Effects of Glucose and other Modifiers of Insulin Release on the Oxidative Metabolism of Amino Acids in Micro-Dissected Pancreatic Islets

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The oxidation of alanine, arginine, leucine, glucose, and pyruvate was studied in microdissected pancreatic islets of obese-hyperglycaemic mice. The following main observations were made. The oxidation of glucose was enhanced severalfold when its concentration was raised from 3 to 20 mM. At the latter concentration the rate was about 65 mmol/h per kg dry wt. The oxidation of 17 mM-pyruvate amounted to 20 mmol/h per kg dry wt. indicating a significant entry of this compound into the β -cells. Leucine oxidation was little affected by concentration changes above 5 mM, the rate at 20 mM corresponding to about 25% of that obtained with 20 mM-glucose. In the absence of glucose, the oxidation of alanine or arginine was barely significant. Glucose stimulated the oxidation of alanine but depressed that of leucine. These effects of glucose were blocked by mannoheptulose or iodoacetamide but were not influenced by adrenaline, diazoxide, dibutyryl 3':5'-cyclic AMP, or glibenclamide. The rate of alanine oxidation was doubled in the presence of 17 mM-pyruvate but was unaffected by citrate or succinate. Succinate depressed the oxidation of leucine. Neither alanine nor leucine significantly affected the oxidation of glucose. It is suggested that the effects of glucose on the oxidation of alanine and leucine were mediated by metabolism of the sugar, and that amino acids do not act as insulin secretagogues by serving as fuels for the β -cells. The results are consistent with the existence of mechanisms auxiliary to glucose metabolism for control of insulin release.

It is well established that amino acids can stimulate insulin release (Fajans, Floyd, Knopf & Conn, 1967). The pancreatic β -cell does not, however, respond equally well to all amino acids. Studies of rabbit (Milner, 1969) and rat (Sussman, Stjernholm & Vaughan, 1967; Malaisse, 1969) pancreas preparations have, for example, indicated that leucine is able to elicit insulin release in a glucose-free medium, whereas in the presence of glucose some other amino acids, including arginine, are also effective (Milner, 1969). It is not clear to what extent the different capacities of individual amino acids to stimulate insulin release are related to differences in their metabolism. In fact, metabolism of amino acids in the pancreatic β -cells has been little studied, relative to the interest focused on glucose degradation in these cells. In the present paper we give results on the effects of glucose and other modifiers of insulin release on the oxidation of alanine, arginine and leucine in micro-dissected islets of obese-hyperglycaemic mice. These islets are characterized by an unusually high proportion of β -cells, which have a well-defined pattern of insulin release in response to the three amino acids studied.

MATERIALS AND METHODS

Materials. Unlabelled L-alanine, L-arginine and L-leucine were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A., and from Mann Research Laboratories, New York, N.Y., U.S.A. D-Glucose was from British Drug Houses Ltd., Poole, Dorset, U.K., and Sigma Chemical Co. supplied us with D-mannoheptulose, sodium citrate, sodium pyruvate, sodium succinate and adrenaline. Diazoxide (7-chloro-3-methyl-1,2,4-benzothiadiazine 1,1-dioxide) was obtained from Schering Corp., Bloomfield, N.J., U.S.A., dibutyryl cyclic AMP (6-N,2'-O-dibutyryl-adenosine 3':5'-cyclic monophosphate) from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany, glibenclamide (*N*-{4-[2-(5-chloro-2-methoxybenzamido)-ethyl]benzenesulphonyl}-*N'*-cyclohexylurea) from Farbwerke Hoechst A.G., Frankfurt/M., Germany, and iodoacetamide from Sigma Chemical Co. Fisher Scientific Co., Fair Lawn, N.J., U.S.A., May and Baker Ltd., Dagenham, Essex, U.K., and E. Merck A.G., Darmstadt, Germany, supplied the inorganic salts. Crystalline human serum albumin was from AB Kabi, Stockholm, Sweden.

Uniformly ^{14}C -labelled L-alanine, L-arginine, L-leucine, D-glucose and sodium pyruvate were from The Radiochemical Centre, Amersham, Bucks., U.K. Batches of labelled D-glucose were also obtained from New England Nuclear Chemicals G.m.b.H., Frankfurt/M., Germany.

The radiochemical purity of the labelled alanine, arginine and leucine was occasionally checked by two-dimensional t.l.c. on pre-coated cellulose plates (Merck A.G.), with methanol-pyridine-acetic acid-water (40:2:3:10, by vol.) as solvent in the first direction and phenol-water (100:39, v/v) supplemented with 3mM-EDTA in the second. Radioautography of the chromatograms revealed no signs of impurity in the amino acid batches tested.

All reagents were of analytical grade. Distilled and deionized water (conductivity less than 10^{-7} mho) was used throughout the experiments.

Animals and micro-dissection of islets. Male obese-hyperglycaemic mice about 5 months old (gene symbol *ob/ob*; Hellman, 1965) from the Umeå colony were starved overnight. For each experiment about 15 fresh pancreatic islets were isolated by micro-dissection at $+2^{\circ}\text{C}$ (Hellerström, 1964) in gassed ($\text{O}_2 + \text{CO}_2$, 95:5) Krebs-Ringer bicarbonate buffer (Umbreit, Burris & Stauffer, 1964) supplemented with 3mM-glucose and 0.3% (w/v) human serum albumin.

Incubations. All incubations were performed with shaking (148 strokes/min, 3.5 cm amplitude) at 37°C . In each experiment the islets were first preincubated for 30 min in a medium containing gassed ($\text{O}_2 + \text{CO}_2$, 95:5) Krebs-Ringer bicarbonate buffer, 0.3% (w/v) human serum albumin and, unless otherwise stated, 3mM-glucose. The islets were then individually incubated for 2 h with uniformly ^{14}C -labelled glucose, alanine, arginine or leucine in media (100 μl) which otherwise had the same basic composition as that used for the preincubation. The concentrations and specific radioactivities of the radioactive substances as well as the presence of other additives in the media are given in the legends of the Figures and Tables. The incubations were performed in liquid scintillation vials equipped with a small glass centre well (Keen, Field & Pastan, 1963). The effects of various additives were assessed by parallel incubations in control and test media, islets from a single animal being used in each run. Blank values were obtained by incubating media without islets. Metabolism was arrested by the injection of 100 μl of 0.1M-HCl into the centre well. The liberated CO_2 was collected in 100 μl of Hyamine, injected on to a circular piece of filter paper which had been placed on the bottom of the outer container vial before incubation. After shaking for 1 h at room temperature, the centre well was removed, 10 ml of scintillation liquid [5 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 1 litre of toluene] was added, and the radioactivity was counted in a liquid-scintillation spectrometer (Packard model 3375).

Weighing of the islets and determination of protein. After washing, the islets were freeze-dried overnight at -40°C and 0.001 mmHg and weighed on a quartz-fibre balance (Lowry, 1953). In a few experiments with homogenized islets, protein was assayed as described by Lowry, Rosebrough, Farr & Randall (1951), with human serum albumin as reference.

Evaluation of results. In each experiment blank values were secured by incubating the complete medium without islet tissue. The radioactivity of these blanks was much smaller than that recorded after incubation with islets. As a typical example it may be mentioned that after incubation with 5mM-leucine (2.6 mCi/mmol) the blank radioactivity was 209 ± 28 c.p.m., whereas the islets

yielded 767 ± 138 c.p.m. (mean values $\pm$ s.e.m. for 9 and 14 vials, respectively). Background radioactivity corresponded to about 30 c.p.m.

The observed c.p.m. values were translated into mmol of substrate oxidized by comparison with external standards the radioactivity of which was counted in parallel with the incubated vials. These standards consisted of 5 μl of incubation medium dissolved in 100 μl of Hyamine. Weighing of the islets made it possible to convert the data further into mmol of glucose or amino acid equivalents oxidized/h per kg dry wt. The statistical probability that the effect of an additive to the incubation medium was due to chance was estimated from the mean difference between test and control incubations in a series of identical but separate experiments. Throughout this paper the expression 'experiment' refers to a set of incubations with islets from a single animal. Within each experiment all factors were usually tested in triplicate incubations.

RESULTS

Effect of incubation time on the oxidation of glucose and amino acids. The production of $^{14}\text{CO}_2$ from uniformly ^{14}C -labelled glucose, alanine or leucine with time is shown in Fig. 1. After an initial lag period a constant oxidation rate was maintained for at least 2 h with all the three substrates.

Relationships between concentration and oxidation of glucose, amino acids and pyruvate. The oxidation of glucose was enhanced severalfold when its concentration was raised from 3 to 20mM. At the concentration of 3mM, the rate of $^{14}\text{CO}_2$ production corresponded to a complete oxidation of 8.8 ± 1.1 mmol/h per kg dry wt. (mean value $\pm$ s.e.m. for five experiments). The corresponding values at 10 and 20mM-glucose were 49.0 ± 5.7 ($n = 5$) and 63.4 ± 9.3 ($n = 6$).

Fig. 2 demonstrates the relationships between the concentration and the rate of oxidation of pyruvate, alanine, arginine and leucine. Formation of $^{14}\text{CO}_2$ from leucine was little affected by concentration changes above 5mM, the apparent K_m being of the order of 1–2mM. The rate of $^{14}\text{CO}_2$ production from 20mM-leucine corresponded to about one-fourth of that obtained with 20mM-glucose. In the absence of glucose only small amounts of $^{14}\text{CO}_2$ were obtained from alanine or arginine (c.p.m. values less than twice the blanks), even when the concentration of these amino acids was as high as 20mM.

Pyruvate was readily oxidized by the pancreatic islets. The production of $^{14}\text{CO}_2$ from 17mM-pyruvate was about one-sixth of that from 20mM-glucose. One experiment was performed to test whether the exogenous pyruvate could be diluted by endogenous pyruvate arising from glucose metabolism. This was apparently the case, since the rate of $^{14}\text{CO}_2$ production from pyruvate was markedly depressed in the presence of 17mM-glucose.

Fig. 1. Effect of time on the oxidation of [U-¹⁴C]glucose (●, 10 mM, 0.9 mCi/mmol), [U-¹⁴C]alanine (○, 5 mM, 1.8 mCi/mmol), and [U-¹⁴C]leucine (■, 5 mM, 2.6 mCi/mmol). The oxidation of alanine was studied in the presence of 17 mM-glucose (non-radioactive). All points represent the mean of two different experiments.

Effects of glucose on amino acid oxidation. Fig. 3 shows that the presence of glucose in the incubation media markedly affected the oxidation of alanine and leucine. There was, however, a principal difference between these two amino acids in that the production of ¹⁴CO₂ from alanine was enhanced, whereas that from leucine was depressed by glucose. Glucose at a concentration of 5 mM markedly stimulated the oxidation of alanine. At glucose concentrations of 10 and 17 mM the formation of ¹⁴CO₂ from alanine was several times more rapid than in a glucose-free medium. The relationship between the rate of alanine oxidation and the glucose concentration appeared to be sigmoidal, sensitivity to changes in the sugar concentration being greatest in the range 5–10 mM-glucose.

Alanine oxidation was also studied with islets homogenized in Krebs–Ringer bicarbonate buffer. When such homogenates were incubated in a glucose-free medium, the rate of ¹⁴CO₂ liberation was 6 μmol/h per kg of protein (mean of two experiments). In the presence of 17 mM-glucose there was an enhanced production of ¹⁴CO₂ corresponding to an oxidation of 25 μmol/h per kg of protein (mean of two experiments).

Fig. 2. Relationships between the concentration and the rate of oxidation for [U-¹⁴C]pyruvate (●, 0.6–17.3 mCi/mmol), [U-¹⁴C]alanine (▲, 0.9–1.8 mCi/mmol), [U-¹⁴C]arginine (△, 0.5–2.0 mCi/mmol), and [U-¹⁴C]leucine (□, 1.3–2.6 mCi/mmol). Each point represents the mean value of different experiments. Listed in order from the lowest concentration to the highest, the numbers of experiments were: for pyruvate 2, 2, 2, 1; for alanine 3, 5, 3, 3; for arginine 5, 6, 5, 5; and for leucine 8, 8, 4, 2. In addition, the result of one experiment showing the effect of 17 mM-glucose on the oxidation of pyruvate is given (○). Because the lability of labelled pyruvate yielded a comparatively high radioactivity blank, the experiments with this compound were performed with up to 20 micro-dissected islets in each incubation vial.

In striking contrast with the results obtained with alanine, 17 mM-glucose depressed the rate of leucine oxidation to only 25% of the value noted in the absence of glucose. Interference with leucine oxidation was apparent with as little as 5 mM-glucose, and a statistically significant inhibition was observed at a glucose concentration of 10 mM.

The rate of arginine oxidation remained at a barely detectable value irrespective of the glucose concentration within the range 0–17 mM.

Effects of citrate, pyruvate and succinate on amino acid oxidation. Whereas the liberation of ¹⁴CO₂ from leucine was strongly depressed by 17 mM-succinate (Table 1), neither succinate nor citrate (Tables 1 and 2) had any effect on the oxidation of alanine. The rate of ¹⁴CO₂ production from alanine was, however, enhanced by about 100% in the presence of 17 mM-pyruvate (Table 2).

Effects of insulin secretagogues on amino acid oxidation. Neither glibenclamide (50 μg/ml) nor dibutyryl cyclic AMP (250 μg/ml) had any significant effects on the oxidation of alanine and leucine,

irrespective of whether or not 5 mM-glucose was present (Table 3).

Compared with glucose, leucine had only a slight, if any, stimulating action on the oxidation of alanine (Table 4).

Effects of inhibitors of insulin release on amino acid oxidation. As shown in Table 5, neither adrenaline (2.0 µg/ml) nor diazoxide (125 µg/ml) affected the oxidation of alanine in the presence of 17 mM-glucose. By contrast, the ability of 10 mM-glucose to enhance alanine oxidation was almost completely blocked by 0.5 mM-iodoacetamide or 10 mM-mannoheptulose.

Mannoheptulose at a concentration of 15 mM

Fig. 3. Effects of glucose on the oxidation of [U-¹⁴C]alanine (●, 5 mM, 1.8 mCi/mmol), [U-¹⁴C]arginine (□, 5 mM, 2.0 mCi/mmol), and [U-¹⁴C]leucine (○, 5 mM, 2.6 mCi/mmol). Results are mean values ± s.e.m. for five (alanine, leucine) and six (arginine) different experiments.

also completely prevented the inhibitory action of 10 mM-glucose on the oxidation of leucine (Table 6). The production of ¹⁴CO₂ from leucine was not, however, affected by mannoheptulose in the absence of glucose.

Effects of alanine and leucine on glucose oxidation. In marked contradistinction to the interference of glucose with the oxidation of alanine and leucine, neither of these amino acids at a concentration of 10 mM significantly affected the oxidation of glucose (Table 7).

DISCUSSION

It has been known for several years that certain amino acids increase serum insulin concentration (Fajans *et al.* 1967). Experiments *in vitro* have made it clear that this is due to a direct stimulation of the pancreatic islets (Milner & Hales, 1967; Sussman *et al.* 1967; Edgar, Rabinowitz, Merimee & Almogela, 1969; Lambert, Jeanrenaud, Junod & Renold, 1969; Malaisse, 1969). There is at present no compelling reason to assume that the primary site of action is not the β-cells themselves, although the possibility that the amino acid effect is mediated or influenced by other types of islet cells cannot be excluded. In particular it should be kept in mind that amino acids can stimulate the release of glucagon (Unger & Eisentraut, 1970), which may in turn trigger insulin release. In the present study advantage was taken of the unusually low proportion of α-cells in the micro-dissected islets from obese-hyperglycaemic mice (Hellman, 1965). Our results are therefore probably representative of the insulin-secreting β-cells.

It is generally accepted that all amino acids are not equally effective stimulants of insulin release, although there seem to be species and age differences between their relative potencies (Fajans *et al.* 1967; Sussman *et al.* 1967; Lambert *et al.* 1969; Milner, 1969). The decision to study alanine, arginine and leucine in the present investigation was based on

Table 1. *Effect of succinate on the oxidation of alanine and leucine by pancreatic islets*

In each experiment micro-dissected islets from a single animal were incubated in media supplemented with 17 mM-succinate as well as in a succinate-free control medium. Specific radioactivities were 1.8 mCi/mmol for [U-¹⁴C]alanine and 2.6 mCi/mmol for [U-¹⁴C]leucine. Rates of ¹⁴CO₂ production are expressed as mmol of amino acid equivalents oxidized/h per kg dry islet wt. Mean values ± s.e.m. are given for each medium as well as for the differences between test and control media.

Labelled amino acid	No. of expts.	Rate of amino acid oxidation			P value for effect of succinate
		Control	Succinate (17 mM)	Succinate minus control	
Alanine, 5 mM	5	1.60 ± 0.66	2.38 ± 1.14	0.78 ± 1.50	>0.05
Leucine, 5 mM	5	9.74 ± 0.96	2.72 ± 1.20	-7.02 ± 0.95	<0.001

Table 2. *Effects of citrate and pyruvate on the oxidation of alanine by pancreatic islets*

Six experiments were performed. In each of these, micro-dissected islets from a single animal were incubated in media supplemented with 17 mM-citrate or 17 mM-pyruvate as well as in a control medium lacking these substances. [^{14}C]Alanine was used at a concentration of 5 mM with a specific radioactivity of 1.8 mCi/mmol. Rates of $^{14}\text{CO}_2$ production are expressed as mmol of alanine equivalents oxidized/h per kg dry islet wt. Mean values $\pm$ S.E.M. are given for each medium as well as for the differences between test and control media.

Test substance	Rate of alanine oxidation		<i>P</i> value for effect of test substance
		Test substance minus control	
None (control)	2.62 $\pm$ 0.39	—	—
Citrate, 17 mM	4.02 $\pm$ 1.23	1.40 $\pm$ 1.34	>0.05
Pyruvate, 17 mM	5.15 $\pm$ 0.59	2.53 $\pm$ 0.94	<0.05

Table 3. *Effects of glibenclamide and dibutyryl cyclic AMP on the oxidation of alanine and leucine by pancreatic islets*

In each experiment micro-dissected islets from a single animal were incubated in media supplemented with glibenclamide (50 $\mu\text{g}/\text{ml}$) or dibutyryl cyclic AMP (DB-cAMP; 250 $\mu\text{g}/\text{ml}$) as well as in a control medium lacking these substances. With both [^{14}C]alanine (5 mM, 1.8 mCi/mmol) and [^{14}C]leucine (5 mM, 2.6 mCi/mmol) two series of experiments were performed, one in the absence and one in the presence of 5 mM-glucose. Rates of $^{14}\text{CO}_2$ production are expressed as mmol of amino acid equivalents oxidized/h per kg dry islet wt. Mean values $\pm$ S.E.M. are given for each medium as well as for the differences between test and control media.

Labelled amino acid (5 mM)	No. of expts.	Concn. of glucose (mM)	Rate of amino acid oxidation				
			Control	Glibenclamide (50 $\mu\text{g}/\text{ml}$)	Glibenclamide minus control	DB-cAMP (250 $\mu\text{g}/\text{ml}$)	DB-cAMP minus control
Alanine	6	0	2.05 $\pm$ 0.31	2.43 $\pm$ 0.65	0.38 $\pm$ 0.65	3.00 $\pm$ 0.57	0.95 $\pm$ 0.54
Alanine	7	5	4.20 $\pm$ 0.84	3.21 $\pm$ 0.57	-0.99 $\pm$ 0.71	6.49 $\pm$ 1.11	2.29 $\pm$ 1.03
Leucine	5	0	8.14 $\pm$ 0.98	6.82 $\pm$ 0.86	-1.32 $\pm$ 0.89	9.36 $\pm$ 1.73	1.22 $\pm$ 1.92
Leucine	5	5	8.38 $\pm$ 1.16	8.36 $\pm$ 0.91	-0.02 $\pm$ 0.51	8.56 $\pm$ 1.38	0.18 $\pm$ 1.51

Table 4. *Effect of leucine on the oxidation of alanine by pancreatic islets*

Five experiments were performed. In each of these, micro-dissected islets from a single animal were incubated in glucose-free media supplemented with 0 mM-, 1 mM- and 10 mM-leucine (unlabelled). Rates of $^{14}\text{CO}_2$ production from 5 mM-[^{14}C]alanine (1.8 mCi/mmol) are expressed as mmol of alanine equivalents oxidized/h per kg dry islet wt. Mean values $\pm$ S.E.M. are given for each medium as well as for the differences between test and control media.

Concn. of leucine (mM)	Rate of alanine oxidation		<i>P</i> value for effect of leucine
		Leucine minus control	
0 (control)	2.46 $\pm$ 0.27	—	—
1	4.98 $\pm$ 0.45	2.52 $\pm$ 0.67	<0.02
10	4.04 $\pm$ 0.64	1.58 $\pm$ 0.88	>0.05

the observation that these amino acids differ markedly in their ability to stimulate the release of insulin from micro-dissected islets of obese-hyperglycaemic mice (Å. Lernmark, unpublished work). Whereas leucine at a concentration of 5 mM is an active secretagogue both in the absence and presence of glucose, the same concentration of

arginine alone fails to stimulate insulin release but is effective in the presence of glucose. In contrast to both leucine and arginine, alanine does not elicit a secretory response whether or not glucose is present. These observations with micro-dissected islets of obese-hyperglycaemic mice are in good agreement with previous results for mature

Table 5. *Effects of adrenaline, diazoxide, iodoacetamide, and mannoheptulose on the glucose-stimulated oxidation of alanine by pancreatic islets*

Five experiments were performed in series I. In each of these, micro-dissected islets from a single animal were incubated in media supplemented with adrenaline (2.0 µg/ml) or diazoxide (125 µg/ml) as well as in a control medium lacking these substances. All media contained 17 mM-glucose, which in this particular series of experiments was also present during the preincubation (see the Materials and Methods section). Series II comprised four experiments. In each of these, micro-dissected islets from a single animal were incubated in media containing 0.5 mM-iodoacetamide or 10 mM-mannoheptulose. All media contained 10 mM-glucose. Preincubation was, however, as usual performed with 3 mM-glucose. For both series of experiments the rates of $^{14}\text{CO}_2$ production from 5 mM-[U- ^{14}C]alanine (1.8 mCi/mmol) are expressed as mmol of alanine equivalents oxidized/h per kg dry islet wt. Mean values $\pm$ S.E.M. are given for each medium as well as for the differences between test and control media.

Test substance	Rate of alanine oxidation		<i>P</i> value for effect of test substance
		Test substance minus control	
Experimental series I			
None (control)	16.0 ± 2.0	—	—
Adrenaline, 2.0 µg/ml	15.6 ± 1.3	-0.4 ± 2.1	>0.05
Diazoxide, 125 µg/ml	15.6 ± 0.6	-0.4 ± 1.6	>0.05
Experimental series II			
None (control)	17.3 ± 2.6	—	—
Iodoacetamide, 0.5 mM	3.4 ± 0.9	-13.9 ± 1.7	<0.005
Mannoheptulose, 10 mM	2.6 ± 0.6	-14.7 ± 2.4	<0.005

Table 6. *Effect of mannoheptulose on the oxidation of leucine by pancreatic islets in the absence and presence of glucose*

In each experiment islets from a single animal were incubated in media supplemented with either 10 mM-glucose or 15 mM-mannoheptulose, or 10 mM-glucose plus 15 mM-mannoheptulose, as well as in a control medium lacking these substances. The rates of $^{14}\text{CO}_2$ production from 5 mM-[U- ^{14}C]leucine (2.6 mCi/mmol) are expressed as mmol of leucine equivalents oxidized/h per kg dry islet wt. In addition to the results of individual experiments, mean values $\pm$ S.E.M. are given for each medium as well as for the differences between media containing or lacking mannoheptulose.

Rate of leucine oxidation						
Expt. no.	Control	Mannoheptulose (15 mM)	Difference mannoheptulose versus control		Glucose (10 mM) + mannoheptulose (15 mM)	
				Glucose (10 mM)		Difference glucose + mannoheptulose versus glucose
1	12.7	8.6	-4.1	3.9	12.9	9.0
2	11.3	8.8	-2.5	4.0	12.7	8.7
3	20.9	14.0	-6.9	7.2	18.5	11.3
4	11.1	15.6	4.5	—	—	—
Mean $\pm$ S.E.M.	14.0 $\pm$ 2.3	11.8 $\pm$ 1.8	-2.2 $\pm$ 2.4 P > 0.05	5.0 $\pm$ 1.1	14.7 $\pm$ 1.9	9.7 $\pm$ 0.8 P < 0.01

β -cells of other species (Sussman *et al.* 1967; Edgar *et al.* 1969; Malaisse, 1969; Milner, 1969).

Information about amino acid oxidation in the pancreatic β -cells is scanty. It has been reported, however, that the islets of fish (Humbel & Renold, 1963) and obese-hyperglycaemic mice (Hellerström, Westman, Marsden & Turner, 1970; Stork, Schmidt, Hellerström & Westman, 1970) can oxidize

leucine. The present investigation revealed distinct differences between the oxidation in β -cells of glucose on the one hand and that of amino acids on the other. Further, strikingly different results were obtained with the three amino acids studied. In agreement with previous reports (Ashcroft, Hedeskov & Randle, 1970) the oxidation of glucose was enhanced severalfold by raising its concentra-

Table 7. *Effects of alanine and leucine on the oxidation of glucose by pancreatic islets*

The effect of leucine was tested in three different series of experiments. In each of these, islets from a single animal were incubated in media supplemented with 10 mM-leucine as well as in a leucine-free control medium. The rates of $^{14}\text{CO}_2$ production from $[\text{U-}^{14}\text{C}]\text{glucose}$ (0.9–2.7 mCi/mmol) in the concentrations listed are expressed as mmol of glucose equivalents oxidized/h per kg dry islet wt. The effect of alanine was tested in a separate series of experiments. In each of these, islets from a single animal were incubated in media containing 3 mM- $[\text{U-}^{14}\text{C}]\text{glucose}$ (2.7 mCi/mmol), 3 mM- $[\text{U-}^{14}\text{C}]\text{glucose}$ plus 10 mM-alanine, 20 mM- $[\text{U-}^{14}\text{C}]\text{glucose}$ (0.9 mCi/mmol), and 20 mM- $[\text{U-}^{14}\text{C}]\text{glucose}$ plus 10 mM-alanine. Oxidation rates are expressed as for the experiments with leucine. In the alanine as well as in the leucine experiments preincubation was performed in a glucose-free medium. Mean values $\pm$ S.E.M. are given for each medium as well as for the differences between test and control media.

Concn. of glucose (mM)	No. of expts.	Rate of glucose oxidation			<i>P</i> value for effect of amino acid
		Control	Amino acid (10mM)	Amino acid minus control	
Effect of alanine					
3	5	7.5 ± 1.3	8.8 ± 1.6	1.3 ± 1.2	>0.05
20	5	51.4 ± 4.7	53.7 ± 5.0	2.3 ± 6.5	>0.05
Effect of leucine					
3	5	8.8 ± 1.1	12.7 ± 1.2	3.9 ± 2.0	>0.05
10	5	49.0 ± 5.7	52.7 ± 3.2	3.7 ± 6.8	>0.05
20	6	63.4 ± 9.3	66.0 ± 8.9	2.6 ± 6.5	>0.05

tion from 3 to 20 mM. The oxidation of leucine was much less sensitive to concentration changes above 5 mM. The rate at 20 mM-leucine amounted to approx. 25% of that recorded for 20 mM-glucose.

In marked contrast with leucine, both alanine and arginine were only slowly oxidized in a glucose-free medium. This difference between alanine and leucine is somewhat surprising in view of the fact that the two amino acids appeared to be equally effective stimulants of oxygen uptake by islets from obese-hyperglycaemic mice (Hellerström *et al.* 1970). Like alanine and leucine, arginine is rapidly taken up by the pancreatic islet cells (B. Hellman, J. Sehlin & I.-B. Täljedal, unpublished work). The very small amounts of $^{14}\text{CO}_2$ obtained from arginine therefore suggest that metabolism of arginine in the pancreatic β -cells does not rapidly give rise to oxidizable intermediates. Little attention seems to have been paid to the oxidation of arginine in other mammalian tissues, but it is worth noting that this amino acid is also poorly oxidized by homogenates (Schepatz, 1963) and slices (Sadasivudu & Lajtha, 1970) of brain.

Islets of obese-hyperglycaemic mice exhibited a concentrative uptake of alanine resulting in distribution ratios much higher than unity (B. Hellman, J. Sehlin & I.-B. Täljedal, unpublished work). At an extracellular concentration of 5 mM, equilibrium was reached within 60 min of incubation, at which time the apparent intracellular concentration was of the order of 50 mM. The very small amounts of $^{14}\text{CO}_2$ obtained from alanine during 120 min of incubation in the present study there-

fore suggest a considerable restriction of the conversion of alanine into pyruvate. This appears to be overcome when glucose is also present, since rates of alanine oxidation equivalent to 17 mmol/h per kg dry wt. were reached with 17 mM-glucose. A similar stimulation of alanine oxidation by glucose has been observed with brain slices (Swaiman & Milstein, 1964; Cory & Rose, 1970; Sadasivudu & Lajtha, 1970). On the other hand, alanine oxidation by rat amnion cells was found to be inhibited by glucose (Ihle, Schultze & Rosenthal, 1966). The present results suggest that the stimulation of alanine oxidation in the pancreatic islets was mediated by glucose metabolism. The rate of alanine oxidation depended on the concentration of glucose in a manner similar to the oxidation of glucose itself. Further, mannoheptulose and iodoacetamide, which are both inhibitors of glucose metabolism in the pancreatic islets (Ashcroft *et al.* 1970; Hellerström *et al.* 1970; B. Hellman, unpublished work), almost completely blocked the stimulating action of glucose on the oxidation of alanine. The site of action of mannoheptulose may be glucose entry (Matschinsky *et al.* 1970) and/or phosphorylation (Ashcroft & Randle, 1970), whereas iodoacetamide in the concentration used seems to inhibit glycolysis at the phosphoglycerdehyde dehydrogenase stage in the islets (B. Hellman, unpublished work) as in other tissues (Webb, 1966).

Since neither citrate nor succinate could replace glucose as a stimulator of alanine oxidation in the β -cells, it seems unlikely that the stimulating action of glucose was mediated by the tricarboxylic acid

cycle. Further, the concentrations of α -keto-glutarate and glutamate in the β -cells have been found to be little influenced by changes in the extracellular glucose concentration (Danielsson, Hellman & Idahl, 1970). The possibility that the stimulated alanine oxidation reflected an enhanced uptake of the amino acid by islets did not receive support from direct studies of this process (B. Hellman, J. Sehlín & I.-B. Täljedal, unpublished work). In addition, the present results indicate that glucose may stimulate alanine oxidation in islet homogenates as well, although the latter results must be interpreted with some caution in view of the low oxidation rates that were obtained in homogenates whether or not glucose was present.

The observation that pyruvate was able to enhance the formation of $^{14}\text{CO}_2$ from alanine could mean that the stimulating effect of glucose was caused by increased concentrations of pyruvate in the β -cells. The interpretation of this pyruvate effect is somewhat difficult, since it cannot be ruled out that some kind of isotope exchange between alanine and pyruvate may have contributed to the results. The small effect obtained with pyruvate compared with that exhibited by glucose might reflect a difference between these two compounds in their ability to penetrate the β -cells. Such an interpretation seems compatible with the observation that formation of $^{14}\text{CO}_2$ from 17 mM-pyruvate was about one-sixth of that from 20 mM-glucose.

The moderately high rate of leucine oxidation in the absence of glucose was depressed by the addition of physiological concentrations of the sugar. Such an effect of glucose has previously been observed also with the β -cell-poor islets of the teleost *Opsanus tau* (Humbel & Renold, 1963). In this respect the islets differ from brain tissue, in which leucine oxidation has been reported to be stimulated by glucose (Sadasivudu & Lajtha, 1970). The inhibitory effect of glucose on the islet oxidation of leucine was probably not due to isotope dilution at the level of acetyl-CoA, since non-radioactive leucine did not diminish the rate of glucose oxidation. It has been suggested that mannoheptulose prevents the entrance of glucose into the β -cells (Matschinsky *et al.* 1970). In the present study mannoheptulose did not inhibit the oxidation, and thus probably not the uptake of leucine by islets incubated in a glucose-free medium. This makes it reasonable to assume that glucose and leucine do not share a common uptake mechanism. Mannoheptulose did, however, prevent glucose from inhibiting leucine oxidation. This strongly suggests that the interference of glucose with the oxidation of this amino acid was mediated by metabolism of the sugar. Such an interpretation is in good agreement with the observation that glucose did not inhibit the uptake of leucine by isolated pancreatic islets

(B. Hellman, J. Sehlín & I.-B. Täljedal, unpublished work).

Since the effects of exogenous glucose on the oxidation of both alanine and leucine appeared to be mediated by metabolism of the sugar, it is notable that the effects of glucose were not significantly altered by adrenaline, diazoxide, dibutyryl cyclic AMP or glibenclamide. These compounds are all potent modifiers of insulin release, but their modes of action are obscure. We have previously shown that inhibitors of insulin release, such as adrenaline and diazoxide, may inhibit glucose oxidation and cause accumulation of early glycolytic intermediates as well as of glycogen in the β -cells (Hellman, Sehlín & Täljedal, 1970; Hellman, 1970). The β -cell glycogen is, on the other hand, mobilized by insulin secretagogues such as dibutyryl cyclic AMP and glibenclamide (Hellman & Idahl, 1970). The interference of these compounds with glucose metabolism was apparently not great enough to be reflected in altered rates of alanine and leucine oxidation. Against this background it can well be questioned whether adrenaline, diazoxide, dibutyryl-cyclic AMP and glibenclamide exert their effects of insulin release primarily by influencing the glucose metabolism in the β -cell.

It has been reported that tolbutamide inhibits the oxidation of leucine in the same kind of islets as used in the present investigation (Stork *et al.* 1970). It should therefore be pointed out that uniformly labelled amino acids were used in the present study, whereas the results of Stork *et al.* (1970) only refer to the decarboxylation of $[1-^{14}\text{C}]$ leucine. If the effect of sulphonylurea were limited to the decarboxylation step, this effect would be less evident in our results.

Evidence has been presented in support of the idea that amino acids may stimulate insulin release without being metabolized (Christensen & Cullen, 1969). These experiments were performed with non-metabolizable artificial compounds and do not rule out the possibility that some natural amino acids act as insulin secretagogues by virtue of their metabolism. The present results suggest, however, that amino acids do not stimulate insulin release simply by serving as fuels for the β -cells. Alanine, which is not a secretagogue in our system, was rapidly oxidized in the presence of glucose. Conversely, the oxidation of leucine was markedly depressed by glucose, although the capacity of this amino acid to stimulate insulin secretion is not reduced by the sugar. Finally, arginine was practically not oxidized at all, although this amino acid elicits insulin release from our islets when glucose is present in the medium.

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