Regulation of insulin secretion by energy metabolism in pancreatic B-cell mitochondria

Studies with a non-metabolizable leucine analogue

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In mouse pancreatic islets the kinetics of insulin secretion and O₂ uptake in response to the non-metabolizable leucine analogue (±)-BCH (2-*endo*-aminonornbornane-2-carboxylic acid) were compared. In addition, the fuel-mobilizing effect of (±)-BCH was studied with a mitochondrial fraction from islets. (1) Within 2 min 20 mM-(±)-BCH markedly enhanced insulin release or O₂ consumption by islets respiring in the absence of exogenous fuels. During prolonged exposure to 20 mM-(±)-BCH secretion declined more rapidly than O₂ uptake. (2) L-Glutamine (10 mM) prevented the decrease of both insulin release and O₂ uptake of islets exposed to 20 mM-(±)-BCH. During the second phase of insulin release in response to 20 mM-(±)-BCH + 10 mM-L-glutamine, the kinetics of secretion and respiration correlated closely. (3) Initial peaks were consistently seen in the (±)-BCH-induced secretory profiles, but never in the respiratory profiles. (4) In contrast with L-glycerol 3-phosphate, L-malate or pyruvate, L-glutamine or L-glutamate maintained low rates of oxidative phosphorylation in B-cell mitochondria. The effects of L-glutamine or L-glutamate were potentiated severalfold by (±)-BCH. (5) The effects of other branched-chain amino acids on oxidative phosphorylation resembled their effects on insulin release, redox state of nicotinamide nucleotides and glutamate dehydrogenase activity. (6) The results support the view that (±)-BCH stimulates insulin secretion via a primary enhancement of hydrogen supply to the respiratory chain of B-cell mitochondria.

It has been suggested that insulin-releasing fuels may initiate the secretory process by changing the B-cell metabolism (Grodsky *et al.*, 1963; Coore & Randle, 1964; Ashcroft *et al.*, 1973; Malaisse *et al.*, 1979b). At least the non-metabolizable leucine analogue (–)-BCH and 4-methyl-2-oxopentanoate do not act by producing specific trigger metabolites (Christensen *et al.*, 1971; Panten *et al.*, 1981; Lenzen *et al.*, 1982), but may operate by enhancing production of hydrogen (reducing equivalents) in the B-cell mitochondria (Holze & Panten, 1979; Hutton *et al.*, 1979; Panten *et al.*, 1980; Sener *et al.*, 1981; Malaisse-Lagae *et al.*, 1982). Two main routes exist along which intramitochondrial hydrogen production could elicit insulin secretion (Panten *et al.*, 1980). The first starts with a transfer of

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

reducing equivalents from the mitochondria to the cytosol via shuttle mechanisms (Sies, 1982) and includes a hypothetical redox-state receptor, presumably coupled to ion-transport mechanisms in the B-cell plasma membrane (Malaisse *et al.*, 1979b). The second is the respiratory chain and processes using the proton electrochemical gradient across the mitochondrial inner membrane, e.g. ATP synthesis and transport of ions (Mitchell, 1976).

Whereas Malaisse *et al.* (1982) favour the first route, there is no need to postulate the existence of an extramitochondrial redox-state receptor mechanism in stimulus–secretion coupling in B-cells. Experimental evidence for such a receptor is lacking. Moreover, a mathematical model has been formulated for ionic and electrical events in B-cells, which includes glucose-induced elevation of mitochondrial membrane potential (Chay &

Keizer, 1983). The latter model is consistent with the more comprehensive hypothesis that the hydrogen supply to the respiratory chain normally governs the proton electrochemical gradient in the mitochondrial inner membrane of B-cells and thereby regulates insulin release.

The leucine analogue BCH has the unique features of stimulating insulin secretion, of being non-metabolizable and of activating glutamate dehydrogenase allosterically (Christensen *et al.*, 1971; Gylfe, 1976; Sener & Malaisse, 1980; Panten & Langer, 1981). The latter effect, which may initiate oxidation of endogenous glutamate, appears to be the only primary mechanism by which BCH could enhance intramitochondrial hydrogen supply. Therefore we used BCH as a tool to elucidate further the role of mitochondrial energy metabolism in the fuel-recognition mechanism both with intact pancreatic islets and with subcellular fractions containing B-cell mitochondria. Some of these observations have been published in abstract form (Panten *et al.*, 1982).

Experimental

Chemicals and media

The following substances were used: L-malate (monosodium salt), L-glutamate (monosodium salt), L-glutamine, imidazole (fluorimetric grade) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49; from *Leuconostoc mesenteroides*) from Sigma, St. Louis, MO, U.S.A.; EGTA from Serva, Heidelberg, Germany; bovine serum albumin (fatty acid-poor) from Miles, Frankfurt, Germany; hexokinase (EC 2.7.1.1; 1500 units of freeze-dried material dissolved in 1 ml of redistilled water and stored frozen; 1 unit is defined as the activity converting 1 μ mol/min at pH 7.4 and 25°C) from Boehringer, Mannheim, Germany. All other reagents were from Boehringer or from the sources detailed previously (Panten & Langer, 1981; Panten & Klein, 1982).

Basal medium for isolation, incubation and perfusion of pancreatic islets was prepared as previously described (Panten *et al.*, 1981), except that for measurements of O_2 consumption it was equilibrated with $O_2/N_2/CO_2$ (12:7:1).

Isolation of pancreatic islets

Male albino mice (NMRI, 11–15 weeks old, fed *ad libitum*) or *ob/ob* (obese-hyperglycaemic) mice (non-inbred, 6–8 months old, deprived of food for 22–25 h) of either sex were used. Pancreatic islets were isolated by collagenase digestion (Lernmark, 1974) in basal medium supplemented with 4 mM-D-glucose.

Measurement of insulin secretion

Batches of 50 albino-mouse islets or 25 medium-sized *ob/ob*-mouse islets were perfused at 0.9 ml/min at 37°C as detailed previously (Panten *et al.*, 1977). At zero time (after pre-perfusion for 48 min with control medium) the distribution valve of the system was switched to control or test medium. Insulin content of 1–4 min fractions was determined by radioimmunoassay with mouse insulin as reference (Joost, 1979).

Measurement of O_2 consumption

O_2 consumption by batches of 120–160 albino-mouse islets or 40–50 medium-sized *ob/ob*-mouse islets was recorded at 37°C as described previously (Panten & Klein, 1982) except that the response time of the measuring system was 15–30 s for 99% change. After preincubation for 40 min, medium with or without 200 mM-($\pm$)-BCH (to give a final 20 mM concentration in the incubation chamber) was injected into the chamber. O_2 consumption by the islets was calculated from the decrease in the pO_2 during 1–4 min intervals and from the amount of medium in the closed incubation chamber, with corrections for atmospheric pressure, water vapour and drift, and with Bunsen's solubility coefficient of 0.0227 ml/ml (at 37°C). The DNA content of the islets was measured by a modification (Panten & Klein, 1982) of the method of Kissane & Robins (1958).

Preparation of mitochondrial fractions

For this, 150–200 medium-sized *ob/ob*-mouse islets were homogenized as described previously (Lenzen & Panten, 1983) except that the homogenization buffer contained 1 mM-EGTA. Nuclei and cell debris were sedimented for 5 min at 600 g at 4°C. The mitochondrial pellet was obtained by centrifugation of the supernatant for 10 min at 8000 g at 4°C and resuspended in 70 μ l of homogenization buffer. The main contaminant of the mitochondrial fraction was insulin-secretory granules (Lenzen & Panten, 1983). More than 95% of cytosol and microsomal fraction were removed.

Incubation of mitochondrial fractions

Portions (10 μ l) of the final mitochondrial suspension were incubated at 37°C after mixing with 10 μ l of incubation medium. Unless stated otherwise this medium contained KCl (70 mM), KH_2PO_4 (10 mM), $MgCl_2$ (10 mM), glucose (20 mM), Tris (10 mM), HCl to give pH 7.4, fatty acid-poor albumin (1 mg/ml), ADP (0.4 mM), hexokinase (5 units/ml) and variable concentrations of test substances (neutralized if necessary) as indicated in the Results section. Iso-osmolality of the media was achieved by additional KCl. Ca^{2+} was trapped by the EGTA (0.5 mM) in the incubations.

Incubations were stopped by pipetting 5 μ l of cold 2.5M-HClO₄ into the incubation tubes (tubes 30/8; Sarstedt, Nümbrecht, Germany) and vortex-mixing. After centrifugation at 0°C (10000g for 2 min), 20 μ l of the supernatant was neutralized with 10 μ l of a cold solution containing KOH (1M), imidazole (0.2M) and KCl (0.2M). After 20 min at 0°C, KClO₄ was sedimented at 10000g for 2 min at 0°C. Supernatant (25 μ l) was used for measurement of glucose 6-phosphate, ADP or AMP.

Measurement of ADP, AMP and glucose 6-phosphate

ADP and AMP were measured as previously described (Williamson & Corkey, 1969). Glucose 6-phosphate was measured by monitoring the increase in NADH fluorescence induced by addition of 0.5 unit of glucose-6-phosphate dehydrogenase to 1 ml of test solution containing 80mM-Tris/HCl, pH 8.0, 10mM-MgCl₂, 4mM-NAD⁺ and sample. Internal standards were made with glucose 6-phosphate.

Measurement of glutamate dehydrogenase

Glutamate dehydrogenase was measured as previously described (Panten & Langer, 1981), except that the reaction mixture contained 20mM-L-leucine. Appropriate dilutions of the mitochondrial suspensions were made with distilled water in plastic tubes, and frozen and thawed twice to disrupt the mitochondria. A m-unit of glutamate dehydrogenase was defined as the activity converting 30nmol of NADH into NAD⁺ per 30 min at 37°C.

Presentation of results

Results are presented as means $\pm$ S.E.M. for independent experiments. Significances were calculated by the two-tailed matched-pairs signed rank test of Wilcoxon. $P < 0.05$ was considered significant.

Results

When *ob/ob*-mouse pancreatic islets, which contain more than 90% B-cells (Gepts *et al.*, 1960), were perfused without substrate and then exposed to 20mM-($\pm$)-BCH, stimulation of insulin release occurred, with a maximum 2–3 min after starting the change of medium (Fig. 1). Simultaneously a steep increase in islet O₂ uptake was observed, amounting to 114% during the second 1 min interval after addition of 20mM-($\pm$)-BCH, and gradually decreasing afterwards (Fig. 1). However, in contrast with the secretory profile, there was no initial overshoot in the respiratory profile. In each single experiment ($\pm$)-BCH-induced insulin secretion or O₂ consumption was higher than the last prestimulation value from 2 min up to 42 min or

Fig. 1. Effects of ($\pm$)-BCH on insulin secretion or O₂ uptake by pancreatic islets isolated from *ob/ob* mice. The experiments were performed with basal medium throughout (○) or with basal medium containing 20mM-($\pm$)-BCH from zero time to 60 min (●). Values in the curves are means $\pm$ S.E.M. of results from five to seven separate experiments and are drawn in the middle of the sampling intervals. The initial overshooting part in the secretion profile is hatched. The broken lines in the O₂-uptake profiles span the interval during which O₂ uptake could not be determined because injections caused pO_2 jumps.

58 min, respectively. The ratio of the increase in O₂ uptake to the increase in insulin release was 58 pmol of O₂/ng of insulin at 10 min, and rose continuously up to 130–140 pmol of O₂/ng of insulin at 54–58 min. The ratios were calculated by assuming that the islets used in the secretion experiments had the same mean DNA content (55.4 ng per islet) as those used in the O₂-uptake measurements. This seemed reasonable, since the islets for both types of experiments were taken from the same daily islet preparation. Efforts to determine the DNA content of islets after perfusions failed because recoveries were poor.

In the presence of 10mM-L-glutamine, ($\pm$)-BCH (20mM) again triggered a first peak of insulin release from *ob/ob*-mouse islets (Fig. 2). However, after this peak the secretory rate slightly increased and then remained constant from 26 to 58 min. In each single experiment the peak rate was higher than all subsequent rates. In conjunction with 10mM-L-glutamine, ($\pm$)-BCH (20mM) enhanced O₂ consumption by *ob/ob*-mouse islets by 129% during the second 1 min interval after addition of ($\pm$)-BCH ($P < 0.005$ for comparison with the last pre-stimulation value) and kept the respiration

Fig. 2. Effects of ($\pm$)-BCH and L-glutamine on insulin secretion or O_2 uptake by pancreatic islets isolated from *ob/ob* mice

All media contained L-glutamine (10mM). From zero time to 60 min 20mM-($\pm$)-BCH was added ($\bullet$). Controls were run in the sole presence of 10mM-L-glutamine ($\circ$). Values shown are means $\pm$ S.E.M. of results from six to ten separate experiments. Further details are the same as described in the legend to Fig. 1.

nearly constant for at least 1 h (Fig. 2). The ratio of the increase in O_2 uptake to the increase in insulin release was 26 pmol of O_2 /ng of insulin at 10 min and afterwards always ranged between 21 and 23 pmol of O_2 /ng of insulin (55.4 ng of DNA/islet), illustrating the close correlation between second phase of insulin release and islet O_2 uptake in the presence of 10 mM-L-glutamine + 20 mM-($\pm$)-BCH.

As an additional test for the relationship between insulin release and O_2 consumption, experiments with albino-mouse islets were included. In the presence of 10 mM-L-glutamine, ($\pm$)-BCH (20 mM) induced slower secretory and respiratory responses (Fig. 3) than in *ob/ob*-mouse islets (Fig. 2). A first secretory maximum, a subsequent gradual increase and a final plateau which was higher than the initial peak were observed in each single experiment with albino-mouse islets. In conjunction with 10 mM-L-glutamine, ($\pm$)-BCH

Fig. 3. Effects of ($\pm$)-BCH and L-glutamine on insulin secretion or O_2 uptake by pancreatic islets isolated from albino mice

Values shown are means $\pm$ S.E.M. of results from five or six separate experiments with ($\bullet$) or without ($\circ$) ($\pm$)-BCH (20 mM). Further details are the same as described in the legend to Fig. 2.

(20 mM) enhanced O_2 consumption by 95% within 2 min ($P < 0.05$ for comparison with the last pre-stimulation value) (Fig. 3). The O_2 uptake during the latter interval was lower than afterwards in each single experiment, and maximal respiratory rates were not seen before 38 min. The close correlation between the second phase of insulin release and O_2 uptake is documented by the ratio of the increase in O_2 uptake to the increase in insulin release, which was 37 pmol of O_2 /ng of insulin at 10 min and afterwards always ranged between 29 and 33 pmol of O_2 /ng of insulin. The ratios were calculated from a DNA content of 24 ng/islet.

The outcome of experiments with intact islets led us to study the fuel-mobilizing effect of ($\pm$)-BCH with a mitochondrial fraction from *ob/ob*-mouse islets. ATP produced by mitochondria was trapped as glucose 6-phosphate and served as a sensitive indicator of the hydrogen supply. Over a 10 min incubation, various substrates enhanced glucose 6-phosphate production with a nearly linear time course (results not shown). Maximally effective concentrations of hexokinase, phosphate and ADP were chosen for the standard procedure, determined by control experiments (results not shown). The ATP trapped in the absence of substrate was mainly produced from the ADP in the incubation media via the mitochondrial myokin-

Table 1. *Effects of malate and pyruvate on ADP, AMP and glucose 6-phosphate contents of incubations containing a mitochondrial fraction from ob/ob-mouse islets*

Portions (10 μ l) of the same mitochondrial suspension were incubated for 10 min in parallel in the presence of the indicated concentrations of test substances. Values shown are means $\pm$ S.E.M. for results from six separate experiments.

Test substances	Content (nmol/20 μ l)		
	ADP	AMP	Glucose 6-phosphate
None	0.76 $\pm$ 0.18	3.00 $\pm$ 0.19	1.47 $\pm$ 0.13
L-Malate (0.5 mM)	0.73 $\pm$ 0.17	3.03 $\pm$ 0.15	3.00 $\pm$ 0.38
L-Malate (2.5 mM) + pyruvate (1 mM)	0.79 $\pm$ 0.17	2.99 $\pm$ 0.16	6.69 $\pm$ 1.12

Table 2. *Effects of different substrates on ATP production by a mitochondrial fraction from ob/ob-mouse islets*

Each experimental series consisted of simultaneous 10 min incubations in the presence of the indicated concentrations of test substances. ATP trapped by the hexokinase reaction is given as glucose 6-phosphate. Values shown are means $\pm$ S.E.M. for results from six separate experiments.

Test substances	Glucose 6-phosphate production (nmol/m-unit of glutamate dehydrogenase activity)
Experimental series I	
A: None	2.54 $\pm$ 0.29
B: Oligomycin (0.4 μ g/ml)	2.34 $\pm$ 0.28
C: L-Glutamine (10 mM)	3.31 $\pm$ 0.31
D: L-Glutamate (10 mM)	3.88 $\pm$ 0.33
E: L-Malate (2.5 mM) + pyruvate (1 mM)	12.31 $\pm$ 1.18
F: L-Malate (2.5 mM) + pyruvate (1 mM) + oligomycin (0.4 μ g/ml)	2.37 $\pm$ 0.36
Experimental series II	
G: None	2.28 $\pm$ 0.12
H: ($\pm$)-BCH (20 mM)	2.24 $\pm$ 0.10
J: L-Malate (2.5 mM)	5.46 $\pm$ 0.43
K: L-Malate (2.5 mM) + ($\pm$)-BCH (20 mM)	5.49 $\pm$ 0.35
L: L-Glycerol 3-phosphate (20 mM)	8.53 $\pm$ 0.24
M: L-Glycerol 3-phosphate (20 mM) + ($\pm$)-BCH (20 mM)	8.59 $\pm$ 0.20

ase reaction. This was documented by the high AMP concentrations in the incubations (Table 1). The small effect of oligomycin in the absence of exogenous substrate (Table 2) indicated that oxidation of endogenous fuels played a minor role only. The blocking effect of oligomycin on substrate-induced glucose 6-phosphate production (Table 2) showed that the latter resulted from oxidative phosphorylation. Owing to ATPases in the incubation, the true rate of oxidative phosphorylation may have been higher than the rate of ATP trapping in response to substrates. ATPase activity was probably indicated by the finding that in the absence of substrates mitochondrial glucose 6-phosphate production was less than AMP production ($P < 0.05$; Table 1).

Pyruvate alone was ineffective, but augmented the ATP production induced by L-malate or L-glutamate (results not shown). L-Glycerol 3-phosphate, L-malate or pyruvate enhanced oxidative phosphorylation by B-cell mitochondria, with half-

maximal effects at concentrations around 7 mM, 0.5 mM and 0.02 mM respectively (results not shown). ATP trapped in response to L-glutamine (10 mM) or L-glutamate (10 mM) amounted to 7.9 or 13.7% of the ATP trapped in response to 2.5 mM-L-malate + 1 mM-pyruvate (Table 2). In each single experiment of series I in Table 2 the response to 10 mM-L-glutamine was less than that to 10 mM-L-glutamate. L-Malate- or L-glycerol 3-phosphate-induced rates of oxidative phosphorylation in islet mitochondria were not changed significantly by 20 mM-($\pm$)-BCH (Table 2). However, 20 mM-($\pm$)-BCH enhanced the ATP production caused by high or low concentrations of L-glutamate 6–8-fold (Table 3). Even at very low concentrations of ADP in the incubation media, 20 mM-($\pm$)-BCH augmented the response to L-glutamate (Table 3). The potentiating effect of ($\pm$)-BCH depended on its concentration in the range 5–20 mM (Table 4). ($\pm$)-BCH (20 mM) also markedly stimulated oxidative phosphorylation in the presence of 10 mM-L-glut-

Table 3. *Effects of BCH on glutamate-induced ATP production by a mitochondrial fraction from ob/ob-mouse islets*

Each experimental series consisted of simultaneous 10 min incubations in the presence of the indicated concentrations of test substances. In groups I and K of series II the ADP concentration in the media was 10-fold lower than usual. ATP trapped by the hexokinase reaction in response to the test substances was calculated by subtracting the glucose 6-phosphate production in the absence of test substances, which was measured in parallel. Values shown are means $\pm$ S.E.M. for results from six separate experiments. Comparison of A versus D, B versus E, C versus F, G versus H or I versus K gave significant differences ($P < 0.05$).

Test substances (mM)	ATP production (nmol/m-unit of glutamate dehydrogenase activity)
Experimental series I	
A: L-Glutamate (0.5)	0.38 $\pm$ 0.10
B: L-Glutamate (2)	0.78 $\pm$ 0.14
C: L-Glutamate (10)	1.28 $\pm$ 0.16
D: L-Glutamate (0.5) + ($\pm$)-BCH (20)	3.20 $\pm$ 0.34
E: L-Glutamate (2) + ($\pm$)-BCH (20)	5.64 $\pm$ 0.60
F: L-Glutamate (10) + ($\pm$)-BCH (20)	7.94 $\pm$ 0.67
Experimental series II	
G: L-Glutamate (10)	1.18 $\pm$ 0.14
H: L-Glutamate (10) + ($\pm$)-BCH (20)	7.56 $\pm$ 0.99
I: L-Glutamate (10)	0.86 $\pm$ 0.13
K: L-Glutamate (10) + ($\pm$)-BCH (20)	2.07 $\pm$ 0.24

Table 4. *Effects of BCH concentration and branched-chain amino acids on ATP production by a mitochondrial fraction from ob/ob-mouse islets*

Each experimental series consisted of simultaneous 10 min incubations in the presence of the indicated concentrations of test substances. ATP trapped by the hexokinase reaction in response to the test substances was calculated by subtracting the glucose 6-phosphate production in the absence of test substances which was measured in parallel. Values shown are means $\pm$ S.E.M. for results from six separate experiments.

Test substances (mM)	ATP production (nmol/m-unit of glutamate dehydrogenase activity)
Experimental series I	
A: L-Glutamate (10)	1.14 $\pm$ 0.15
B: L-Glutamate (10) + ($\pm$)-BCH (5)	3.30 $\pm$ 0.62
C: L-Glutamate (10) + ($\pm$)-BCH (10)	4.37 $\pm$ 0.66
D: L-Glutamate (10) + ($\pm$)-BCH (20)	5.01 $\pm$ 0.61
E: L-Glutamate (10) + ($\pm$)-BCH (40)	5.57 $\pm$ 0.32
Experimental series II	
F: L-Glutamine (10)	0.85 $\pm$ 0.14
G: L-Glutamine (10) + ($\pm$)-BCH (20)	5.17 $\pm$ 0.69
H: L-Glutamine (10) + L-leucine (10)	4.58 $\pm$ 0.70
I: L-Glutamine (10) + L-isoleucine (10)	1.28 $\pm$ 0.32
K: L-Glutamine (10) + L-valine (10)	0.75 $\pm$ 0.16

amine (Table 4). L-Leucine (10mM) was nearly as effective as ($\pm$)-BCH (20mM), L-isoleucine was only weakly effective and L-valine was ineffective (Table 4). Except for the relationship between the values of groups D and E, the patterns of effects represented by the mean values were seen in each single experiment of series I and II in Table 4.

Discussion

The main difference between the secretory and respiratory profiles was the lack of O₂-uptake peaks corresponding to the secretory maxima which occurred during the third or fourth 1 min

interval after switching to BCH-containing media (Figs. 1–3). This dissociation means that enhanced production of high-energy phosphates is probably not the cause of initiation of insulin release. During the second phase of insulin release in the presence of glutamine (10mM), the kinetics of the secretory and respiratory responses to ($\pm$)-BCH (20mM) correlated closely (see the Results section). This coincidence mainly indicates a parallel activation of ATP-consuming reactions by stimulation of ion fluxes and exocytosis, since the energy requirement for Ca²⁺ cycling across the mitochondrial inner membrane is probably too modest (Åkerman & Nicholls, 1983) to account for the strong BCH-

induced O_2 consumption by islets. However, the secretory process cannot be the only determinant for the respiratory response to $(\pm)$ -BCH (20 mM), because O_2 consumption per ng of insulin released during the second phase of insulin secretion was much higher in the absence than in the presence of 10 mM-glutamine (see the Results section). The low ATP/ADP ratio in pancreatic islets preincubated in substrate-free media (Ashcroft *et al.*, 1973; Malaisse *et al.*, 1979a) represents an additional motive force which can stimulate respiration if hydrogen supply to the respiratory chain is enhanced. There are indications that $(\pm)$ -BCH may enhance total fuel oxidation in islets (Malaisse-Lagae *et al.*, 1982). However, only a complete separation of the insulin-releasing function from the fuel-mobilizing function of $(\pm)$ -BCH can rule out the possibility that stimulation of the secretory process is a basic requirement for an increase in fuel oxidation.

The fuel supply of mitochondria in intact B-cells respiring in the sole presence of 10 mM-glutamine and utilizing only minor amounts of glycogen and endogenous fatty acids (Idahl & Hellman, 1971; Malaisse *et al.*, 1980) resembles the fuel supply of isolated mitochondria incubated with high concentrations of glutamine or glutamate. The latter amino acids maintained low rates of oxidative phosphorylation in B-cell mitochondria, which were markedly enhanced by $(\pm)$ -BCH at both high and low ADP concentrations in the media (Tables 2–4). In mouse islets a close parallelism is demonstrated between the effects of structurally related neutral amino acids on oxidative phosphorylation on the one hand and on glutamate dehydrogenase activity, the reduction of nicotinamide nucleotides or insulin release on the other hand (Table 4; Panten & Christians, 1973; Panten, 1975; Panten & Langer, 1981). As a result the experiments favour the view that glutamate dehydrogenase activity in B-cell mitochondria limits the rate at which hydrogen is derived from glutamate or glutamine. This bottleneck can be overcome directly by $(\pm)$ -BCH in the absence of Ca^{2+} and without any change in substrate or ADP supply to B-cell mitochondria (Tables 1–4).

The data presented support the hypothesis that the hydrogen supply to the respiratory chain governs B-cell function. This does not mean that an increase in the cytosolic $[ATP]/[ADP] \cdot [P_i]$ ratio must be the main event in stimulus–secretion coupling in B-cells. Indeed, changes in the total contents of ATP or ADP in islets are usually not seen during fuel-induced insulin secretion (Ashcroft *et al.*, 1973; Malaisse *et al.*, 1979a). These measurements may reflect values in the cytosol, because the mitochondrial volume accounts for 4% of the B-cell volume only (Dean, 1973). However, mechanisms

can be formulated which integrate Ca^{2+} handling by mitochondria into the fuel-recognition process of B-cells. Enhanced hydrogen supply could drive both Ca^{2+} influx and Ca^{2+} efflux in the mitochondria via an elevation of the transmembrane potential (Åkerman & Nicholls, 1983; Bernardi & Azzone, 1983). In case the effect on efflux dominates, the cytosolic Ca^{2+} concentration could rise readily. Alternatively stimulation of net Ca^{2+} uptake by mitochondria might initiate and maintain depolarisation of the B-cell plasma membrane and thereby might increase Ca^{2+} influx through the voltage-gated Ca^{2+} channel (Chay & Keizer, 1983). In view of the low mitochondrial content of pancreatic B-cells (Dean, 1973), persistent enhancement of hydrogen supply would be necessary to prevent the increase in oxidative phosphorylation (Figs. 1–3) lowering the membrane potential of B-cell mitochondria to values insufficient for high rates of Ca^{2+} transport.

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