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Dynamics of O_2 Consumption in Rat Pancreatic Islets

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Summary. The O_2 consumption of rat pancreatic islets was determined by monitoring pO_2 in the perifusate from groups of 200–300 islets. Basal respiration was maintained for up to 2 h. The insulin secretagogues, glucose and 4-methyl-2-oxopentanoate, provoked an immediate (<5 s) increase in islet respiration which attained a new steady-state within 10–40 min. The respiratory changes were immediately reversible upon removal of the substrate and were parallelled by changes in insulin release and substrate oxidation. The concentration dependence of glucose-induced respiratory changes was sigmoidal with a threshold at 3 mmol/l. The concentration dependence with 4-methyl-2-oxopentanoate was characterised by a hyperbolic relationship. The weak insulin secretagogues 3-methyl-2-oxobutyrate and d,l-3-methyl-2-oxopentanoate, although stimulating islet respiration were not more effective than 4-methyl-2-oxopentanoate at non-insulinotropic concentrations. Rotenone, antimycin and oligomycin inhibited both basal O_2 consumption and the ability of glucose and 4-methyl-2-oxopentanoate to increase islet respiration. 2,4-Dinitrophenol increased islet O_2 consumption. The omission of Ca^{2+} and Mg^{2+} from the perifusing media, or the addition of the ionophore A23187, had little effect on respiration. The omission of K^+ inhibited glucose-induced changes but had a lesser effect in the absence of substrate or in the presence of 4-methyl-2-oxopentanoate. The omission of HCO_3^- reduced both basal and secretagogue-induced changes in islet respiration. It is concluded that mitochondrial O_2 consumption linked to oxidative phosphorylation is a major component in the respiratory response, and that some energy consuming process in the islets depends on the availability of HCO_3^-. Mitochondrial reactions may generate a signal initiating the secretory process.

Key words: Islets, insulin release, glucose, 2-oxo acids, O_2 consumption, respiration.

The importance of oxidative metabolism in the stimulus-secretion coupling of insulin release is illustrated by the finding that nutrient secretagogues increase islet respiration [1–4], and that agents which interfere with mitochondrial electron transport or oxidative phosphorylation are potent inhibitors of insulin release [5–7]. The rates of oxidation of various carbohydrate homologues and anomers are correlated to their secretory capacities [8–10], though such a correlation is not observed when comparing metabolisable secretagogues of differing chemical structure [11, 12]. The question remains as to whether oxidative metabolism is important in the generation of a signal initiating insulin release or merely plays a supportive role in the stimulus-secretion coupling mechanism by providing metabolic energy for as yet undefined reactions possibly associated with ion transport or biosynthetic activity.

The present communication investigates the temporal relationship between changes in islet respiratory activity, substrate oxidation and insulin secretion in perifused rat pancreatic islets. The two principal secretagogues presently used, glucose and 4-methyl-2-oxopentanoate (2-ketoisocaproate; KIC) were chosen to represent metabolisable nutrients of high insulinotropic potency which share only the tricarboxylic acid cycle as a common metabolic pathway. The nature of the O_2 consuming reactions was investigated by using a number of inhibitors or modifiers of insulin release.

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Materials and Methods

Chemicals

Enzymes, cofactors, oligomycin and antimycin were obtained from Boehringer, Mannheim, Germany; 2,4-dinitrophenol from BDH, Poole, U. K., and 2 keto acids, valinomycin, luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), cycloheximide, rotenone and bovine serum albumin from Sigma, St. Louis, MO, USA. The ionophore A23187 was a gift from Eli Lilly, Indianapolis, IN, USA. All other reagents were of analytical reagent grade and obtained from Merck, Darmstadt, Germany. Radioisotopes were obtained from The Radiochemical Centre, Amersham, Bucks, U. K. With the exception of [2-^{14}C]pyruvate, all labelled 2-keto acids were prepared by the enzymic oxidation of the corresponding 1-amino acid using 1-amino acid oxidase (EC 1.4.3.2) in the presence of catalase (EC 1.11.1.6) [13]. [U-^{14}C] isovalerate was prepared by the oxidation of [U-^{14}C] KIC with ceric sulphate [14].

Islets and Perifusion Media

Islets were isolated from the pancreases of fully fed 200 g female Wistar rats by the collagenase digestion technique [15]. Groups of 200 islets were collected in ice-cold Hank's saline supplemented with 3.3 mmol/l glucose. The islets were rinsed three times in fresh medium and then transferred to the perifusion chamber. The perifusion medium in most experiments was a Krebs-bicarbonate buffer, pH 7.5, with bovine serum albumin 5 mg/ml [16]. The Cl^- content of the media ranged from 84–124 mmol/l since NaCl in many cases was replaced by the sodium salts of acidic substrates in order to maintain iso-osmolarity. Equilibration with a 95% air, 5% CO_2 (v/v) atmosphere was achieved by passing a stream of humidified gas at 100–200 ml/min above the media contained in a 250 ml Erlenmeyer flask. This step and all subsequent operations were performed in a chamber maintained at between 36.9 and 37.1 °C. Bicarbonate-free media were prepared by substitution of HCO_3^- by 25 mmol/l Hepes (2-(*N*-2'-hydroxyethyl piperazine-*N'*-yl-ethane sulphonate) with appropriate adjustments being made in NaCl content to maintain iso-osmolarity; the final pH was adjusted to 7.5 with carbonate-free NaOH, and the gas phase was ambient air.

Dynamics of O_2 Consumption

The perifusion chamber was a 2 cm length of 6 mm i. d. glass tubing into which was inserted at both ends a stainless-steel cannula (0.58 mm i. d., 1 mm o. d.) covered by successive layers of silicone rubber tubing to achieve an o. d. of 6.05 mm. The internal volume of the chamber was 30 µl, and the islets were placed in the centre of the chamber between two 6 mm glass fibre discs (Whatman GF/A) separated by a 0.6 mm × 6 mm o. d. spacer ring of cellulose nitrate. The chamber was connected on one side to a medium reservoir (75 mm × 10 mm i. d. tube) and on the other to a Clark-type O_2 electrode assembly (Radiometer E5046 in a D616 thermostatised cell; Radiometer, Copenhagen, Denmark) the outlet of which was connected via a perifusate pump (LKB 12000, Stockholm, Sweden) to a fraction collector (LKB 7000). All connections were made with short pieces of 0.41 mm i. d., 2 mm o. d. tubing (Technicon, NY, USA). The overall volume of the system was 180 µl, the flow rate 100 µl/min, and the dead space between the reservoir and electrode 60 µl.

The O_2 electrode response was monitored with a Radiometer PHM 72 blood gas analyser (Radiometer, Copenhagen, Denmark), the output of which was registered on a potentiometric chart recorder (Servegor S, Goer 2, F. R. G.) equipped for scale expansion and background suppression. An initial recording of the media pO_2 was attained using an empty perifusion chamber. The chamber was then replaced with one containing islets, and after a steady state was attained (20–30 min), the experiment was performed. The islet oxygen consumption was calculated from the preset flow rate and the observed decrease in the pO_2 in the perifusate. The relationship between pO_2 and the O_2 content of the media was established from O_2 solubility data [17] with appropriate corrections for atmospheric pressure and H_2O vapour pressure.

Dynamics of Insulin Release and Islet Acetoacetate Production

Insulin in the effluent was assayed by the procedure of Wright et al. [18]. Acetoacetate was determined enzymatically on $HClO_4$-deproteinised extracts of the perifusate by a fluorimetric technique [19]; the recommended procedure was modified in that a smaller volume of enzymatic reagent (100 µl) was used and that the reaction product NAD^+ was determined by the alkali-induced fluorescence method [20].

Dynamics of $^{14}CO_2$ Production

Radioactive CO_2 produced by the islets from uniformly ^{14}C-labelled substrates was trapped as $Ba^{14}CO_3$ by collecting the effluent perifusate (2-min sample) in 300 µl of a solution which contained initially 20 mmol/l NaOH and 150 mmol/l $BaCl_2$. The precipitated material was isolated by centrifugation (2000 g for 10 min) and washed twice with the alkaline $BaCl_2$ solution. The tube (3.5 cm × 6 mm i. d.) with the final precipitate was placed in a liquid-scintillation counting vial which contained 0.5 ml hyamine hydroxide (Packard Instrument Co., Downer Groves, IL, USA). The vial was sealed with a rubber septum and 100 µl of 0.2 mol/l HCl injected into the tube. The vials were shaken for 1 h at 37 °C and the radioactivity in the hyamine hydroxide phase was determined by liquid-scintillation counting after adding 10 ml of a toluene based scintillation fluid (Lipoluma, Lumac Ind., Basel, Switzerland). Samples of the perifusate which contained either the labelled substrate or $NaH^{14}CO_3$ were passed through the entire procedure in order to attain a blank determination (about 60% of the observed incorporation) and to correct for losses of $^{14}CO_2$ (69.7 ± 2.1% recovery, mean ± SEM, n = 4) in the procedure.

Analyses Performed in Long-term Incubations

Insulin released from groups of 8 islets incubated for 90 min in 1 ml buffer, and $^{14}CO_2$ formed from ^{14}C-labelled substrates by groups of 15 islets incubated for 120 min in 50 µl buffer, were determined by methods previously described [9, 16]. In these experiments the gas phase used was 95% O_2/5% CO_2.

H_2O_2 Production

Groups of 30–50 islets placed in 30 mm × 5 mm i. d. pyrex tubes were incubated for 2 h at 37° in 50 µl medium. The medium was then withdrawn and injected into 300 µl of a solution which contained 0.5 mmol/l luminol and 0.1 mol/l NaOH. Luminescence was determined (Chem-Glow Photometer; American Instrument Co., Silver Spring, MD, USA) and recorded on a moving paper chart. The steady-state luminescence recorded 30 s after initiation of the reaction was calibrated by the subsequent injection of standard H_2O_2 solutions (0.1–10 nmol). Blank determinations were made from incubations not containing islet tissue.

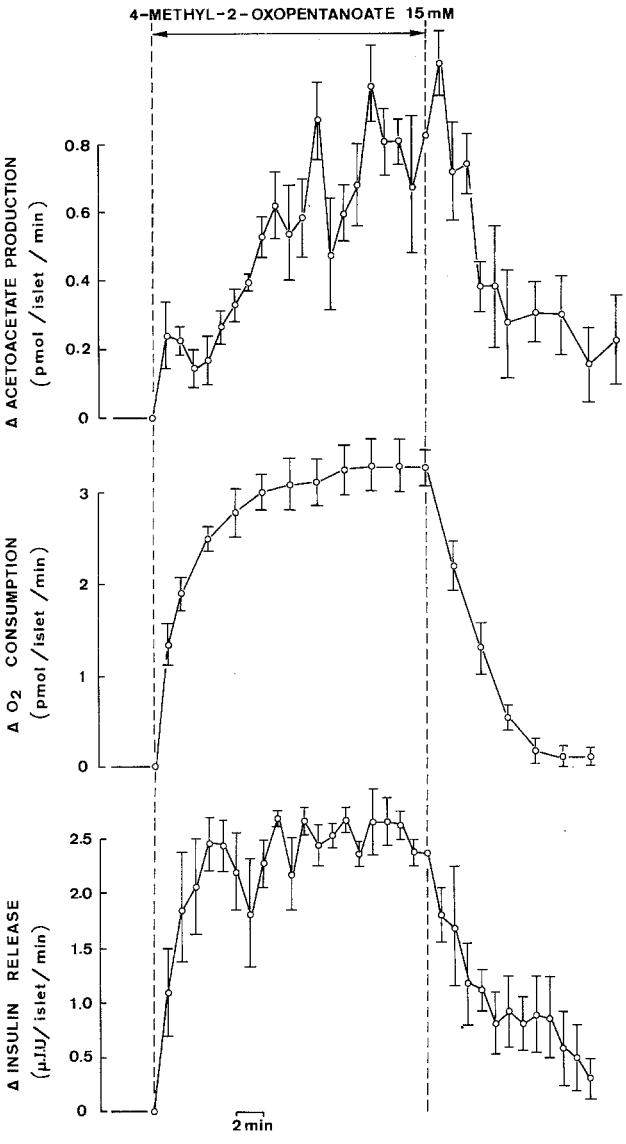

Fig. 1. Dynamic response in islet acetoacetate production, O_2 consumption and insulin release on stimulation with KIC. Groups of 200–300 islets were perifused for 30 min the absence of substrate and then exposed to 15 mmol/l KIC for 20 min. The plotted values are the mean ± SEM of results obtained in 5 separate experiments. Each variable is shown as the increment observed in the presence of the substrate. Basal O_2 consumption was 8.1 ± 0.8 pmol/min/islet, basal insulin release 0.14 ± 0.13 μU/min/islet and basal acetoacetate production 0.49 ± 0.13 pmol/min/islet

Presentation of Results

Determinations of islet respiration were performed on at least 4 separate batches of islets; however, the results shown are actual recordings from single experiments which illustrate the results obtained in the overall experimental series. Tabulated values of O_2 consumption correspond to the steady-state value 10–30 min after the change in medium composition. Results are expressed as the mean ± SEM with the number of separate observations indicated in parentheses. The statistical significance of differences was estimated by two-tailed Student's *t* test.

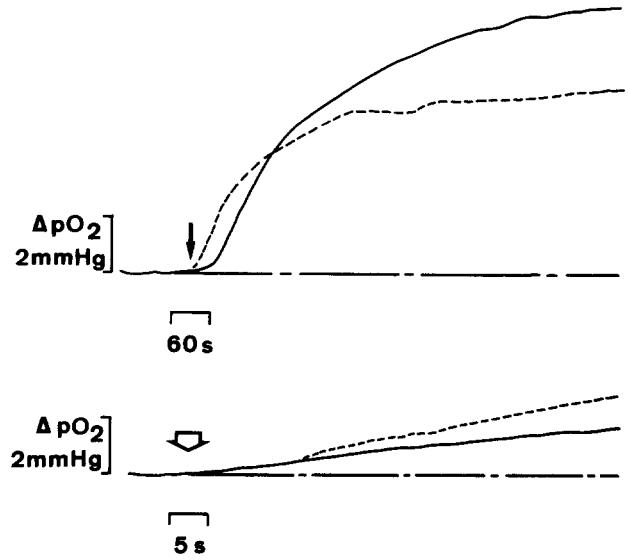

Fig. 2. Islet respiratory response to glucose and KIC. Groups of 200 rat pancreatic islets were perifused with a substrate-free medium for 20 min and then exposed to either 27.8 mmol/l glucose (solid line) or 15 mmol/l KIC (dotted line). The results shown were obtained in two separate experiments and illustrate differences which were observed in the short term and long-term respiratory response of islets to these substrates. The change in pO_2 is shown relative to the pO_2 recorded prior to the introduction of the substrate (----)

Results

Technical Considerations

The drift in the pO_2 signal recorded from the assembled perifusion system was <2%/h when no tissue was present and <4%/h in the presence of tissue. The decrement in pO_2 caused by the introductions of tissue was approx 15 to 20% of the initial pO_2. Respiration was maintained for up to 2 hours in the absence of substrate but thereafter declined rapidly. A perturbation in the respiration rate of as little as 5% was detectable provided this occurred over a time interval of 5 min or less.

The use of air/CO_2 rather than O_2/CO_2 as the gas phase was dictated by the greater stability of media prepared at close to ambient pO_2 and the greater relative change in pO_2 induced by islet respiration. Lowering the medium pO_2 to this extent did not alter significantly the oxidation of [U-^{14}C] labelled substrates or the insulinotropic action of glucose (16.7 mmol/l) and KIC (10 mmol/l).

The Dynamic Response to KIC

Exposure of islets to KIC provoked an immediate (<5 s) change in islet respiration which increased

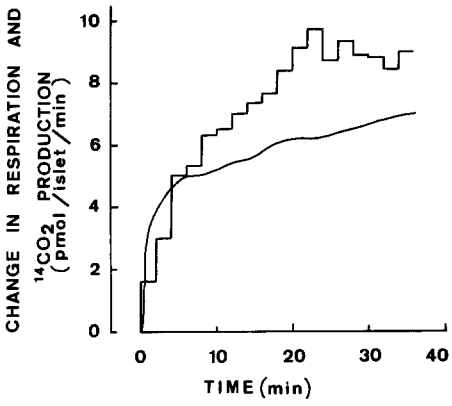

Fig. 3. Dynamic response of changes in islet respiration and $^{14}CO_2$ production in the presence of [U-^{14}C] KIC. Islets were perifused with substrate-free buffer for 30 min prior to the introduction of [U-^{14}C] KIC (25 mmol/l, 10 μCi/ml) into the perifusate. Results of a typical experiment are shown in which islet O_2 consumption (continuous trace) and $^{14}CO_2$ formation (discontinuous trace) were determined simultaneously. The basal O_2 consumption in this experiment was 15.7 pmol/min per islet

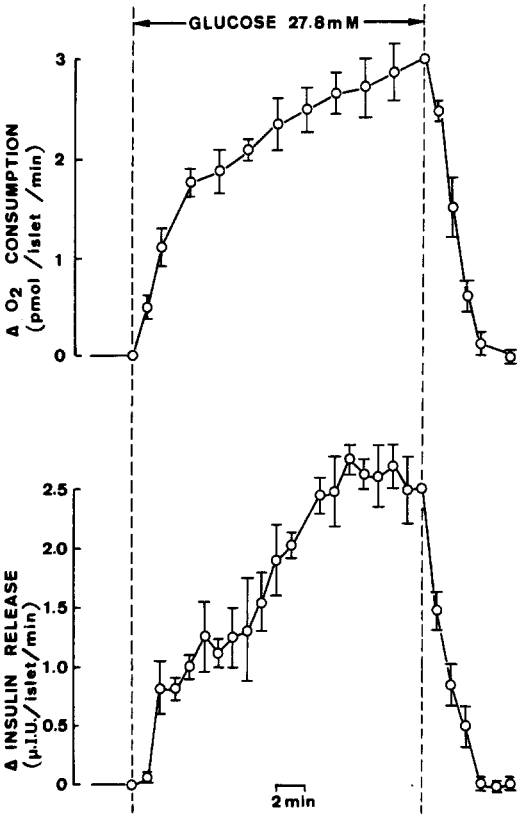

Fig. 4. Dynamic response of islet O_2 consumption and insulin release to 27.8 mmol/l glucose. Groups of 200–300 islets were perifused for 30 min in a substrate-free medium and then exposed to 27.8 mmol/l glucose over a 20 min interval. The plotted values are the mean ± SEM of results obtained in 3 separate experiments. Each variable is shown as the increment observed in the presence of the substrate. Basal O_2 consumption was 7.7 ± 0.6 pmol/min per islet, and basal insulin release 0.34 ± 0.13 μU/min per islet

usually in a monophasic manner to attain a new steady-state after 10 to 20 min (Figs. 1 and 2). Removal of KIC invoked a rapid decrease in islet O_2 consumption, the respiratory rate then returning to that observed before the substrate was added. The half-time for the on-response was 1.7 min and for the off response 2.5 min. The half-time of the response of the perifusion system tested with square wave changes in medium pO_2 was 20 s.

Changes in the insulin and acetoacetate content of the perifusate evoked by KIC followed a time course similar to the changes in islet respiration (Fig. 1). The acetoacetate response, although immediate, developed more slowly and was less rapidly reversed than either respiration or insulin release. As shown in Figure 3, the time course of $^{14}CO_2$ production from 25 mmol/l [U-^{14}C] KIC resembled that of O_2 uptake. The apparent respiratory quotient (mole $^{14}CO_2$/mole O_2) was about 1.3, a value comparable to that expected on the basis of combination of 3 moles of O_2 with one mole of [U-^{14}C] KIC to yield one mole of acetoacetate and 3 moles of $^{14}CO_2$.

The Dynamic Response to Glucose and Other Substrates

The respiratory response to glucose was rapid in onset (less than 5 s), sustained, and rapidly reversible (Figs. 2 and 4). Compared to the respiratory response to KIC that obtained with glucose initially developed more slowly and did not reach a true steady-state until after 40 min. These differences were reflected in the time course of insulin release invoked by glucose; insulin release was not observed within 1 min of exposure to glucose, and the rate of secretion increased progressively. The decrease in respiratory rate and insulin release upon removal of glucose was more rapid than with KIC.

The dynamic responses of islet respiration to all other 2-oxo acids and amino acids tested (Table 1) were qualitatively similar to that observed with KIC in that the response was initiated as rapidly, and attained a steady-state within 10–20 min after the change in medium composition.

Concentration-dependence of Respiratory Changes

Glucose at a concentration below 2.8 mmol/l did not cause a significant change in islet O_2 consumption within 15 min, but higher concentrations provoked a marked increase in respiratory activity (Fig. 5). Representation of the results shown in Figure 5 on a logarithmic concentration scale yielded a linear relationship, which when extrapolated to a zero incre-

Table 1. Relationship between respiratory rate, substrate oxidation and insulin release. The increment in islet respiratory rate observed 10–20 min after the introduction of the indicated substrates into the perifusate is expressed relative to the increment in respiratory rate induced by 10 mmol/l KIC within the same experiment. The mean basal O_2 consumption was 486 ± 43 pmol O_2/h/islet (n = 18), and the mean increment induced by 10 mmol/l KIC was 200 ± 19 pmol O_2/h/islet (n = 18). The rate of substrate oxidation was determined over a 120 min incubation in the presence of ^{14}C-labelled substrates. The rates of incorporation of radioactivity into $^{14}CO_2$ calculated as pmol substrate were multiplied by the number of carbon atoms in each compound and expressed relative to the value observed with 10 mmol/l KIC in the same experiment. All substrates were uniformly labelled except for succinate, [2,3-^{14}C], and pyruvate, [2-^{14}C]. The rate of oxidation of KMV was determined with the 1-stereoisomer of the radioisotope but in the presence of a racemic mixture of unlabelled substrate; results are expressed as if both stereoisomers were equivalently oxidised. The mean rate of [U-^{14}C]-KIC oxidation observed in these experiments was 164 ± 10 pmol $^{14}CO_2$/h/islet (n = 40). Insulin release was determined over a 90 min incubation. Results are expressed relative to those obtained with 10 mmol/l KIC in the same experiment. The mean basal secretory rate was 22.3 ± 1.4 μU/90 min/islet (n = 50) and the rate observed in the presence of 10 mmol/l KIC 155 ± 9 μU/90 min/islet (n = 63). Each tabulated value is the mean ± SEM of results obtained with separate batches of islets, the number of batches used being shown in parentheses

Substrate	Increment in O_2 consumption	Substrate oxidation	Insulin release
KIC 10 mmol/l	100.0± 9.5 (18)	100.0± 6.0 (40)	100.0±5.0 (63)
KIC 4 mmol/l	48.4± 6.2 (4)	73.8± 4.1 (16)	< 20 (16)
Glucose 11 mmol/l	68.9±10.5 (8)	78.0± 4.7 (8)	106.0±4.0 (16)
Leucine 40 mmol/l	69.0± 6.2 (3)	80.2±17.9 (19)	46.3±2.3 (16)
KMV 20 mmol/l	57.0± 3.2 (5)	62.0± 9.6 (8)	37.2±3.2 (30)
KIV 20 mmol/l	52.0± 6.1 (4)	59.4±12.1 (14)	< 20 (16)
Pyruvate 10 mmol/l	25.2± 1.2 (4)	58.0±10.6 (8)	< 20 (16)
Glutamine 10 mmol/l	22.7± 2.7 (3)	136.0±11.3 (8)	< 20 (16)
Isovalerate 20 mmol/l	~ 0 (3)	30.3± 5.7 (8)	< 20 (16)
Succinate 20 mmol/l	~ 0 (3)	25.7± 5.7 (7)	< 20 (16)

ment in islet respiration indicated that the threshold of the glucose-induced changes occurred between 3 and 3.5 mmol/l.

The concentration dependence of the respiratory response to KIC (Fig. 5) was hyperbolic, there being no evidence of a threshold response.

The finding that marked changes in O_2 consumption were induced by KIC at concentrations well below those provoking insulin secretion [21] was further investigated by comparing the effects of this 2-keto acid with the effects of the weak insulin secretagogues 3-methyl-2-oxobutyrate (2-ketoisovalerate; KIV) and d,1-3-methyl-2-oxopentanoate (d,1-2-keto-3-methyl valerate; KMV) [14]. The concentration dependencies of KIV- and KMV-induced respiration were identical to KIC-induced changes but only up to a concentration of 3.3 mmol/l whereafter only KIC induced a further increment in O_2 uptake (Fig. 6). It is interesting that the threshold for KIC-induced insulin release lies at 4 mmol/l [21]. The rate of respiration in the presence of 20 mmol/l KMV was equivalent to that in the presence of 6.0 mmol/l glucose.

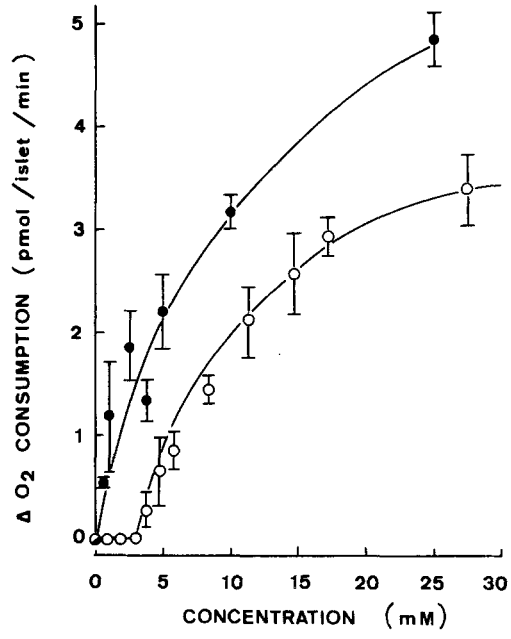

Fig. 5. Response of islet respiration to variation in the concentration of glucose and KIC. Islets were perifused for 30–40 min with substrate-free medium and then exposed to either glucose (open circles) or KIC (closed circles) until a new steady-state respiratory rate was observed (about 20 min). The medium substrate concentration was then increased or decreased in 3 or 4 successive steps before returning to a substrate-free medium and terminating the experiment. Each plotted value is the mean ± SEM of results obtained in 8–16 separate experiments. The mean basal respiration in these experiments was 8.1 ± 0.8 pmol/min per islet (n = 18)

Relationship of Respiratory Changes to Substrate Oxidation and Insulin Release

Comparisons of the respiratory response on islets to various substrates, and to the rates of oxidation and insulinotropic effect of these substrates, established

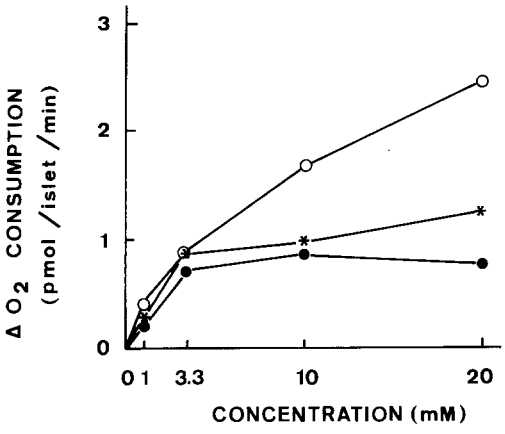

Fig. 6. Effect of related 2 keto acids upon islet respiration. Batches of 200 islets were perifused for 20 min in the absence of substrate and then exposed for a 10 min interval successively to KIV (closed circles), KMV (asterisks) and KIC (open circles) at each of the concentrations indicated. Each result is the mean of four determinations performed in two separate experiments

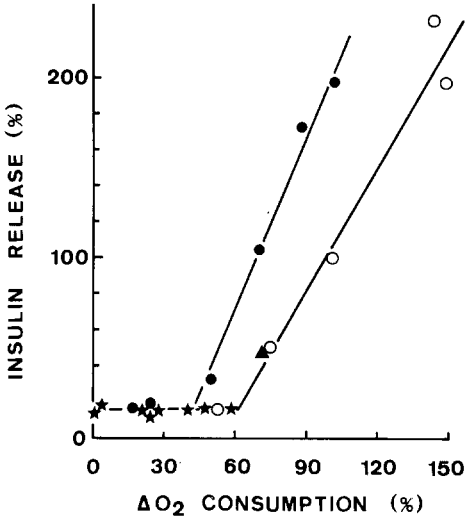

Fig. 7. Relationship between the effect of various substrates on islet respiration and insulin release. The respiratory response of islets was determined in the perifusion system presently described in the presence of KIC (open circles), d-glucose (closed circles), l-leucine (triangle) and non-secretagogue nutrients (stars; see Table 1). Insulin release determined over a 90 min incubation is taken from references 14 and 21. Each result is expressed relative to the effect observed in the presence of 10 mmol/l KIC

in batch-type incubations, are shown in Table 1. All the substrates examined were oxidised by islets, but their oxidation did not parallel their effects on respiration. This difference could not be attributed simply to differences in the O_2/CO_2 stoichiometry of the respective metabolic pathways, certainly not in the case of glutamine, pyruvate, isovalerate and succinate for which wide discrepancies between oxidation and respiration were observed. As found by previous authors [8–12] a general correlation between rates of insulin release and substrate oxidation under such conditions was observed. However there were exceptions, exemplified in the present experiment by glutamine.

A much closer correlation was seen between respiratory response and insulin release. Compounds which were insulin secretagogues provoked larger increases in O_2 consumption than did non-secretagogues; indeed, no substrate was encountered which markedly stimulated islet respiration but not insulin release. The correlation of the insulinotropic and respiratory response was also born out from studies of the concentration dependencies of KIC- and glucose-induced changes (Fig. 7). However, at any given rate of insulin secretion the respiratory response to KIC was about 1.4 × that to glucose.

Effect of Pharmacological Agents

Rotenone 3 μmol/l, or 5 μmol/l antimycin A, rapidly inhibited the respiration of islets leading to a new steady-state at 0–30% of the basal rate within 10–15 min (Fig. 8). Inhibition was seen in the absence of substrate and in the presence of glucose (2.8–28 mmol/l) or KIC (3.3–25 mmol/l).

2,4-Dinitrophenol, 0.1–0.2 mmol/l, provoked an immediate and marked increase in islet respiration which occasionally persisted for 10 min or more before being replaced by an inhibitory effect of increasing intensity. The stimulatory response to 2,4-dinitrophenol was augmented in the presence of glucose or KIC or a combination of these substrates. The magnitude of the response to 2,4-dinitrophenol was proportionate to the extent to which the substrate(s) itself stimulated respiration (Fig. 9).

In the absence of substrate or in the presence of KIC, 1–10 μmol/l oligomycin immediately though progressively inhibited islet respiration. The subsequent addition of either 0.1 mmol/l 2,4-dinitrophenol or 1 μmol/l valinomycin caused an increase in respiration, but the rate attained was less than that observed in the presence of uncoupler alone.

The ionophore A23187, 0.1–12 μmol/l, in the presence of 10 mmol/l KIC did not affect islet respiration over 20 min (Fig. 10). Cycloheximide, 36 μmol/l, caused only a 7% fall in respiratory rate when used in the presence of 10 mmol/l KIC (data not shown).

Effect of Modification of the Ionic Composition of the Perifusate

The effect of the omission of Ca^{2+}, Mg^{2+} or K^+ from the perifusate was tested in several different protocols which included the simultaneous removal of

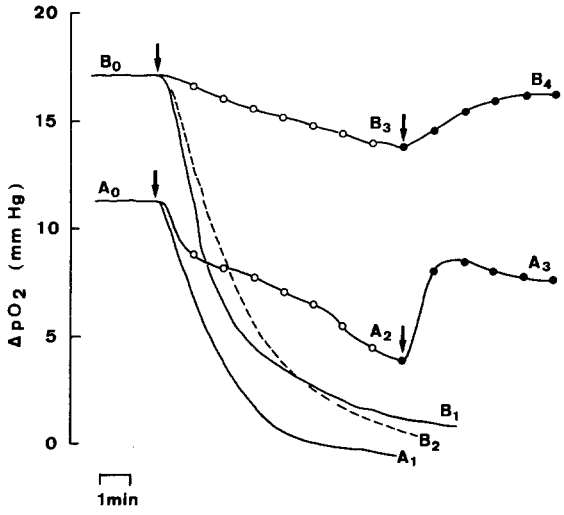

Fig. 8. Effect of antimycin A, rotenone and oligomycin on islet respiration. Groups of 200 islets were perifused either in the absence of substrate (A) or in the presence of 20 mmol/l KIC (B). At the time indicated by the first arrow (↓) 5 μmol/l antimycin A (A_1 & B_1), 3 μmol/l rotenone (B_2) or 1 μmol/l oligomycin (A_2 & B_3) was added to the perifusate. From the time indicated by the second arrow (↓) 0.1 μmol/l valinomycin was combined with 1 μmol/l oligomycin (A_3 & B_4). The results shown are traces of direct recordings of perifusate pO_2 obtained in 5 separate experiments. The results are shown with a common baseline (Ao or Bo) which was the mean change in pO_2 observed under each substrate condition. Circles were superimposed on the traces obtained in the presence of either oligomycin (○) or both oligomycin and valinomycin (●)

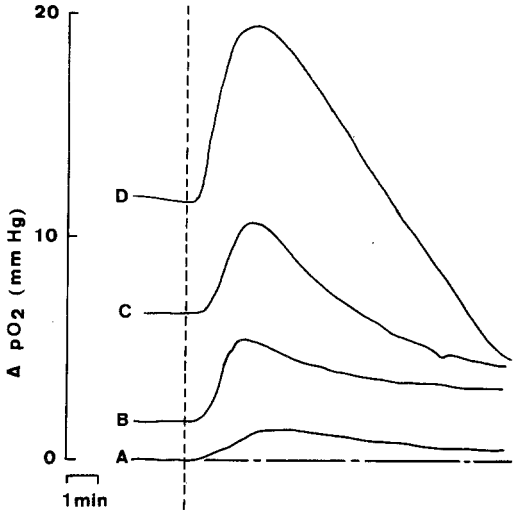

Fig. 9. Effect of 2,4-dinitrophenol on islet respiration. Groups of 200 islets were perifused in the absence of metabolisable substrate for 20 min and then in the presence of either glucose or KIC. When a steady-state of respiration was reached (10–30 min) 0.2 mmol/l 2,4-dinitrophenol was introduced in the perifusate. The results shown are direct recordings from experiments with 4 different batches of islets. In each case the change in pO_2 is shown relative to the pO_2 recorded prior to the introduction of substrate (---). The time of introduction of 2,4-dinitrophenol is indicated by the vertical dashed line. (A) No exogenous substrate; (B) 5 mmol/l glucose; (C) 10 mmol/l KIC; (D) 20 mmol/l KIC combined with 20 mmol/l glucose

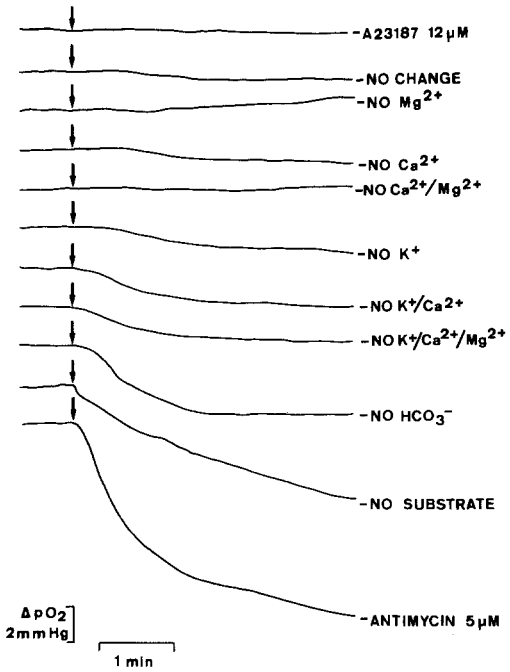

Fig. 10. Effect of various modifications in buffer ionic composition and the presence of antimycin A or the ionophore A23187 upon islet respiration. The results illustrated were taken from the same or different experiments all conducted in the presence of 20 mmol/l KIC. Groups of 200 islets were perifused with the complete media for 5–10 min before the media of altered composition was introduced (↓). Experiments which investigated the effects of K^+, Mg^{2+} and Ca^{2+} were performed in the same batch of islets. Experiments with HCO_3^- were conducted in the presence of 25 mmol/l Hepes buffer containing initially 2.5 mmol/l $NaHCO_3$

the three ions, their removal individually or their removal in pairs. Experiments were conducted either in the absence of substrate or in the presence of 10–20 mmol/l KIC or 27.8 mmol/l glucose. Alternatively, islets were perifused in the absence of substrate, the ion of interest then omitted from the medium for 10 min, the substrate of interest then added, and finally the cation readmitted. All experimental approaches yielded essentially the same results and are summarised in Table 2 and Figure 10.

The omission of Ca^{2+} or Mg^{2+} in any given protocol did not markedly affect the basal rate of respiration or the changes induced by glucose or KIC.

The omission of K^+ resulted in a rapid though small decrease in the basal respiratory rate which attained, within 5 min, a new steady-state value. The effect of K^+ removal was fully reversed within 10 min of the readmission of the cation into the perifusate. The effect of K^+ omission in the presence of KIC was quantitatively similar to the effect observed in the absence of substrate, there being a diminution in the respiratory rate of about 10%. In contrast, K^+ omission in the presence of glucose resulted in a marked decreased in the respiratory rate such that the new

Table 2. Effect of media K^+ and bicarbonate concentration on islet respiration in the absence of substrate or in the presence of glucose and/or KIC. The effect of K^+ omission was investigated in the presence of a bicarbonate buffer (24 mmol/l) equilibrated with 95% air, 5% CO_2 (by vol.) (buffer A), and the effect of bicarbonate omission in the presence of a Hepes buffer (25 mmol/l) equilibrated with ambient air (buffer B). Unless specified the K^+ concentration of both buffers was 5 mmol/l, and the bicarbonate concentration in buffer B was 2.5 mmol/l. The islet rate of respiration observed in the complete media is expressed in absolute terms. The rate of respiration observed 10 min after removal of the relevant ion is expressed in percent of the paired control value. Each value is the mean ± SEM of results obtained in separate experiments; the number of such experiments is shown in parentheses. The statistical indices refer either to the difference in control value attributable to the buffer used, or to the effect attributable to the removal of K^+ or bicarbonate ([a] $P < 0.01$; [b] $P < 0.02$; [c] $P < 0.05$; [d] $P < 0.10$). N.D. not determined

Substrate	Buffer A		Buffer B	
	Control (pmol/min per islet)	No K^+ %	Control (pmol/min per islet)	No HCO_3^- %
None	8.5±1.5 (4)	89.3±1.5[a]	5.5±0.4 (3)	72.7±9.3[d]
Glucose 27.8 mmol/l	12.2±1.2 (4)	71.2±5.0[b]	8.9±0.7 (3)	77.1±5.7[d]
KIC 10.0 mmol/l	11.1±1.0 (4)	89.0±1.1[a]	8.2±0.5 (3)	83.4±6.0
Glucose 20 mmol/l and KIC 20 mmol/l	14.8±0.7 (3)	N.D.	11.5±0.8[c] (3)	84.7±3.0[c]

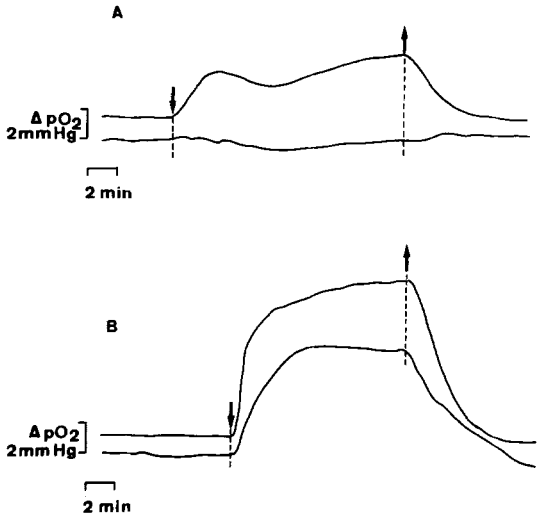

Fig. 11 A and B. Effect of media cation composition on the respiratory response evoked by glucose and KIC. Groups of 300 islets were perifused for 10 min with substrate-free media which was either of normal ionic composition (upper trace) or depleted of Ca^{2+}, Mg^{2+} and K^+ (lower trace). At the indicated time (↓) either 11.1 mmol/l glucose **A** or 10 mmol/l KIC **B** was introduced into the perifusate. The substrate was removed at the time indicated by the second arrow (↑)

steady-state value was no greater than the basal respiratory rate before glucose was added. Glucose added to a perifusate depleted of K^+, Ca^{2+} and Mg^{2+} likewise failed to stimulate islet respiration, again in contrast to the effect of KIC (Fig. 11).

The effect of HCO_3^- was investigated using a perifusate buffered with 25 mmol/l Hepes. Control experiments and published data [22] suggested that 2.5 mmol/l HCO_3^- was sufficient for the close-to-full expression of the insulinotropic potential of the substrates presently used. The minor variation in medium pH and pO_2 which occurred as a result of changing the HCO_3^- concentration did not significantly influence the results.

Removal of HCO_3^- from a perifusate previously containing 2.5 mmol/l $NaHCO_3$ caused an immediate (< 5 s) and rapidly developing inhibition of islet respiration which reached a new steady-state within 10 min (Figs. 10 and 12). The relative magnitude of the inhibition was similar in the absence of substrate and in the presence of 5–27.8 mmol/l glucose or 10–20 mmol/l KIC (Table 2). The respiratory rate was fully restored by the subsequent addition of 2.5 mmol/l $NaHCO_3$ and partially restored by 0.25 mmol/l $NaHCO_3$. The finding that such a low concentration of HCO_3^- was effective suggested that the effect of HCO_3^- omission may have been underestimated since contamination of the medium with atmospheric CO_2 was unavoidable.

H_2O_2 Production

Islet production of H_2O_2 was not detected (i. e. < 20 pmol/h per islet) in the absence of substrate or in the presence of 20 mmol/l glucose or 20 mmol/l KIC. In the case of KIC, however, a substantial proportion of any H_2O_2 produced would have been eliminated through chemical reaction with the substrate.

Discussion

The present results obtained by continuous monitoring of effluent pO_2 in a perifusion system confirm, in several respects, previous findings obtained by the Cartesian diver method [1–3]. Thus, islet tissue maintained a significant rate of endogenous respiration for up to 2 h, and insulin secretagogues pro-

voked increases in O_2 consumption, which were commensurate with their insulinotropic potencies. However, the basal rate of islet respiration presently observed (about 8 pmol/min per μg dry wt) was 2 to 5 times higher than that previously reported [1, 2], although the relative increase induced by glucose (about 43% of basal at 27.8 mmol/l) was of similar magnitude. It was not possible to confirm the previous observation [23] that succinate, in contradistinction to its effect on islet insulin release, was a powerful stimulus to islet respiratory activity.

Relationship between Exogenous Substrate Oxidation and Respiratory Changes

A general correlation was observed between the rates of ^{14}C-labelled substrate oxidation and the effects of these substrates on islet respiration and insulin release. However, notable exceptions existed. In these experiments, no attempt was made to measure the specific radioactivity of the $^{14}CO_2$ formed. Thus, no account could be made of the extent to which exogenous substrate oxidation replaced that of endogenous nutrient. That such replacement may occur or that the exogenous substrate may undergo radioisotopic exchange with an endogenous pool of a related metabolite was suggested by the finding that rate of $^{14}CO_2$ production from any substrate was invariably greater than that which may have been predicted from its effect on O_2 consumption (Table 1). Although the radioisotopes used were nominally pure, it should also be remembered that the radioactivity recovered as $^{14}CO_2$ in most cases represented less than 0.1% of that present in the incubation medium. The possibility of artifactual changes in $^{14}CO_2$ production due to radioisotopic contamination should not be overlooked.

The difference between the observed rate of $^{14}CO_2$ production and that predicted from O_2 consumption measurements was less evident at higher rates of respiration. The results in Table 1 and Figure 3 suggest that the increase in respiration induced by glucose and KIC was associated with oxidation of the secretagogue itself rather than with any major change in the rate of oxidation of endogenous nutrients.

Concentration-dependence of Substrate-induced Changes in Respiration

The finding that a threshold concentration of glucose existed below which respiratory changes were not apparent mimics in many ways the effect of glucose on various other indices of islet function [24]. In this respect, the minimal concentration of glucose required to evoke respiratory changes (about 3 mmol/l) corresponded closely to the threshold for

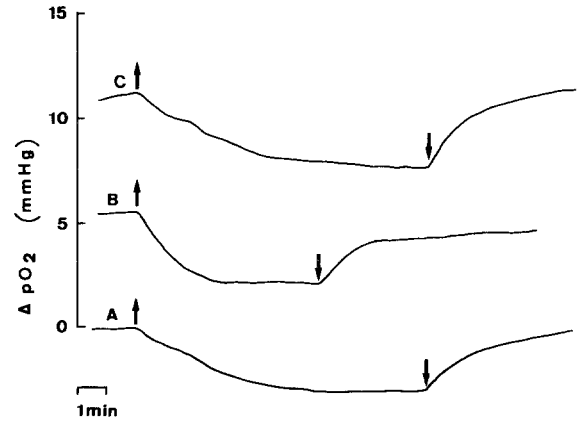

Fig. 12. Effect of bicarbonate upon islet respiratory activity. Groups of 200 islets were perifused with media buffered with 25 mmol/l Hepes, pH 7.5, either in the absence of substrate (A) or in the presence of 27.8 mmol/l glucose (B) or 20 mmol/l glucose combined with 20 mmol/l KIC (C). The media initially contained 2.5 mmol/l $NaHCO_3$ which was omitted at the time indicated (↑) and then latter reintroduced (↓)

changes in insulin biosynthesis, ^{86}Rb or ^{45}Ca net uptake, but was lower than that required to stimulate insulin release from islets such as those presently used [24]. Insulin release, bioelectrical activity and changes in ^{45}Ca movements induced by KIC also exhibit threshold responses to increasing concentrations [21]; however, the respiratory response to KIC, unlike that to glucose, appeared to be a continuous function of its concentration (Fig. 5).

The respiratory response to KIC was greater than that to glucose, at the same insulinotropic concentration. A greater proportion of the reducing equivalents formed in the metabolism of glucose, however, pass via pyridine rather than flavin nucleotides than is the case for KIC, thus giving a higher P/O ratio when glucose is the substrate. Furthermore glucose, but not KIC, stimulates glycolysis in islet tissue [25, 26]. When available quantitative data were considered [25, 26], it appeared that the rate of ATP generation was the same at equivalent insulinotropic concentrations of glucose and KIC.

The Dynamic Response in Islet Respiration to Insulin Secretagogues

The finding that large increases in respiratory activity were provoked only by insulin secretagogues, and that the effects of glucose and KIC on insulin release parallelled their effects on respiration and $^{14}CO_2$ production supports the hypothesis that a metabolite or related cofactor [21, 27], rather than the secretagogue molecule itself [28], provides the signal which initiates the secretory response. The findings that glucose or KIC exerted an immediate effect on islet

respiration and that the reversal of respiratory changes were accompanied by a decline in insulin release (Figs. 1 and 4) are also consistent with this hypothesis. The changes induced in islet respiration by KIC developed more rapidly than with glucose and were more slowly reversible, differences which parallelled the effects of these compounds on insulin release.

The Nature of the Reactions Coupled to O_2 Consumption

Insulin secretory granules, like the storage granules of the adrenal gland and other secretory tissues, are reported to contain significant amounts of adenine nucleotides [29, 30]. In chromaffin granules [31] such accumulation may be associated with the presence of an uncoupler-sensitive respiratory chain. Thus the question arises as to whether extramitochondrial respiration may be an important component of the respiratory response of islet tissue to insulin secretagogues. Phagocytosing cells which, like islet tissue, respond to specific stimuli by an increased rate of O_2 consumption produce significant quantities of H_2O_2 through extramitochondrial single electron transfer reactions [32]. However, it was not possible to detect H_2O_2 production from islets incubated in the absence of substrate or in the presence of glucose. The finding that 2,4-dinitrophenol provoked an increased respiratory response of the same relative magnitude at different concentrations of secretagogues (Fig. 9), and the finding that antimycin A, oligomycin and rotenone inhibited islet respiratory activity (Fig. 8) suggest that islet O_2 consumption was to a major extent linked to oxidative phosphorylation.

The finding that the omission of Ca^{2+} (Table 2) from the medium or the promotion of the uptake of divalent cations by the ionophore A23187 did not markedly affect islet respiratory activity suggested that the reactions involved in the transport or sequestering of Ca^{2+} were not responsible for secretagogue-induced changes in islet respiration. The finding that cycloheximide failed to affect markedly the respiratory response to KIC suggested that changes in protein synthesis were not important in determining the respiratory response.

The possibility that the handling of K^+ by islets is a major site of energy expenditure was investigated in experiments on the effects of omission of this cation from the media. The finding of a marked K^+ dependency for glucose-induced changes in respiration contrasted with the effects of K^+ removal in the presence of KIC (Figs. 10 and 11, Table 2). These differences are perhaps accounted for by the presence of the K^+-sensitive enzyme pyruvate kinase (EC 2.7.1.40) in the pathway of glucose metabolism [34].

The finding that bicarbonate removal exerted a marked effect on islet respiration both in the absence of substrate or in the presence of metabolisable secretagogues (Fig. 12, Table 2) may account for the present observation of a much higher rate of O_2 consumption than has been previously reported using the Cartesian diver technique [1, 2]. Frankel et al. [4] have similarly reported high rates of O_2 consumption in islets maintained in tissue culture medium.

Islet tissue like several other tissues of the gastrointestinal tract [35] contains a HCO_3^--activated ATPase [36], a finding consistent with the present sensitivity of islet respiration to the presence of bicarbonate. Given the magnitude of the bicarbonate effect on respiration and the observation that bicarbonate is essential for insulin release [22], it is conceivable that the handling of this anion constitutes an important physiological process in the stimulus-secretion coupling mechanism.

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