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Research Report

Regulation of Mct1 by cAMP-dependent internalization in rat brain endothelial cells

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ABSTRACT

In the cerebrovascular endothelium, monocarboxylic acid transporter 1 (Mct1) controls blood–brain transport of short chain monocarboxylic and keto acids, including pyruvate and lactate, to support brain energy metabolism. Mct1 function is acutely decreased in rat brain cerebrovascular endothelial cells by β-adrenergic signaling through cyclic adenosine monophosphate (cAMP); however, the mechanism for this acute reduction in transport capacity is unknown. In this report, we demonstrate that cAMP induces the dephosphorylation and internalization of Mct1 from the plasma membrane into caveolae and early endosomes in the RBE4 rat brain cerebrovascular endothelial cell line. Additionally, we provide evidence that Mct1 constitutively cycles through clathrin vesicles and recycling endosomes in a pathway that is not dependent upon cAMP signaling in these cells. Our results are important because they show for the first time the regulated and unregulated vesicular trafficking of Mct1 in cerebrovascular endothelial cells; processes which have significance for better understanding normal brain energy metabolism, and the etiology and potential therapeutic approaches to treating brain diseases, such as stroke, in which lactic acidosis is a key component.

1. Introduction

Cerebral lactic acid levels are dependent on the activity of the monocarboxylic acid transporter 1 (Mct1) in the cerebral microvasculature where it is the only facilitator of lactic acid transport across the blood–brain barrier (Daneman et al., 2010; Enerson and Drewes, 2006). Because lactate is a critical energy substrate that has important roles in brain health and disease, understanding Mct1's physiological regulation in cerebrovascular endothelial cells is of great significance (Gerhart et al., 1997; Smith and Drewes, 2006). This is illustrated by the importance of Mct1 in controlling post-ischemic brain lactate concentrations, a key biochemical indicator of outcomes following stroke and brain injury (Coon et al., 2006; Frykholm et al., 2005; Wagner et al., 1992, 1998; Wass and Lanier, 1996). Mct1 is further implicated in metabolic coupling between astrocytes

Abbreviations: Mct1, monocarboxylic acid transporter 1; 8-BrcAMP, 8-bromo-cyclic 3′,5′-adenosine mono phosphate; cAMP, cyclic 3′,5′-adenosine mono phosphate; db-cAMP, dibutyryl cyclic 3′,5′-adenosine mono phosphate; MbCD, methyl-beta-cyclodextrin; RBE4, rat brain endothelial-4.

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and neurons for brain energy metabolism, for regulating tumor growth, inflammatory responses, and for long term memory consolidation (Belanger et al., 2011; Coon et al., 2006; Erlichman et al., 2008; Frykholm et al., 2005; Murray et al., 2005; Sonveaux et al., 2008; Suzuki et al., 2011; Wagner et al., 1992; Wass and Lanier, 1996).

Previously, we have shown that the function of Mct1 is acutely regulated in a cerebrovascular endothelial cell line (RBE4) by a β-adrenergic receptor-mediated pathway that signals through adenylyl cyclase, cyclic adenosine monophosphate (cAMP), and protein kinase A (PKA). The regulation reduced the Michaelis–Menten parameter V_{max}, but not K_M, consistent with stimulus dependent reduction in the number of functional transporters on the cell surface (Smith and Drewes, 2006). However, the mechanism linking PKA activation to reduced Mct1 function was not elucidated and questions remain regarding Mct1's plasma membrane location, phosphorylation status and trafficking.

PKA-dependent phosphorylation has been well characterized in having direct effects on the gating and kinetic function of various ion channels and transporters. This type of regulation is often associated with changes in K_M; however, this was not observed in our previous studies with RBE4 cells. Alternatively, phosphorylation-dependent vesicular trafficking to and from the plasma membrane also controls the cellular function of a diversity of membrane proteins and transporters, and is a plausible hypothesis for the cAMP-dependent changes in Mct1's V_{max} that we observed (Balklava and Grant, 2005; Ceresa and Schmid, 2000; Dart, 2010; Delom and Fessart, 2011; Melikian, 2004). Included among the transporters that are regulated by vesicular trafficking are monoamine transporters, aquaporins, sodium–hydrogen exchangers, proton pumps, and glucose transporters (Jayanthi et al., 2004; Lau et al., 2009; Lukashova et al., 2011; Moeller et al., 2011; Mortensen et al., 2008; Reinhardt et al., 2002; Yang and Holman, 2006). Vesicular trafficking occurs by different mechanisms including most notably the clathrin and caveolin-dependent vesicular pathways. The caveolar pathway has been particularly well characterized in capillary endothelial cells, where it mediates not only the surface expression of proteins but also transcytosis of molecules between the luminal and abluminal surfaces of the endothelial barrier (Demeule et al., 2000; Frank et al., 2003; Stewart, 2000). Both clathrin- and caveolin-dependent endocytosis deliver protein cargos to various internal compartments including lysosomes, trans-golgi, endoplasmic reticulum, and Rab 11 positive recycling endosomes. The specificity of protein trafficking to these organelles is controlled through processes involving early-sorting endosomes, which are identified by their expression of the small GTPase Rab5, and EEA1. Therefore, we predicted that treatment of RBE4 cells with cAMP analogs would reduce the levels of Mct1 on the plasma membrane and increase Mct1 in clathrin-coated pits or caveoli, early-sorting endosomes, and targeted cytoplasmic organelles. Identification of such a regulatory pathway, and whether direct Mct1 phosphorylation is involved, would greatly increase our understanding of the mechanism linking PKA activation to reduced Mct1 function, and could contribute to the development of new treatments for diseases in which monocarboxylate transport across the cerebral microvasculature is important.

2. Results

2.1. Mct1 phosphorylation and surface expression were reduced by cAMP

Mct1 transport function was reduced by more than 25% when RBE4 cells were treated for 10 min with membrane-permeant cAMP analogs (8-BrcAMP or db-cAMP), a result that is consistent with our previous report (Smith and Drewes, 2006; see also Fig. 6). Because we previously determined that this reduction was PKA-dependent, we assessed whether treatment with cAMP analogs causes a change in Mct1's phosphorylation status. This was achieved by purifying cellular membrane phosphoproteins from treated and untreated RBE4 cell cultures followed by Western blot analysis and densitometry. Under basal conditions, 41% of the Mct1 signal was present in the phosphoprotein fraction and cAMP analogs reduced the signal to 29% (Fig. 1A). This reduction was statistically significant ($p<0.05$), thus, cAMP signaling via PKA does not directly phosphorylate Mct1, but rather reduces the phosphorylation of Mct1.

Because the phosphorylation status of membrane proteins can control their surface expression, and because we previously showed that adrenergic signaling through cAMP decreases Mct1's Michaelis–Menten parameter V_{max} (Smith and Drewes, 2006), we next assessed whether the surface expression of Mct1 was affected by elevated cytoplasmic cAMP. This was accomplished by brief treatment of RBE4 cells with cAMP analogs followed by biotinylation of the cell-surface proteins that were isolated on streptavidin columns and subjected to Western blot detection and densitometry. Treatment resulted in a 16% reduction ($p<0.02$) in the level of Mct1 detected at the cell surface when compared to untreated controls or non-trafficking controls that were treated at 4 °C (Fig. 1B). Therefore, our combined results (Fig. 1) demonstrate that elevated cytoplasmic cAMP causes dephosphorylation of Mct1 and that it becomes internalized from the surface of RBE4 cells. Thus, the cAMP dependent reduction of V_{max} is a result of Mct1 dephosphorylation and internalization.

2.2. Colocalization of Mct1 with caveolin-1, but not clathrin, was increased by cAMP

To explore further the pathway for internalization of Mct1, we used dual immunostaining and fluorescence confocal imaging of Mct1 combined with either clathrin or caveolin-1 in RBE4 cells (Fig. 2). Widespread expression of Mct1 on the plasma membrane and especially at the leading edge of lamellipodia was observed. Mct1 was also detected across the cytoplasmic face in numerous puncta of different sizes and shapes as well as in a large perinuclear compartment that stained very brightly in most cells (Figs. 2 and 3, green). Clathrin-immunoreactivity (Fig. 2A, red) was present in numerous round puncta that were distributed across the cytoplasmic face and in the large perinuclear compartment. Overlap between Mct1 and clathrin (yellow) was present in a subset of the round puncta that appeared to be very close to the plasma membrane, within the cytoplasm, and especially prominent in the brightly staining large central