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Label-free quantitative proteomics of rat hypothalamus under fever induced by LPS and PGE_2

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ABSTRACT

Fever is a brain-mediated increase in body temperature mainly during inflammatory or infectious challenges. Although there is considerable data regarding the inflammation pathways involved in fever, metabolic alterations necessary to orchestrate the complex inflammatory response are not totally understood. We performed proteomic analysis of rat hypothalamus using label-free LC–MS/MS in a model of fever induced by lipopolysaccharide (LPS) or prostaglandin E_2 (PGE_2). In total, 7021 proteins were identified. As far as we know, this is the largest rat hypothalamus proteome dataset available to date. Pathway analysis showed proteins from both stimuli associated with inflammatory and metabolic pathways. Concerning metabolic pathways, rats exposed to LPS or PGE_2 presented lower relative abundance of proteins involved in glycolysis, pentose phosphate pathway and tricarboxylic acid cycle. Mitochondrial function may also be altered by both stimuli because significant downregulation of several proteins was found, mainly in complexes I and IV. LPS was able to induce downregulation of important proteins in the enzymatic antioxidant system, thereby contributing to oxidative stress. The results offered comprehensive information about fever responses and helped to reveal new insights into proteins potentially involved in inflammatory signaling and metabolic changes in the hypothalamus during systemic LPS and central PGE_2 administration.

Significance: The evolutionary persistence of fever, despite the elevated cost for maintenance of this response, suggests that elevation in core temperature may represent an interesting strategy for survival. Fever response is achieved through the integrated behavioral, physiological, immunological and biochemical processes that determine the balance between heat generation and elimination. The development of such complex response arouses interest in studying how the cell metabolism responds or even contributes to promote fever. Our results offered comprehensive information about fever responses, including metabolic and inflammatory pathways, providing new insights into candidate proteins potentially involved in inflammatory signaling and metabolic changes in the hypothalamus during fever induced by systemic LPS and central PGE_2 perturbation.

1. Introduction

Fever is a complex physiological response developed against inflammatory or infectious insults [1–3]. Several evidence indicates that the controlled increase in body temperature, characteristic of febrile response, is associated with improvement in immunity and, consequently, increase of survival and resolution of infections [3, 4]. However fever is not always beneficial, since uncontrolled fever is associated with worse prognostics in patient that present sepsis or neuronal injury [4, 5].

The global analgesics market, including antipyretics, is expected to earn US$ 26.4 billion by 2022 as forecast by Allied Market Research.

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The forecast for the market compound annual growth rate (CAGR) is of 7.1% during 2015–2022. The pharmaceutical arsenal for control of febrile response includes nonsteroidal anti-inflammatory/antipyretic drugs (NSAIDs, such as ibuprofen, aspirin and indomethacin), acetaminophen (paracetamol) and metamizol (dipyrone) [2]. Aspirin is one of the most used medications globally, with over 44,000 tons (50 to 120 billion pills) consumed per year [6]. To its turn, paracetamol is the most widely employed medication for pain and fever in both the United States and Europe [7]. The principal action of these drugs rests in their ability to inhibit cyclooxygenase (COX) activity, and to interrupt the synthesis of inflammatory prostaglandins [2]. Besides, COX-independent mechanisms such as reduction in proinflammatory mediators and increase in anti-inflammatory signaling were also attributed to NSAIDs action [8]. For dipyrone or paracetamol, there is a possibility for other central mediators involvement in addition to PGE_2 [2, 9]. Although the complex action mechanism of antipyretics are well investigated in general, the indications for their correct clinical applications for a better prognosis are less clear [8]. The comprehension of the proteins and metabolic pathways involved in mechanisms of fever production is fundamental to understand this complex response, and may consequently facilitate the development of new, more specific drugs by biotechnological and pharmaceutical research groups and companies.

Systemic administration of lipopolysaccharide (LPS) is widely used in experimental models to induce fever, since it reproduces what naturally occurs during infectious and inflammatory processes [2]. LPS binding to the toll-like receptor (TLR) member TLR4, presents in cells from both peripheral and central nervous system [10, 11], triggers an intracellular cascade of events, from activation of transcription factors up to production and release of pro-inflammatory mediators, such as cytokines, chemokines and prostaglandins. Such molecules work together in a cascade of interacting mediators that coordinate the response for fever development [1, 12].

Stimulation of macrophages by LPS induces a rapid transcription (0.5 to 2.0 h) of several genes related to inflammation [10]. This response is regulated by transcription factors that are constitutively expressed by many cell types, activated post-translationally by TLR signaling, such as nuclear factor-κB (NFκB), IFN-regulator factors (IRFs), activator protein 1(AP-1) and cAMP-responsive-element-binding protein 1 (CREB1) [10, 13, 14]. In the brain, NFκB and the signal transducer and activator of transcription-3 (STAT3) are the first inflammatory transcription factors induced by systemic challenge with LPS (1 to 2 h) [15–17]. These transcription factors are involved in the regulation of cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1) [15, 16, 18], both responsible for the production of prostaglandin E_2 (PGE_2), a metabolite of arachidonic acid (AA) [2, 19] and the main mediator of fever induced by LPS [20–22].

It is widely accepted that the central mechanism of fever induction is triggered by the action of PGE_2 on EP3 receptors (EP3R) expressed on neurons in preoptic area of the anterior hypothalamus, the brain structure responsible for controlling thermoregulatory mechanisms in normal and febrile response [21, 23, 24]. Multiple splice variants of EP3R allow the coupling to several G proteins, triggering different signaling pathways that, in turn, lead to the activation of peripheral mechanisms such as cutaneous vasoconstriction and brown adipose tissue thermogenesis to ultimately increase body temperature [25, 26]. Despite this, the knowledge about the exact downstream signaling activated by PGE_2/EP3R interaction is not completely elucidated. It was recently demonstrated that febrile response to peripheral inflammation induced by LPS is dependent on local synthesis of PGE_2 in its target hypothalamic neurons and not on the overall PGE_2 production in the brain [20].

Although there is a considerable amount of data regarding the inflammatory pathway involved in febrile response, the exact mechanisms of downstream PGE_2 production for the stimulation of innate and adaptive immune responses are not totally understood. Since there is an overlap between systemic physiology and neuronal circuitry in the febrile response, there is growing interest in studying how the cell metabolism responds to and interacts with the inflammatory process. Therefore, the analysis of whole proteome changes in hypothalamus after LPS or PGE_2 exposure may provide advanced and comprehensive answers to these questions. In this study, we compared inflammatory and metabolic changes during fever induced by a peripheral, LPS, and a central, PGE_2, pyrogenic stimuli. Using combined mass spectrometry-based proteomic analysis with label-free quantification, it was possible to identify proteins, many of them not previously described in the present experimental setting, as well as signaling pathways associated with febrile response.

2. Methods

2.1. Animals

Animals were obtained from the Animal House of the Institute of Biological Sciences, University of Brasilia. Experiments were conducted using male Wistar rats (180 ± 20 g body weight), housed in a constant temperature room (24 ± 1 °C) and 50% humidity on an 12:12 h light/dark cycle (lights on at 7 am), with free access to food and water. Each animal was used only once. The study was approved by the Animal Research Ethics Committee of the University of Brasília (Protocol. nr. 30,652/2014). The care and use of the animals were in full compliance with the Guide for the Care and Use of Laboratory Animals of the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and the Guide for the Care and Use of Laboratory Animals of the Institute for Laboratory Animal Research [27].

2.2. Intracerebral cannula implantation

For intracerebroventricular (i.c.v.) injections, a 22-gauge stainless steel guide cannula (0.7 mm OD, 10 mm in length) was stereotaxically (Insight Equipments®, Ribeirão Preto, Brazil) implanted into the right lateral ventricle under ketamine (60 $mg.kg^{-1}$) and xylazine (10 $mg.kg^{-1}$) anesthesia under aseptic conditions. The stereotaxic coordinates were 1.6 mm lateral to the midline, 1.5 mm posterior to bregma and 2.5 mm below the brain surface, with the incisor bar lowered by 2.5 mm below the horizontal zero [28]. Cannulas were fixed to the skull with screws and dental acrylic. All animals received oxytetracycline hydrochloride (400 $mg.kg^{-1}$ intramuscularly) and dexamethasone (1 $mg.kg^{-1}$ intramuscularly) after surgery and were kept at rest for one week prior to the experiments.

2.3. Core temperature measurement

Body core temperature was measured in conscious unrestrained rats using data loggers (Subcue, Calgary, Canada) surgically implanted intra–abdominally at the time of the i.c.v. cannulation. Experimental measurements were conducted at the thermoneutral zone for rats [29], in a temperature-controlled room (28 ± 1 °C). Temperature was continuously monitored and recorded at 10 or 15 min intervals from 2 h before any injection until 1.5 h or 6 h after the injection of the pyrogenic stimuli. For proteomic and metabolite analysis of hypothalamus, samples were collected 30 min after PGE_2 injection and 2.5 h after LPS injection. The time point of 2.5 h was chosen based on the early phase of LPS-induced fever whereas the time point of 30 min represents the peak of PGE_2 induced fever. The increase in core temperatures of animals at the time of collection related to basal values was expressed as ΔT (°C).

2.4. Fever induction by LPS and PGE_2

Independent groups of rats received intravenous (i.v.) injections of LPS (5 $\mu g.kg^{-1}$ in sterile saline solution) derived from *Escherichia coli*,