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Stress-induced anhedonia is associated with the activation of the inflammatory system in the rat brain: Restorative effect of pharmacological intervention

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Imipramine: 3-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-*N*,*N*-dimethylpropan-1-amine
Agomelatine: *N*-[2-(7-methoxynaphthalen-1-yl) ethyl]acetamide
Lurasidone: (3aR,4S,7R,7aS)-2-{(1R,2R)-2-[4-(1,2-benzisothiazol-3-yl) piperazin-1-ylmethyl] cyclohexylmethyl} hexahydro-4,7-methano-2H-isoindole-1,3-dione

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ABSTRACT

Major depression is a complex disease that originates from the interaction between a genetic background of susceptibility and environmental factors such as stress. At molecular level, it is characterized by dysfunctions of multiple systems including neurotransmitters, hormones, signalling pathways, neurotrophic and neuroplastic molecules and – more recently – inflammatory mediators. Accordingly, in the present study we used the chronic mild stress (CMS) paradigm in the rat to elucidate to what extent brain inflammation may contribute to the development and/or the maintenance of an anhedonic phenotype and how pharmacological intervention may interfere with such behavioral and molecular stress-induced alterations.

To this aim, adult male rats were exposed to CMS for 2 weeks and the cerebral expression of several mediators of the inflammatory system was evaluated in the hippocampus and prefrontal cortex of both stressed and control animals in parallel with the sucrose intake. Next, the animals that showed a decreased sucrose consumption were exposed to five further weeks of CMS and treated with the antidepressants imipramine or agomelatine, or the antipsychotic lurasidone. Our results demonstrate that only the stressed animals that were characterized by a deficit in sucrose intake showed increased expression of the pro-inflammatory cytokines IL-1β, IL-6 and up-regulation of markers and mediators of microglia activation such as CD11b, CX3CL1 and its receptor CX3CR1 in comparison with stress-resilient animals. Some of these molecular alterations persisted also after longer stress exposure and were modulated, similarly to the behavioral effects of CMS, by chronic pharmacological treatment. These data suggest that neuroinflammation may have a key role in the pathological consequences of stress exposure, thus contributing to the subject's vulnerability for depression.

1. Introduction

Major depression is a severe psychiatric disorder estimated to become the second leading cause of disability in the world by 2020 [1]. Although its etiology has not yet been fully elucidated, it is known that the exposure to stressful events may significantly contribute to the development of the disease [2–4]. However, even if depression occurs in a significant percentage of stress-exposed subjects, most of them are able to successfully cope with the adverse situation and avoid such psychopathology [5,6]. The nature of this differential vulnerability is probably multi-factorial and involves a complex interplay between stress and the genetic and biological personal background.

Over the past decade, there has been increasing attention to the involvement of the inflammatory system in the etiology of depression [7–9]. In particular, it has been reported that depressed subjects exhibit increased levels of inflammatory mark-

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ers both in the periphery and in brain [10] and several pathologies associated with a moderate grade of inflammation present high co-morbidity with depression [11]. Furthermore, a high percentage of patients with cancer or hepatitis C receiving immunotherapy with interferon-alpha develop major depression [12], suggesting that the activation of the immune system may effectively contribute to the onset of the disease. In addition, it has been described that stress may activate pro-inflammatory mediators at both peripheral and central level. For example, an increased inflammatory response has been observed in depressed subjects who experienced early life adversities [13–15] and similar effects were reported in laboratory animals exposed to different stress paradigms [16–19]. However, whether the neuroinflammation plays a pathogenic role in the insurgence of depression or it represents a merely epiphenomena is still elusive.

In order to clarify this issue, in the present study we evaluated to what extent the development of a stress-induced anhedonic-like phenotype is associated with brain inflammation. To this purpose we exposed adult male rats to a chronic mild stress (CMS) paradigm, an experimental procedure that takes into account the naturally occurring variation in the stress response. Indeed, CMS leads to two distinct behavioral responses in the rat: a "susceptible" response characterized by anhedonic-like symptoms as well as a "resilient" response where the animals appear able to avoid the pathological consequences of the stress exposure [20]. Given that, it is thought to be a well-established model of depression and has been widely used to evaluate stress-related molecular mechanisms [21–23].

On these bases, we first exposed the animals to 2 weeks of CMS, a period sufficient to identify rats that were "susceptible" or "resilient" to the development of a decrease in the sucrose intake, a test used as measure of anhedonia in the CMS [24] as well as in other animal models of depression [25]. We then assessed the contribution of specific mediators of the immune/inflammatory system during this initial phase of stress by a detailed analysis of the expression of pro- and anti-inflammatory cytokines and markers of microglia activation and regulation in the hippocampus and prefrontal cortex, two brain regions that play a critical role in the pathophysiology of depression [26,27]. Next, we established if these molecular changes persisted following exposure to an additional 5 weeks of CMS. Last, we used two antidepressant drugs characterized by different primary mechanism of action, namely the classic tricyclic imipramine and agomelatine. Imipramine was chosen as a gold standard inhibitor of monoamine uptake, whereas agomelatine was selected based on its novel mechanism as melatonergic (MT1/MT2) agonist and serotonergic (5HT2c) antagonist. Moreover, a separate cohort of animals received the antipsychotic lurasidone, to evaluate to what extent pharmacological intervention with different class of drugs could normalize the behavioral and molecular consequences set in motion by CMS.

2. Methods

General reagents were purchased from Sigma–Aldrich (Milan, Italy) whereas molecular biology reagents were obtained from Applied Biosystem Italia (Monza, Italy), Eurofins MWG-Operon (Ebersberg, Germany) and Bio-Rad Laboratories S.r.l. (Segrate, Italy). Imipramine was purchased by Sigma–Aldrich (Milan, Italy), whereas agomelatine and lurasidone were kindly provided by Servier (Suresnes, France), and by Sumitomo Dainippon Pharma Co., Ltd. (Japan), respectively.

2.1. Animals

Adult male Wistar rats (Charles River, Germany) were brought into the laboratory one month before the start of the experiment. Except as described below, the animals were singly housed with food and water freely available, and were maintained on a 12-h light/dark cycle in a constant temperature ($22 \pm 2\,^{\circ}C$) and humidity ($50 \pm 5\%$) conditions. All procedures used in this study are conformed to the rules and principles of the 2010/63/EU Directive, were approved by the Local Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland. All efforts were made to minimize animal suffering and to reduce the number of animals used ($n = 10$ each experimental groups).

2.2. Stress procedure and pharmacological treatment

After a period of adaptation to laboratory and housing conditions, the animals (220 ± 7 g) were trained to consume a 1% sucrose solution. Training consisted of nine 1 h-baseline tests, in which sucrose was presented in the home cage, following 14 h of food and water deprivation. The sucrose intake was measured at the end of the test by weighing pre-weighed bottles (300 ml Polythene bottles equipped with Stainless steel ball sippers, North Kent Plastics, UK) containing the sucrose solution. Subsequently, sucrose consumption was monitored, under similar conditions, at weekly intervals throughout the whole experiments. On the basis of their sucrose intake in the final baseline test, animals were divided into two matched groups to be subjected to a chronic mild stress procedure [28] for a period of two (Experiment 1) or seven (Experiment 2) weeks. Each week of the stress regime consisted of two periods of food or water deprivation, two periods of 45 degree cage tilt, two periods of intermittent illumination (lights on and off every 2 h), two periods of soiled cage (250 ml water in sawdust bedding), one period of paired housing, two periods of low intensity stroboscopic illumination (150 flashes/min), and three periods of no stress. All stressors were 10–14 h of duration and were applied individually and continuously, day and night. Control animals were housed in separate rooms and had no contact with the stressed animals. They were deprived of food and water for 14 h preceding each sucrose test, but otherwise food and water were freely available in the home cage. According with these procedures, two separated sets of experiments have been performed:

Experiment 1. Animals were subjected to the stress procedure for two weeks, tested for the sucrose consumption and then killed by decapitation 24 h after the final sucrose test. Based on the results of this test, the stressed animals were divided into 2 groups: "stress-reactive" (i.e. showing at least 50% decrease of sucrose consumption) and "stress-non reactive" (i.e. showing small or no decrease of sucrose consumption) to be compared versus unstressed rats. This experimental design implied three groups of animals: unstressed rats used as control group ($n = 10$ animals); stressed animals that showed a decrease in sucrose consumption ("stress-reactive" animals, $n = 10$); stressed animals that were resilient to the CMS ("stress-non reactive", $n = 10$). The brains were removed and the hippocampus (dorsal and ventral) and the prefrontal cortex were dissected as fresh tissues. Specifically, the dorsal hippocampus corresponds to the Plates 25–33 according to the atlas of Paxinos and Watson [29], whereas the ventral hippocampus corresponds to the Plates 34–43. These two hippocampal subregions were dissected from the whole brain whereas the prefrontal cortex (defined as Cg1, Cg3, and IL subregions corresponding to the Plates 6–10 according to the atlas of Paxinos and Watson) was dissected from 2-mm-thick slices. The brain specimens were then rapidly frozen in dry ice/isopentane and stored at -80 °C for the molecular analyses.

Experiment 2. Animals were subjected to the stress procedure for 7 weeks. Based on the results of the final sucrose test carried out following the first 2 weeks of stress, both control and stress-reactive groups were further divided into matched subgroups and for the subsequent five weeks they received intraperitoneal injec-