Ketone bodies and islet function : 86Rb handling and metabolic data

W.J. Malaisse, P. Lebrun, J. Rasschaert, Francois F. Blachier, Tuncay Yilmaz, A. Sener

▶ **To cite this version:**

W.J. Malaisse, P. Lebrun, J. Rasschaert, Francois F. Blachier, Tuncay Yilmaz, et al.. Ketone bodies and islet function : 86Rb handling and metabolic data. AJP - Endocrinology and Metabolism, 1990, 22 (1), pp.E123-E130. hal-02705911

HAL Id: hal-02705911
https://hal.inrae.fr/hal-02705911

Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Ketone bodies and islet function: 86Rb handling and metabolic data

W. J. Malaisse, P. Lebrun, J. Rasschaert, F. Blachier, T. Yilmaz and A. Sener

Am J Physiol Endocrinol Metab 259:E123-E130, 1990. ;

You might find this additional info useful...

This article has been cited by 2 other HighWire-hosted articles:
http://ajpendo.physiology.org/content/259/1/E123#cited-by

Updated information and services including high resolution figures, can be found at:
http://ajpendo.physiology.org/content/259/1/E123.full

Additional material and information about ***American Journal of Physiology - Endocrinology and Metabolism*** can be found at:
http://www.the-aps.org/publications/ajpendo

This information is current as of January 24, 2013.

American Journal of Physiology - Endocrinology and Metabolism publishes results of original studies about endocrine and metabolic systems on any level of organization. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 1990 the American Physiological Society. ISSN: 0193-1849, ESSN: 1522-1555. Visit our website at http://www.the-aps.org/.

Downloaded from http://ajpendo.physiology.org/ by Renaud Beauwens on January 24, 2013

Ketone bodies and islet function: ^{86}Rb handling and metabolic data

WILLY J. MALAISSE, PHILIPPE LEBRUN, JOANNE RASSCHAERT, FRANÇOIS BLACHIER, TEMEL YILMAZ, AND ABDULLAH SENER
Laboratory of Experimental Medicine, Brussels Free University, B-1000 Brussels, Belgium

MALAISSE, WILLY J., PHILIPPE LEBRUN, JOANNE RASSCHAERT, FRANÇOIS BLACHIER, TEMEL YILMAZ, AND ABDULLAH SENER. *Ketone bodies and islet function: ^{86}Rb handling and metabolic data.* Am. J. Physiol. 259 (Endocrinol. Metab. 22): E123–E130, 1990.—The metabolism of ketone bodies was investigated in rat pancreatic islets incubated in the absence or presence of D-glucose. The generation of $^{14}CO_2$ from 3-^{14}C-labeled ketone bodies, the interconversion of D-(−)-β-hydroxybutyrate and acetoacetate (AcAc), the reciprocal effects of ketone bodies and D-glucose on their respective catabolism, and the influence of these exogenous nutrients on the output of $^{14}CO_2$ from islets preincubated with either L-[U-^{14}C]glutamine or [U-^{14}C]palmitate provided an estimation of the nutrient-induced changes in O_2 uptake that was in fair agreement with the observed modifications of islet respiration. There was a close correlation between such changes and the corresponding values for insulin output. Because the stimulation of insulin release by ketone bodies also coincided with a decrease in ^{86}Rb outflow from prelabeled islets, these findings suggest that the insulinotropic action of ketone bodies is causally linked to their catabolism through an increase in ATP generation rate and a subsequent decrease in K^+ conductance. A complementary participation of changes in mitochondrial redox state to stimulus-secretion coupling is considered, however, in the light of comparisons between the effects of D-(−)-β-hydroxybutyrate and AcAc, respectively, on mitochondrial NADH generation, ^{45}Ca net uptake, and D-[6-^{14}C]glucose oxidation.

pancreatic islets; D-(−)-β-hydroxybutyrate; acetoacetate; rubidium-86

THE EFFECTS of ketone bodies on insulin release, calcium handling, and biosynthetic activity in pancreatic islets are compatible with the view that the insulinotropic action of these nutrients may be causally linked to their catabolism in the islets (26). Such a hypothesis was further investigated in the present study by exploring both the metabolism of ketone bodies in isolated rat islets and the resulting changes in ^{86}Rb outflow from prelabeled islets.

MATERIALS AND METHODS

D-(−)-β-Hydroxy-[3-^{14}C]butyrate {D-(−)-β-[3-^{14}C]-OHB} and ethyl[3-^{14}C]acetoacetate were purchased from Amersham (Buckinghamshire, UK). The [^{14}C]acetoacetate (AcAc) was freshly prepared from its ester, in the presence of unlabeled ethyl AcAc, as described in the preceding report (26).

All experiments were performed with islets prepared from fed female Wistar albino rats (32). The methods used to measure the uptake of nutrients (37), their oxidation (9) and their effects on O_2 consumption (6, 36), and the output of $^{14}CO_2$ from islets prelabeled with either [U-^{14}C]palmitate (38) or L-[U-^{14}C]glutamine (30, 40) are described in the cited references. In the measurement of [3-^{14}C]AcAc oxidation, which was made with groups of 20–25 islets each, the blank value was unusually high, representing 0.81 ± 0.19% ($n = 4$) of the total radioactive content of the incubation media (40 μl). The incorporation of metabolic poisons (KCN, 5.0 mM and antimycin A, 0.01 mM) in the incubation medium failed to decrease such a blank value. The procedures used to measure the generation of [^{14}C]AcAc (16) and the efflux of ^{86}Rb from prelabeled islets (8) were also previously reported.

To measure the content of the islets and incubation media in D-(−)-β-hydroxybutyrate [D-(−)-β-OHB], groups of 40 islets each were incubated for 120 min at 37°C in 40 μl of our usual bicarbonate-buffered medium (32). The incubation was halted by addition of 10 μl perchloric acid (PCA; 12.5%, vol/vol) and, after freezing in liquid N_2, the islets were disrupted by mechanical agitation (23). After heating for 5 min at 85°C and centrifugation for 3 min at 5,000 *g*, aliquots of the PCA extract (40 μl) were neutralized with 20 μl of a solution of KOH (1.0 M) and tris(hydroxymethyl)aminomethane (Tris; 0.2 M) and stored at −20°C. The next day, the samples were again centrifuged, and aliquots of the supernatant solution (40 μl) were mixed with 30 μl Tris·HCl buffer (100 mM, pH 8.5) containing EDTA (2.6 mM), hydrazinium hydroxide (1.0 M), NAD^+ (1.0 mM), and β-hydroxybutyrate dehydrogenase (0.15 U/ml) from *Rhodopseudomonas spheroides* (Boehringer, Mannheim, FRG). After 60-min incubation at 20°C, the reaction was halted by heating for 10 min at 65°C. After addition of 140 μl *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-NaOH buffer (100 mM, pH 7.0) containing ammonium acetate (100 mM), ADP (1.0 mM), 2-ketoglutarate (0.1 mM) mixed with a tracer amount of 2-[U-^{14}C]ketoglutarate, and beef liver glutamate dehydrogenase (6 U/ml; Boehringer) and further incubation for 30 min at 20°C, the reaction was halted by addition of 0.9 ml iced H_2O and ^{14}C-labeled glutamate separated by ion-exchange chromatography (30). The results were corrected for the blank value found in the absence of islets and corrected for the recovery of standards of D-(−)-β-OHB prepared in the same incubation medium and treated throughout in the same manner.

0193-1849/90 $1.50 Copyright © 1990 the American Physiological Society

Downloaded from http://ajpendo.physiology.org/ by Renaud Beauwens on January 24, 2013

For measuring the activity of β-hydroxybutyrate dehydrogenase, groups of 500 islets were sonicated (3 × 10 s) in 0.6 ml Tris·HCl buffer (50 mM; pH 7.4). Aliquots (30 μl) of the homogenate were mixed with 20 μl of the same buffer containing increasing concentrations of D-(−)-β-OHB and 50 μl of a reaction mixture (18) consisting of the same buffer enriched with 1.0 mM EDTA, 2.0 mM L-cysteine, 0.25 mM rotenone, 2.0 mM NAD^+, and bovine serum albumin (0.1 mg/ml). After 10 to 30 min incubation at 20°C, the reaction was halted by heating for 10 min at 80°C. The NADH formed and the standards of NADH (2–8 nmol/sample) were then measured as described above through the generation of L-[U-^{14}C]-glutamate from 2-[U-^{14}C]ketoglutarate. The generation of NADH was constant with time (10–30 min incubation) and proportional to the number of islets (5–25 islets/sample).

All results are expressed as means ± SE together with the number of individual determinations (n) or degrees of freedom (df). The SE on the sum, difference, or ratio between mean values was calculated as described elsewhere (39, 41). The statistical significance of differences between mean values was assessed with Student's t test.

RESULTS

Uptake and oxidation of ketone bodies. During 10-min incubation and after correction for extracellular contamination, as judged from the space of distribution of L-[1-^{14}C]glucose (2.0 mM) measured in paired groups of islets, the net uptake of D-(−)-β-[3-^{14}C]OHB and [3-^{14}C]AcAc (10.0 mM each) amounted to 12.8 ± 4.2 and 10.4 ± 4.4 pmol/islet, respectively (n = 16 and 17). Considering the intracellular space of the islets (12, 13), this suggests that the concentration of ketone bodies was close to reaching equilibrium across the islet cell plasma membrane.

In the absence of D-glucose, the oxidation of D-(−)-β-[3-^{14}C]OHB (10 mM) averaged 17.1 ± 1.6 pmol·120 min^{-1}·$islet^{-1}$. Assuming full oxidation of the ketone body, the yield of CO_2 represented only 58.2 ± 6.2% of that recorded within the same experiments in the presence of 7.0 mM D-[U-^{14}C]glucose (Table 1). The hexose augmented markedly ($P < 0.001$) D-(−)-β-[3-^{14}C]OHB oxidation, which was concentration-related in the 2.5–10.0 mM range. The association of KCN and antimycin A abolished D-(−)-β-[3-^{14}C]OHB oxidation in either the absence or presence of D-glucose.

The oxidation of [3-^{14}C]AcAc was not significantly different in the absence or presence of D-glucose, and also suppressed by KCN and antimycin A. In the presence of D-glucose (7 mM), the oxidation of D-(−)-β-[3-^{14}C]OHB and [3-^{14}C]AcAc (each 10 mM) occurred at similar rates. Neither D-(−)-β-OHB nor AcAc (each 10 mM) affected significantly the oxidation of D-[U-^{14}C]-glucose (7 mM).

Activity of β-hydroxybutyrate dehydrogenase and interconversion of ketone bodies. In a series of seven experiments, the generation of NADH by islet homogenates incubated in the presence of 10 mM D-(−)-β-OHB and 1.0 mM NAD^+ averaged 1.19 ± 0.27 pmol·min^{-1}·$islet^{-1}$. The K_m for D-(−)-β-OHB was close to 1.9 mM (Fig. 1).

In the absence of D-glucose, the production of [^{14}C]-AcAc from exogenous D-(−)-β-[3-^{14}C]OHB (10 mM) averaged 7.75 ± 0.38 pmol·120 min^{-1}·$islet^{-1}$ (Table 2). It was increased by ~60% ($P < 0.001$) in the presence of D-glucose (7 mM). Whether in the presence or absence of the hexose, the generation of [^{14}C]AcAc was abolished by the combination of KCN (5 mM) and antimycin A (0.01 mM), the reading recorded in the presence of these metabolic poisons not exceeding 0.83 ± 1.51 pmol·120 min^{-1}·$islet^{-1}$ (n = 8).

The comparison between the production of $^{14}CO_2$ and [^{14}C]AcAc, respectively, by islets exposed to D-(−)-β-[3-^{14}C]OHB (10 mM) in the absence of D-glucose, suggested that the oxidation of [^{14}C]AcAc accounted for 68.8 ± 6.6% of its total generation rate. This contrasts with the situation previously documented in islets exposed to L-leucine or 2-ketoisocaproate, in which case the major fraction of AcAc generated from the branched-chain amino or keto acid apparently escapes either oxidation or conversion of D-(−)-β-OHB (16, 22, 31). For instance, in the presence of L-leucine (10 mM), the comparison between the amino acid-induced increment in unlabeled AcAc production and the oxidation of L-[1-^{14}C]leucine suggests that 77.7 ± 6.6% of the leucine-derived AcAc is not further metabolized (31). Similarly, in the present

TABLE 1. *Reciprocal effects of ketone bodies and D-glucose on their respective oxidation*

^{14}C-Labeled Nutrients, mM	Unlabeled Agents, mM	Oxidation Rate, pmol·120 min^{-1}·$islet^{-1}$	n
10.0 D-(−)-β-[3-^{14}C]OHB		17.06±1.64	55
10.0 D-(−)-β-[3-^{14}C]OHB	5 KCN + 0.01 AA	0.82±0.08	4
2.5 D-(−)-β-[3-^{14}C]OHB	7.0 D-Glucose	12.11±0.80	24
10.0 D-(−)-β-[3-^{14}C]OHB	7.0 D-Glucose	28.88±1.62	32
10.0 D-(−)-β-[3-^{14}C]OHB	7.0 D-Glucose +5 KCN + 0.01 AA	0.17±0.07	4
10.0 [3-^{14}C]AcAc		28.52±4.10	27
10.0 [3-^{14}C]AcAc	5 KCN + 0.01 AA	−3.37±3.30	16
10.0 [3-^{14}C]AcAc	7.0 D-Glucose	32.41±8.20	18
7.0 D-[U-^{14}C]glucose		19.54±0.90	83
7.0 D-[U-^{14}C]glucose	10.0 D-(−)-β-OHB	19.55±0.82	14
7.0 D-[U-^{14}C]glucose	10.0 AcAc	18.39±0.96	15
16.7 D-[U-^{14}C]glucose		42.26±2.00	9

Values for oxidation rate are means ± SE; n, no. of individual determinations. AA, antimycin A.

Downloaded from http://ajpendo.physiology.org/ by Renaud Beauwens on January 24, 2013

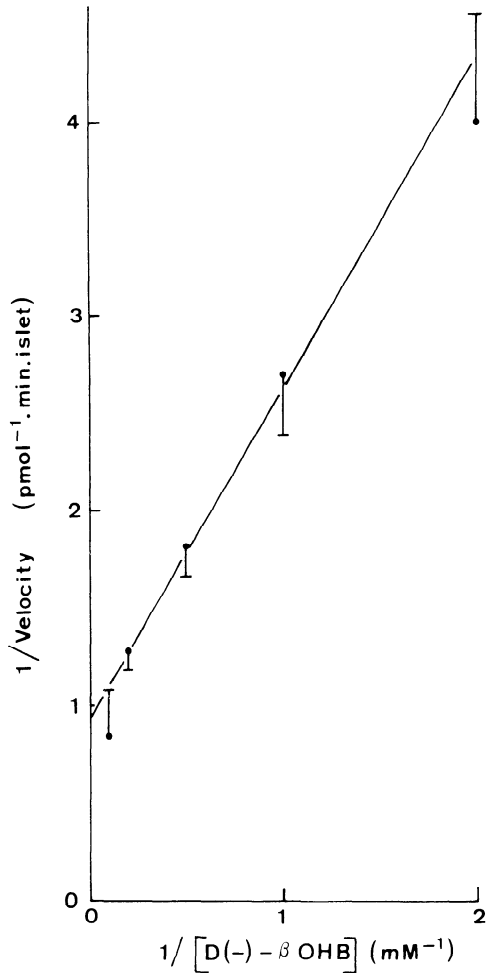

FIG. 1. Double reciprocal plot for activity of β-OHB dehydrogenase in islet homogenates. Values are means ± SE, derived from duplicate measurements collected in 4 separate experiments.

TABLE 2. *Interconversion of ketone bodies in the absence or presence of* D-*glucose*

Exogenous Nutrient(s), mM			Production, pmol·120 min^{-1}·islet^{-1}			
D-Glucose	D-(−)-β-[3-^{14}C]OHB	AcAc	[^{14}C]AcAc	n	D-(−)-β-OHB	n
					0.65±0.14	20
	10.0		7.75±0.38	8		
		10.0			8.91±0.40	20
7.0					1.25±0.10	14
7.0	10.0		3.22±0.56	8		
7.0		10.0			11.74±0.51	14

Values for production means ± SE.

series of experiments, when both the generation of $^{14}CO_2$ and [^{14}C]AcAc were simultaneously measured in islets exposed to L-[U-^{14}C]leucine (10 mM), the results indicated that at least 71.7 ± 8.8% (n = 12) of the molecules of AcAc generated from exogenous L-leucine escaped further metabolism. To facilitate the interpretation of these contrasting behaviors, we examined the effects of L-leucine and 2-ketoisocaproate on the oxidation of D-(−)-β-[3-^{14}C]OHB. As shown in Table 3, the branched-chain amino or keto acid (each 10 mM) decreased by 30–50% the generation of $^{14}CO_2$ from D-(−)-β-[3-^{14}C]OHB

TABLE 3. *Reciprocal effect of* D-(−)-β-*OHB and* L-*leucine on respective oxidation*

^{14}C-Labeled Nutrient, mM	Unlabeled Agent(s), mM	Oxidation Rate, pmol·120 min^{-1}·islet^{-1}	n
10 D-(−)-β-[3-^{14}C]OHB		18.76±1.80	27
10 D-(−)-β-[3-^{14}C]OHB	10 L-Leucine	12.83±0.94	27
10 D-(−)-β-[3-^{14}C]OHB	10 2-Ketoisocaproate	9.21±1.16	26
		82.35±4.58	16
10 L-[1-^{14}C]leucine	10 D-(−)-β-OHB	75.02±4.69	9
10 L-[1-^{14}C]leucine			
10 L-[U-^{14}C]leucine		33.91±1.85	15
10 L-[U-^{14}C]leucine	10 D-(−)-β-OHB	32.37±1.29	16
10 L-[U-^{14}C]leucine	5 KCN + 0.01 AA	0.45±0.20	4

Values for oxidation rate are means ± SE. AA, antimycin A.

(also 10 mM). It should be underlined, however, that unlabeled D-(−)-β-OHB failed to affect significantly the oxidation of either L-[1-^{14}C]leucine or L-[U-^{14}C]leucine (Table 3). These findings indicate that, although the ketone body does not alter the catabolism of L-leucine, the oxidation of AcAc derived from exogenous D-(−)-β-OHB is markedly inhibited in islets exposed to L-leucine or 2-ketoisocaproate. These results may reflect, in part at least, reciprocal isotopic dilution, e.g., of the acetyl-CoA pool.

The generation of D-(−)-β-OHB from exogenous AcAc (10 mM) amounted, in the absence of D-glucose and after correction for the basal reading, to 8.26 ± 0.42 pmol·120 min^{-1}·islet^{-1} (Table 2). In the absence of exogenous AcAc, D-glucose (7 mM) doubled ($P < 0.005$) the islet content and/or output of D-(−)-β-OHB. The hexose, however, increased the AcAc-induced increment in D-(−)-β-OHB production by only 27.0 ± 8.8%.

From the data summarized in Table 2, it can be calculated that, in terms of the net generation of NADH attributable to the interconversion of ketone bodies, the difference between islets exposed to D-(−)-β-OHB and AcAc, respectively, amounts to 16.0 ± 0.8 pmol·120 min^{-1}·islet^{-1} in the absence of D-glucose and 13.7 ± 1.0 pmol·120 min^{-1}·islet^{-1} in the presence of the hexose. These calculations are restricted to the fraction of the ketone bodies that escaped further oxidation. However, if the same calculation is applied to both unoxidized and oxidized ketone bodies, the difference in NADH generation at the level of the reaction catalyzed by β-hydroxybutyrate dehydrogenase would amount to 33.1 ± 1.1 and 42.6 ± 1.0 pmol·120 min^{-1}·islet^{-1}, in the absence and presence of D-glucose, respectively.

Catabolism of endogenous nutrients and O_2 uptake. To investigate the possible interference of ketone bodies with the catabolism of endogenous nutrients, we examined the effects of D-(−)-β-OHB and AcAc on the production of $^{14}CO_2$ by islets prelabeled with either L-[U-^{14}C]glutamine or [U-^{14}C]palmitate, the oxidation of the latter two nutrients accounting for a major fraction of the basal respiration by islets deprived of exogenous nutrient (20, 30).

When islets were preincubated for 30 min with L-[U-^{14}C]glutamine (1.0 mM), the radioactive content of the islets averaged 13.5 ± 0.5 pmol of glutamine-equivalent per islet (Table 4). After correction for the readings

Downloaded from http://ajpendo.physiology.org/ by Renaud Beauwens on January 24, 2013

TABLE 4. *Effect of ketone bodies and D-glucose on oxidation of endogenous nutrients*

	L-[U-^{14}C]glutamine[a] (1.0 mM)	n	[U-^{14}C]palmitate[b] (0.3 mM)	n
Net uptake of ^{14}C-labeled nutrients, pmol/islet[c]	13.53±0.54	106	1.73±0.08	140
$^{14}CO_2$ output, %content				
Control[d]	15.93±0.80	24	6.93±0.62	30
Metabolic poisons[e]	0.86±0.07	14	0.19±0.04	17
Effect of exogenous nutrient(s) on $^{14}CO_2$ output, %control[f]				
No exogenous nutrient	100.0±5.0	23	100.0±7.3	30
10 D-(−)-β-OHB	96.2±6.9	12	71.1±4.8	18
10 AcAc	110.7±7.5	11	71.8±6.0	12
7 D-Glucose	123.2±5.9	23	83.3±7.9	32
7 D-Glucose + 10 D-(−)-β-OHB	137.8±8.5	12	63.3±4.8	18
7 D-Glucose + 10 AcAc	132.1±9.4	11	68.6±7.8	13

Values are means ± SE. [a] Islets were preincubated for 30 min with 1.0 mM L-[U-^{14}C]glutamine, then incubated for 30 min in absence or presence of unlabeled exogenous nutrient(s). [b] Islets were preincubated for 120 min with 0.3 mM [U-^{14}C]palmitate in presence of 8.3 mM D-glucose, then incubated for 120 min in absence or presence of unlabeled exogenous nutrient(s). [c] Net uptake of ^{14}C-labeled nutrients was calculated at the onset of the final incubation. [d] Control value for output of $^{14}CO_2$ during final incubation performed in absence of exogenous nutrient was already corrected for readings recorded in presence of metabolic poisons. [e] Metabolic poisons included 5.0 mM KCN, 0.01 mM antimycin A, and 0.01 mM rotenone. [f] All readings were corrected for $^{14}CO_2$ output recorded in presence of metabolic poisons.

collected in the presence of metabolic poisons (KCN, antimycin A, and rotenone), the output of $^{14}CO_2$ during a further incubation of 30 min performed in the absence of any exogenous nutrient represented 15.9 ± 0.8% of the islet radioactive content. Neither D-(−)-β-OHB nor AcAc (each 10 mM) significantly affected the $^{14}CO_2$ output during the final incubation, whether in the absence or presence of D-glucose (7 mM). The hexose, however, significantly increased the production of $^{14}CO_2$ ($P < 0.005$). For reasons discussed elsewhere (20), this effect of D-glucose does not coincide with any sizable change in O_2 uptake attributable to the catabolism of endogenous amino acids.

When islets were preincubated for 120 min with [U-^{14}C]palmitate (0.3 mM) in the presence of D-glucose (8.3 mM), the radioactive content of the islets amounted to 1.73 ± 0.08 pmol of palmitate-equivalent per islet (Table 4). After correction for the readings recorded in the presence of metabolic poisons, the ouput of $^{14}CO_2$ during a further incubation of 120 min conducted in the absence of exogenous nutrient represented 6.91 ± 0.62% of the initial radioactive content of the islets. Relative to such a control value, D-(−)-β-OHB and AcAc (10 mM each) inhibited $^{14}CO_2$ output by 28–29% ($P < 0.025$). D-Glucose (7 mM) failed to significantly affect ($P > 0.1$) the output of $^{14}CO_2$, which was decreased by 31–34% ($P < 0.02$) in the concomitant presence of the hexose and ketone bodies (Table 4). Incidentally, when islets were immediately exposed for 120 min to [U-^{14}C]palmitate (0.1 mM) in the presence of D-glucose (7 mM), the oxidation of the fatty acid (0.28 ± 0.03 pmol·120 min^{-1}·islet^{-1}; $n = 7$) failed to be affected by D-(−)-β-OHB (10 mM; data not shown), suggesting that the sparing action of ketone bodies on $^{14}CO_2$ output from islets prelabeled with [U-^{14}C]palmitate may be due, to a large extent, to inhibition of lipolysis. This proposal is consistent with both the large esterification of [U-^{14}C]palmitate during the preincubation period (38) and the well-known antilipolytic effects of ketone bodies in other cell types (1, 5, 10).

Taken as a whole, the findings summarized in Table 4 suggest that ketone bodies fail to affect the catabolism of endogenous glutamate (derived from L-[U-^{14}C]glutamine), but exert a significant sparing action on the catabolism of endogenous fatty acids. Hence, the latter effect was taken into account to assess the overall effect of ketone bodies on O_2 consumption by the islets (Table 5).

The effect of ketone bodies on islet respiration was also explored through the direct measurement of O_2 uptake by polarography. The basal rate of O_2 uptake averaged 4.16 ± 0.56 pmol·min^{-1}·islet^{-1} ($n = 13$). As shown in Fig. 2, the administration of D-glucose (7 mM) to islets first deprived of exogenous nutrient increased O_2 consumption by 57.3 ± 11.4% ($n = 10$; $P < 0.001$). Similarly, when the islets were first exposed to either D-(−)-β-OHB or AcAc (10 mM each), the addition of D-glucose (7 mM) provoked an increase in O_2 uptake that represented 60.4 ± 16.0% ($n = 10$; $P < 0.005$) of the paired basal respiration. In the absence of D-glucose, D-(−)-β-OHB and AcAc (each 10 mM) increased O_2 uptake by 19.1 ± 4.1% ($n = 6$; $P < 0.005$) and 25.4 ± 3.9% ($n = 4$; $P < 0.01$), respectively (Fig. 2). When the islets were first exposed to D-glucose (7 mM), the administration of AcAc (10 mM) failed to cause any sizable change in respiration ($n = 6$; $P > 0.1$), whereas D-(−)-β-OHB further augmented by 15.0 ± 4.6% ($n = 8$; $P < 0.02$) the glucose-induced paired increase in O_2 consumption. As shown in Fig. 2*A*, there was a fair correlation ($r = 0.922$; $P < 0.01$) between the observed changes in respiration and the theoretical values derived from both the interconversion of ketone bodies and oxidative data on the fate of exogenous and endogenous ^{14}C-labeled nutrients (Table 5).

ATP generation and insulin release. When the rate of insulin release was compared with either the experimental or calculated changes in O_2 uptake, a typical relationship (Fig. 2*B*) characterized by a threshold value for the stimulation of insulin release as a function of islet respiration (15, 30) was observed. In considering this relationship, it should be realized that the respiratory data do not fully inform on the high-energy yield because of differences in the P/O ratio for the oxidation of distinct nutrients. For instance, the extramitochondrial net generation of ATP associated with anaerobic glycolysis does not lead to a change in O_2 uptake. Similarly, the conversion of AcAc to acetoacetyl CoA in the reaction catalyzed by acetoacetate-succinate CoA-transferase occurs at the expense of guanosine 5′-triphosphate (GTP) that would otherwise be formed through the succinyl-CoA synthetase reaction in the citric acid cycle (33). Because the generation of ATP, or more precisely the cytosolic ATP-to-ADP ratio, is currently thought to control the closing of ATP-responsive K^+ channels located at the B-cell plasma membrane (28, 35), the next series of experiments

Downloaded from http://ajpendo.physiology.org/ by Renaud Beauwens on January 24, 2013

TABLE 5. *Changes in O_2 uptake calculated from fate of ^{14}C-labeled nutrient(s)*

	Nutrients, mM				
	10 D-(−)-β-OHB	10 AcAc	7 D-Glucose	7 D-Glucose + 10 D-(−)-β-OHB	7 D-Glucose + 10 AcAc
Oxidation of exogenous nutrient(s)	+76.8±7.4	+114.1±16.4	+117.2±5.4	+247.3±8.8	+240.0±33.3
Oxidation of endogenous nutrients	−57.8±9.6	−56.4±12.0	−33.4±15.8	−67.4±9.6	−62.8±15.6
Interconversion of ketone bodies	+3.9±0.2	−4.3±0.2	0	+1.6±0.3	−5.2±0.3
Calculated change in O_2 uptake	+22.9±12.1	+53.4±20.3	+83.8±16.7	+181.5±13.0	+172.0±36.8

Values are means ± SE in pmol $O_2 \cdot 120\ min^{-1} \cdot islet^{-1}$. We converted sparing action of exogenous nutrient(s) on $^{14}CO_2$ output from islets prelabeled with [U-^{14}C]palmitate to a respiratory rate, assuming that endogenous fatty acids account for 40% of basal O_2 uptake (see Ref. 30).

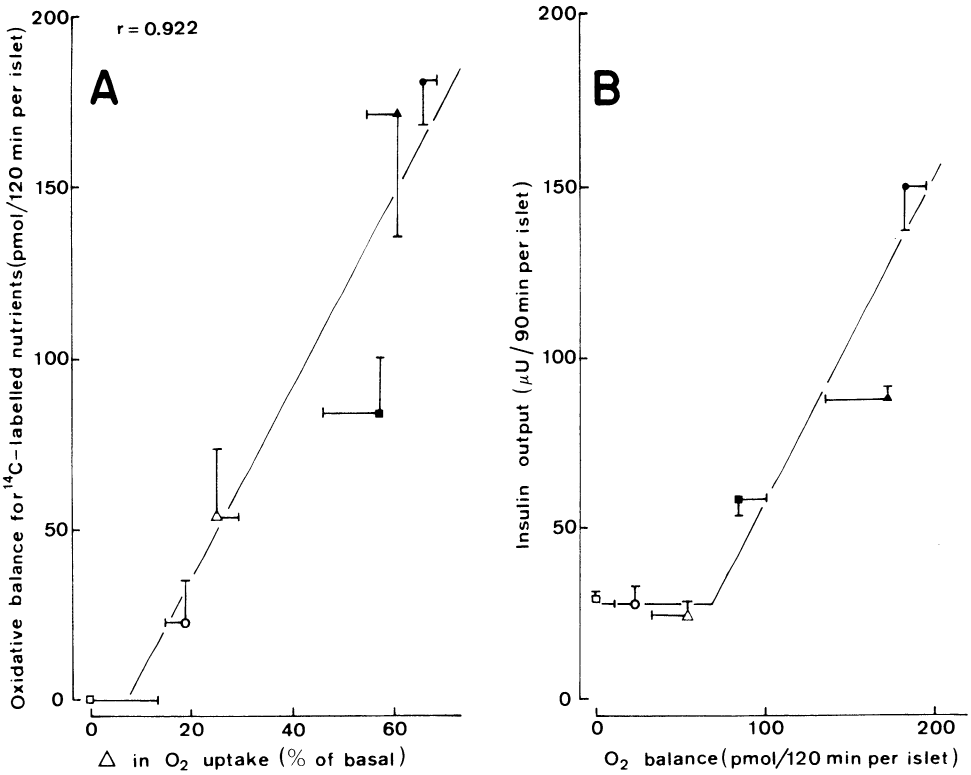

FIG. 2. *A*: correlation between observed changes in O_2 uptake and oxidative data collected with ^{14}C-labeled nutrients (Table 5) in islets incubated in absence (squares) or presence of either D-(−)-β-hydroxybutyrate (circles) or acetoacetate (triangles) tested at a concentration of 10 mM with (closed symbols) or without (open symbols) D-glucose (7 mM). *B*: relationship between insulin release and oxidative data (same presentation as in *A*).

aimed at investigating the effect of ketone bodies on ^{86}Rb outflow from prelabeled islets.

^{86}Rb outflow. The effect of ketone bodies on ^{86}Rb outflow was examined both in the absence and presence of D-glucose (7 mM).

In the absence of the hexose, the increase in O_2 uptake evoked by D-(−)-β-OHB (10 mM) coincided with a modest but significant decrease in both ^{86}Rb and ^{45}Ca fractional outflow rate and a paradoxical early decrease in insulin output (Fig. 3). As judged from the changes in either effluent radioactivity or insulin output recorded just before (*min 40* vs. *44*) and after (*min 44* vs. *48*) introduction of D-(−)-β-OHB, the inhibitory action of the ketone body on cationic fluxes and hormonal secretion was highly significant in all cases ($P < 0.001$). The fall in ^{86}Rb outflow, which was observed in 12 separate experiments, was reminiscent of, but less pronounced than, that evoked by D-glucose when tested at concentrations below that required for stimulation of insulin release (7). Hence, it could result from a modest increase in cytosolic ATP-to-ADP ratio (28) and/or the partial inactivation of a Ca^{2+}-dependent modality of K^+ outflow (11, 25). The latter hypothesis takes into account the fact that D-(−)-β-OHB caused an early decrease in basal insulin output, suggesting that the ketone body may lower cytosolic Ca^{2+} activity. Because D-(−)-β-OHB also inhibited ^{45}Ca outflow from the islets perifused in the absence of D-glucose but presence of extracellular Ca^{2+}, the postulated lowering of cytosolic Ca^{2+} concentration could conceivably result from stimulation of Ca^{2+} uptake by intracellular organelles (14).

In the presence of D-glucose (7 mM), both D-(−)-β-OHB and AcAc (10 mM) provoked a rapid and rapidly reversible inhibition of ^{86}Rb outflow (Fig. 4). At normal extracellular Ca^{2+} concentration (1.0 mM), the initial fall in effluent radioactivity was followed, after 5- to 6-min exposure to the ketone bodies by a modest but systematic reascension in ^{86}Rb outflow. As shown in Fig. 4*B*, such a phenomenon failed to occur when the experiments were repeated in the absence of extracellular $CaCl_2$ and presence of ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) (0.5 mM). Thus, between the 60th and 68th min of perifusion (i.e., after 16-min exposure to AcAc), the ^{86}Rb fractional outflow rate was significantly lower ($P < 0.05$) in the absence than in the presence of extracellular Ca^{2+}, although such

Downloaded from http://ajpendo.physiology.org/ by Renaud Beauwens on January 24, 2013

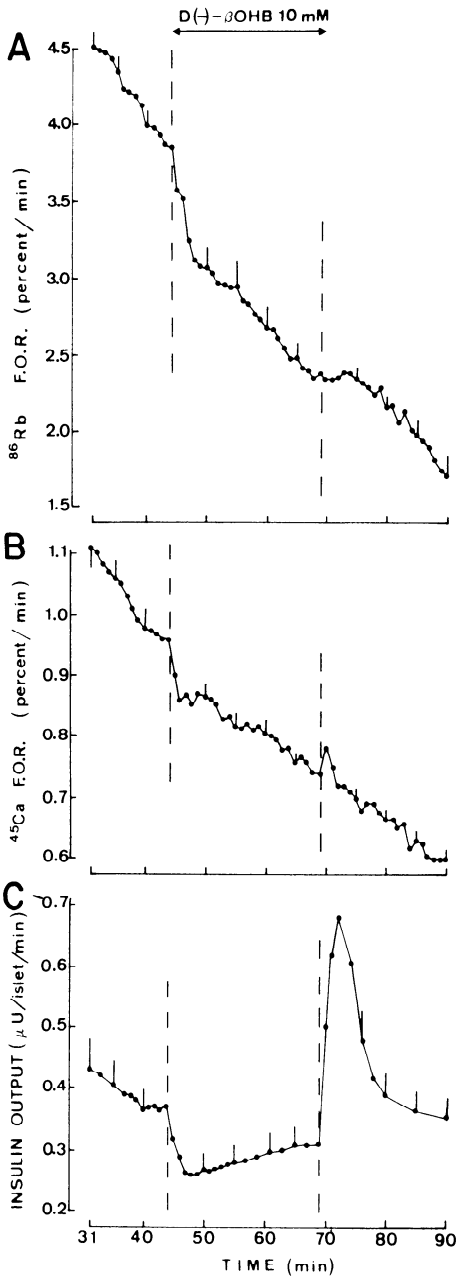

FIG. 3. Effect of D-(−)-β-hydroxybutyrate [D-(−)-β-OHB] (10 mM) on ^{86}Rb fractional outflow rate (FOR; *A*), ^{45}Ca fractional outflow rate (*B*), and insulin release (*C*) from islets perifused throughout in absence of glucose at normal Ca^{2+} concentration (1.0 mM). Values (means ± SE) refer to 8–9 individual experiments.

was not the case before administration of the ketone body. These findings are compatible with the view that the secondary reascension in ^{86}Rb outflow reflects a Ca^{2+}-dependent activation of K^+ channels.

Mitochondrial redox state and oxidation of D-[6-^{14}C]-glucose. Our measurements concerning the generation of both D-(−)-β-OHB from unlabeled exogenous AcAc and [^{14}C]AcAc from exogenous D-(−)-β-[3-^{14}C]OHB refer to the islet content and output of each metabolite. They do not inform therefore on the mitochondrial NADH-to-NAD^+ ratio (42). The interconversion of ketone bodies could conceivably affect the latter ratio. It was recently proposed (21, 27) that the mitochondrial accumulation of Ca^{2+}, as ruled inter alia by the mitochondrial redox state (19), plays a critical role in regulating the activity of key mitochondrial dehydrogenases (e.g., pyruvate dehydrogenase, isocitrate dehydrogenase, and 2-ketoglutarate dehydrogenase) in islet cells. The effect of ketone bodies on the generation of $^{14}CO_2$ from D-[6-^{14}C]glucose was assessed, therefore, to investigate specifically the oxidation in the tricarboxylic acid cycle of acetyl residues derived from exogenous D-glucose. Exogenous AcAc (10 mM) decreased ($P < 0.025$) and exogenous D-(−)-β-OHB (also 10 mM) increased ($P < 0.02$) D-[6-^{14}C]glucose oxidation from a control value of 17.1 ± 0.7 to, respectively, 14.2 ± 1.0 and 20.8 ± 1.3 pmol·120 min^{-1}·$islet^{-1}$ (n = 15–16 in all cases).

DISCUSSION

The present data indicate that ketone bodies penetrate into islet cells, undergo interconversion in the reaction catalyzed by β-hydroxybutyrate dehydrogenase, and are converted to CO_2 (2), the latter two processes presumably taking place in mitochondria. The extent of AcAc oxidation, relative to its availability or generation rate, appears markedly affected by environmental factors such as the presence of D-glucose or L-leucine. Thus, whereas L-leucine decreases the oxidation of D-(−)-β-[3-^{14}C]-OHB, D-glucose exerts an opposite and stimulatory effect on $^{14}CO_2$ output by islets exposed to D-(−)-β-[3-^{14}C]-OHB. The latter stimulation was already observed in several other cell types (17, 34), but we are not aware of any documented explanation for such a phenomenon.

The close correlation observed in the present study between the rates of O_2 uptake and insulin release by the islets strongly suggests that the insulinotropic action of ketone bodies is causally related to their catabolism, possibly through changes in ATP generation rate.

As already noted in a prior study (4), there was no tight relationship between the oxidation of ^{14}C-labeled exogenous nutrients, tested alone or in combination and the corresponding secretory data. However, such an observation is not sufficient to deny the cause-to-effect link between the catabolism of these nutrients and their insulinotropic action. Indeed, allowance must also be made for both the reciprocal effects of exogenous nutrients on their respective metabolic fate and their sparing action on the utilization of endogenous nutrients in the islet cells. Moreover, the secretory response to ketone bodies may also depend on the relative extent of their interconversion in the reaction catalyzed by β-hydroxybutyrate dehydrogenase (3).

In these respects, the situation found in the present study is similar to that previously documented when the effect of pyruvate and lactate on the net generation of reducing equivalents in islets (24, 38) is considered. These two situations differ, however, from one another by the subcellular location of the relevant dehydrogenase. It is likely that the interconversion of ketone bodies affects primarily the mitochondrial rather than cytosolic redox state. It was recently proposed that the mitochondrial redox state may, through modulating the calcium content of mitochondria (19), control the activity of mitochondrial enzymes such as glycerol phosphate de-

Downloaded from http://ajpendo.physiology.org/ by Renaud Beauwens on January 24, 2013

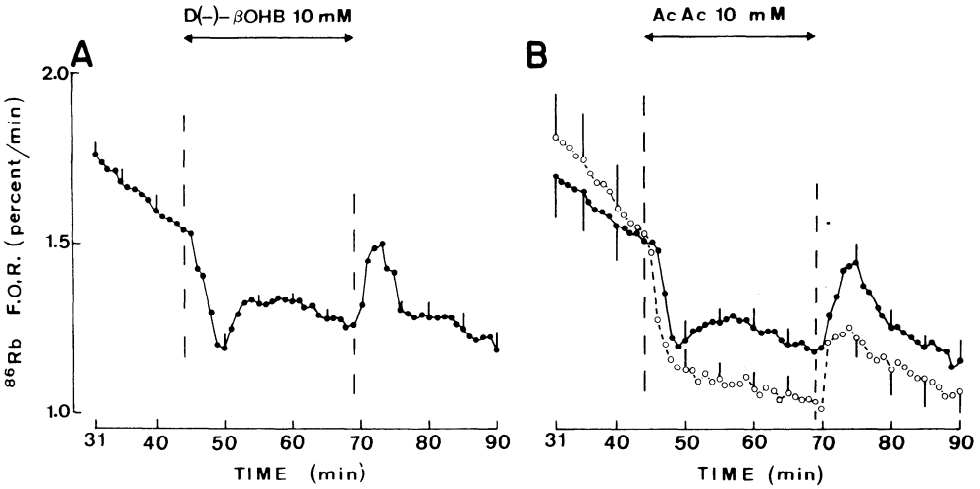

FIG. 4. Effect of D-(−)-β-hydroxybutyrate [D-(−)-β-OHB; 10 mM; *A*] or AcAc (10 mM; *B*) on ^{86}Rb FOR from islets perifused throughout in presence of 7 mM D-glucose. Basal media either contained 1.0 mM Ca^{2+} (closed circles and solid line) or were deprived of Ca^{2+} and enriched with 0.5 mM EGTA (open circles and dashed line). Values (means ± SE) refer to 4–6 individual experiments.

hydrogenase, isocitrate dehydrogenase, and 2-ketoglutarate dehydrogenase (21, 27). The modest but significant changes in D-[6-^{14}C]glucose oxidation evoked by either D-(−)-β-OHB or AcAc affords further support to such a proposal.

A further argument in support of a cause-to-effect link between the metabolic and secretory response to ketone bodies consists in the finding that these nutrients inhibit ^{86}Rb outflow from prelabeled islets. In the process of nutrient-stimulated insulin release, the closing of ATP-responsive K^+ channels, as induced by a rise in the cytosolic ATP-to-ADP ratio, is currently thought to play an essential role in coupling the catabolism of nutrient secretagogues to the depolarization of the plasma membrane and further gating of voltage-sensitive Ca^{2+} channels (28, 35). The resulting increase in cytosolic Ca^{2+} activity may then not solely trigger insulin release but also favor the exit of K^+ from the islet cells through Ca^{2+}-responsive channels (11, 25). The present data indeed suggest that the changes in ^{86}Rb outflow evoked by ketone bodies in the presence of D-glucose involve both a Ca^{2+}-independent decrease and a Ca^{2+}-dependent increase in K^+ conductance.

The cationic and secretory data collected in perifused islets exposed to D-(−)-β-OHB in the absence of D-glucose suggest that the ketone body may lower cytosolic Ca^{2+} concentration by favoring the entry of the cation into intracellular organelles. Therefore, the increase in ^{45}Ca net uptake evoked by D-(−)-β-OHB in islets first exposed to ^{45}Ca in the absence of D-glucose and then submitted at low temperature to extensive washes to remove extracellular radioactivity (26) could correspond, in part at least, to an intracellular redistribution of the tracer cation. The fact that, under the same experimental conditions, AcAc failed to significantly affect ^{45}Ca net uptake, would then again point to the changes in mitochondrial redox state as a regulatory factor in the control of mitochondrial calcium content.

Taken as a whole, these considerations merely emphasize the view that some caution should be exerted when considering the possible link between the metabolism of ketone bodies in islet cells and their insulinotropic action. In this respect, a further factor that could conceivably participate in the stimulus-secretion coupling might consist in changes in intracellular pH, as resulting for instance from the possible transport of undissociated β-hydroxybutyric or acetoacetic acid into the islet cells.

The changes in insulin release evoked by ketone bodies differ vastly as a function of the nature and concentration of other nutrients (e.g., L-leucine instead of D-glucose) concomitantly used to stimulate the B-cell (4). It should be duly underlined therefore that the present study was purposely restricted to the effect of ketone bodies in islets incubated in the absence or presence of D-glucose.

Within these limits, the comparison of oxidative, biosynthetic, cationic, and secretory data strongly suggests that the insulinotropic potential of ketone bodies is tightly and causally linked to their catabolism in islet cells. The present work extends therefore to ketone bodies the fuel concept (29) for the stimulation of insulin release by nutrient secretagogues.

We are grateful to M. Mahy, J. Schoonheydt, M. Urbain, and G. Vandenbroeck for technical assistance and C. Demesmaeker for secretarial help.

This study was supported by grants from the Belgian Foundation for Scientific Medical Research and Belgian Ministry of Scientific Policy and a predoctoral fellowship of the Belgian Institute for Scientific Research in Industry and Agriculture to J. Rasschaert.

Address for reprint requests: W. J. Malaisse, Laboratory of Experimental Medicine, Brussels Free University, 115 Blvd. de Waterloo, B-1000 Brussels, Belgium.

Received 25 September 1989; accepted in final form 1 March 1990.

REFERENCES

1. BALASSE, E. O., AND H. A. OOMS. Changes in the concentration of glucose, free fatty acids, insulin and ketone bodies in the blood during sodium β-hydroxybutyrate infusions in man. *Diabetologia* 4: 133–135, 1968.
2. BERNE, C. The metabolism of lipids in mouse pancreatic islets. The oxidation of fatty acids and ketone bodies. *Biochem. J.* 152: 661–666, 1975.
3. BERNE, C. Determination of D-3-hydroxybutyrate dehydrogenase in mouse pancreatic islets with a photokinetic technique using bacterial luciferase. *Enzyme* 21: 127–136, 1976.
4. BIDEN, T. J., AND K. W. TAYLOR. Effects of ketone bodies on insulin release and islet-cell metabolism in the rat. *Biochem. J.* 212: 371–377, 1983.
5. BJÖRNTORP, P., AND T. SCHERSTEN. Effect of β-hydroxybutyrate

Downloaded from http://ajpendo.physiology.org/ by Renaud Beauwens on January 24, 2013

on lipid mobilization. *Am. J. Physiol.* 250: 683–687, 1967.
6. BLACHIER, F., A. SENER, AND W. J. MALAISSE. Interference of a nonmetabolized analogue of L-leucine with lipid metabolism in tumoral pancreatic islet cells. *Biochim. Biophys. Acta* 921: 494–501, 1987.
7. CARPINELLI, A. R., AND W. J. MALAISSE. Regulation of ^{86}Rb outflow from pancreatic islets: the dual effect of nutrient secretagogues. *J. Physiol. Lond.* 315: 143–156, 1981.
8. CARPINELLI, A. R., AND W. J. MALAISSE. Regulation of $^{86}Rb^+$ efflux from pancreatic islets. I. Reciprocal changes in the response to glucose, tetraethylammonium and quinine. *Mol. Cell. Endocrinol.* 17: 103–110, 1980.
9. CARPINELLI, A. R., A. SENER, A. HERCHUELZ, AND W. J. MALAISSE. The stimulus-secretion coupling of glucose-induced insulin release. XLI. Effect of intracellular acidification on calcium efflux from islet cells. *Metabolism* 29: 540–545, 1980.
10. FERY, F., AND E. O. BALASSE. Differential effects of sodium acetoacetate and acetoacetic acid infusions on alanine and glutamine metabolism in man. *J. Clin. Invest.* 66: 323–331, 1980.
11. FINDLAY, I., M. J. DUNNE, AND O. H. PETERSEN. High-conductance K^+ channel in pancreatic islet cells can be activated and inactivated by internal calcium. *J. Membr. Biol.* 83: 169–175, 1985.
12. GIROIX, M.-H., A. SENER, AND W. J. MALAISSE. Artefactual and true uptake of labeled sucrose by rat pancreatic islets. *Comp. Biochem. Physiol. A Comp. Physiol.* 85: 289–296, 1986.
13. GIROIX, M.-H., A. SENER, D. G. PIPELEERS, AND W. J. MALAISSE. Hexose metabolism in pancreatic islets. Inhibition of hexokinase. *Biochem. J.* 223: 447–453, 1984.
14. HELLMAN, B. β-Cell cytoplasmic Ca^{2+} balance as a determinant for glucose stimulated insulin release. *Diabetologia* 28: 494 501, 1985.
15. HUTTON, J. C., AND W. J. MALAISSE. Dynamics of O_2 consumption in rat pancreatic islets. *Diabetologia* 18: 394–405, 1980.
16. HUTTON, J. C., A. SENER, AND W. J. MALAISSE. The metabolism of 4-methyl-2-oxopentanoate in rat pancreatic islets. *Biochem. J.* 184: 291–301, 1979.
17. IDE, T., J. STEINKE, AND G. F. CAHILL, JR. Metabolic interactions of glucose, lactate, and β-hydroxybutyrate in rat brain slices. *Am. J. Physiol.* 217: 784–792, 1969.
18. KREBS, H. A., D. H. WILLIAMSON, AND H. Ü. BERGMEYER. 3-Hydroxybutyrate dehydrogenase. In: *Methods in Enzymology*, edited by J. M. Lowenstein. New York: Academic, 1969, vol. 14, p. 222–227.
19. LEHNINGER, A. L., B. REYNAFARJE, A. VERCESI, AND W. P. TEW. Transport and accumulation of calcium in mitochondria. *Ann. NY Acad. Sci.* 307: 160–174, 1978.
20. MALAISSE, W. J., L. BEST, S. KAWAZU, F. MALAISSE-LAGAE, AND A. SENER. The stimulus-secretion coupling of glucose-induced insulin release: fuel metabolism in islets deprived of exogenous nutrient. *Arch. Biochem. Biophys.* 224: 102–110, 1983.
21. MALAISSE, W. J., F. BLACHIER, R. POCHET, B. MANUEL, Y. KEENOY, AND A. SENER. Calmodulin and calbindin in pancreatic islet cells. In: *Calcium Binding Proteins in Normal and Transformed Cells*, edited by D. E. M. Lawson and R. Pochet. New York: Plenum, 1989, p. 127–131.
22. MALAISSE, W. J., J. C. HUTTON, A. R. CARPINELLI, A. HERCHUELZ, AND A. SENER. The stimulus-secretion coupling of amino acid-induced insulin release. Metabolism and cationic effects of leucine. *Diabetes* 29: 431–437, 1980.
23. MALAISSE, W. J., J. C. HUTTON, S. KAWAZU, AND A. SENER. The stimulus-secretion coupling of glucose-induced insulin release. Metabolic effects of menadione in isolated islets. *Eur. J. Biochem.* 87: 121–130, 1978.
24. MALAISSE, W. J., S. KAWAZU, A. HERCHUELZ, J. C. HUTTON, G. SOMERS, G. DEVIS, AND A. SENER. The stimulus-secretion coupling of glucose-induced insulin release. XXXIV. Effect of lactate on islet function. *Arch. Biochem. Biophys.* 194: 49–62, 1979.
25. MALAISSE, W. J., P. LEBRUN, AND A. HERCHUELZ. Ionic determinants of bioelectrical spiking activity in the pancreatic B-cell. *Pfluegers Arch.* 395: 201–203, 1982.
26. MALAISSE, W. J., P. LEBRUN, B. YAYLALI, J. CAMARA, I. VALVERDE, AND A. SENER. Ketone bodies and islet function: ^{45}Ca handling, insulin synthesis, and release. *Am. J. Physiol.* 259 *(Endocrinol. Metab.* 22): E117–E122, 1990.
27. MALAISSE, W. J., F. MALAISSE-LAGAE, J. RASSCHAERT, D. ZÄHNER, A. SENER, D. R. DAVIES, AND E. VAN SCHAFTINGEN. The fuel concept for insulin release: regulation of glucose phosphorylation in pancreatic islets. *Biochem. Soc. Trans.* 18: 107–108, 1990.
28. MALAISSE, W. J., AND A. SENER. Glucose-induced changes in cytosolic ATP content in pancreatic islets. *Biochim. Biophys. Acta* 927: 190–195, 1987.
29. MALAISSE, W. J., A. SENER, A. HERCHUELZ, AND J. C. HUTTON. Insulin release: the fuel hypothesis. *Metabolism* 28: 373–386, 1979.
30. MALAISSE, W. J., A. SENER, F. MALAISSE-LAGAE, J. C. HUTTON, AND J. CHRISTOPHE. The stimulus-secretion coupling of amino acid-induced insulin release. VI. Metabolic interaction of L-glutamine and 2-ketoisocaproate in pancreatic islets. *Biochim. Biophys. Acta* 677: 39–49, 1981.
31. MALAISSE, W. J., A. SENER, F. MALAISSE-LAGAE, M. WELSH, D. E. MATTHEWS, D. M. BIER, AND C. HELLERSTRÖM. The stimulus-secretion coupling of amino acid-induced insulin release. X. Metabolic response of pancreatic islet to L-glutamine and L-leucine. *J. Biol. Chem.* 257: 8731–8737, 1982.
32. MALAISSE-LAGAE, F., AND W. J. MALAISSE. Insulin release by pancreatic islets. In: *Methods in Diabetes Research*, edited by J. Larner and S. L. Pohl. New York: Wiley, 1984, p. 147–152.
33. MCGILVERY, R. W. *Biochemistry, a Functional Approach.* Philadelphia, PA: Saunders, 1970, p. 373.
34. OPENSHAW, H., AND W. M. BORTZ. Oxidation of glucose, acetoacetate, and palmitate in brain mince of normal and ketotic rats. *Diabetes* 17: 90–95, 1968.
35. RORSMAN, P., AND G. TRUBE. Glucose dependent K^+-channels in pancreatic β-cells are regulated by intracellular ATP. *Pfluegers Arch.* 45: 305–309, 1985.
36. SENER, A., F. BLACHIER, J. RASSCHAERT, A. MOURTADA, F. MALAISSE-LAGAE, AND W. J. MALAISSE. Stimulus-secretion coupling of arginine-induced insulin release: comparison with lysine-induced insulin secretion. *Endocrinology* 124: 2558–2567, 1989.
37. SENER, A., M.-H. GIROIX, AND W. J. MALAISSE. Impaired uptake of D-glucose by tumoral insulin-producing cells. *Biochem. Int.* 12: 913–919, 1986.
38. SENER, A., S. KAWAZU, J. C. HUTTON, A. C. BOSCHERO, G. DEVIS, G. SOMERS, A. HERCHUELZ, AND W. J. MALAISSE. The stimulus-secretion coupling of glucose-induced insulin release. XXXIII. Effect of exogenous pyruvate on islet function. *Biochem. J.* 176: 217–232, 1978.
39. SENER, A., F. MALAISSE-LAGAE, S. P. DUFRANE, AND W. J. MALAISSE. The coupling of metabolic to secretory events in pancreatic islets. The cytosolic redox state. *Biochem. J.* 220: 433–440, 1984.
40. SENER, A., F. MALAISSE-LAGAE, AND W. J. MALAISSE. Stimulation of islet metabolism and insulin release by a nonmetabolizable amino acid. *Proc. Natl. Acad. Sci. USA* 78: 5460–5464, 1981.
41. SENER, A., F. MALAISSE-LAGAE, AND W. J. MALAISSE. The stimulus-secretion coupling of glucose-induced insulin release. II. Environmental influences on L-glutamine oxidation in pancreatic islets. *Biochem. J.* 202: 309–316, 1982.
42. WILLIAMSON, D. H., P. LUND, AND H. A. KREBS. The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. *Biochem. J.* 103: 514–527, 1967.

Downloaded from http://ajpendo.physiology.org/ by Renaud Beauwens on January 24, 2013