Effect of cytochalasin B on 3-O-[^{14}C]-methyl-D-glucose or D-[U-^{14}C]glucose handling by BRIN-BD11 cells

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Abstract. The present study aimed to investigate the effects of cytochalasin B (20 μM) on the uptake of 3-O-[^{14}C]-methyl-D-glucose or D-[U-^{14}C]glucose (8.3 mM each) by BRIN-BD11 cells. Taking into account the distribution space of tritiated water (^{3}HOH), which was unexpectedly increased shortly after exposure of the cells to cytochalasin B and then progressively returned to its control values, and that of L-[1-^{14}C]glucose, used as an extracellular marker, it was demonstrated that cytochalasin B caused a modest, but significant inhibition of the uptake of D-glucose and its non-metabolized analog by the BRIN-BD11 cells. These findings resemble those observed in acinar or ductal cells of the rat submaxillary gland and displayed a relative magnitude comparable to that found for the inhibition of D-glucose metabolism by cytochalasin B in purified pancreatic islet B cells. These findings reinforce the view that the primary site of action of cytochalasin B is located at the level of the plasma membrane.

Introduction

The insulin-producing tumoral BRIN-BD11 cell line was produced by electrofusion of NEDH rat islet B cells with immortal RINm5F cells (1). The insulin-secreting BRIN-BD11 cell line has provided a model for the study of pancreatic B-cell functions, including glucose, amino acid and hypotonicity-induced insulin secretion (1-7), expression and the role of the adenosine triphosphate (ATP)-sensitive potassium channels (8), the electrogenic Na^{+}-HCO_3^{-} cotransporter NBCe1 (9), plasma membrane Ca^{2+}-ATPase (10), aquaglyceroporin 7 (11) and the malate-aspartate NADH shuttle (12). BRIN-BD11 cells express glucose transporter-2 and display an improved metabolic response to glucose (13). In view of the latter observations, the uptake of D-glucose and its non-metabolized analog, 3-O-methyl-D-glucose, as well as the effects of cytochalasin B were investigated in these cells in the present study.

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Materials and methods

Materials. L-[1-^{14}C]glucose, 3-O-[^{14}C]-methyl-D-glucose (labelled with ^{14}C in the methyl group) and D-[U-^{14}C]glucose were purchased from PerkinElmer, Inc. (Boston, MA, USA). Cytochalasin B, L-glucose, 3-O-methyl-D-glucose and RPMI-1640 medium were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ca^{2+}- and-Mg^{2+}-free Hank's balanced salt solution (HBSS) and trypsin-EDTA were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA).

The BRIN-BD11 cells that were kindly provided by Professors R. Beauwens and R. Crutzen (Laboratory of Molecular Physiology, Brussels Free University, Brussels, Belgium) were grown at 37˚C in a humidified incubator, with an atmosphere of 5% CO_2 in air, and cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 50 IU/ml penicillin and 50 μg/ml streptomycin (Invitrogen Life Technologies). The D-glucose (Sigma-Aldrich) and L-glutamine concentrations of the culture medium were 11.1 and 2.0 mM, respectively.

The BRIN-BD11 cells were gently washed with 5 ml Ca^{2+}-and-Mg^{2+}-free HBSS for 30 sec at room temperature (20˚C) prior to being detached from the tissue culture flask with 3 ml trypsin-EDTA (0.05%) solution. Subsequent to being washed with culture medium, the cells were counted and suspended in Krebs-Ringer bicarbonate buffer (1.5-2.0x10^6 cells/ml) (14).

Methods. In order to take measurements of the net uptake of 3-O-[^{14}C]-methyl-D-glucose (1.0 μCi/ml) and D-[U-^{14}C]glucose (1.0 μCi/ml) by the BRIN-BD11 cells, 50 μl cell suspension (50-75x10^3 cells) was mixed with 50 μl of a bicarbonate- and HEPES-buffered salt-balanced medium containing bovine serum albumin (1 mg/ml), 2.0 mM L-glucose, 16.7 mM D-glucose or 3-O-methyl-D-glucose in the absence or presence of cytochalasin B (40.0 μM). The cells were incubated for 5-30 min at 37˚C in incubation medium containing tritiated water (^{3}HOH) (4.2 μCi/ml; New England Nuclear, Boston, MA, USA). For evaluation of the extracellular space and the total water space, 50x10^3 cells were also

incubated for 5-30 min at 37˚C in 0.1 ml of a bicarbonate- and HEPES-buffered salt-balanced medium containing L-[1-^{14}C]glucose (1.3 μCi/ml) and ^{3}HOH (4.2 μCi/ml). Following incubation, 0.15 ml of a mixture of dibutylphthalate and di-isononylphthalate (10:3, v/v; Sigma-Aldrich) was added to each polyethylene tube (Beckman Coulter, Fulterton, CA, USA). This was then centrifuged for 3 min at 5,000 x g. The bottom of the tube (polyethylene Bechman microfuge tube) containing the cell pellet was then cut, placed in a counting vial containing 5.0 ml of scintillation fluid (ICN Biomedicals, Costa Mesa, CA, USA) and, after mixing, examined for its radioactive content in a double channel (^{14}C/^{3}H) beta counter (TRI-CARB 2810 TR, PerkinElmer). Following correction for the blank value found under the same experimental conditions in the absence of cells, the results were expressed as nl/10^{3} cells.

Statistical analysis. All data are expressed as the mean ± standard error of the mean together with either the number of individual determinations (n) or the degree of freedom (df). The statistically significant differences (P<0.05) between the mean values was assessed by Student's t-test.

Results

Measurement of ^{3}HOH space in the presence/absence of cytochalasin B. In the absence of cytochalasin B, the apparent distribution space of ^{3}HOH progressively increased over the first 15 min of incubation, reaching a steady-state averaging 1.82±0.15 nl/10^{3} cells (n=18; Fig. 1). The presence of cytochalasin B (20 μM) increased the ^{3}HOH space, which then gradually decreased in an exponential manner during incubation (Fig. 1). Thus, a negative correlation coefficient (r=-0.9873) was found between the length of incubation and the logarithmic values for the cytochalasin B-induced increase in ^{3}HOH space, expressed relative to the mean control values recorded at the same time of incubation in the same experiments. As indicated from such logarithmic values, the time required to provoke a 50% decrease in the response to cytochalasin B was ~850 sec, and therefore no significant difference between the results recorded in the presence/absence of cytochalasin B was observed after the 30-min incubation period.

The extracellular space, as indicated from the distribution space of L-[1-^{14}C]glucose, used as an extracellular marker, averaged over 10-30 min of incubation 0.84±0.13 nl/10^{3} cells (n=18), representing 43.8±3.5% (n=18) of the total ^{3}HOH space. In the absence of cytochalasin B, the intracellular space, as indicated from the paired difference between the distribution space of ^{3}HOH and that of L-[1-^{14}C]glucose, averaged 1.11±0.20 nl/10^{3} cells (n=18).

Pooling all available control data collected after 10-30 min of incubation, the paired ratio between the distribution space of either 3-O-[^{14}C]-methyl-D-glucose or D-[U-^{14}C]glucose (8.3 mM each) and that of ^{3}HOH averaged to 62.7±6.4% (n=26). As summarized in Table I, there was no significant difference (df, 24; P>0.5) between the mean paired ratio for the distribution space of 3-O-[^{14}C]-methyl-D-glucose or D-(U-^{14}C)glucose and that of ^{3}HOH. Moreover, as determined from data collected in each individual experiment, the difference between the mean paired ratio for the distribution space of either 3-O-[^{14}C]-methyl-D-glucose or D-[U-^{14}C]glucose and that of ^{3}HOH, as well as the difference between the mean paired ratio for the distribution space of L-[1-^{14}C]glucose and that of ^{3}HOH, did not differ significantly from one another (P>0.87) compared with the results recorded with either D-glucose or its non-metabolized analogue; and in both cases, yielded mean values significantly different from zero (P<0.02). Furthermore, as previously observed in intact pancreatic islets (15), the intracellular space accessible to D-glucose or its non-metabolized analog in the BRIN-BD11 cells represented approximately half of the total intracellular space.

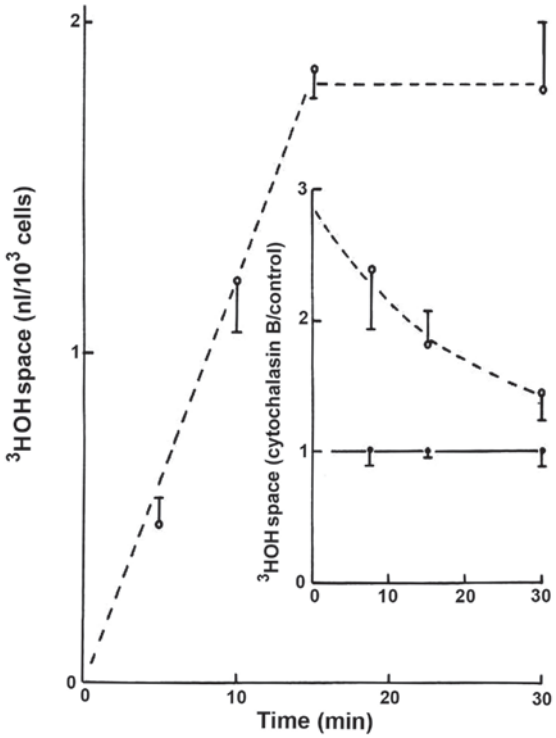

Figure 1. Timecourse for the measurement of ^{3}HOH space in the BRIN-BD11 cells incubated in the absence of cytochalasin B. The inset refers to the results recorded in the presence of cytochalasin B, expressed relative to the control values found at the same time within the same experiment(s). Mean values ± standard error of the mean refer to 5-16 individual determinations.

Effects of cytochalasin B. The paired ratio between the distribution space of either 3-O-[^{14}C]-methyl-D-glucose or D-[U-^{14}C]glucose (8.3 mM each) and that of ^{3}HOH averaged over 5-30 min of incubation in the absence of cytochalasin B 67.1±7.3% (n=21). As expected, this was significantly higher (P>0.01) compared with the paired value between the distribution space of L-[1-^{14}C]glucose and ^{3}HOH in the absence of cytochalasin B. The latter mould metabolite decreased the paired ratio between the distribution space of either 3-O-[^{14}C]-methyl-D-glucose or D-[U-^{14}C]glucose and that of ^{3}HOH (P<0.02). Thus, relative to the control values for such a ratio recorded in the absence of cytochalasin B (100.0±5.5%; n=21), the measurements made in the presence of cytochalasin B averaged to 83.2±3.2% (n=21). It should be stressed that the mean values for the latter variable were virtually identical in all cases, averaging to 84.1±4.6 (n=3)

Table I. Mean control values recorded in the absence of cytochalasin B.

Variables	Incubation time, min	Mean control values
^{3}HOH space, nl/10^{3} cells	15-30	1.82±0.15 (n=18)
L-[1-^{14}C]glucose space, nl/10^{3} cells	10-30	0.84±0.13 (n=18)
L-[1-^{14}C]glucose space/^{3}HOH space, %	10-30	43.8±3.5 (n=18)
^{3}HOH space minus L-[1-^{14}C]glucose space, nl/10^{3} cells	10-30	1.11±0.20 (n=18)
3-O-[^{14}C]-methyl-D-glucose space/^{3}HOH space, %	10-30	65.9±9.7 (n=16)
D-[U-^{14}C]-D-glucose space/^{3}HOH space, %	10-30	57.5±6.1 (n=10)
3-O-[^{14}C]-methyl-D-glucose space/^{3}HOH space minus L-[1-^{14}C]glucose space/^{3}HOH space, %	10-30	18.6±7.1 (df=24)
D-[U-^{14}C]-D-glucose space/^{3}HOH space minus L-[1-^{14}C]glucose space/^{3}HOH space, %	10-30	20.6±7.4 (df=16)

n, number of individual determinations; df, degree of freedom.

Table II. Effects of cytochalasin B on D-glucose metabolism in rat islets, dispersed islet cells and purified islet B cells.

	D-glucose, % (n)		
Cell type	2.8 mM	8.3 mM	16.7 mM
Control (no cytochalasin B)			
Islets	-	100.0±3.2 (48)	100.0±2.4 (46)
Dispersed islet cells	100.0±6.2 (26)	-	100.0±5.7 (26)
B cells	100.0±4.9 (49)	-	100.0±5.0 (55)
Cytochalasin B (21 μM)			
Islets	-	73.2±2.5 (46)	83.9±2.4 (46)
Dispersed islet cells	66.5±4.0 (27)	-	84.2±3.6 (29)
B cells	87.4±6.7 (49)	-	91.8±4.7 (54)

The results refer to the pooled measurements of D-[5-^{3}H]glucose utilization and D-[U-^{14}C]glucose conversion to $^{14}CO_2$ and radioactive acidic metabolites, and are expressed relative to the mean corresponding control values.

and 84.3±2.3% (n=3), after 10 and 30 min of incubation, respectively, in the presence of 3-O-[^{14}C]-methyl-D-glucose, and to 83.4±10.7 (n=5), 82.8±6.7 (n=5) and 82.4±6.5% (n=5) after 5, 15 and 30 min of incubation, respectively, in the presence of D-[U-^{14}C]glucose.

Discussion

The time-related increase in the ^{3}HOH space provoked by cytochalasin B in the BRIN-BD11 cells was an expected finding. In ductal cells from submandibular salivary glands, no significant difference (df, 18; P>0.25) in such a space is observed after 20 min of incubation in the absence or presence of cytochalasin B (unpublished data). Similarly, in acinar cells from the same salivary gland, the ^{3}HOH space measured after 20 min of incubation in the presence of cytochalasin B averages to 102.5±8.3% (n=15; P>0.85) of the mean corresponding control values recorded within the same experiments (100.0±9.8%; n=15) (unpublished data). The findings observed in the BRIN-BD11 cells suggest that cytochalasin B transiently affects the access of ^{3}HOH (and either 3-O-[^{14}C]-methyl-D-glucose or D-[U-^{14}C]glucose) to the intracellular space.

The present study demonstrated that cytochalasin B inhibits the uptake of D-glucose or its non-metabolized analog by BRIN-BD11 cells, which is in accordance with recent observations in acinar and ductal cells of the rat submandibular salivary glands. For instance, in the acinar cells the paired ratio between the distribution space of either 3-O-[^{14}C]-methyl-D-glucose or D-[U-^{14}C]glucose and that of ^{3}HOH averaged after 20 min of incubation 83.4±3.6% (n=18) of the mean corresponding control values. The latter finding is virtually identical to that found in the BRIN-BD11 cells.

The present findings concerning the cytochalasin B-induced inhibition of either 3-O-[^{14}C]-methyl-D-glucose or D-[U-^{14}C] glucose uptake by the BRIN-BD11 cells is in accordance with the findings of a previous study on the inhibition of D-glucose uptake, utilization, oxidation, glucose-stimulated lactate output and D-glucose conversion to acidic metabolites by the mould metabolite in rat-isolated pancreatic islets (16). The data listed in Table II, which were computed from primary data provided in a previous study (17), document that the

relative magnitude of the inhibitory action of cytochalasin B, used at the same concentration as in the present study, on D-glucose catabolism, as determined by three distinct metabolic criteria (D-[5-^{3}H]glucose conversion to ^{3}HOH and D-[U-^{14}C]glucose conversion to both $^{14}CO_2$ and radioactive acidic metabolites), was in purified islet B cells comparable to those recorded in the present study for the uptake of D-glucose and its non-metabolized analog by BRIN-BD11 cells. This analogy reinforces the view that the primary site of action of cytochalasin B on the handling of D-glucose concerns hexose transport across the plasma membrane. The data summarized in Table II further illustrates two additional characteristics with regard to the effect of cytochalasin B on glucose handling by islet cells. First, in either intact islets or dispersed islet cells, the relative magnitude of the inhibitory action of cytochalasin B progressively decreased as the extracellular concentration of D-glucose increased from 2.8 to 8.3 and 16.7 mM. Expressed relative to the corresponding control values (no cytochalasin B), the experimental results recorded in the presence of the mould metabolite averaged 66.5±4.0 (n=27), 73.2±2.5 (n=46) and 84.0±3.0% (n=75) at 2.8, 8.3 and 16.7 mM D-glucose, respectively. Second, at the same D-glucose concentration (2.8 or 16.7 mM), the relative magnitude of the inhibitory action of cytochalasin B was less pronounced in purified islet B cells than either intact islet or dispersed islet cells (17,18). Thus, the percentage inhibition recorded in the purified B cells represented 44.8±18.0% (n=103; P<0.008) of the mean corresponding value found at the same D-glucose concentration in intact islets and/or dispersed islet cells (100.0±9.7%; n=102). Such a difference coincides with the fact that purified B cells are much less sensitive to the inhibitory action of cytochalasin B on D-glucose catabolism compared with non-B islet cells (17).

In conclusion, the present study extends to BRIN-BD11 cells the knowledge that cytochalasin B impairs the uptake of D-glucose and that of one of its non-metabolized analogs. The identity of the glucose transporter(s) affected by cytochalasin B requires further investigation.

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