European Journal of Pharmacology 659 (2011) 259–264

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Immunopharmacology and Inflammation

Fructose 1,6 biphosphate administration to rats prevents metabolic acidosis and oxidative stress induced by deep hypothermia and rewarming

Norma Alva [a,*], Teresa Carbonell [a], Teresa Roig [b], Jordi Bermúdez [b], Jesús Palomeque [a]

[a] Departament de Fisiologia (Biologia), Universitat de Barcelona, Barcelona, Spain
[b] Departament de Ciències Fisiològiques II, Universitat de Barcelona, Barcelona, Spain

ARTICLE INFO

Article history:
Received 29 October 2010
Received in revised form 16 February 2011
Accepted 9 March 2011
Available online 2 April 2011

Keywords:
Hypothermia
Rewarming
Acidosis
Fructose 1,6-biphosphate
Oxidative stress
Nitric oxide

ABSTRACT

Fructose 1,6 biphosphate (F1,6BP) exerts a protective effect in several *in vitro* models of induced injury and in isolated organs; however, few studies have been performed using *in vivo* hypothermia. Here we studied the effects of deep hypothermia (21 ºC) and rewarming in anaesthetised rats after F1,6BP administration (2 g/kg body weight). Acid–base and oxidative stress parameters (plasma malondialdehyde and glutathione, and erythrocyte antioxidant enzymes) were evaluated. Erythrocyte and leukocyte numbers in blood and plasma nitric oxide were also measured 3 h after F1,6BP administration in normothermia animals. In the absence of F1,6BP metabolic acidosis developed after rewarming. Oxidative stress was also evident after rewarming, as shown by a decrease in thiol groups and in erythrocyte superoxide dismutase, catalase and GSH-peroxidase, which corresponded to an increase in AST in rewarmed animals. These effects were reverted in rats treated with F1,6BP. Blood samples of F1,6BP-treated animals showed a significant increase in plasma nitric oxide 3 h after administration, coinciding with a significant rise in leukocyte number. F1,6BP protection may be due to the decrease in oxidative stress and to the preservation of the antioxidant pool. In addition, we propose that the reduction in extracellular acidosis may be due to improved tissue perfusion during rewarming and that nitric oxide may play a central role.

1. Introduction

Fructose 1,6 biphosphate (F1,6BP) is a metabolite commonly found in cells. Although it was first studied as an intermediate of the glycolytic pathway, several regulatory functions and protective effects have recently been attributed to this molecule. Exogenous F1,6BP exerts protective effects in a wide variety of animal models, ranging from cell cultures, isolated tissues or organs to *in vivo* models. Regarding the metabolic effects of this metabolite, evidence points to a multitarget effect. The most consistent effects of F1,6BP include the following: it decreases ATP consumption by increasing metabolic efficiency (Espanol et al., 1998); limits passive and active membrane transport (ion channel arrest) (Roig et al., 1997; Cuesta et al., 2006); exerts chelating properties (decreasing intracellular calcium) thus regulating cell death (increasing or decreasing apoptosis) (Calafell et al., 2009); and even modulates second messenger release by decreasing cytokine secretion.

These beneficial effects are specific for F1,6BP because the use of fructose 1 phosphate or 6 phosphate fails to induce protection (de Oliveira et al., 2007).

This protection has been demonstrated against ischemia reperfusion damage in heart, intestine, brain (Cardenas et al., 2000), liver (Sano et al., 1995) and also against other insults like galactosamine-induced hepatitis, and cold-induced damage during the preservation of isolated organs (Cuesta et al., 2006).

During transplantation, organs are injured by cold storage, ischemia, reoxygenation, and, once implanted, by rewarming. F1,6BP has been found to protect against cold-induced damage in isolated organs when it is added to the preservation medium. We propose that F1,6BP also prevents oxidative stress induced by deep hypothermia–rewarming *in vivo*. In a previous assay we described that rewarming rats from 21 °C hypothermia induces pronounced oxidative damage in liver, by increasing lipid peroxidation products and decreasing the antioxidant pool (also causing acute metabolic acidosis) (Alva et al., 2009).

Although many studies have addressed the protective effect of F1,6BP in cell cultures and isolated organs, few have examined hypothermia *in vivo*. Moreover, there are no reports describing the acid–base impact of F1,6BP in spite of the importance of this parameter during surgery.

In critical clinical protocols (cardiopulmonary bypass and intracranial surgery) deep hypothermia is often used even the side-effects include oxidative damage. Given the therapeutic potential of F1,6BP, here we designed an assay to test whether F1,6BP pre-treatment confers protection against the oxidative stress induced by deep hypothermia/

* Corresponding author at: Departament de Fisiologia (Biologia), Universitat de Barcelona, Avda Diagonal 645, 08028 Barcelona, Spain. Tel.: +34 93 4039635; fax: +34 93 4110358.
E-mail address: nvalva@ub.edu (N. Alva).

0014-2999/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2011.03.034

rewarming. Previous studies have established that hypothermia and rewarming protocol have an impact on acid base status, hence, the parameters of acid–base balance were continuously recorded during cooling and rewarming.

Recently, the protective role of F1,6BP has been associated to nitric oxide (NO) overproduction in isolated hepatocytes injured by galactosamine (Calafell et al., 2009). But to our knowledge there are not evidences of NO response to FBP administration *in vivo*. Considering the powerful vasodilatory role of NO and its free radical nature, the concentrations of NO during 3 h post F1,6BP administration have been analysed.

2. Material and methods

The experimental protocol was reviewed and approved by the Institutional Committee of Animal Care and Research of the University of Barcelona in accordance with European Community guidelines. Sprague–Dawley rats (200–250 g body weight) were maintained in a controlled environment with free access to water and food, under a 12 h light–dark cycle and at a room temperature of 20 °C.

All animals were fasted overnight and given tap water *ad libitum* before surgery. Animals were anaesthetised by sodium pentobarbital (60 mg/kg body weight intraperitoneally).

At the end of the protocol all animals were killed by anaesthesia overdose.

The study included two experiments (Fig. 1). The first experiment was designed to test whether F1,6BP protects against hypothermia- and rewarming-induced damage whilst the second one tested if NO production is induced by acute F1,6BP treatment in an *in vivo* model. Variations in the concentration of plasma NO during 3 h after F1,6BP administration were monitored in normothermia in order to avoid hypothermia-induced interferences.

2.1. Effects of F1,6BP administration during hypothermia and rewarming

Rats were placed on a cooling/rewarming device as described elsewhere (Alva et al., 2009). After cannulation of the right common carotid artery, a polyethylene catheter (PE 50) was inserted for blood sampling and acid–base monitoring. Tracheal intubation was then performed and respiration was supported by a volume and frequency controlled small-animal respirator (Harvard Apparatus Limited, Kent, United Kingdom) using room air. Whilst cooling and rewarming the animals, we adjusted the respiratory frequency following alpha stat principles to maintain arterial pH close to 7.4 and P_{CO_2} between 4.0 and 5.0 kPa at 37 °C (Romsi et al., 2003). A small longitudinal incision (2–3 cm) was made in the abdominal cavity and loops of coil polyethylene tube connected to a cold/warm circulating pump were placed into the cavity and under the surgery table. Two thermocouples were inserted: one next to the carotid artery (internal neck temperature) and the other near the liver, inside the peritoneal cavity (abdominal temperature). The average of the two was considered core temperature. The time interval from anaesthesia induction until the end of surgery never exceeded 45 min.

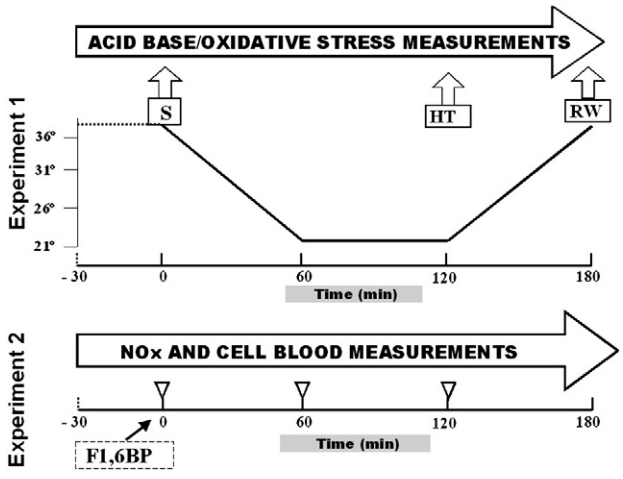

Fig. 1. Experimental design. Experiment 1 was designed to test the effects of F1,6BP on hypothermia and rewarming. It is shown in the upper scheme. Arrows represent the time at which the animals in each group were killed. S, sham; HT, hypothermia; and RW, rewarming. Experiment 2 was designed to measure the haematological parameters and NOx levels in normothermia animals during 3 h post F1,6BP administration. It is shown in the lower scheme. Empty inverted triangles represent blood extractions during the experiment.

Thirty-six animals were divided into two groups: control and F1,6BP-treated rats receiving 1 ml of saline or F1,6BP solution (2 g/kg of body weight as described elsewhere) (Gamez et al., 2008) respectively by intraperitoneal injection 30 min before anaesthesia induction.

The experimental design is summarised in Fig. 1. In the first experiment, each group was further divided and subjected to 3 protocols:

In the sham group, animals were killed immediately after surgery. In the hypothermia (HT) group, animals were cooled for 1 h and kept in cold (21 °C) for another h. In the rewarming (RW) group, animals first underwent the hypothermia protocol and were then warmed to 37 °C.

Capillary blood samples (40 µl) were obtained every 30 min for arterial blood gas monitoring.

The percentage of oxyhemoglobin was determined using hemoximeter (OSM3, Radiometer, Copenhagen, Denmark). Partial pressures of arterial blood gases P_{O_2}, P_{CO_2}, and pH were measured with a microgas analyser Radiometer ABL 5 acid–base laboratory (Radiometer, Copenhagen, Denmark) at 37 °C and then corrected to body temperature. Calculations were made following Severinghaus (1979) for P_{O_2}, and using the nomograms by Kelman and Nunn (1966) for P_{CO_2}. We selected three parameters: pH, the relative alkalinity ratio ($[OH^-]/[H^+]$) and standard excess of buffer bases (SBE), calculated by Severinghaus' alogarithms, as the main acid–base balance indicators. Ionic concentrations of sodium (Na^+) and chloride (Cl^-) were measured by an electrolyte-metabolite analyser (EML100 Radiometer, Copenhagen, Denmark) in heparinised blood samples.

Respiratory frequency was adjusted during cooling and rewarming to keep P_{CO_2} values (measured at 37 °C) constant following alpha stat strategy of blood gas management (Duebener et al., 2002). F1,6BP-treated and non-treated animals were subjected to the same ventilatory conditions, which were established on the basis of body weight.

2.1.1. Sample processing

Arterial blood samples were centrifuged at 1000×*g* for 10 min at 4 °C to separate plasma from erythrocytes.

The concentration of plasma aspartate aminotransferase (AST) was used as an indicator of tissue injury. AST enzymatic activity was determined using commercial kits from Merck (3397 Merckotest). Results were expressed as IU/ml.

Plasmatic pro-oxidant status was measured by the formation of malondialdehyde as determined by thiobarbituric acid-reactive substances concentration (TBARS), following Yagi's technique with minor modifications (Yagi, 1984). The chain breaking antioxidant butylated hydroxytoluene (BHT) and the iron chelator EDTA were added to fresh plasma samples to prevent peroxidation amplification during the assay. Results were expressed as concentration in µM compared to a standard obtained by acid hydrolysis of tetraethoxypropane.

The antioxidant status of plasma samples was estimated by measuring the oxidation of glutathione and other protein thiol groups using spectrophotometric determination at 414 nm, following Hu (1994). Total thiol groups were expressed in µM.