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ORIGINAL ARTICLES

Characterization and Modulation of Glucose Uptake in a Human Blood–Brain Barrier Model

Manuela Meireles · Fátima Martel · João Araújo · Celestino Santos-Buelga · Susana Gonzalez-Manzano · Montserrat Dueñas · Victor de Freitas · Nuno Mateus · Conceição Calhau · Ana Faria

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Abstract The blood–brain barrier (BBB) plays a key role in limiting and regulating glucose access to glial and neuronal cells. In this work glucose uptake on a human BBB cell model (the hCMEC/D3 cell line) was characterized. The influence of some hormones and diet components on glucose uptake was also studied. ^{3}H-2-deoxy-D-glucose ([^{3}H]-DG) uptake for hCMEC/D3 cells was evaluated in the presence or absence of tested compounds. [^{3}H]-DG uptake was sodium- and energy-independent. [^{3}H]-DG uptake was regulated by Ca^{2+} and calmodulin but not by MAPK kinase pathways. PKC, PKA and protein tyrosine kinase also seem to be involved in glucose uptake modulation. Progesterone and estrone were found to decrease ^{3}H-DG uptake. Catechin and epicatechin did not have any effect, but their methylated metabolites increased [^{3}H]-DG uptake. Quercetin and myricetin decreased [^{3}H]-DG uptake, and glucuronic acid-conjugated quercetin did not have any effect. These cells expressed GLUT1, GLUT3 and SGLT1 mRNA.

Keywords Blood–brain barrier · Flavonoid · Glucose · Hormone · Signaling pathway

M. Meireles · F. Martel · J. Araújo · C. Calhau · A. Faria (✉)
Department of Biochemistry (U38.FCT), Faculty of Medicine, University of Porto, Al. Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
e-mail: anafaria@med.up.pt

C. Santos-Buelga · S. Gonzalez-Manzano · M. Dueñas · A. Faria
Grupo de Investigación en Polifenoles (GIP-USAL), Universidad de Salamanca, 37007 Salamanca, Spain

V. de Freitas · N. Mateus
Chemistry Investigation Center (CIQ), Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal

C. Calhau
CINTESIS—Center for Research in Health Technologies and Information Systems, University of Porto, 4200-319 Porto, Portugal

A. Faria
Faculty of Nutrition and Food Sciences, University of Porto, 4200-465 Porto, Portugal

Introduction

The brain requires a constant and substantial energy supply to maintain its main functions. Beyond infancy, and under normal conditions, brain energy requirements are met almost exclusively by the breakdown of glucose. During hypoglycemia, other tissues will stop using glucose altogether in order to increase glucose availability to the brain (Owen and Sunram-Lea 2011).

Compared to other organs, the brain is particularly vulnerable to small and transient changes in its energy supply. Interrupted delivery leads within seconds to unconsciousness and within minutes may cause irreparable brain damage, which makes brain function highly dependent on the availability and metabolism of glucose and oxygen resources (Owen and Sunram-Lea 2011).

Free exchange between the blood and interstitial fluid occurs in nearly all organs of the body with the exception of the capillaries in the brain, which have evolved to constrain the movement of molecules and cells between the blood and brain. This characteristic, besides fulfilling a protective role in providing a natural defense against toxic or infective agents circulating in the blood, has also the crucial role of supplying essential nutrients, hormones and drugs to the brain and eliminating metabolites from the brain (Abbott et al. 2010). The blood–brain barrier (BBB)

is made up of three cell types, endothelial cells, astrocytes and pericytes; and the barrier properties are conferred by cell adhesion molecules that allow endothelial cells to form tight junctions.

Brain glucose levels are maintained through the balance of glucose's utilization in neural cells and its entry from the peripheral circulation via enhanced cerebral blood flow (Kiyatkin and Lenoir 2012). Since most of the energy required to sustain normal brain function is provided by blood glucose, glucose utilization in the brain may be limited by BBB glucose transport (Maher et al. 1994).

Glucose transport through membranes must involve a transport system, and thus, it is subject to its expression, function and regulation with consequences to brain glucose availability. Glucose transporter type 1 deficiency syndrome is a treatable epileptic encephalopathy, caused by a defect of glucose transport mediated by GLUT1 across the BBB and into brain cells. It is characterized by an infantile-onset epileptic encephalopathy associated with delayed neurological development, deceleration of head growth, acquired microcephaly, incoordination and spasticity (Verrotti et al. 2012).

Several substances, e.g., dietary components, hormones, therapeutic drugs and drugs of abuse, have been shown to influence different transporters at different levels (e.g., enterocyte, colon, placenta) (Araujo et al. 2010; Goncalves et al. 2009, 2011; Keating et al. 2007, 2009; Martel et al. 2010). Particularly, glucose transport was shown to be modified by flavonoids, vitamins and drugs of abuse (Araujo et al. 2008; Faria et al. 2009; Keating et al. 2009; Martel et al. 2010).

Flavonoids (a major group of polyphenols) are ubiquitously found in nature and important components in the human diet. Epidemiological and dietary intervention studies in humans and other animals have indicated that flavonoid consumption may be capable of promoting neuronal health (Aquilano et al. 2008; Lau et al. 2006; Singh et al. 2008; Spencer 2009b, c; Spencer et al. 2009). However, flavonoids' mechanisms of action are not fully understood: whether they have a direct effect or interfere with cellular mechanisms causing biological effects is not completely clarified.

Because the BBB is a critical barrier in controlling brain glucose homeostasis and because glucose transport is influenced by several substances, increasing our knowledge of glucose uptake characterization in a human BBB model and its modulation by hormonal and dietary compounds is urgent.

The development of an immortalized cell line of human brain capillary endothelial cells which display many of the properties of the BBB cells in vivo provides a good model for glucose transport across the BBB in vitro (Ohtsuki et al. 2013; Weksler et al. 2005).

Materials and Methods

Chemicals

^{3}H-2-deoxy-D-glucose ([^{3}H]-DG, specific activity 40–50 Ci/mmol) was from American Radiolabeled Chemicals (St. Louis, MO); (+)-catechin, (−)-epicatechin, quercetin, myricetin, neomycin, 4′,5,7-trihydroxyisoflavone (genistein), 5,5′-dimethyl-BAPTA-AM, calmidazolium, chelerythrine chloride, H-89 dihydrochloride hydrate, KN-62, dinitrophenol, phloridzin, rapamycin, LY294002, progesterone, testosterone, estradiol, estrone, corticosterone, pregnanediol, aldosterone, penicillin G, amphotericin B, streptomycin, HEPES, trypsin–EDTA and collagen type I from rat tail were from Sigma-Aldrich (Madrid, Spain); PD 98058, SB 203580 and SP600125 were from Research Biochemicals International (Natick, MA); dimethylsulfoxide (DMSO) and triton X-100 were from Merck (Darmstadt, Germany); fetal bovine serum (FBS), basic fibroblast growth factor (FGF) and Hanks balanced salt solution (HBSS) were from GIBCO (Barcelona, Spain); EBM-2 medium, vascular endothelial growth factor (VEGF), IGF-I, epidermal growth factor (EGF), basic FGF, hydrocortisone, ascorbate and gentamycin were from Clonetics (Wokingham, UK).

3′-*O*-Methylcatechin and 3′- and 4′-*O*-methylepicatechin were prepared by hemisynthesis as described (Gonzalez-Manzano et al. 2009). Quercetin-3-*O*-glucuronide was isolated from green bean pods as reported (Duenas et al. 2011).

Cell and Culture Conditions

The hCMEC/D3 cell line was kindly supplied by Dr. Pierre-Olivier Couraud (INSERM U.567, Université René Descartes, Paris, France). Cells were maintained in a humidified atmosphere of 5 % CO_2–95 % air at 37 °C, between passages 26 and 34. Cells were grown in EBM-2 medium supplemented with VEGF, IGF-I, EGF, basic FGF, hydrocortisone, ascorbate, gentamycin and 2.5 % FBS as recommended by the manufacturer, as well as 100 U/ml penicillin G, 0.25 mg/ml amphotericin B and 100 mg/ml streptomycin. The cell medium was changed every 48 h, and cells reached confluence after 5–6 days in culture. For subculturing, cells were dissociated with 0.25 % trypsin–EDTA, diluted 1:5 and subcultured in Petri dishes collagen-coated with a 21-cm^{2} growth area (Corning Costar, Badhoevedorp, the Netherlands).

For the experiments, cells were seeded on 24-well collagen-coated plates (Corning Costar). All experiments were performed 7–9 days after initial seeding.

[^{3}H]DG Uptake

The culture medium was aspirated, and cells were washed with 0.3 ml buffer at 37 °C. Then, hCMEC/D3 cells were deprived of glucose for 2 h (preincubation period) by incubation with glucose-free HEPES buffered saline (containing [in mM] 140 NaCl, 5 KCl, 1 pyruvate, 2.5 $MgSO_4$, 1 $CaCl_2$ and 20 HEPES, pH 7.4). Uptake was initiated by the addition of 0.3 ml medium at 37 °C containing 50 nM [^{3}H]-DG. At the end of the incubation period, incubation was stopped by placing the cells on ice and rinsing them with 0.5 ml ice-cold buffer. Cells were then solubilized with 0.3 ml 0.1 % (v/v) Triton X-100 (in 5 mM Tris–HCl, pH 7.4) and placed at 37 °C overnight. Radioactivity in the cells was measured by liquid scintillation counting. Compounds to be tested were present during the last 50 or 30 min of the preincubation period and during the incubation period. Controls were made with 0.1 % of solvent.

Quantitative RT-PCR

RNA was extracted from the hCMEC/D3 cells using the Tripure Isolation Reagent (Roche, Indianapolis, IN), according to the manufacturer's instructions. RNA was dissolved in water (diethylpyrocarbonate-treated) and stored at −80 °C.

Total RNA was treated with DNase I (Invitrogen, Carlsbad, CA), and 0.5 µg of the resulting DNA-free RNA was reverse-transcribed using Superscript Reverse Transcriptase II and random hexamer primers (Invitrogen) in 20 µl of final reaction volume, according to the manufacturer's instructions. The resulting cDNA was treated with RNase H (Invitrogen) to degrade unreacted RNA. For real-time quantitative PCR, 2 µl of the 20 µl reverse transcription reaction mixture was used. For the calibration curve, hCMEC/D3 standard cDNA was diluted in six different concentrations.

QueryReal-time PCR was carried out using a LightCycler (Roche, Nutley, NJ). Twenty-microliter reactions were set up in microcapillary tubes using 0.5 µM of each primer and 4 µl of SYBRGreen master mix (LightCycler FastStart DNA MasterPlus SYBR Green I; Roche, Indianapolis, IN). Cycling conditions were as follows: denaturation (95 °C for 5 min), amplification and quantification (95 °C for 10 s, annealing temperature [AT] for 5 s and 72 °C for 10 s, with a single fluorescence measurement at the end of the 72 °C for a 10-s segment) repeated 45 times, a melting curve program ([AT+10] °C for 15 s and 95 °C with a heating rate of 0.1 °C/s and continuous fluorescence measurement) and a cooling step to 40 °C. Annealing temperatures and sequences of primers are indicated in Table 1. Data were analyzed using LightCycler software. Results are presented as the expression of the gene of interest relative to hypoxanthine-guanine phosphoribosyltransferase (HPRT).

Protein Determination

The protein content of cell monolayers was determined by Bradford's (1976) method, using human serum albumin as standard.

Statistical Analysis

All assays were performed at least in two independent experiments with $n \geq 3$ replicates. Values are expressed as the arithmetic mean ± SEM. Statistical significance of the difference between various groups was evaluated by one-way analysis of variance, followed by the Bonferroni test. For comparison between two groups, Student's t test was used. Differences were considered significant when $p < 0.05$.

Results

Characterization of [^{3}H]-DG Uptake by hCMEC/D3 Cells

Cellular uptake of [^{3}H]-DG was determined using hCMEC/D3 cells as a model of the human BBB. [^{3}H]-DG was taken up by the cells in a time-dependent way, and uptake was linear with time for up to 5 min of incubation (data not shown). Thus, all subsequent experiments were performed using a 1-min incubation period, in the presence or absence of compounds, in order to determine initial rates of uptake.

Sodium and Energy Dependence of [^{3}H]-DG Uptake

Replacement of NaCl by LiCl in both the preincubation and incubation media significantly increased [^{3}H]-DG

Table 1 Primer sequences and annealing temperatures used in qRT-PCR

Gene name	Primer sequence (5′–3′)	AT (°C)
GLUT1	GAT GAT GCG GGA GAA GAA GGT	65
	ACA GCG TTG ATG CCA GAC AG	
GLUT2	CAG GAC TAT ATT GTG GGC TAA	65
	CTG ATG AAA AGT GCC AAG T	
GLUT3	CTT CCC CTC CGC TGC TCA CTA	64
	CAA AAG TCC TGC CAC GGG TCT	
SGLT1	TGG CAA TCA CTG CCC TTT A	60
	TGC AAG GTG TCC GTG TAA AT	
HPRT	GGT CAA GGT CGC AAG C	65
	GGG CAT ATC CTA CAA CAA ACT	

uptake. However, when NaCl was replaced by choline chloride (CoCl), no significant changes were induced in [^{3}H]-DG uptake (Fig. 1a).

Dinitrophenol, which inhibits ATP production, and phloridzin, an inhibitor of the sodium-dependent glucose transporter, were also tested in order to characterize [^{3}H]-DG uptake in hCMEC/D3 cells (Fig. 1b). No differences in [^{3}H]-DG uptake were found with either of these compounds, revealing that [^{3}H]-DG is energy-independent and that the involvement of SGLT1 in this transport can be excluded.

mRNA Expression of Glucose Transporters

The mRNA expression of glucose transporters in hCMEC/D3 cells was analyzed by qRT-PCR. As previously described (Cardoso et al. 2011; Uchida et al. 2011), GLUT1 was the most expressed glucose transporter; but interestingly, SGLT1 and GLUT3 were also present in this cell line. GLUT2 was not expressed (Fig. 2).

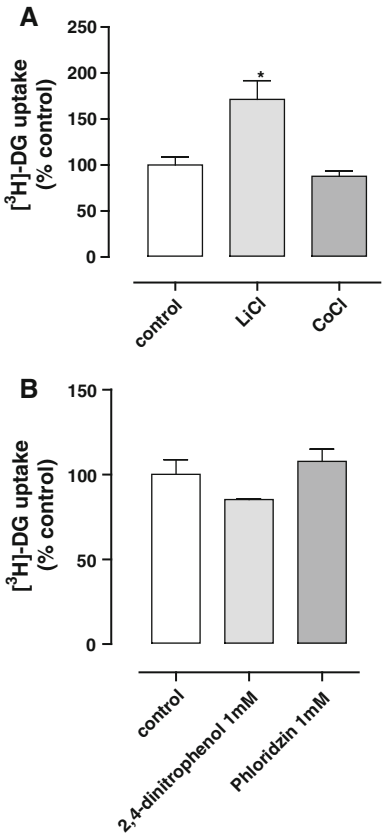

Fig. 1 **a** Effect of Na^{+} substitution by LiCl and choline chloride (CoCl) on [^{3}H]-DG uptake by hCMEC/D3 cells. Cells were preincubated for 2 h with glucose-free buffer and then incubated with [^{3}H]-DG (50 nM) for 1 min. The tested compounds were present in the last 30 min of preincubation and in the incubation. **b** Effect of dinitrophenol and phloridzin on [^{3}H]-DG uptake by hCMEC/D3 cells. Cells were preincubated for 2 h with glucose-free buffer and then incubated with [^{3}H]-DG (50 nM) for 1 min. The tested compounds were present in the last 30 min of preincubation and in the incubation. $^{*}p < 0.05$ versus control

Regulation of [^{3}H]-DG Uptake by Intracellular Signaling Pathways

The intracellular signaling mechanisms regulating [^{3}H]-DG uptake in hCMEC/D3 cells were next studied. For this, the effect of a 50-min exposure of hCMEC/D3 cells to modulators of intracellular signaling pathways was assessed.

First, the role of intracellular Ca^{2+} was studied by testing the effect of the Ca^{2+} chelator BAPTA-AM and of calmidazolium, a Ca^{2+}/calmodulin (CaM) inhibitor. There was a significant decrease in glucose uptake in the presence of these compounds (Fig. 3a). These results suggested the involvement of Ca^{2+} in modulating glucose uptake. On the other hand, KN-62, a CaM-dependent protein kinase II (CaMK II) inhibitor, did not have any effect on glucose uptake in the hCMEC/D3 cell line (Fig. 3a).

The involvement of MAP kinase (MAPK) was investigated by testing the effect of specific inhibitors of MAPK, ERK1/2 (PD 98059), p38 MAPK (SB 203580) and JNK (SP 600125). None of these compounds significantly interfered with [^{3}H]-DG uptake, suggesting it is not dependent on MAPK regulation (Fig. 3b).

The involvement of protein kinase pathways was tested using chelerythrine, a selective inhibitor of PKC, and H89, a selective inhibitor of PKA. Both compounds interfered with glucose uptake but in opposite directions: inhibition of PKC led to a decrease in [^{3}H]-DG uptake and inhibition of PKA resulted in an increase in [^{3}H]-DG uptake (Fig. 3c). Genistein, an inhibitor of protein tyrosine kinases (PTKs), was also tested and found to increase [^{3}H]-DG uptake. LY294002, a known inhibitor of phosphoinositide

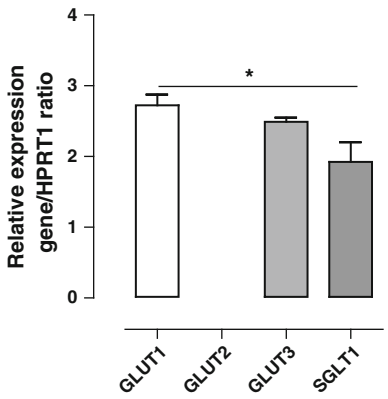

Fig. 2 Quantification of mRNA levels of GLUT1, -2, -3 and SGLT1 by qRT-PCR in hCMEC/D3 cells. Results are shown as the expression of the gene of interest relative to HPRT (arithmetic mean ± SEM). $^{*}p < 0.05$

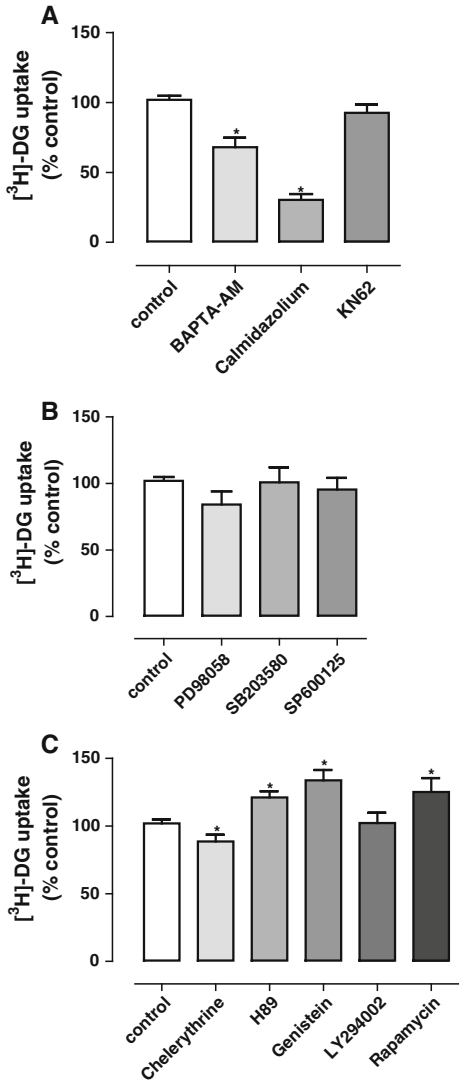

Fig. 3 Effect of several signaling pathway inhibitors on [^{3}H]-DG uptake by hCMEC/D3 cells. Cells were preincubated for 2 h with glucose-free buffer and then incubated at 37 °C with [^{3}H]-DG (50 nM) for 1 min. The tested compounds were present in the last 50 min of preincubation and in the incubation. **a** Effect of BAPTA-AM (50 μM), calmidazolium (25 μM) and KN-62 (10 μM). **b** Effect of PD 98059, SB 203580 and SP 600125 (25 μM). **c** Effect of chelerythrine (40 μM), H89 (10 μM), genistein (10 μM), LY294002 (10 μM) and rapamycin (500 nM). *$p < 0.05$ versus control

3-kinases (PI3 K), did not have any effect on [^{3}H]-DG uptake; but rapamycin, an inhibitor of the mTOR pathway, increased [^{3}H]-DG uptake in this cell line (Fig. 3c).

Hormonal Modulation of [^{3}H]-DG Uptake

In order to assess a possible endocrine modulation of ^{3}H-DG uptake, several hormones were tested: progesterone, testosterone, estradiol, estrone, corticosterone, pregnanediol and aldosterone (Table 2). Of these, only progesterone and estrone were able to influence [^{3}H]-DG uptake, decreasing it.

Nutritional Modulation of [^{3}H]-DG Uptake

A possible nutritional modulation of [^{3}H]-DG uptake was also assessed. Polyphenols are important dietary components since several beneficial neuroprotective effects have been attributed to their consumption. Catechin and epicatechin, two flavan-3-ols, as well as their methylated metabolites were tested. The unconjugated polyphenols had no effect on [^{3}H]-DG uptake, but interestingly, their methylated forms were able to increase [^{3}H]-DG uptake (Fig. 4).

Quercetin and myricetin, two flavonols, were also tested along with a quercetin–glucuronic acid conjugate (Fig. 5). The flavonols decreased [^{3}H]-DG uptake in a concentration-dependent manner, but the conjugate had no effect on [^{3}H]-DG uptake.

Table 2 Effect of several hormones on [^{3}H]-DG uptake by hCMEC/D3 cells

Compound	[^{3}H]-DG (% control)
Control	100.0 ± 2.2
Progesterone (μM)	
50	53.3 ± 12.0*
100	71.9 ± 8.59*
200	54.5 ± 15.8*
Testosterone (μM)	
50	92.4 ± 7.4
100	99.7 ± 6.7
200	112.8 ± 17.0
Estradiol (μM)	
100	82.4 ± 4.3
250	94.9 ± 11.6
500	118.7 ± 19.0
Estrone (μM)	
50	86.2 ± 3.89
100	78.8 ± 7.9
200	70.8 ± 1.6*
Corticosterone (μM)	
100	99.2 ± 7.3
Pregnanediol (μM)	
100	89.3 ± 15.3
Aldosterone (μM)	
100	102.7 ± 7.4

Cells were preincubated for 2 h with glucose-free buffer and then incubated at 37 °C with [^{3}H]-DG (50 nM) for 1 min. The tested compounds were present in the last 30 min of the preincubation period and in the incubation

* $p < 0.05$ versus control

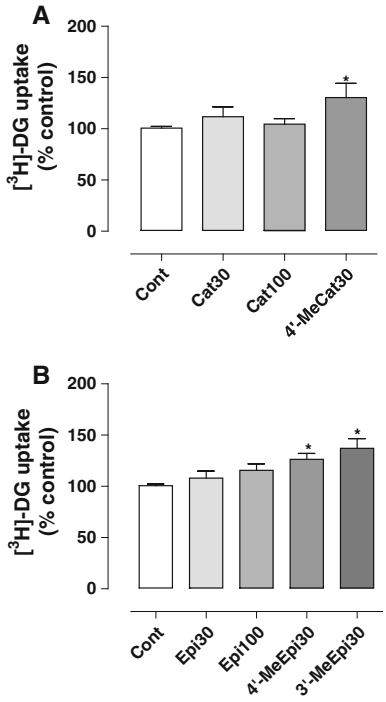

Fig. 4 Effect of catechin and epicatechin and their methylated metabolites on [^{3}H]-DG uptake by hCMEC/D3 cells. Cells were preincubated for 2 h with glucose-free buffer and then incubated at 37 °C with [^{3}H]-DG (50 nM) for 1 min. The tested compounds were present in the last 30 min of preincubation and in the incubation. **a** Effect of catechin (*Cat*, 30 and 100 μM) and of 4′-methylcatechin (*4′-MeCat*, 30 μM) on [^{3}H]-DG uptake by hCMEC/D3 cells. **b** Effect of epicatechin (*Epi*, 30 and 100 μM), 4′-methylepicatechin (*4′-MeEpi*, 30 μM) and 3′-methylepicatechin (*3′-MeEpi*, 30 μM) on [^{3}H]-DG uptake by hCMEC/D3 cells. $*p < 0.05$ versus control

Discussion

Glucose is an essential substrate to the organism, in particular to the brain as it is the main energetic substrate to neurons; therefore, glucose supply is crucial to maintain the normal functions of the brain. However, due to its hydrophilic nature, it cannot freely cross cellular membranes and, thus, needs specialized transporters to facilitate its transport. The first limiting barrier to glucose access into the brain is the BBB. Therefore, uptake of glucose at the BBB level and its modulation are of great importance since they will have consequences upon brain glucose availability.

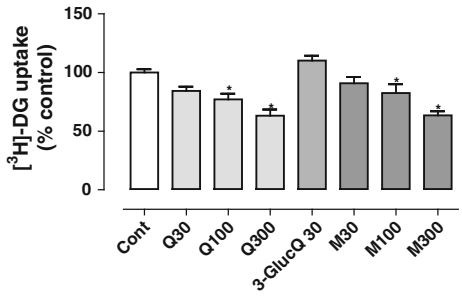

Fig. 5 Effect of quercetin (*Q*; 30, 100 and 300 μM), 3-glucuronilquercetin (*3-GlucQ*, 30 μM) and myricetin (*M*; 30, 100 and 300 μM) on [^{3}H]-DG uptake by hCMEC/D3 cells. Cells were preincubated for 2 h with glucose-free buffer and then incubated at 37 °C with [^{3}H]-DG (50 nM) for 1 min. The tested compounds were present in the last 30 min of preincubation and in the incubation. $*p < 0.05$ versus control

In this work, a human BBB cell line, hCMEC/D3, was used as a model of the BBB to study glucose uptake. This cell line is well characterized and retains most of the morphological and functional features of brain endothelial cells, even without coculture with glia cells (Ohtsuki et al. 2013; Weksler et al. 2005).

First, a functional characterization of glucose uptake into these cells was made, allowing us to conclude that glucose uptake into hCMEC/D3 cells was sodium-independent. Isosmolar substitution of extracellular NaCl by LiCl led to an increase in this uptake, but this is not consistent with Na^{+} dependence and does not seem enough to support the conclusion that this transport is sodium-dependent. Importantly, taking into account the therapeutic use of lithium as a mood enhancer (Malhi et al. 2012) and the first-choice therapy for bipolar disorder, for example, we have to consider this effect on glucose uptake as potentially having a clinical expression.

In addition, the lack of effect of dinitrophenol, an inhibitor of ATP production, supports the conclusion of the energy-independent nature of glucose uptake into these cells.

According to the literature, GLUT1 is the main transporter responsible for glucose transport at the BBB level (Cardoso et al. 2011; Simpson et al. 2007; Uchida et al. 2011). However, the presence of other glucose transporters cannot be excluded. In the present work, the mRNA expression of some glucose transporters, namely, GLUT1, GLUT2, GLUT3 and the sodium-dependent glucose transporter 1 (SGLT1), was quantified; and with the exception of GLUT2, all were found to be expressed by hCMEC/D3 cells. GLUT1 had the highest expression, but surprisingly, GLUT3 also had a very high expression, not even statistically different from GLUT1. GLUT3 is usually associated with glucose uptake at the neuronal level but not at the BBB level (Cardoso et al. 2011; Simpson et al. 2007). However, a recent study described the expression of GLUT3 and GLUT14 in human brain microvessels (Uchida et al. 2011), which is in line with the finding, some years ago, of GLUT3 presence in dog BBB (Gerhart et al. 1995). Nevertheless, one could not exclude a relation of this result with the high energy requirements of these cells due to their potent proliferative nature. Moreover, in a murine cell line, the presence of GLUT8 and GLUT9 was also detected (Cura and Carruthers 2010). Because GLUT3

mRNA is present in a significant amount in hCMEC/D3 cells, it should not be excluded or not taken into consideration when studying glucose transport at the BBB. Interesting, SGLT1 also had remarkable levels of expression. This could be valuable information to fully understand glucose uptake at the BBB level, especially in situations associated with low glucose availability, where SGLT1, as a high-affinity glucose transporter (Ferraris 2001), could have an important role in determining glucose availability to the brain. In this study, DG was used as a substrate, and because this compound is not a good substrate for SGLT1, the contribution of SGLT1 for the studied transport could be neglected. This was confirmed by the functional characterization of [^{3}H]-DG uptake (see above).

Inhibitors of several pathways were studied in order to understand which intracellular signaling pathways may be involved in the regulation of glucose uptake by hCMEC/D3 cells. Recently, it was described that AMP kinase activation directs the trafficking of GLUT1 to the plasma membrane, having a direct role in regulating the glucose transport capacity of the BBB (Cura and Carruthers 2012). Also, according to the literature, Ca^{2+} appears to be involved in GLUT1 translocation and calmodulin is involved in GLUT1 stimulation (Maraldi et al. 2006).

These findings are in line with the literature as Ca^{2+} quelation and the inhibition of calmodulin interfered with [^{3}H]-DG uptake (Maraldi et al. 2006). Specific inhibitors of MAPK pathways were also used, but no effect on [^{3}H]-DG uptake was observed, excluding them from having a role in GLUT regulation. On the other hand, several protein kinases seem to be involved in this transport regulation: PKA and PTK are glucose-uptake inhibitors, and PKC is an activator of this transport. Interestingly, it is known that intracellular Ca^{2+} (Ikeda et al. 1997) and total PKC activation (Fleegal et al. 2005) increase gradually during hypoxia in BBB cells, which could positively influence glucose uptake in these cells in this extreme condition. mTOR has been negatively related with GLUT4 trafficking (Jiang et al. 2008), and in this work the mTOR inhibitor rapamycin increased glucose uptake. This could suggest a possible involvement of this factor in membrane localization of the glucose transporter.

Taking into account that hormone levels have an impact on cognition, learning and memory (Bimonte-Nelson et al. 2010; Foy et al. 2010; Maggio et al. 2012), we recognized the relevance of investigating the effect of several endogenous hormones on [^{3}H]-DG uptake by hCMEC/D3 cells. Of all of the hormones tested, only progesterone and estrone were able to influence [^{3}H]-DG uptake, by decreasing it. Progesterone has been characterized as an activator of GLUT expression in chronic treatment (Frolova et al. 2009; Medina et al. 2004), but herein the opposite effect was observed with acute treatment; one could hypothesize that this cell response could be part of a compensation mechanism. Interestingly, the two hormones that have an effect on [^{3}H]-DG uptake are present in high levels during pregnancy (Brett and Baxendale 2001), which could suggest a relationship between decreased brain glucose levels and memory impairment during this phase (Henry and Rendell 2007).

Polyphenols have received attention from the scientific community due to their beneficial health properties, in particular at the neuronal level (Gutierrez-Merino et al. 2011). Several epidemiological and dietary intervention studies in humans and other animals indicate that polyphenol consumption is important for neuronal health (Assuncao et al. 2011; Krikorian et al. 2010; Spencer 2009a; Youdim et al. 2004). Interference with glucose uptake at the neuronal level, allowing a greater influx of glucose into the brain, could be an important mechanism contributing to the beneficial effect of polyphenols for neuronal health. Nevertheless, there is evidence supporting the extensive biotransformation of polyphenols to different conjugated derivatives (*O*-methylated, glucuronides, sulfates), which would be the main circulating forms, able to reach metabolic targets (Crozier et al. 2009). Two classes of the most abundant flavonoids were tested, and different effects were achieved. The flavan-3-ols catechin and epicatechin did not have any effect on [^{3}H]-DG uptake. However, it was noted that the methylated forms of catechin and epicatechin were able to increase [^{3}H]-DG uptake. Bearing in mind that these are probably among the most abundant forms circulating in vivo, an increase of glucose uptake through the BBB should be expected. The effects of flavonols were in a different direction: both quercetin and myricetin decreased [^{3}H]-DG uptake in a concentration-dependent manner. However, the glucuronic acid conjugate of quercetin, one of the most abundant circulating metabolites of quercetin after ingestion of a quercetin-rich meal (Mullen et al. 2004), produced no effect on [^{3}H]-DG uptake by hCMEC/D3 cells, which could support a reduced impact of quercetin ingestion on glucose uptake.

Overall, this work showed that DG uptake in a human BBB model (1) is sodium- and energy-independent; (2) probably involves GLUT1 and/or GLUT3; (3) is regulated by Ca^{2+}-dependent pathways; (4) is regulated by kinase proteins such as PKC, PKA and PTK; and (5) is regulated by flavonoids but especially by their in vivo methylated metabolites.

This work is expected to contribute to the clarification of glucose transporter modulation by dietary compounds or hormones, which could be critical to supporting nutritional recommendations in the future and to understanding metabolic dysfunctions.

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