Opposite Effects of D-Glucose and a Nonmetabolized Analogue of L-Leucine on Respiration and Secretion in Insulin-Producing Tumoral Cells (RINm5F)

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SUMMARY

D-Glucose increased the cytosolic NADH/NAD^+ ratio (but not the cytosolic NADPH/$NADP^+$ ratio), augmented O_2 uptake, raised the ATP/ADP ratio, decreased ^{86}Rb outflow, and stimulated insulin release in tumoral insulin-producing cells of the RINm5F line. L-Leucine and 4-methyl-2-oxopentanoate also stimulated insulin secretion. In the RINm5F cells, as in normal islet cells, the nonmetabolized analogue of L-leucine, 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid (BCH), activated glutamate dehydrogenase, augmented L-[U-^{14}C]glutamine oxidation, and induced a more reduced state of cytosolic redox couples. However, in sharp contrast to either its effect in normal islet cells or that of D-glucose in the tumoral cells, BCH severely decreased O_2 uptake, lowered the ATP/ADP ratio, increased ^{86}Rb outflow, and inhibited insulin release in the RINm5F cells. These findings are interpreted to support the concept that the rate of ATP generation represents an essential determinant of the secretory response of insulin-producing cells to nutrient secretagogues. *Diabetes* 36:187–92, 1987

Tumoral insulin-producing cells of the RINm5F cell line are currently used as a model for the study of biochemical and biophysical events associated with the stimulation of insulin release. These cells, which can easily be obtained in large amounts, display a positive secretory response to most secretagogues known to stimulate insulin release from normal pancreatic islet β-cells (1–3). Although the tumoral cells were first thought to be poorly or nonresponsive to D-glucose, it is now established that the hexose causes a modest but definite increase in insulin output from the RINm5F cells, such an increment reaching its maximal value at a concentration of D-glucose close to 2.8 mM (3). However, at variance with the situation found in normal β-cells, our report reveals that the nonmetabolized analogue of L-leucine, 2-aminobicyclo[2,2,1]-heptane-2-carboxylic acid (BCH), inhibits O_2 uptake and insulin release in the RINm5F cells.

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MATERIALS AND METHODS

The L-leucine analogue BCH was purchased as the (±)stereoisomer mixture from Calbiochem AG (Lucerne, Switzerland). Tumoral insulin-producing cells of the RINm5F line were cultured, harvested, and counted as described elsewhere (4). The methods used to measure amino acid aminotransferase (5) and glutamate dehydrogenase activity in cell homogenates (6), and nutrient oxidation (7) and insulin release (8) by intact cells were identical to those described.

To measure cytosolic redox states, groups of 300–500 × 10^3 cells were incubated for 30 min at 37°C in 0.2 ml of bicarbonate-buffered media (8). After removal of the incubation medium and addition of perchloric acid (0.1 ml; 2.5%, vol/vol), the tubes containing the cells were placed in liquid N_2, and the cells were disrupted by mechanical vibration (9). An aliquot (80 μl) of the perchloric extract was neutralized to pH 7.8–8.0 by mixing with 40 μl of Tris-KOH (0.2–1.0 M) and centrifuged for 3 min at 5000 × *g*. Aliquots of 5 μl were used for the assay of malate (10), 50 μl for the assay of oxalacetate (11), and 50 μl for the assay of pyruvate. In the latter case, the 50 μl of supernatant solution were mixed with 50 μl of a reaction mixture containing triethanolamine-HCl (100 mM, pH 7.0), NADH (1.0 mM), ammonium acetate (100 mM), ADP (0.1 mM), and beef liver glutamate dehydrogenase (EC 1.4.1.3; 0.1 μg/μl). After 20 min incubation at 22°C to remove 2-ketoglutarate (10), the reaction was halted by heating for 5 min at 70°C, and the sample was mixed with an equal volume (100 μl) of a second reaction mixture containing Tris-HCl (100 mM, pH 8.0), L-[1-^{14}C]-glutamate (0.05 mM), and pig heart glutamate-pyruvate transaminase (EC 2.6.1.2; 0.1 μg/μl). After 45 min incubation

TABLE 1
Effect of BCH on amino acid aminotransferase activity

Amino acid (10 mM)	No BCH (pmol · min^{-1} · 10^{-3} cells)	BCH (20 mM) (pmol · min^{-1} · 10^{-3} cells)
Nil	0.23 ± 0.02	0.23 ± 0.01
L-Glutamate	26.55 ± 0.64	26.49 ± 0.26
L-Alanine	1.34 ± 0.14	1.25 ± 0.01
L-Leucine	0.68 ± 0.04	0.63 ± 0.01
L-Aspartate	9.31 ± 0.62	9.41 ± 0.04

BCH, 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid.
N = 3 in all cases.

at 22°C, the sample was diluted with 2.0 ml of iced H_2O and placed on a Dowex 50 (H^+ form) column so that the [1-^{14}C]-2-ketoglutarate generated by transamination could be separated from L-[1-^{14}C]glutamate and counted by liquid scintillation. The results were corrected for blank values found in the absence of cells and expressed by reference to the readings obtained with standard amounts of pyruvate (12.5–100.0 pmol/sample) prepared in neutralized perchloric acid and treated in the same manner as the cell samples. In this procedure the recovery of pyruvate averaged 97.6 ± 4.3%. The cytosolic NADH/NAD^+ and NADPH/$NADP^+$ ratios were judged from the malate/oxalacetate and malate/pyruvate islet contents, respectively (12).

The respiratory rate of the RINm5F cells was assayed with the aid of the Cartesian diver respirometer as modified by Hellerström (13,14). For this purpose RIN cells were seeded at a density of 3000 cells/ml in 25-ml tissue culture flasks (Nunclon; Nunc, Roskilde, Denmark), each containing 10-ml tissue culture medium RPMI-1640 (11.1 mM glucose) supplemented with 10% fetal calf serum. After culture for 4–7 days, the medium was changed and the cells detached 24 h later by treatment for 4–6 min with 3 ml of a trypsin solution (Trypsin 10×, Gibco, Grand Island, NY) diluted 1:10 in Ca^{2+}- and Mg^{2+}-free Hanks' solution (CMF). The suspended cells were sedimented by gentle centrifugation (500 rpm) and resuspended in 6 ml of CMF before being counted in a hemocytometer. After another centrifugation the supernatant was carefully removed and the cells resuspended in an appropriate volume of a modified Krebs-Ringer phosphate medium (ionic composition: Na^+, 150.8 mM; K^+, 5.9 mM; Mg^{2+}, 1.2 mM; Ca^{2+}, 1.3 mM; Cl^-, 125.8 mM; SO_4^{2-}, 1.2 mM; PO_4^{3-}, 17.3 mM) containing 1% (wt/vol) human serum albumin (Miles, Elkhart, IN). The concentration of respiring cells varied between 5000 and 20,000 cells/μl, but most experiments were conducted with 10,000 cells/μl. Immediately before transfer to a Cartesian diver respirometer, the cells were carefully resuspended in the medium by gentle pipetting. The cells were then deposited in a diver in a final volume of 1.0 μl Krebs-Ringer medium. A side drop (0.5 μl) containing glucose (8.4 or 50.1 mM) or BCH (30 mM) but no cells was deposited immediately below the cell suspension. After measurements for ~60 min in substrate-free Krebs-Ringer medium, the side drop was added to the cell suspension by increasing the pressure on the diver. The concentrations of test substances presented to the respiring cells then became 2.8 mM or 16.7 mM for glucose and 10 mM for BCH. The CO_2 evolved during respiration was trapped in alkali (370 mM KOH). At equilibrium pressure the gas phase of each diver consisted of ~10 μl ambient air. At the end of the experiment the equilibrium pressure of each diver was plotted against time, a line fitted, and the O_2 consumption calculated as pmol · 10^3 cells^{-1} · h^{-1}.

The adenine nucleotide content of the cells was measured after 30 min incubation, as described elsewhere (9). Briefly, groups of 150–300 × 10^3 cells were preincubated for 30 min at 37°C in the absence of exogenous nutrient and then incubated for 30 min at 37°C in 50 μl of incubation medium containing the agent to be tested. The medium was mixed with an equal volume of perchloric acid (5%). The tubes were placed in liquid N_2 and the cells disrupted by mechanical vibration (9). The cell extract was heated for 5 min at 70°C and centrifuged; an aliquot (80 μl) of the supernatant was then neutralized by addition of 40 μl of a solution of KOH (1.0 M) and Tris (0.2 M). The following steps in the procedure were previously detailed (9).

For measurement of ^{86}Rb outflow, groups of cells (783 ± 16 × 10^3 cells; N = 12) were preincubated for 60 min at 37°C in the presence of ^{86}Rb (62.5 μCi/ml; 0.23 mM) and D-glucose (16.7 mM), washed three times with a non-radioactive medium, and loaded on columns of Bio-Gel beads (P_2, Bio-Rad, Richmond, CA) preswollen in perifusion medium at 4°C overnight. The release of ^{86}Rb from the prelabeled cells was expressed as an instantaneous fractional outflow rate (15), taking into account the radioactive content of the column at the end of the perifusion period.

All results are expressed as the mean ± SE and statistical significance of differences between mean values as assessed by Student's t test.

RESULTS

Enzymatic data. We first investigated whether BCH could act as a transamination partner in RINm5F cells. Cell homogenates were incubated for 30 min at 37°C in the presence of 2-keto-[1-^{14}C]glutarate (2.5 mM); labeled glutamate was then separated by ion-exchange chromatography (Table 1). In the absence of exogenous amino acid, the generation of L-[1-^{14}C]glutamate would correspond to the transamination of endogenous amino acids in an amount close to 6.9 ± 0.6 pmol/10^3 cells. In the presence of exogenous unlabeled glutamate, the cell homogenate catalyzed the interconversion between this amino acid and 2-ketoglutarate. In the presence of other exogenous amino acids, the reaction velocity was much higher with L-asparate than with either L-alanine or L-leucine. BCH failed to act as a transamination partner and failed to affect the reaction velocity in the presence of exogenous amino acids.

The effect of BCH on glutamate dehydrogenase activity in RINm5F cell homogenates is documented in Table 2. BCH

TABLE 2
Effect of BCH on glutamate dehydrogenase activity

Activator (mM)	NADH (0.2 mM) (pmol · min^{-1} · 10^{-3} cells)	NADPH (0.2 mM) (pmol · min^{-1} · 10^{-3} cells)
Nil	6.29 ± 1.31	1.65 ± 0.41
L-Leucine (20)	21.14 ± 0.71	11.45 ± 0.79
BCH (20)	22.43 ± 0.95	11.23 ± 0.78
ADP (1)	26.32 ± 1.39	19.71 ± 1.03

N = 3 in all cases.

TABLE 3
Effect of BCH and other secretagogues on cytosolic redox state

Secretagogue (mM)	Malate (fmol/10^3 cells)	Oxalacetate (fmol/10^3 cells)	Pyruvate (fmol/10^3 cells)	NADH-NAD$^+$ (ratio × 10^3)	NADPH-NADP$^+$ (ratio)
Nil	156.8 ± 6.2 (35)	5.14 ± 0.38 (35)	109.6 ± 19.5 (31)	1.03 ± 0.09 (35)	69.99 ± 8.30 (31)
			(Percent of basal)		
D-Glucose (2.8)	327.1 ± 16.6 (44)*	37.2 ± 1.6 (44)*	382.2 ± 25.8 (42)*	849.2 ± 29.2 (44)*	119.7 ± 9.4 (42)
BCH (10)	249.1 ± 10.5 (7)*	59.4 ± 2.9 (7)†	176.4 ± 20.8 (7)‡	396.6 ± 33.5 (7)*	136.0 ± 10.5 (7)‡
L-Leucine (10)	314.1 ± 5.3 (12)*	115.2 ± 5.5 (12)‡	289.4 ± 49.5 (12)†	274.5 ± 13.9 (12)*	138.9 ± 24.9 (12)
4-Methyl-2-oxopentanoate (10)	402.8 ± 25.2 (12)*	193.8 ± 14.8 (12)*	321.8 ± 55.5 (11)*	207.0 ± 16.1 (12)*	142.0 ± 22.5 (11)
TPA (0.002)	130.1 ± 7.7 (6)§	113.5 ± 10.8 (6)	72.2 ± 11.7 (6)	114.3 ± 10.4 (6)	187.6 ± 19.7 (6)†
Ba^{2+} (2) + theophylline (1.4); no Ca^{2+}	171.5 ± 13.4 (7)*	96.2 ± 12.4 (7)	149.6 ± 47.9 (7)	179.3 ± 12.4 (7)*	122.3 ± 19.4 (7)

TPA, 12-*O*-tetradecanoylphorbol13-acetate.
Statistical significance of differences from mean basal values: *$P < .001$; †$P < .005$; ‡$P < .05$; §$P < .02$.
Numbers in parentheses denote *N*.

(20 mM) augmented the reaction velocity to the same extent as L-leucine (also 20 mM), whether in the presence of NADH or NADPH. A somewhat more pronounced increase in reaction velocity was recorded when ADP (1.0 mM) was used instead of L-leucine or its nonmetabolized analogue as enzyme activator. The reaction velocity was invariably lower ($P < .05$ or less) in the presence of NADPH than NADH.

Oxidative data. In RINm5F cells, BCH (10 mM) increased ($P < .001$) the oxidation of L-[U-^{14}C]glutamine (1.0 mM) by 50%, from 7.32 ± 0.38 to 10.92 ± 0.77 pmol · 10^3 cells^{-1} · 90 min^{-1} ($N = 15$ in both cases). For purpose of comparison, the oxidation of D-[U-^{14}C]-glucose (2.8 mM) averaged 6.69 ± 0.45 pmol · 10^3 cells^{-1} · 90 min^{-1} ($N = 15$) within the same experiments.

Cytosolic redox state. Relative to protein content (3), the basal value for the malate and oxalacetate content of RINm5F cells was comparable to that of rat pancreatic islets (11). The pyruvate content of the RINm5F cells could not be compared with that of pancreatic islets, because in our study we used a more specific radioisotopic assay that had not been used in our previous study with pancreatic islets. The basal value for the cytosolic NADH/NAD$^+$ and NADPH/NADP$^+$ ratios in RINm5F cells (Table 3) was virtually identical to that recorded by Veech et al. (12) in the freeze-clamped livers of rats fed a standard diet. This similarity suggests that the conditions required to assess the cytosolic redox state from the cell content in selected substrates, e.g., the appropriate subcellular location of enzymes, the near-equilibrium state of those reactions concerned, and the approximation between total and cytosolic concentrations of each substrate, were satisfied in the RINm5F cells.

D-Glucose (2.8 mM) augmented strikingly the malate and pyruvate content of RINm5F cells while lowering their oxalacetate content. The cytosolic NADH/NAD$^+$ ratio was dramatically increased, but the cytosolic NADPH/NADP$^+$ ratio was not significantly affected in RINm5F cells exposed to D-glucose. BCH (10 mM) changed the malate, oxalacetate, and pyruvate content of the RINm5F cells in the same direction as D-glucose but to a lesser extent than the hexose. BCH significantly augmented both the cytosolic NADH/NAD$^+$ and NADPH/NADP$^+$ ratios. Like BCH, L-leucine or 4-methyl-2-oxopentanoate (10 mM each) augmented both the cell content in malate and pyruvate and the malate-oxalacetate and malate-pyruvate ratios, the latter increment achieving statistical significance ($P < .05$ or less) in only one ($N = 5–6$) of two experiments. At variance with BCH, however, L-leucine and 4-methyl-2-oxopentanoate failed to lower and instead increased the oxalacetate content of the RINm5F cells.

For the purpose of comparison with secretory data, we also examined the effect of nonnutrient secretagogues on cytosolic redox state. Both 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (2.0 μM) and the combination of Ba^{2+} (2.0 mM) and theophylline (1.4 mM), tested in the absence of Ca^{2+}, caused a modest increment in the malate content of the cells and apparently increased either the NADH/NAD$^+$ or NADPH/NADP$^+$ ratio.

Respiratory data. The rate of respiration, whether measured in the absence or presence of D-glucose, was constant over at least 2 h. A plot of the regression of O_2 uptake on cell concentration (Fig. 1) indicates that in the range of 0.5–2.0 × 10^4 cells/μl there was no obvious effect of the cell concentration on O_2 uptake.

D-Glucose (2.8 mM) augmented O_2 uptake from a basal value of 220 ± 20 to 324 ± 23 pmol · 10^3 cells^{-1} · 60 min^{-1} ($N = 8$ in each case), corresponding to a 51 ± 12% paired increase in respiration ($P < .005$). On the contrary, BCH (10 mM) inhibited respiration, the consumption of O_2 falling from a basal value of 198 ± 20 to 117 ± 10 pmol · 10^3 cells^{-1} · 60 min^{-1} ($N = 4$ in each case), corresponding to a 40 ± 5% paired decrease in respiration ($P < .005$).

Adenine nucleotide content. Over 30 min incubation, D-glucose slightly increased the total adenine nucleotide content of the RINm5F cells (Table 4) from 2.41 ± 0.10 to 2.75 ± 0.10 pmol/10^3 cells ($P < .05$) and raised both the ATP/ADP ratio ($P < .001$) and adenylate charge ($P < .02$). On the contrary, BCH caused dramatic decreases in total adenine nucleotide content, to 1.73 ± 0.09 pmol/10^3 cells, ATP/ADP ratio, and adenylate charge ($P < .001$ in all cases).

Cationic data. When the RINm5F cells were preincubated for 60 min in the presence of ^{86}Rb (0.23 mM) and D-glucose (16.7 mM) and then placed in a perifusion device, the fractional outflow rate for ^{86}Rb displayed a fairly stable value during the initial period of perifusion (min 15 to 31), which was always conducted in the absence of exogenous nutrient (Fig. 2). At min 30–31, such a fractional outflow rate aver-

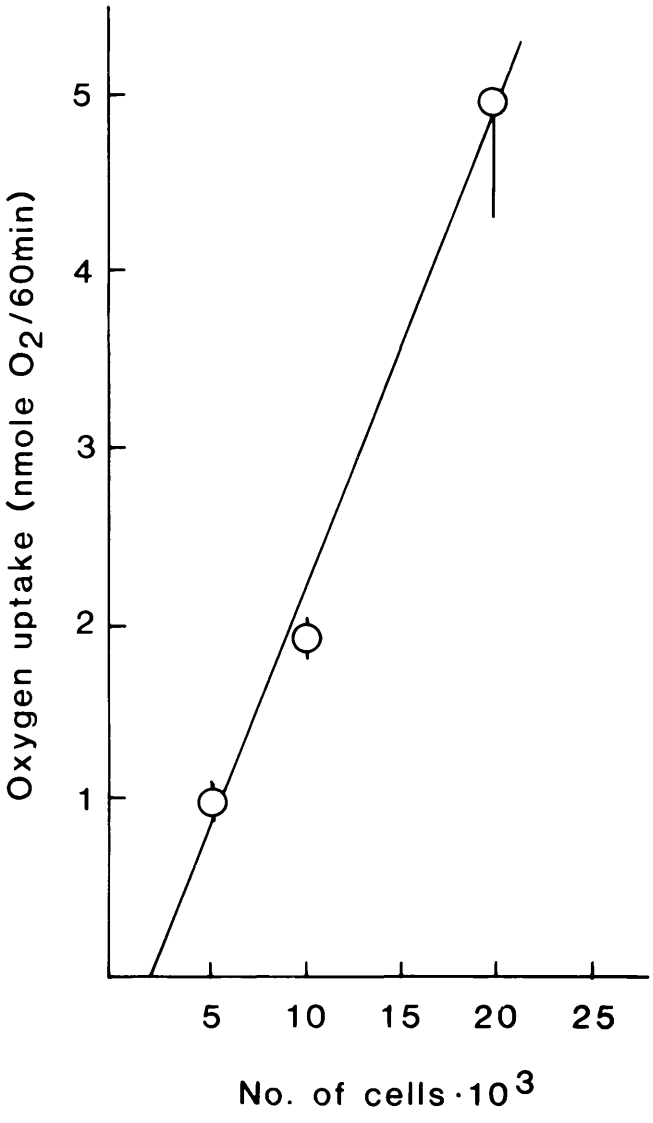

FIG. 1. Correlation between O_2 uptake and number of RINm5F cells per microliter incubation medium (r = .992). Each *point* represents mean (±SE) of 4–10 observations.

aged 2.692 ± 0.130%/min (N = 12). In the control experiments performed throughout in the absence of exogenous nutrient, there was a slight fall in ^{86}Rb fractional outflow rate between min 31 and 55, the value reached at the latter time averaging 92.0 ± 3.9% (N = 3) of that recorded at the former time. The administration of D-glucose (16.7 mM) caused a rapid and sustained decrease in ^{86}Rb outflow. In the cells exposed to D-glucose, the difference in ^{86}Rb fractional outflow rate between min 30–31 and 55 (0.915 ± 0.019 %/min; P < .02) was much higher (P < .01) than that recorded in the control experiments (0.203 ± 0.083 %/min; P < .1). When D-glucose was removed from the perifusate, a limited reascension in ^{86}Rb outflow was observed, but the fractional outflow rate remained lower than the initial or control value. At variance with D-glucose, BCH (10 mM) failed to decrease ^{86}Rb outflow. Instead of the slight decrease in ^{86}Rb fractional outflow rate recorded between min 30–31 and 55 in control experiments (−0.203 ± 0.083 %/min), BCH provoked a modest increase in ^{86}Rb fractional outflow rate (+0.193 ± 0.071 %/min). Because the difference between these two mean results is significant (P < .025), it can be concluded that BCH augments ^{86}Rb fractional outflow rate. Such an effect is reminiscent of, albeit much less marked than, that evoked by antimycin A (0.01 mM), which caused a dramatic increase in ^{86}Rb outflow from the perifused RINm5F cells (Fig. 2).

Secretory data. Relative to the mean basal value for insulin release measured within the same experiments, D-glucose increased insulin output by 32.4 ± 4.5% (N = 61). Gliclazide augmented modestly but significantly (P < .001) glucose-stimulated insulin release (Table 5). L-Leucine or 4-methyl-2-oxopentanoate (10 mM each) also significantly increased insulin output above basal value. At variance with the situation found in pancreatic islets, the effect of L-leucine was more marked (P < .001) than that evoked by 4-methyl-2-oxopentanoate in the same experiments. The secretory response to these nutrient secretagogues was less marked than that evoked, in the absence of exogenous nutrient, either by the tumor-promoting phorbol ester TPA or by the combination of Ba^{2+} and theophylline (tested in the absence of extracellular Ca^{2+}).

BCH caused a dramatic inhibition of insulin release. As little as 5 mM BCH was sufficient to decrease insulin output to a barely detectable level (Table 5). Within the same series of experiments, BCH (10 mM) augmented insulin release from rat pancreatic islets incubated in the absence of glucose from a basal value of 13.8 ± 1.1 to 32.7 ± 3.5 $\mu U \cdot islet^{-1} \cdot 90\ min^{-1}$ (P < .001, N = 8 in both cases).

DISCUSSION

Our results indicate that D-glucose at a concentration of 2.8 mM increases the cytosolic NADH/NAD^+ ratio (but not the cytosolic NADPH/$NADP^+$ ratio), augments O_2 uptake, inhibits ^{86}Rb outflow, and stimulates insulin release in RINm5F cells. Incidentally, the response to D-glucose (2.8 mM) in terms of either D-[U-^{14}C]glucose oxidation, cytosolic redox state, O_2 uptake, or insulin release was not further enhanced

TABLE 4
Effect of D-glucose and BCH on adenine nucleotide content of RINm5F cells

Secretagogue (mM)	Nil	D-Glucose (2.8)	BCH (10)
ATP (pmol/10^3 cells)	1.22 ± 0.08 (24)	1.56 ± 0.08 (24)	0.71 ± 0.08 (24)
ADP (pmol/10^3 cells)	0.79 ± 0.03 (24)	0.77 ± 0.04 (24)	0.54 ± 0.03 (24)
AMP (pmol/10^3 cells)	0.40 ± 0.04 (8)	0.42 ± 0.05 (8)	0.48 ± 0.03 (8)
ATP/ADP (ratio)	1.61 ± 0.06 (24)	2.17 ± 0.12 (24)	1.31 ± 0.05 (24)
Adenylate charge	0.647 ± 0.012 (8)	0.693 ± 0.010 (8)	0.544 ± 0.011 (8)

Numbers in parentheses denote N.

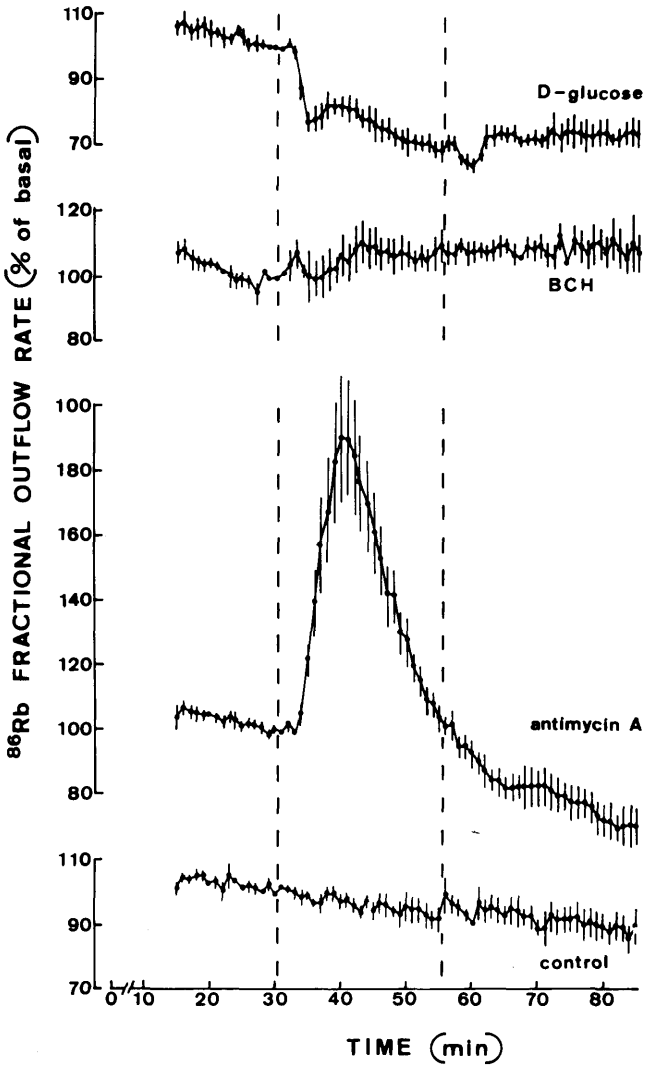

FIG. 2. Effect of D-glucose (16.7 mM), BCH (10.0 mM), or antimycin A (0.01 mM) administered from min 31 to 55 on ^{86}Rb fractional outflow rate from prelabeled RINm5F cells. *Vertical dotted lines* indicate time at which composition of perifusate entering perifusion column was changed, with no correction for dead space (corresponding to delay of ~2 min) introduced. Mean values (± SE) are expressed in percent of mean paired basal value (min 30–31) and refer to 3 individual experiments in each case. Basal ^{86}Rb fractional outflow rate averaged 2.692 ± 0.130 %/min (*N* = 12).

at higher hexose concentrations, e.g., in the presence of 16.7 mM D-glucose (data not shown). At first glance, these data are compatible with the knowledge that the insulinotropic action of D-glucose is causally linked to its metabolism in a sequence of events involving an increased generation of reducing equivalents and ATP and a subsequent decrease in K^+ conductance (16).

In terms of the secretory response, two points need to be stressed. First, the stimulant action of D-glucose on insulin secretion was rather modest relative to the high basal insulin output. Such a modest effect does not correspond, however, to the maximal secretory capacity of the RINm5F cells. Indeed, our data indicate that the release of insulin evoked by D-glucose can be further enhanced by a hypoglycemic sulphonylurea and is much lower than that evoked by other secretagogues, e.g., the tumor-promoting phorbol ester TPA or the combination of Ba^{2+} and theophylline (tested in the absence of Ca^{2+}). Second, the high basal insulin output, which corresponds to an active process of secretion inhibited in the absence of Ca^{2+} or presence of metabolic poisons and enhanced in the presence of cytochalasin B (17), coincided with a high basal rate of O_2 consumption (3.54 ± 0.24 pmol · 10^3 cells^{-1} · min^{-1}). Indeed, relative to protein content (3), the basal respiratory rate in the RINm5F cells was much higher than that recorded by the same technique in rat pancreatic islets (11). Thus, it could be proposed that even in the absence of exogenous nutrient the RINm5F cells are already engaged in an active state of secretion induced by a high oxidation rate of endogenous nutrients. This proposal is supported by the much lower basal ^{86}Rb fractional outflow rate in RINm5F cells (2.69 ± 0.13 %/min) than normal rat islets (5.54 ± 0.29 %/min) (18).

Nutrients other than D-glucose, e.g., L-leucine and 4-methyl-2-oxopentanoate, also augmented insulin release from the RINm5F cells, an effect again associated with a more reduced state of cytosolic redox couples. From previous work it is known that in normal β-cells, the nonmetabolized analogue of L-leucine, BCH, activates glutamate dehydrogenase (6), stimulates the catabolism of endogenous amino acids (19), augments O_2 uptake (20), inhibits ^{86}Rb outflow (21), and hence mimics the features encountered in the process of nutrient-induced insulin release. This study reveals that, in RINm5F cells as in normal islet cells, BCH could not act as a transamination partner, but it did activate glutamate dehydrogenase in cell homogenates, stimulate the oxidation of L-[U-^{14}C]glutamine in intact cells, and increase the cytosolic NADH/NAD^+ and NADPH/$NADP^+$ ratios. Quite unexpectedly, however, BCH inhibited respiration, augmented ^{86}Rb outflow in a manner comparable to that seen with uncoupling agents, and markedly decreased basal insulin release in the RINm5F cells. We feel that it is premature to speculate on the mechanism by which BCH decreased O_2 uptake. Further work is required to identify those endogenous nutrients (e.g., fatty acids) the oxidation of which is prevented in cells exposed to BCH.

Whatever the mechanism responsible for the inhibition of cell respiration by BCH, our data document the existence of a close correlation between O_2 uptake and insulin release in the RINm5F cells. D-Glucose augmented both respiration and insulin secretion, whereas BCH inhibited both O_2 consumption and insulin release in the tumoral cells. In our opin-

TABLE 5
Effect of BCH and other secretagogues on insulin release

Secretagogue (mM)	Insulin output (nU · 90 min^{-1} · 10^{-3} cells)
Nil	84.0 ± 3.0 (87)
D-Glucose (2.8)	112.4 ± 2.9 (61)
D-Glucose (2.8) + gliclazide (0.06)	130.7 ± 3.2 (22)
BCH (5)	13.4 ± 3.6 (8)
BCH (10)	4.8 ± 4.7 (25)
BCH (20)	5.0 ± 1.7 (15)
L-Leucine (10)	127.8 ± 2.9 (38)
4-Methyl-2-oxopentanoate (10)	107.2 ± 2.0 (22)
TPA (0.002)	156.0 ± 1.7 (14)
Ba^{2+} (2) + theophylline (1.4); no Ca^{2+}	212.9 ± 11.2 (24)

Numbers in parentheses denote *N*.

ion, these findings may contribute to a better understanding of the process of stimulus-secretion coupling in insulin-producing cells. It is currently believed that the stimulation of insulin release by nutrient secretagogues is causally linked to an increase in oxidative fluxes in the islet cells (22). However, the nature of the messengers coupling metabolic to more distal events in the secretory sequence remains a matter of debate (23).

We have previously defended the view that such a coupling mechanism represents a multifactorial process, involving changes in the generation rate of protons, reducing equivalents, and ATP (24). The present data indicate that there is not always a close correlation between the effect of nutrient secretagogues on cytosolic redox couples and insulin output, respectively. Moreover, the cytosolic redox state is apparently affected by nonnutrient secretagogues, a situation that could reflect the feedback control of metabolic events by nonmetabolic, e.g., ionic, variables (25). From recent electrophysiological work performed with the patch-clamp technique, it would appear that an ATP-responsive K^+ channel may play a key role in the sequence of cationic events associated with the process of nutrient-induced insulin release (26,27). The present data indicate that BCH lowers the ATP/ADP ratio and adenylate charge in the RINm5F cells. BCH also lowered the total adenine nucleotide content, a situation reminiscent of that found in normal islet cells whenever the oxidation of nutrients is severely decreased (24). Our data are compatible with the view that the rate of ATP generation represents an essential determinant of the secretory response to nutrient secretagogues, as already proposed (28). If the availability of ATP indeed controls the gating of K^+ channels located in the plasma membrane, it may then be important to assess the influence of nutrient secretagogues on the cytosolic concentration of ATP in both normal and tumoral insulin-producing cells.

In conclusion, the opposite effects of D-glucose and BCH, respectively, on O_2 uptake and insulin release in RINm5F cells as revealed by this study apparently ascribes to the rate of ATP generation a pivotal role in the control of insulin release by nutrient secretagogues. This conclusion is not meant to deny that the oxidation of nutrients coincides with changes in the availability or concentration of metabolic intermediates and cofactors other than ATP, which may also participate in the fine tuning of secretory activity in insulin-producing cells.

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