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Research Report

Glycogen metabolism in rat ependymal primary cultures: Regulation by serotonin

Stephan Verleysdonk [a,*], Steffen Kistner [a], Brigitte Pfeiffer-Guglielmi [a], John Wellard [a], Adrian Lupescu [b], Julia Laske [c], Florian Lang [b], Mirna Rapp [d], Bernd Hamprecht [a]

[a] *Interfaculty Institute for Biochemistry, University of Tübingen, Hoppe-Seyler-Str. 4, D-72076 Tübingen, Germany*
[b] *Institute of Physiology, University of Tübingen, Gmelinstr. 5, D-72076 Tübingen, Germany*
[c] *Department of Pharmacology and Toxicology, University of Tübingen, Wilhelmstr. 56, D-72074 Tübingen, Germany*
[d] *Aventis Behring GmbH, Emil-von-Behring-Str. 76, D-35041 Marburg, Germany*

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Abstract

Ependymal primary cultures are a model for studying ependymal energy metabolism. Intracellular glycogen is built up in the cultures dependent on culture age and the presence of glucose and glutamate. This energy store is mobilized upon glucose withdrawal, stimulation with isoproterenol, forskolin or serotonin and after uncoupling of oxidative phosphorylation from ATP production. Serotonin regulates ependymal glycogen metabolism predominantly via 5-HT receptor (5-HTR) 7, which elicits an increase in the level of ependymal cyclic AMP. Although the most abundant mRNAs for serotonin receptors are those of 5-HTR 2B and 5-HTR 3A, ependymal cells in primary culture do not respond to serotonin with an increase in their concentration of cytosolic calcium ions. The mRNAs of 5-HTRs 1A, 6, 1B, 5B, 7, 1/2C and 5A are also detectable in order of decreasing abundance. The mRNAs for 5-HTRs 1D, 1F, 3B and 4 are absent from the cultured cells. The ability of serotonin to mobilize ependymal glycogen depends on the culture age and the time allowed for glycogen buildup. During glycogen buildup time, glutamate is consumed by the cells. An increased ability of 5-HT to mobilize ependymal glycogen stores is noticed after the depletion of glutamate from the glycogen buildup medium. In ependymal primary cultures, cilia are colocalized with glycogen phosphorylase isozyme BB, while the MM isoform is not expressed. It is known from the literature that an increase in the concentration of cytosolic cAMP in ependymal cells leads to a decrease in ciliary beat frequency. Therefore, the present data point towards a function for ependymal glycogen other than supplying energy for the movement of cilia.

Theme: Cellular and molecular biology
Topic: Neuroglia and myelin

Keywords: 5-HT; Energy metabolism; Glycogen phosphorylase; Kinocilia

1. Introduction

Glycogen is a cellular energy store which supplies glucosyl residues in times of increased cellular energy demand and regenerates in times of rest. Recent research suggests that this is also true in the brain (for review, see [19]). Although the precise functions of cerebral glycogen have not yet been firmly assessed, it seems to be important for such diverse events like astrocyte activation during ischemia [27], defense against reactive oxygen species [47], maintenance of the wake state [29] and learning [25]. The enzyme necessary for glycogen mobilization, glycogen phosphorylase (GP), occurs in three genetically distinct homodimeric isoforms

* Corresponding author. Fax: +49 7071 29 5360.
E-mail address: Stephan.Verleysdonk@uni-tuebingen.de (S. Verleysdonk).

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named brain (BB), muscle (MM) and liver (LL) types according to the tissues in which they predominate (for review, see [39]). The cells showing the highest level of GP expression throughout the brain are the ependymal cells, in which only the BB isoform is present [43].

Ependymal cells line the ventricles of the vertebrate brain by forming a single-layered, kinocilia-bearing epithelium (for review, see [4]). Their functions are yet to be elucidated but have been speculated to include secretion of molecules into the cerebrospinal fluid (CSF) [34], movement of cellular debris in the direction of bulk CSF flow [5], local mixing of the CSF to optimize dispersion of components in this fluid [48] and formation of a protective barrier between brain and CSF [6]. Ependymal activity appears to be regulated by messenger molecules originating from the varicosities of axons that innervate the ventricular area. Many of these axons are derived from serotoninergic neuronal perikarya in the Raphe nuclei, a complex of cells topographically organized with respect to the ventricular system [37,53]. The ependymal cells respond to the released transmitter serotonin (5-HT) by mobilizing intracellular glycogen stores [45] and increasing the ciliary beat frequency (CBF) [41]. Presumably, the glycogen supplies the glucosyl residues necessary for sustaining the augmented axonemal movement. While increased ciliary motility may be taken as a general sign of ependymal activation, the nature of the energy-requiring processes encompassed in this activity is likely to exceed the mere movement of cell appendages. This is illustrated by lung airway epithelial cells, besides ependymocytes, another type of kinocilia-bearing epithelial cells, which utilize glycogen to acquire the energy and building blocks for surfactant biosynthesis [13]. The study of ependymal glycogen metabolism is therefore expected to lead to a better understanding of ependymal cell function.

Ependymal primary cultures (EPC) have been introduced as a model system for studying ependymal glycogen metabolism [45]. In the current study, we examine the properties of the model with respect to glycogen synthesis in different culture media, GP isotype expression and glycogen degradation under the control of the neurotransmitter 5-HT. The 5-HT receptors (5-HTR) can be grouped into several classes according to their mode of action [31]. The five members (5-HTR 1A to 5-HTR 1F) of the 5-HTR 1 group as well as 5-HTR 5A prevent increases in cellular cAMP levels via the inhibitory G protein G_i. The 5-HTR 2 group (three members) is coupled to G_q and the inositol-1,4,5-trisphosphate signal transduction pathway. The two 5-HTR 3 types are ion channels permeable to Na^+ and Ca^{2+}, and the remaining members 5-HTR 4, 6 and 7 are likely to be all coupled to G_s and to lead to an increase in the level of intracellular cAMP. The mechanism by which 5-HT exerts control over the ependymal glycogen level is investigated.

2. Experimental procedure

2.1. Materials

Minimal Essential Medium (MEM; [10]) was purchased from Invitrogen Life Technologies (Eggenstein, Germany). Waymouth medium (WM, type 705/1; [59]), fatty acid-free bovine serum albumin (BSA), transferrin, insulin, hydrazine dihydrochloride, *R*-4-iodo-2,5-dimethoxyphenylisopropylamine hydrochloride (*R*-DOI) and 6-methyl-(1-methyl-ethyl)ergoline-8β-carboxylic acid 2-hydroxy-1-methylpropyl ester (LY53857) maleate salt were from Sigma (Deisenhofen, Germany). Thrombin was from Aventis Behring (Marburg, Germany). Fibronectin was purified from bovine plasma (obtained at the local abattoir) according to a published procedure [36]. 5-HT hydrochloride was obtained from Alexis Biochemicals (Grünberg, Germany). Carbonyl cyanide 3-chlorophenylhydrazone (CCCP), 8-hydroxy-2-propylaminotetralin (8-hydroxy-DPAT) hydrobromide, 5-carboxamidotryptamine (5-CT) maleate salt, (*S*)-WAY 100135 ((*S*)-*N*-*tert*-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide) and methysergide were from Tocris (via Biotrend, Cologne, Germany). Glutamate dehydrogenase, amyloglucosidase, hexokinase, glucose-6-phosphate dehydrogenase, phosphoglucomutase, ATP and NADP were from Roche Biochemicals (Mannheim, Germany). Fura-2 acetoxymethyl ester and thapsigargin were purchased from Molecular Probes, Göttingen, Germany. All other chemicals were obtained from Merck Eurolab (Bruchsal, Germany). “Basic medium” (BM), a simplified medium for incubations of cell cultures, was prepared by weighing in compounds to give the following concentrations: 135 mM NaCl, 5 mM KCl, 0.8 mM $CaCl_2$, 1.4 mM $MgSO_4$, 1.3 mM Na_2HPO_4, 25 mM $NaHCO_3$. “Falcon” cell culture dishes, 35 mm in diameter, were from Becton Dickinson (Schubert Medizinprodukte, Wackersdorf, Germany). Avian myoblastosis virus reverse transcriptase (RT) was from Peqlab, Erlangen, Germany. Random hexamer primers (Cat. No. C1181), oligo$(dT)_{15}$ primer (Cat. No. C1101), dATP (Cat. No. U1211), dCTP (Cat. No. U1231), dGTP (Cat. No. U1221) and dTTP (Cat. No. U1201) were from Promega, Mannheim, Germany. PAN script NH_4 reaction buffer was from PAN Biotech, Aidenbach, Germany. The LightCycler® instrument, the “FastStart DNA Master Sybergreen” kit and cAMP were from Roche Diagnostics, Penzberg, Germany. [5′,8′-^{3}H]Adenosine 3′,5′-cyclic phosphate, NH_4^+ salt was from Amersham Biosciences Europe, Freiburg, Germany. The “RNeasy” RNA isolation kit was obtained from Qiagen, Hilden, Germany.

2.2. Cytosolic Ca^{2+} imaging

Measurements of the cytosolic Ca^{2+} concentration were performed as described previously [55]. Briefly, the cells were loaded with fura-2 (2.5 μM) at 37 °C for 25 min. Fluorescence measurements were carried out with an inverted