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Functional Role of Aquaglyceroporin 7 Expression in the Pancreatic Beta-Cell Line BRIN-BD11

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Both mouse and rat pancreatic islet β-cells were recently found to express aquaglyceroporin 7 (AQP7). In the present study, the expression and role of AQP7 in the function of BRIN-BD11 cells were investigated. AQP7 mRNA and protein were detected by RT-PCR and Western blot analysis, respectively. In an isoosmolar medium, the net uptake of [2-^{3}H]glycerol displayed an exponential time course reaching an equilibrium plateau value close to its extracellular concentration. Within 2 min of incubation in a hypotonic medium (caused by a 50 mM decrease in NaCl concentration), the [2-^{3}H]glycerol uptake averaged 143.2 ± 3.8% (n = 24; $P < 0.001$) of its control value in isotonic medium, declining thereafter consistently with previously demonstrated volume regulatory decrease. When isoosmolarity was restored by the addition of 100 mM urea to the hypotonic medium, [2-^{3}H]glycerol uptake remained higher (112.1 ± 2.8%, n = 24; $P < 0.001$) than its matched control under isotonic conditions, indicating rapid entry of urea and water. Insulin release by BRIN-BD11 cells was 3 times higher in hypotonic than in isotonic medium. When glycerol (100 mM) or urea (100 mM) were incorporated in the hypotonic medium, the insulin release remained significantly higher than that found in the control isotonic medium, averaging respectively 120.2 ± 4.2 and 107.0 ± 3.8% of the paired value recorded in the hypotonic medium. These findings document the rapid entry of glycerol and urea in BRIN-BD11 cells, likely mediated by AQP7.

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Mammalian aquaporins (AQPs) are a family of water channels consisting of 13 members, AQP0 to AQP12 (Agre, 2004). AQPs are small hydrophobic integral membrane proteins of about 270 amino acids presenting six transmembrane domains in each monomer, as well as three extracellular and two intracellular loops (Preston and Agre, 1991). Two repeating Asn-Pro-Ala (NPA) sequences, present in the first intracellular and third extracellular loop, represent the amino acid signature sequence motifs of AQPs (Agre, 2004). Based on their permeability characteristics and their amino acid sequence homologies, AQPs can be divided in three groups: the aquaporins, the aquaglyceroporins, and the aquaporins containing unusual NPA motifs. The aquaporins (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, AQP8) are primarily permeable to water, whereas the aquaglyceroporins (AQP3, AQP7, AQP9, AQP10) also transport glycerol and small solutes (Agre, 2004; King et al., 2004; Takata et al., 2004; Hara-Chikuma and Verkman, 2006; Krane and Goldstein, 2007; Rojek et al., 2008). The aquaporins containing unusual NPA motifs appear to be more distantly related to the other mammalian aquaporins and aquaglyceroporins (Agre, 2004; Ishibashi, 2006). Recent reports have drawn attention to the presence of AQP7 in pancreatic islet insulin-producing cells in mice (Matsumura et al., 2007) and rats (Best et al., 2009). AQP7 is permeable to water and to certain small osmolytes, notably glycerol and urea (Ishibashi et al., 1997), and thought to be involved in the transport of glycerol across the plasma membrane (Hara-Chikuma and Verkman, 2006; Rojek et al., 2008). Pancreatic islets from AQP7$^{-/-}$ knockout mice were reported to contain elevated islet cell glycerol and triglyceride content and to exhibit increased rates of basal and glucose-stimulated insulin release, reduced insulin content and reduced islet cell number and β-cell mass (Matsumura et al., 2007). In rat pancreatic islets, urea and glycerol can activate β-cells via their uptake across the plasma membrane possibly via AQP7, resulting in cell swelling, VRAC activation, electrical activity and insulin release; glycerol, in contrast to urea, appears to exert an additional effect, possibly related to its intracellular metabolism (Best et al., 2009). Taken together, these data suggest that AQP7 could be important in normal pancreatic β-cell function.

The major aim of the present study was to determine whether AQP7 is present in tumoral insulin-producing cells of the BRIN-BD11 line and to explore its functional significance in these cells. The BRIN-BD11 cell line is an insulin-secreting cell line established by electrofusion of normal pancreatic β-cell from New England Deaconess Hospital with immortalized RINm5F cells (McClenaghan et al., 1996). These cells display an abnormal D-glucose metabolism and an impaired response to the hexose in terms of changes in cell ATP content (Rasschaert et al., 1996; Kamagate et al., 2008). Yet they exhibit an acute

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release of insulin in response to exposure to extracellular hypoosmolality (Beauwens et al., 2006). They were selected for the present study as representative of tumoral, as distinct from normal, insulin-producing cells.

Materials and Methods

Material

Tissue culture materials and media were obtained from Sarstedt (Numbrecht, Germany), and Invitrogen Life Technologies (Carlsbad, CA), respectively. All pharmacological agents were purchased from Sigma (St. Louis, MO).

Cell culture

BRIN-BD11 cells, kindly provided by Prof. A. Herchuelz (Laboratory of Pharmacology, Brussels Free University, Brussels, Belgium), were grown at 37°C in a humidified incubator gassed with 5% CO_2 in air, and grown in RPMI 1640 medium (cat. No. 21875-034, Gibco, Merelbeke, Belgium) supplemented with 10% (v/v) heat-inactivated FBS, 50 IU/ml penicillin and 50 μg/ml streptomycin. Groups of 10^6 cells each were subcultured onto 6-well plates (10 cm^2) 24 h before the experiments.

RT-PCR

Total RNA from rat tissues and BRIN-BD11 cells were extracted using a AURUM™ total RNA fatty acid and tissue kit (Bio-Rad Laboratories, Hercules, CA). The purified total RNA was quantified on Nanodrop spectrophotometer (NanoDrop Technologies, Inc, Wilmington, DE). Prior to the reverse transcription from 1 μg of total RNA using a Revert Aid™ first strand cDNA synthesis kit (Fermentas, St. Leon-Rot, Germany), the quality of total RNA was verified using an Experion Automated Electrophoresis System (Bio-Rad Laboratories). The primers used for amplification of rat AQP7 cDNA were for sense primer 5′-ACCACTATGCAGGTGGAGAACT-3′ (nt 629–650) and antisense primer 5′-TGAAGTGCTGGACTGTTCAACT-3′ (nt 788-809) (in accession no. NM_019157, amplicon: 181 bp), of rat AQP3 sense primer 5′-CATCTACACACTGGCACAGACC-3′ (nt 382–403) and antisense primer 5′-CAGGGTTGTTATAAGGGTCAACA-3′ (nt 610–632) (in accession no. NM_031703, amplicon: 251 bp), of rat AQP9 sense primer 5′-AATGCAACAGCATTCATTTTTG -3′ (nt 887–908) and antisense primer 5′-ACGACAGGTATCCACCAGAAGT-3′ (nt 1,188–1,209) (in accession no. NM_022960, amplicon: 323 bp), and of ß-actin cDNA sense primer 5′-TGACGGGGTCACCCACACTGTGCCCGTC-3′ (nt 539–566) and antisense 5′-CTAGAAGCATTAGCGGTGGACGATGGAGG-3′ (nt 1,171–1,199) (in accession no. BC002409, amplicon: 661 bp). All PCR reactions were performed in a total volume of 20 μl containing 1 μl of cDNA, 0.5 U GoTaq DNA polymerase (Promega, Madison, WI), 0.2 mM dNTP, 0.5 μM of each primer, and 4 μl GoTaq Green 5× buffer using My iCycler System (Bio-Rad Laboratories). The PCR conditions were 95°C for 1.5 min followed by 35 cycles of 30 sec at 95°C, 30 sec at 60°C and 1 min at 72°C. To exclude amplification from contaminating genomic DNA, DNAse treatment was included during the RNA purification procedure. The PCR amplification products were submitted to electrophoresis on a 1.2% agarose gel in TAE buffer: 40 mM Tris, 40 mM Acetate, 1 mM EDTA in the presence of 0.5 μg/ml of ethidium bromide. Gels were visualized by UV translumination using a GelDoc apparatus (Bio-Rad Laboratories).

Western blot analysis

Total proteins were prepared from BRIN-BD11 cells and visceral adipose tissue by homogenization of cells in lysis buffer (50 mM HEPES, 150 mM NaCl, 10 mM EDTA, 10 mM tetrasodium pyrophosphate, 1.5% CHAPS, and a protease inhibitor cocktail (Complete EDTA-free (Roche Diagnostics, Mannheim, Germany), 1 tablet per 25 ml of buffer), followed by centrifugation of the homogenate at 17,000*g* and finally collecting the supernatant. Total proteins were used for SDS–polyacrylamide gel electrophoresis (SDS–PAGE). 5% of β-mercaptoethanol was added to the sample buffer. The samples were analyzed by SDS–PAGE using 12% polyacrylamide gels. Proteins were transferred to polyvinyldiene difluoride membranes and immunolabeled using an affinity-purified antibody to AQP7 at a 1:500 dilution (Alomone, Jerusalem, Israel). The bound antibodies were detected by using the ECL chemiluminescent method (GE Healthcare, Little Chalfont, UK).

[2-^{3}H]glycerol uptake

For measuring [2-^{3}H]glycerol uptake, BRIN-BD11 cells grown at confluence were preincubated for 15 min at 37°C and then incubated for 1–20 min at 37°C in medium containing 0.5 mM glycerol mixed with a tracer amount of [2-^{3}H]glycerol (GE Healthcare UK Ltd., Amersham, Buckinghamshire, UK). The cells were then washed 4 times with 1.0 ml of a medium containing 10 mM glycerol, extracted twice at room temperature with 1.0 ml of HNO_3 (100 mM), and eventually examined for their radioactive content by liquid scintillation.

Insulin release measurement

For measuring insulin release, the BRIN-BD11 cells were preincubated for 30–90 min and eventually incubated for 15–30 min at 37°C. Except if otherwise mentioned, the incubation medium used to measure insulin release (1.0 ml) contained 10 mM Hepes (pH 7.4), 111 mM NaCl, 24 mM $NaHCO_3$, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1.1 mM D-glucose, and 0.5 mg/ml bovine serum albumin, with an estimated osmolarity close to 300 mOsm/L. The medium was equilibrated against a mixture of O_2/CO_2 (19/1; v/v). Hypotonicity was achieved by decreasing the NaCl concentration to 61 mM. The insulin content of the incubation medium was measured by a method previously described (Leclercq-Meyer et al., 1985).

Statistical analysis

All results are presented as mean values (±SEM), together with the number of separate determinations (n) or degree of freedom (df). The statistical significance of differences between mean values was assessed by use of Student's *t*-test.

Results

AQP7 mRNA expression in BRIN-BD11 cells

AQP7 mRNA was detected by RT-PCR in BRIN BD11 cells, as well as in visceral adipose tissue (TAV) and pancreas, used as positive controls (Fig. 1A). The 212 bp amplicon detected in the BRIN BD11 cells is admittedly much fainter than that detected in the positive control, but no attempt was made to quantify the mRNA. RT-PCR for the amplification of AQP3 and AQP9 mRNA did not revealed any amplification product in BRIN-BD11 cells (Fig. 1B,C), while an amplification product was detected in the kidney and liver, used as respective positive controls for the detection of AQP3 and AQP9 mRNA. RT-PCR for the amplification β-actin mRNA using rat visceral adipose tissue, pancreas, kidney and BRIN-BD11 cells showed a PCR product of the expected size (661 bp; data not shown).

AQP7 protein expression in BRIN-BD11 cells

Western blot analysis with anti-AQP7 antibody revealed a band of approximately 37 kDa in total proteins from visceral adipose tissue, used as a positive control, and from BRIN-BD11 cells (Fig. 2).

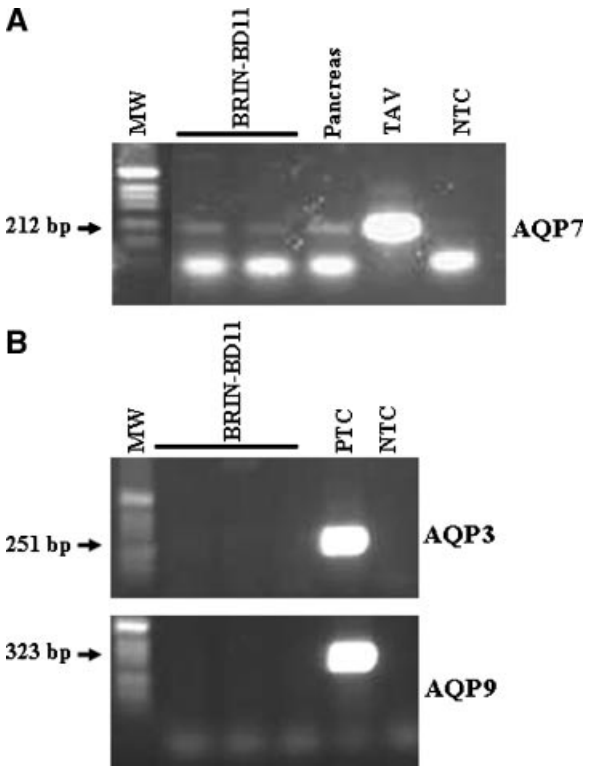

Fig. 1. RT-PCR detection of AQP7 mRNA. A: PCR amplicons using AQP7 primers on cDNAs from BRIN BD11 cells, visceral adipose tissue (TAV) and pancreas used as positive controls. B: PCR amplicons using AQP3 primers and AQP9 primers on cDNA from BRIN BD11 cells, and from rat kidney and liver used as respective positive control (PTC). MW: 1 kb molecular weight marker. NTC: non-target control of the PCR reaction.

[2-^{3}H]glycerol uptake by BRIN-BD11 cells

When incubated in an isotonic medium, the time course for the net uptake of [2-^{3}H]glycerol (0.5 mM) by BRIN-BD11 cells displayed an exponential pattern, defined by the equation $U = U_{max}(1 - e^{-kt})$. In this equation, U represents the net uptake of [2-^{3}H]glycerol after t min of incubation, U_{max} the equilibrium value for such an uptake, and K the fractional turnover rate (expressed as min^{-1}) for the cellular pool of [2-^{3}H]glycerol. As illustrated in the upper inset of Figure 3, the difference between the mean value for [2-^{3}H]glycerol uptake and a theoretical equilibrium value (1.45 nmol/well) slightly in excess of that recorded after 20 min incubation (1.39 ± 0.09 nmol/cell; $n = 4$) indeed yielded in semi-logarithmic coordinates a straight line with a half-life close to 220 sec, corresponding to a K close to 0.19 min^{-1}.

When the same experiments were conducted in a hypotonic medium, as provoked by a decrease in NaCl concentration of 50 mM, higher values for [2-^{3}H]glycerol uptake were observed than in the isotonic medium (Fig. 3). Relative to the paired value recorded within the same experiment and at the same time of incubation in the control isotonic medium, the peak value for

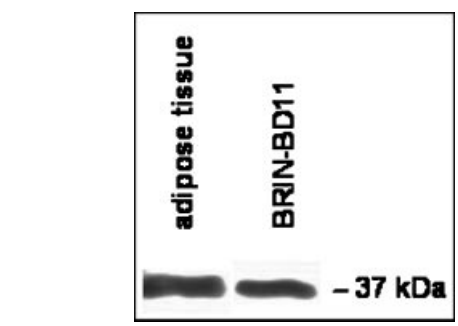

Fig. 2. AQP7 protein expression in BRIN-BD11 cells. Western blot analysis was performed using 20 μg of total proteins from rat visceral adipose tissue, used as positive control, and BRIN-BD11 cells and anti-AQP7 antibodies as described in Materials and Methods Section.

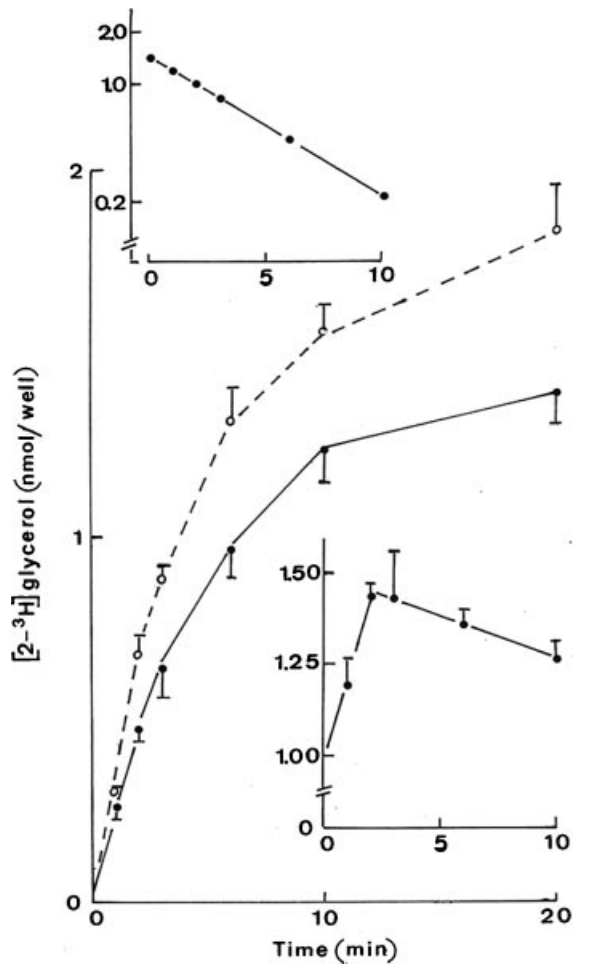

Fig. 3. Time course for the net uptake of [2-^{3}H]glycerol (0.5 mM) by BRIN-BD11 cells incubated in a control isotonic medium (closed circles and solid line) or a hypotonic medium deprived of 50 mM NaCl (open circles and dashed line). Mean values (± SEM) refer to four individual experiments in each case. The upper part illustrates, in semi-logarithmic coordinates, the time-related decrease in the difference between a theoretical equilibrium value for [2-^{3}H]glycerol uptake (1.45 nmol/well) and the observed mean values for such an uptake by cells incubated in the isotonic medium. The lower inset depicts the time-related changes in [2-^{3}H]glycerol uptake by cells incubated in the hypotonic medium, the results found in these cells being expressed relative to those found within the same experiments and at the same time of incubations in cells incubated in the control isotonic medium; mean values (±SEM) refer to the same 4 experiments as those illustrated in the main figure.

the hypotonicity-induced increase in [2-^{3}H]glycerol uptake was already reached after 2 min incubation (Fig. 3, lower inset). At that time, the measurements made in the hypotonic medium averaged 143.2 ± 3.8% (n = 4; $P < 0.005$) of the corresponding control values. Thereafter, such a percentage progressively decreased, the values recorded after 10 min (126.3 ± 4.5%; n = 4) and 20 min incubation (131.5 ± 2.0%; n = 4) being significantly lower ($P < 0.04$ or less) than those found after only 2 min incubation.

Likewise, when the BRIN-BD11 cells were incubated in the medium deprived of 50 mM NaCl, but enriched with 100 mM urea in order to restore extracellular isotonicity, higher values for [2-^{3}H]glycerol uptake were recorded than in the normal control medium. In a series of four experiments, the measurements made between 1 and 20 min incubation in the NaCl-depleted but urea-enriched medium averaged 112.1 ± 2.8% (n = 4; $P < 0.001$) of the corresponding control values recorded within the same experiment and at the same time of incubation in the normal control medium. The latter value was lower ($P < 0.001$), however, than that recorded in the hypotonic medium (132.8 ± 3.2%; n = 24). Moreover, and as possibly related to the more modest response to the NaCl-depleted by urea-enriched medium, the time course for the increase in [2-^{3}H]glycerol uptake caused by the latter medium appeared somewhat more variable, not allowing to detect the same pattern as that illustrated in the lower inset of Figure 3.

When $HgCl_2$ (5.0 μM) was incorporated in the normal isotonic medium, it failed to significantly affect [2-^{3}H]glycerol net uptake. The results recorded in the presence of the mercury salt indeed averaged, after 1–20 min incubation, 97.3 ± 2.5% (n = 12) of the corresponding values found within the same experiment and at the same time of incubation in its absence. When much higher concentrations of $HgCl_2$ (50–100 μM) were used, the uptake of [2-^{3}H]glycerol could not be reliably assessed, because the BRIN-BD11 cells did no more remain attached to the bottom of the well.

The following considerations suggest that the equilibrium value for the net uptake of [2-^{3}H]glycerol by BRIN-BD11 cells exposed to an isotonic medium yields an intracellular concentration of glycerol close to its extracellular concentration. In a series of five experiments, the protein content of the BRIN-BD11 cells exposed to an isotonic medium averaged 714 ± 51 μg per well. At the 10th min of incubation, the protein content of BRIN-BD11 cells exposed to the hypotonic medium and to a medium deprived of 50 mM NaCl but enriched with 100 mM urea represented, respectively, 101.8 ± 2.5 and 102.0 ± 2.6% (n = 4 and $P > 0.5$ in both cases) of the paired value found within the same experiment(s) in the isotonic medium. When both the protein content and number of BRIN-BD11 cells were measured, the results yielded a mean value of 283 pg protein per cell. Assuming an intracellular protein concentration of 1.0 pg per 5 fl, the mean value just mentioned would correspond to cell volume close to 1.42 pl/cell. The modal value for the diameter of the BRIN-BD11 cells, as assessed in a coulter counter was close to 15 μm, yielding, on the assumption of a spherical cell, a volume close to 1.44 pl/cell, in perfect agreement with the value of 1.42 pl/cell mentioned above. In turn, taking into account both the above-mentioned mean value for the protein content and equilibrium value for [2-^{3}H]glycerol net uptake by BRIN-BD11 cells exposed to an isotonic medium, that is, 1.45 nmol per well, their radioactive content would be close to 2.03 ± 0.14 pmol of [2-^{3}H]glycerol equivalent per μg protein or 0.41 ± 0.03 mM. The latter value is indeed close to the extracellular concentration of glycerol in these experiments (0.5 mM).

Insulin release by BRIN-BD11 cells

In a first series of five experiments, the release of insulin (μU/30 min per well) increased ($P < 0.001$) from a basal value in an isotonic medium of 49.8 ± 2.2 to 168.2 ± 12.2 in a hypotonic medium deprived of 50 mM NaCl (Table 1). When glycerol (100 mM) was incorporated in the latter medium in order to restore extracellular isoosmolarity, the output of insulin still represented 120.2 ± 4.2% (n = 5; $P < 0.02$) of the paired value recorded in the hypotonic medium. The release of insulin from BRIN-BD11 cells exposed to the isoosmolar medium deprived of 50 mM NaCl but containing 100 mM glycerol was suppressed by NPPB (5-nitro-2-(3-phenylpropylamino)benzoate, 0.1 mM), under which condition it only averaged 60.4 ± 5.6 μU per 30 min (n = 5), that is, a value no more significantly different ($P > 0.08$) of basal insulin output.

Likewise, in a second series of five experiments, the output of insulin (μU per 30 min) increased ($P < 0.001$) from a basal value of 61.2 ± 2.6 in an isotonic medium to 159.1 ± 7.0 in the hypotonic medium deprived of 50 mM NaCl (Table 1). When urea (100 mM) was added to the latter medium, the release of insulin (170.7 ± 9.7) remained significantly higher ($P < 0.001$) than basal value, averaging 107.0 ± 3.8% (n = 5; $P > 0.13$) of the paired value recorded in the hypotonic medium. Once again, NPPB (0.1 mM) suppressed the secretory response of the BRIN-BD11 cells to the isoosmolar medium deprived of 50 mM NaCl but enriched with 100 mM urea. Indeed, when the latter medium also contained NPPB, the release of insulin (69.3 ± 6.2 μU per 30 min) was no more significantly different ($P > 0.5$) of basal value.

When dimethylurea (100 mM) was added, instead of urea, to the hypotonic medium deprived of 50 mM NaCl, the increment in insulin output above the paired value recorded in an isotonic medium only represented 46.2 ± 10.6% (n = 7; $P < 0.005$) of that found, within the same experiment(s) when urea (100 mM) was added to the hypotonic medium.

Two further sets of experiments were designed to assess the possible effect upon insulin release of potential inhibitors of the aquaglyceroporin channel. In the first of these further sets of experiments, $HgCl_2$ (0.3–3.0 mM) was found to abolish both basal insulin release and the secretory response to extracellular hypoosmolarity (Table 2). In the BRIN-BD11 cells exposed to an isotonic medium, the release of insulin (μU per 15 min) averaged 25.4 ± 2.1 (n = 5) in the absence of $HgCl_2$, as

TABLE 1. Effects of glycerol, urea and dimethylurea upon insulin release

					Insulin output (μU/30 min per well)	
NaCl (mM)	Glycerol (mM)	Urea (mM)	Dimethylurea (mM)	NPPB (mM)	Experiment 1	Experiment 2
115	—	—	—	—	49.8 ± 2.2 (5)	61.2 ± 2.6 (5)
65	—	—	—	—	168.2 ± 12.2 (5)	159.1 ± 7.0 (5)
65	100	—	—	—	202.5 ± 15.9 (5)	
65	100	—	—	0.1	60.4 ± 5.6 (5)	
65	—	100	—	—		170.7 ± 9.1 (5)
65	—	100	—	0.1		69.3 ± 6.2 (5)
65	—	—	100	—		128.6 ± 6.1 (5)

TABLE 2. Effects of $HgCl_2$ upon insulin release

NaCl (mM)	$HgCl_2$ (mM)	Insulin output (μU/15 min per well)
115	—	25.4 ± 2.1 (5)
115	0.3	5.3 ± 1.1 (5)
115	3.0	11.4 ± 0.9 (5)
65	—	110.1 ± 18.9 (5)
65	0.3	25.2 ± 7.9 (5)
65	3.0	14.2 ± 1.1 (5)

compared ($P < 0.001$) to only 8.4 ± 1.2 (n = 10) in its presence. When the BRIN-BD11 cells were incubated in a hypotonic medium, as provoked by a 50 mM decrease in the NaCl concentration, the release of insulin was increased ($P < 0.005$) to 110.1 ± 18.9 μU per 15 min (n = 5). Once again, $HgCl_2$ (0.3–3.0 mM) decreased ($P < 0.005$ or less) such a release to 25.2 ± 7.9 and 14.2 ± 1.1 μU per 15 min, respectively (n = 5 in both cases). The secretion of insulin remained significantly higher ($P < 0.05$) in the hypotonic medium than in the isotonic medium in the presence of 0.3 mM $HgCl_2$, but no more so ($P > 0.09$) in the presence of 3.0 mM $HgCl_2$.

In the second set of further experiments, the release of insulin was again 4.2 ± 1.9 times higher (n = 5; $P < 0.005$) in the hypotonic medium than in the isotonic medium (Table 3). In this set of experiments, Ag sulfadiazine (50 and 100 μM) apparently increased basal insulin release ($P < 0.001$) to 204.3 ± 23.8% (n = 10) of paired basal control measurements, whilst decreasing the secretion of insulin recorded in the hypotonic medium ($P < 0.001$) to 45.7 ± 5.0% (n = 10) of its control value. As a result of these two opposite effects, the absolute value for insulin release found in the presence of Ag sulfadiazine (50 and 100 μM) were virtually identical ($P > 0.7$) in the isotonic and hypotonic media. In the presence of Ag sulfadiazine, the output of insulin in the hypotonic medium indeed averaged 95.4 ± 10.3% (n = 10) of that found in the isotonic medium (100.0 ± 8.9%; n = 10).

Discussion

AQP7 is an aquaglyceroporin permeable to water and glycerol (Krane and Goldstein, 2007; Rojek et al., 2008). From the three aquaglyceroporins cloned so far, the present study demonstrated the expression of only AQP7, and not of AQP3 and AQP9, in BRIN-BD11 cells using RT-PCR and Western blot analysis. Besides, our measurements of [2-^{3}H]glycerol uptake are compatible with a role for AQP7 in mediating the entry of glycerol in the BRIN-BD11 cells, eventually leading to equilibration between the extracellular and intracellular concentrations of glycerol. In this respect, the results illustrated in the lower inset of Figure 1 may well document the time course for the increase in cell volume provoked by the exposure of the BRIN-BD11 cells to a hypotonic medium. Indeed, the initial peak value for the ratio between [2-^{3}H]glycerol net uptake in the cells exposed to the hypotonic/isotonic medium was close to that previously found (1.25–1.40) under the same experimental conditions, when the cell volume was directly measured (Beauwens et al., 2006). Moreover, the later progressive decrease in such a ratio displayed a time course quite similar to that characterizing the regulatory volume decrease in BRIN-BD11 cells exposed to a hypotonic medium, a half-maximal decrease in the latter cell volume or in the hypotonic/isotonic ratio for [2-^{3}H]glycerol uptake being reached after about 10–12 min of exposure to the hypotonic medium. With this interpretation of the data illustrated in Figure 1 in mind, the present results suggest that the cell volume also increases when the BRIN-BD11 cells are exposed to a medium depleted of 50 mM NaCl but enriched with 100 mM urea.

TABLE 3. Effects of Ag sulfadiazine upon insulin release

NaCl (mM)	Ag sulfadiazine (mM)	Insulin output (μU/30 min per well)
115	—	54.3 ± 4.2 (5)
115	0.05	117.8 ± 17.1 (5)
115	0.10	108.9 ± 12.7 (5)
65	—	252.9 ± 72.4 (5)
65	0.05	112.1 ± 18.7 (5)
65	0.10	104.2 ± 16.2 (5)

The present insulin secretion data reinforce the view that, under conditions of extracellular isotonicity, both glycerol and urea accumulate rapidly in BRIN-BD11 cells, leading to cell swelling, subsequent activation of volume-sensitive anion channels, and eventual stimulation of insulin release. The secretion of insulin recorded in media deprived of 50 mM NaCl but enriched with 100 mM glycerol, urea or dimethylurea, when expressed relatively to that recorded within the same experiment(s) in cells exposed to a hypotonic medium deprived of 50 mM NaCl, averaged 120.2 ± 4.2% (n = 5) in the case of glycerol and 107.0 ± 3.8% (n = 5) in the case of urea, as distinct ($P < 0.001$) from only 76.0 ± 3.5% (n = 7) in the case of dimethylurea. The much lower value recorded in the case of dimethylurea, as distinct form urea, may well reflect the low permeability of the aquaglyceroporin channel to dimethylurea. The trend ($P < 0.05$) towards a higher response to glycerol to urea, as suggested by results obtained in separate experiments, could conceivably point to a modest metabolic component of the secretory response to glycerol (Yilmaz et al., 1987). The postulated involvement of volume-sensitive anion channels in the secretory responses to either glycerol or urea is supported by the present finding that 5-nitro-2-(3-phenylpropylamino)benzoate suppressed such responses (Beauwens et al., 2006).

Further attempts to address the participation of the aquaglyceroporin channel in the functional response to glycerol or extracellular hypotonicity were made by using two potential inhibitors of such channels, namely $HgCl_2$ and Ag sulfadiazine. However, the results of these experiments raise serious concern on the specificity of the latter two agents as inhibitors of aquaglyceroporin. A comparable conclusion was recently reached in a study conducted in normal insulin-producing cells (Best et al., 2009).

In conclusion, the present work suggests that BRIN-BD11 cells, although known to display typical features of tumoral cells, such as a vastly different pattern of glucose metabolism when compared to normal islet cells (Rasschaert et al., 1996), may nevertheless represent a useful model for further investigations on the participation of AQP7 in the functional response of insulin-producing cells to various secretagogues, including glucose. Like that of other nutrient secretagogues, the insulinotropic action of glucose indeed also involves cell swelling and subsequent activation of volume-sensitive anion channels (Malaisse et al., 2008).

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Literature Cited

Agre P. 2004. Aquaporin water channels (Nobel Lecture). Angew Chem Int Ed Engl 43:4278–4290.

Beauwens R, Best L, Markadieu N, Crutzen R, Louchami K, Brown P, Yates AP, Malaisse WJ, Sener A. 2006. Stimulus-secretion coupling of hypotonicity-induced insulin release in BRIN-BD11 cells. Endocrine 30:353–363.

Best L, Brown PD, Yates AP, Perret J, Virreira M, Beauwens R, Malaisse WJ, Sener A, Delporte C. 2009. Contrasting effects of glycerol and urea transport on rat pancreatic β-cell function. Cell Physiol Biochem 23:255–264.
Hara-Chikuma M, Verkman AS. 2006. Physiological roles of glycerol-transporting aquaporins: The aquaglyceroporins. Cell Mol Life Sci 63:1386–1392.
Ishibashi K. 2006. Aquaporin subfamily with unusual NPA boxes. Biochim Biophys Acta 1758:989–993.
Ishibashi K, Kuwahara M, Kageyama Y, Tohsaka A, Marumo F, Sasaki S. 1997. Cloning and functional expression of a second new aquaporin abundantly expressed in testis. Biochem Biophys Res Commun 237:714–718.
Kamagate A, Sener A, Courtois P, Malaisse WJ, Herchuelz A. 2008. Effects of plasma membrane Ca^{2+}-ATPase overexpression upon D-glucose metabolism in insulin-producing BRIN-BD11 cells. Biosci Rep 28:251–258.
King LS, Kozono D, Agre P. 2004. From structure to disease: The evolving tale of aquaporin biology. Nat Rev Mol Cell Biol 5:687–698.
Krane CM, Goldstein DL. 2007. Comparative functional analysis of aquaporins/glyceroporins in mammals and anurans. Mamm Genome 18:452–462.
Leclercq-Meyer V, Marchand J, Woussen-Colle MC, Malaisse WJ. 1985. Multiple effects of leucine on glucagon, insulin and somatostatin secretion from the perfused rat pancreas. Endocrinology 116:1168–1174.
Malaisse WJ, Best L, Beauwens R, Sener A. 2008. Ionic determinants of the insulinotropic action of glucose: The anion channel hypothesis. Metab Funct Res Diab 1:2–6.
Matsumura K, Chang BHJ, Fujimiya M, Chen W, Kulkarni RN, Eguchi Y, Kimura H, Kojima H, Chan L. 2007. Aquaporin 7 is a beta-cell protein and regulator of intraislet glycerol content and glycerol kinase activity, beta-cell mass, and insulin production and secretion. Mol Cell Biol 27:6026–6037.
McClenaghan NH, Barnett CR, O'Harte FP, Flatt PR. 1996. Mechanisms of amino acid-induced insulin secretion from the glucose-responsive BRIN-BD11 pancreatic B-cell line. J Endocrinol 151:349–357.
Preston GM, Agre P. 1991. Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: Member of an ancient channel family. Proc Natl Acad Sci USA 88:11110–11114.
Rasschaert J, Flatt PR, Barnett CR, McClenaghan NH, Malaisse WJ. 1996. D-glucose metabolism in BRIN-BD11 islet cells. Biochem Mol Med 57:97–105.
Rojek A, Praetorius J, Frokiaer J, Nielsen S, Fenton RA. 2008. A current view of the mammalian aquaglyceroporins. Annu Rev Physiol 70:301–327.
Takata K, Matsuzaki T, Tajika Y. 2004. Aquaporins: Water channel proteins of the cell membrane. Prog Histochem Cytoc 39:1–83.
Yilmaz MT, Sener A, Malaisse WJ. 1987. Glycerol phosphorylation and oxidation in pancreatic islets. Mol Cell Endocrinol 52:251–256.