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Nrf2 participates in depressive disorders through an anti-inflammatory mechanism

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Summary A causative relationship between inflammation and depression is gradually gaining consistency. Because Nrf2 participates in inflammation, we hypothesized that Nrf2 could play a role in depressive disorders. In this study, we have observed that Nrf2 deletion in mice results in: (i) a depressive-like behavior evaluated as an increase in the immobility time in the tail-suspension test and by a decrease in the grooming time in the splash test, (ii) reduced levels of dopamine and serotonin and increased levels of glutamate in the prefrontal cortex, (iii) altered levels of proteins associated to depression such as VEGF and synaptophysin and (iv) microgliosis. Furthermore, treatment of Nrf2 knockout mice with the anti-inflammatory drug rofecoxib reversed their depressive-like behavior, while induction of Nrf2 by sulforaphane, in an inflammatory model of depression elicited by LPS, afforded antidepressant-like effects. In conclusion, our results indicate that chronic inflammation due to a deletion of Nrf2 can lead to a depressive-like phenotype while induction of Nrf2 could become a new and interesting target to develop novel antidepressive drugs.

1. Introduction

A relationship between depression and immunity has been described for almost 25 years (Irwin and Miller, 2007). In 1991 Smith proposed the macrophage theory of depression

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(Smith, 1991) which postulates that excessive secretion of activated macrophage monokines is implicated in the pathophysiology of depression. Almost at the same time, the group of Maes described a relationship between inflammation and depression (Maes et al., 1992, 1993). Supporting these observations, (i) immune activation with lipopolysaccharide (LPS) is known to produce a set of behavioral and cognitive alterations (anhedonia, anorexia, memory deficits, among others) that resemble depression both in animals and in humans (Yirmiya et al., 2000; Reichenberg et al., 2010); (ii) depression is commonly accompanied by an inflammatory response expressed as an increase in serum levels of TNF-α, IFN-γ, IL-6, IL-1β and C-reactive protein (Maes et al., 1997; Mikova et al., 2001; Howren et al., 2009). These cytokines are known to cause behavioral changes (Asnis and De La Garza, 2006; Kaster et al., 2012), affect neurotransmitter metabolism (Guillemin et al., 2001; Sakash et al., 2002; Barrientos et al., 2004) and decrease neuroplasticity (Barrientos et al., 2004; Ben Menachem-Zidon et al., 2008; Goshen et al., 2008; Koo and Duman, 2008). Of note, patients who suffer from refractory depression (30—40%) show higher levels of acute phase response markers (IL-6, reactive C protein, *etc.*) (Sluzewska et al., 1995; Maes et al., 1997), therefore inflammation could become a new therapeutic target for this particular subset of patients.

Nuclear factor (erythroid 2-derived)-like 2 (Nrf2) is a transcription factor that plays a central role in cellular defense against oxidative and electrophilic insults. Nrf2 binds to antioxidant response elements (ARE) located in the promoter region of genes encoding many phase II detoxifying or antioxidant enzymes and related stress-responsive proteins. It has been recently demonstrated that Nrf2-ARE signaling is also involved in attenuating inflammation-associated pathogenesis, such as autoimmune diseases, rheumatoid arthritis, asthma, emphysema, gastritis, colitis and atherosclerosis (Lee and Johnson, 2004). Thus, disruption or loss of Nrf2 signaling causes enhanced susceptibility not only to oxidative and electrophilic stresses but also to inflammatory injuries. Furthermore, a relationship between Nrf2 and protective effects have also been described in the central nervous system (CNS) (Innamorato et al., 2008).

Since Nrf2 has been recently described to play a crucial role in regulating inflammation, and inflammation has been related to depression, this study was designed to determine if Nrf2 could participate in the pathogenesis of depression through an inflammatory mechanism and/or whether it could be considered a pharmacological target against depression. Our results show for the first time that Nrf2 deficient mice exhibit depressive-like behavior and that this phenotype is reversed by an anti-inflammatory drug such as rofecoxib. Furthermore, Nrf2 knockout (KO) mice showed significant changes in serotonin, glutamate and dopamine levels in the prefrontal cortex, compared to Nrf2 (+/+) mice. We have also demonstrated that the Nrf2 activator sulforaphane can reverse the depressive phenotype in an inflammatory model of depression elicited by lipopolysaccharide (LPS) administration. Taken together, our results support the hypothesis that Nrf2 deletion can lead to depressive-like behavior and that activation of Nrf2 could become a new therapeutical target against depression.

2. Methods and materials

2.1. Animals

Male wild-type C57BL/6 mice (Nrf2 (+/+)) and Nrf2 KO (Nrf2 −/−)) and male Swiss mice (3—4 months) were housed at room temperature under a 12 h light—dark cycle. Food and water were provided *ad libitum*. All experimental procedures with animals were approved by the Ethical Committee of the *Hospital La Paz Health Research Institute (IdiPAZ)*, for the care and use of animals in research, in accordance with the European Community Council Directive of November 24 1986 (86/609/EEC) and with the Spanish Real Decreto (RD-1201/2005).

Nrf2 KO mice and their wild-type littermates were obtained thanks to the courtesy of Dr. Masayuki Yamamoto (Tohoku University, Graduate School of Medicine, Sendai, Japan) (Itoh et al., 1997).

2.2. Drugs and treatments

The non-steroidal anti-inflammatory drug (NSAID), rofecoxib (Toronto Research Chemicals, Canada), was dissolved in saline and administered by the intraperitoneal (IP) route; it was administered once daily during 7 days at a dose of 2 mg/kg based on previous studies (Jain et al., 2001; Singal et al., 2004). Twenty-four hours after the last administration, the tail-suspension test (TST), the splash test (ST) or the open-field test (OFT) were carried out. After this, animals were sacrificed under deep anesthesia with isoflourane.

R,S-sulforaphane (SFN) was from LKT Laboratories (Minnesota, USA) and LPS (Serotype O26:B6) from Sigma (St. Louis, USA); they were prepared in saline solution. Mice received SFN (1 mg/kg, IP) for 7 consecutive days and the day after, LPS (0.1 mg/kg, IP) was injected. Once the experimental schedule was completed, animals were deeply anesthetized with isofluorane and sacrificed.

2.3. Behavioral tests

The behavioral tests were performed by blind experimented researchers under faint light conditions and were scored manually. The open field test was performed after the tail suspension test or after the splash test (4—5 min interval).

2.4. Tail-suspension test (TST)

The total duration of immobility induced by tail-suspension was measured according to the method described elsewhere (Steru et al., 1985). Briefly, mice, both acoustically and visually isolated, were suspended 50 cm above the floor by an adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Mice were considered immobile only when they hung passively and completely motionless.

2.5. Splash test (ST)

The ST consisted in squirting a 1% sucrose solution on the dorsal coat of a mouse placed in a 14 cm glass cylinder. The

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