Neuroscience Letters 508 (2012) 114–118

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Interleukin-1beta does not affect the energy metabolism of rat organotypic hippocampal-slice cultures

Raman Saggu[a,*], Barclay Morrison III[b,1], John P. Lowe[a,2], Ashley K. Pringle[b,3]

[a] Department of Biochemistry, South Parks Road, University of Oxford, South Parks Road, OX1 3QU, UK
[b] Centre for Neuroscience, University of Southampton, Boldrewood Building, Bassett Crescent East, Southampton SO16 7PX, UK

ARTICLE INFO

Article history:
Received 28 September 2011
Received in revised form
16 December 2011
Accepted 19 December 2011

Keywords:
Organotypic hippocampal-slice culture
Interleukin-1beta
Apparent diffusion coefficient
Phosphorus magnetic resonance spectroscopy
Energy metabolism
Brain

ABSTRACT

The aim of this study was to examine the effect of the archetypal pro-inflammatory cytokine, interleukin-1beta (IL-1β), on high-energy phosphate levels within an *ex vivo* rat organotypic hippocampal-slice culture (OHSC) preparation using phosphorus (^{31}P) magnetic resonance spectroscopy (MRS). Intrastriatal microinjection of IL-1β induces a chronic reduction in the apparent diffusion coefficient (ADC) of tissue water, which may be indicative of metabolic failure as established by *in vivo* models of acute cerebral ischaemia. The OHSC preparation enables examination of the effects of IL-1β on brain parenchyma *per se*, independent of the potentially confounding effects encountered *in vivo* such as perfusion changes, blood–brain barrier (BBB) breakdown and leukocyte recruitment. ^{31}P MRS is a technique that can detect multiple high-energy phosphate metabolites within a sample non-invasively. Here, for the first time, we characterise the energy metabolism of OHSCs using ^{31}P MRS and demonstrate that IL-1β does not compromise high-energy phosphate metabolism. Thus, the chronic reduction in ADC observed *in vivo* is unlikely to be a consequence of metabolic failure.

1. Introduction

The pro-inflammatory cytokine, interleukin-1beta (IL-1β), is implicated in the pathogenesis of several neurological diseases including stroke, Alzheimer's disease (AD) and Parkinson's disease (PD) (for review, see [1]). Intrastriatal microinjection of IL-1β *in vivo* induces a chronic reduction in the apparent diffusion coefficient (ADC) of tissue water on magnetic resonance imaging (MRI) [9]. In acute cerebral ischaemia, reduced ADC is associated with compromised cerebral blood flow (CBF) and correlates with the loss of high-energy phosphorus metabolites detected using phosphorus magnetic resonance spectroscopy (^{31}P MRS) [6,12,13,24,25,36]. Reduced oxygen and glucose delivery by the circulation leads to a decline in intracellular ATP synthesis, precipitating the failure of ATP-dependent ion pumps such as the Na^+/K^+-ATPase that regulate cell volume (for review, see [5]). The increase in intracellular Na^+ concentration promotes the osmotically driven movement of water from the extracellular to intracellular compartment [8] causing cell swelling, reducing the extracellular space, and the ADC [17,21,38].

Interestingly, despite inducing a persistently depressed ADC, classically associated with energy failure, IL-1β induces intraparenchymal vessel dilation [4] and increases regional cerebral blood volume (rCBV), with no evidence of an increase in lactate [9], which appears to be inconsistent with an ischaemic event. However, the ^{31}P MRS-detectable energy status of the brain parenchyma when ADC first becomes reduced following IL-1β challenge has not previously been examined, and may assist in clarifying the origins of these tissue water diffusion changes.

It can be difficult to decipher the precise role of IL-1β on central nervous system (CNS) pathophysiology, and *in vivo* studies are complicated by IL-1β-mediated changes in cerebral perfusion, blood–brain barrier (BBB) breakdown, and leukocyte recruitment [3,4,9]. Dissociated neuronal and/or astrocytic cultures also present a significant drawback in that they do not mimic *in vivo* cellular

Abbreviations: OHSC, organotypic hippocampal-slice culture; IL-1β, interleukin-1beta; ADC, apparent diffusion coefficient; ^{31}P MRS, phosphorus magnetic resonance spectroscopy; BBB, blood–brain barrier; OGD, oxygen-glucose deprivation; ATP, adenosine triphosphate; PCr, phosphocreatine; CK, creatine kinase; γ, gamma; MRS, magnetic resonance spectroscopy; ^{31}P, phosphorus; CBV, cerebral blood volume.

* Corresponding author.
E-mail addresses: raman.saggu@wolfson.oxon.org (R. Saggu), bm2119@columbia.edu (B. Morrison III), j.lowe@bath.ac.uk (J.P. Lowe), akp1@soton.ac.uk (A.K. Pringle).
[1] Present address: Biomedical Engineering, Columbia University, 351 Engineering Terrace, MC 8904, 1210 Amsterdam Avenue, New York, NY 10027, United States.
[2] Present address: University of Bath, Bath, BA2 7AY, UK.
[3] Present address: Faculty of Medicine and Health and Life Sciences, School of Medicine, Clinical Neurosciences, Southampton General Hospital, Southampton SO16 6YD, UK.

0304-3940/$ – see front matter
doi:10.1016/j.neulet.2011.12.032

interactions of heterogeneous cell populations. However, organotypic hippocampal-slice cultures (OHSCs) advantageously preserve the synaptic connectivity and cellular organisation of the rodent hippocampus without the complexity of a functional vascular component [34]. In the present study, the OHSC model was utilised to examine the metabolic response of brain parenchyma *per se* to IL-1β challenge, independent of confounding factors related to the vasculature encountered *in vivo* such as perfusion changes, BBB breakdown and leukocyte recruitment.

Phosphorus metabolism is essential to the energy status of all cells. The ^{31}P nucleus is used in MRS to detect intracellular levels of phosphocreatine (PCr) and adenosine triphosphate (ATP) non-invasively under physiological or pathological conditions. Creatine kinase (CK) is a high-activity enzyme that maintains the following reaction, weighted strongly in the favour of ATP synthesis (equilibrium constant, $K_{eq} = 1.66 \times 10^9$), at near-equilibrium essentially at all times [22,33,35]:

$$H^+ + ADP + PCr \overset{CK}{\longleftrightarrow} Cr + ATP.$$

Metabolic biochemists consider the PCr to ATP ratio to be an important indicator of the energy status of any tissue containing PCr and displaying an aerobic metabolic capacity [19]. Thus, ^{31}P MRS-detectable PCr to ATP ratios were measured in control, oxygen-glucose deprivation (OGD)-challenged and IL-1β-challenged OHSCs.

2. Methods

2.1. Preparation of organotypic hippocampal-slice cultures (OHSCs)

OHSCs were prepared based on the method described by Stoppini et al. [34]. Briefly, eight to ten-day-old male Wistar rat pups (bred in-house, Biological Services Unit, University of Southampton) were decapitated without anaesthesia, and the hippocampi bluntly dissected out. Transverse sections (400 μm) were prepared using a McIlwain tissue chopper and separated under ice-cold Gey's balanced salt solution (supplemented with 5 mg/ml glucose and 1.5% fungizone). Slices were plated onto semi-porous membranes (Millipore, Watford, UK) at 9 per membrane and maintained at 37 °C, in 5% CO_2. The maintenance medium consisted of 50% minimum essential medium supplemented with Earle's salts (MEM), 25% Hanks' balanced salt solution (HBBS) and 25% heat-inactivated horse serum (ICN Flow, High Wycombe, UK) supplemented with 5 mg/ml glucose, 1 mM glutamine, and 1.5% fungizone. The media was changed every 3 or 4 days and experiments were performed after 14 days *in vitro* (DIV). Cultures were randomly placed into treatment and control groups, each comprising 10 membranes/90 slices obtained from four different animals.

2.2. The OHSC challenges

2.2.1. Oxygen-glucose deprivation (OGD)

OHSCs were challenged with oxygen-glucose deprivation (OGD) by rapidly replacing the maintenance medium with glucose-free medium [100% glucose-free MEM (Life Technologies) supplemented with 1 mM glutamine and 1.5% fungizone] saturated with 95% N_2:5% CO_2. The culture plates were sealed inside an airtight incubation chamber in which the atmosphere was saturated with 95% N_2:5% CO_2 for 10 min. The chamber was then transferred to an incubator for a further 50 min. Negative controls comprised cultures transferred to fresh maintenance medium and incubated for an equivalent period.

2.2.2. Interleukin-1beta (IL-1β) challenge

All IL-1β experiments were performed in a filtered composite of serum-free medium (SFM) [containing 75% MEM, 25% HBBS, 5 mg/ml glucose, supplemented with 1 mM glutamine and 1.5% fungizone] and bovine serum albumin (BSA) [0.1% solution]. Cultures were incubated with IL-1β, 100 ng/ml for 6 h. Time points were chosen based on published data reporting a reduction in ADC 6 h following intrastriatal IL-1β challenge [9]. Negative controls comprised of cultures transferred to and incubated in SFM and BSA mixture for an equivalent period.

2.3. Preparation of OHSCs for phosphorus (^{31}P) magnetic resonance spectroscopy (MRS)

When ready for harvesting, cultures were snap-frozen in liquid nitrogen and the metabolites extracted using the PCA extraction method. Once freeze-dried, the sample was re-suspended in 400 μl of deuterium oxide (D_2O), vortexed and centrifuged at 3500 rpm for 10 min in a chilled centrifuge. The supernatant was transferred to a 1.5 ml eppendorf. The sample was transferred to a 5 mm diameter NMR tube for ^{31}P MRS. The residue was stored at −80 °C.

2.4. Magnetic resonance sequences and parameters for ^{31}P MRS of OHSCs

Phosphorus spectra were acquired on a 400 MHz vertical bore spectrometer (Varian Inova Plus) at a ^{31}P frequency of 161 MHz. The following parameters were applied: 90° pulse width, 2.5 s inter-pulse delay, 22,000 scans at 30 °C.

2.4.1. ^{31}P MRS spectral analysis

^{31}P MRS spectral data was exponentially line broadened (20 Hz), Fourier transformed, manually phase-corrected and analysed using the 1D WIN NMR (Bruker-Franzen Analytek GmbH) programme. Phosphorus resonances were assigned with reference to published chemical shift data. Spectral peak areas for phosphocreatine (PCr) and gamma adenosine triphosphate (γATP) were calculated. The energy status of tissue in control and test experiments was compared using the PCr to γATP ratio. PCr and γATP saturation areas were corrected using their respective T_1 relaxation times (PCr = 2.75 s, γATP = 0.74 s) and their ratios calculated from the T_1-corrected values.

2.5. ATP assay

Tris buffer was prepared as described [15]. Spectrophotometric determination of the ATP concentration was performed according to manufacturer's instructions (Abcam assay kit).

2.6. Statistical analysis

Statistical analysis was performed using the GraphPad InStat programme with unpaired *t*-tests of *p* value <0.05 considered significant. Results are expressed as mean ± SD.

3. Results

Typical ^{31}P MRS spectra from control OHSCs (Fig. 1a) exhibit peaks from the phosphomonoesters (PME): phosphoryl ethanolamine (PEth) and phosphoryl choline (PCho); inorganic phosphate (Pi), which is the breakdown product of the phosphorus energy metabolites; the phosphodiesters (PDE); phosphocreatine (PCr), and the three resonances of the gamma (γ), alpha (α) and beta (β) phosphates of adenosine triphosphate (ATP).

OGD challenge (Fig. 1a) induced a significant reduction in the PCr to γATP ratio ($p = 0.021$; unpaired *t*-test), as determined by ^{31}P