Atypical L-type channels are down-regulated in hypoxia

L. Callinan*, T.V. McCarthy*, Y. Maulet† and J.J. Mackrill*

†Department of Biochemistry, University College Cork, College Road, Cork, Ireland, and †Neurobiologie des Canaux Ioniques, INSERM U641, Institut Federatif de Recherche Jean Roche, Marseilles, France

Abstract

One type of cellular response to hypoxia is an increase in cytosolic Ca$^{2+}$. VDCCs (voltage-dependent calcium channels) open upon membrane depolarization allowing inward current of Ca$^{2+}$ ions. Two of the so-called L-type VDCC α1 subunits, Ca1.2 and Ca1.3, are found in the brain. We sought to investigate the effect of chronic hypoxia or treatment with a hypoxia-mimicking agent DFX (desferrioxamine mesylate) on expression of L-type VDCC in the SH-SYSY neuroblastoma cell line. Western blotting identified two atypical forms of the L-type channel with apparent molecular masses of approx. 100 and 150 kDa, compared with typical forms of approx. 200 kDa. Immunofluorescence microscopy shows the approx. 100 kDa protein located within the cell and on the cell surface, while the approx. 150 kDa protein is intracellular with punctate staining. Further analysis revealed that this approx. 150 kDa protein co-localizes with nuclear proteins but not with markers for other intracellular compartments. In addition, these proteins are both down-regulated in DFX-treated and hypoxic cells, suggesting that the mechanism of down-regulation is along the HIF (hypoxia-inducible factor) pathway. This atypical localization of the 150 kDa protein suggests that it might play a role in nuclear calcium signalling in health and disease.

Introduction

The brain has the highest metabolic rate of all organs, consuming a large proportion of the body’s oxygen rendering it susceptible to ischaemic conditions. Even transient oxygen deficits can lead to irreversible cellular damage [1]. Disorders that result from ischaemia include stroke, myocardial infarction and cerebral palsy. Reports have revealed that disorders susceptible to ischaemic conditions. Even transient oxygen deficits can lead to irreversible cellular damage [1]. Disorders that result from ischaemia include stroke, myocardial infarction and cerebral palsy. Reports have revealed that disorders susceptible to ischaemic conditions.

The Cav1.2 and Cav1.3 subunits are found in the brain. We sought to investigate the effect of chronic hypoxia on expression of L-type calcium channels in the SH-SYSY neuroblastoma cell line.

Methods

Cells were cultured under normoxic or hypoxic conditions as described previously by another laboratory [2]. Cells were also treated with 100 µM DFX (desferrioxamine mesylate), a hypoxia-mimicking agent, for 24 h prior to harvesting. SDS/PAGE and electroblotting on to nitrocellulose of treated and untreated cell lysates was carried out according to previously described standard procedures [4]. Membranes were incubated with primary antibodies; either anti-human Ca1.2 against the N-terminus of the Ca1.2 isoform or Ab70 directed against the N-terminus of both the Ca1.2 and Ca1.3 isoforms [4]. The secondary antibody was rabbit IgG peroxidase conjugate. Immunofluorescence microscopy was also carried out according to a previously described method [5]. In brief, cells were grown on 10 mm round glass coverslips and fixed with methanol at −20°C. Primary antibodies used were as described above, secondary antibodies were Cy2-conjugated donkey anti-rabbit IgG and Cy3-conjugated donkey anti-mouse IgG.

Results

Using Western blotting, we examined the expression of L-type VDCC in SH-SYSY cell lysates. Two atypical forms of the channel were identified. A protein of approx. 150 kDa was detected by Ab70, while anti-Ca1.2 recognized a protein of approx. 100 kDa. In addition to being of

Key words: calcium channel, desferrioxamine mesylate (DFX), hypoxia, L-type channel, nucleus, SH-SYSY neuroblastoma cell line, voltage-dependent calcium channel (VDCC).

Abbreviations used: DFX, desferrioxamine mesylate; HIF-1, hypoxia-inducible factor 1 complex; SERCA, sarcoplasmic/endoplasmic-reticulum Ca$^{2+}$-ATPase; VDCC, voltage-dependent calcium channel.

*To whom correspondence should be addressed (email lauracallinan@yahoo.com).
Figure 1 | Down-regulation of L-type calcium channels

Lysates extracted from normal (lanes 1 and 3), hypoxic- (lane 2) and DFX-treated (lane 4) cells were resolved by SDS/PAGE (7.5% polyacrylamide), transferred on to nitrocellulose and probed with antibodies specific for L-type VDCC (Ab70 and anti-Ca1.2). This demonstrates the atypical apparent molecular masses of these L-type VDCCs as well as their down-regulation by hypoxia and DFX treatment. SERCA is used as a loading control and each blot is representative of at least three experiments.

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lower molecular mass than expected, these proteins were also both down-regulated in hypoxic- and DFX-treated cells (Figure 1). The SERCA (sarcoplasmic/endoplasmic reticulum Ca2+-ATPase) pump is used as a loading control as its expression is not altered with treatment. Using immunofluorescence microscopy, the subcellular localization of these proteins was investigated (Figure 2). The approx. 100 kDa protein recognized by anti-Ca1.2 appears to be localized at the surface of the cell (Figure 2A). However, the approx. 150 kDa protein has an unexpected localization, since one would expect it to be detected in a pattern similar to the approx. 100 kDa protein. Instead, this protein is intracellular with distinct punctate staining (Figure 2B). Further analysis has revealed that this protein is not detected at the plasma membrane or within the endoplasmic reticulum, mitochondria, Golgi or the recycling pathway. It does however appear to be localized within the nucleus of the cell.

Discussion

Hypoxia is manifested by significant disturbances in ionic homoeostasis and large increases in cell calcium levels have been recorded. In comparison with other channel types, L-type VDCC activity displays a particularly high sensitivity to hypoxia. Calcium plays an important role in the regulation of the channel, with involvement in both activation and inactivation [3,6]. Here we report the down-regulation of the L-type channel in chronically hypoxic cells, and this result was found to be mimicked in DFX-treated cells. DFX is a hypoxia-mimicking agent that inhibits HIF-1α (hypoxia-inducible factor 1α complex) degradation allowing HIF-1α to translocate to the nucleus, bind to HIF-1β and form HIF-1. This is a transcriptional activator of many genes including erythropoietin and vascular endothelial growth factor [3]. The down-regulation of the L-type channel in both hypoxic- and DFX-treated cells indicates that the mechanism of down-regulation is via the HIF pathway and may act as a neuroprotective mechanism in hypoxia. The proteins detected by the antibodies are also of a lower molecular mass than the full-length channel; this may be due to alternative splicing, initiation or post-translational modifications [7]. Further analysis is required to determine the primary structures of the channels detected. Immunofluorescence microscopy experiments localized the protein detected by Ca1.2 to the cell surface but the approx. 150 kDa protein was intracellular, it did not co-localize with markers for the endoplasmic reticulum, Golgi, plasma membrane, recycling pathway (transferrin) or the mitochondria. However, immunofluorescence microscopy does indicate its presence in the nucleus. Additional analysis is required to determine its exact location within the nucleus as well as its function.

Conclusions

Two atypical L-type channels were down-regulated by hypoxia in a neuroblastoma cell line; the mechanism of this down-regulation is potentially via the HIF pathway. Subcellular localization of one of these channels indicates its presence in the nucleus of the cell, allowing for its possible involvement in nuclear calcium signalling.
References

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