rate indeed evoked by 3-phenylpyruvate in islets deprived of exogenous nutrient (Sener *et al.*, 1983).

After 30 min incubation in the presence of L-glutamine (10 mM), 3-phenylpyruvate increased the islet content in NADH and NADPH and caused a modest increase in the ATP/ADP ratio, which remained much lower, however, than in glucose-stimulated islets (Table 6). We have previously shown that L-glutamate exerts little if any effect upon the islet content in NADH and NADPH and ATP/ADP ratio relative to the basal value found in islets deprived of exogenous nutrient (Malaisse *et al.*, 1980, 1982).

The effect of 3-phenylpyruvate upon the ATP/ADP ratio in islets exposed to L-glutamine coin-

Fig. 1. Effect of 3-phenylpyruvate (10 mM), administered during the period shown by the horizontal arrow, upon the radioactive efflux (●) and insulin output from islets prelabelled with [2-³H]adenosine (—)

The perfusate circulated throughout the experiment contained 10 mM-L-glutamine. Results are means $\pm$ S.E.M. for four individual experiments.

cided with a rapid, sustained and reversible fall in the radioactive efflux from islets prelabelled with [2-³H]adenosine (Fig. 1). For reasons detailed by Welsh (1982), such a fall probably reflects a change in the intracellular concentration of AMP and ADP. The decrease in the efflux of radioactive nucleotides occurred concomitantly with stimulation of insulin release, which was characterized by an elevated early peak and a more modest late secretory phase (Fig. 1).

Discussion

The present results indicate that the islets are enzymically equipped for the conversion of 3-phenylpyruvate into phenylalanine by transamination and that such a conversion indeed takes place in islets exposed to 3-phenylpyruvate. This 2-oxo acid enters rapidly into the islet cells and its conversion into phenylalanine is associated with a fall in the islet content of several amino acids (especially aspartate and glutamate), the accumulation of 2-oxoglutarate and a modest fall in NH_4^+ production rate. The latter fall could reflect a decreased availability of endogenous amino acids channelled into transamination reactions and could be favoured by the accumulation of 2-oxoglutarate, through inhibition of glutamate dehydrogenase (Sener *et al.*, 1982).

In good agreement with a prior study (Lenzen & Panten, 1981), the rate of 3-phenylpyruvate oxida-

tion was much too low to account for the increase in O_2 uptake evoked by this 2-oxo acid. Moreover, L-glutamine, which decreases the rate of oxidation of most exogenous nutrients (including L-phenylalanine) and presumably increased the rate of 3-phenylpyruvate transamination, failed to augment 3-phenylpyruvate oxidation, although strikingly increasing the secretory response to the 2-oxo-acid. These findings clearly indicate that such a secretory response cannot be accounted for by the catabolism of 3-phenylpyruvate in the islet cells. Nevertheless, a modest contributive role of endogenously formed phenylalanine in the secretory response to 3-phenylpyruvate should not be ruled out, since L-phenylalanine augments insulin release evoked by D-glucose or L-leucine (Landgraf-Leurs *et al.*, 1975; Sener *et al.*, 1981, 1983). The most conservative view on the insulin-releasing capacity of L-phenylalanine would be that the metabolic effects of this amino acid in islet cells are not sufficiently marked to stimulate insulin release in the absence of another exogenous nutrient and yet sufficient to enhance insulin release evoked by suitable exogenous nutrients (Malaisse, 1973).

The most important catabolic event in islets exposed to 3-phenylpyruvate apparently consisted in the increased utilization of 2-oxo acids derived from endogenous amino acids. This phenomenon was documented by the 3-phenylpyruvate-induced increase in $^{14}\text{CO}_2$ output from islets prelabelled or incubated with L-[U- ^{14}C]glutamine, and shown to be commensurate with the 3-phenylpyruvate-induced increase in O_2 consumption. Our measurements of adenine and nicotinamide nucleotides in the islets and the data on the efflux of radioactivity from islets prelabelled with [2-³H]adenosine support the view that, at least in the presence of L-glutamine, the secretory response to 3-phenylpyruvate coincides with a higher generation rate of reducing equivalents and ATP.

In conclusion, the present work indicates that the capacity of 3-phenylpyruvate to stimulate insulin secretion is perfectly compatible with the fuel hypothesis for insulin release (Malaisse *et al.*, 1979b). This hypothesis postulates that the secretory response to nutrient secretagogues reflects their capacity to augment catabolic fluxes in the islet cells, with emphasis on the role of reducing equivalents as a major coupling factor between metabolic and ionic events in the secretory sequence.

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