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Emotional, endocrine and brain anandamide response to social challenge in infant male rats

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Summary Individual response to stress is orchestrated by hypothalamus–pituitary axis corticosteroids, although critically modulated by the central endocannabinoid (eCB) system. Whilst the role of the eCB system in stress response and emotional homeostasis in adult animals has been extensively studied, it has only been scarcely investigated in developing animals. Herein, we aimed to investigate the participation of eCB ligands in the stress responses of neonate rats. Twelve days-old *Wistar* male rats were exposed to a social challenge (repeated brief isolations from dam and littermates), which resulted in a significant increase in serum corticosterone levels. This stressful social challenge also decreased spontaneous rat pups' behaviours and augmented isolation-induced ultrasonic vocalizations. Notably, a specific decrease in anandamide content (not 2-AG) was observed within the hippocampus (not in the striatum). However, the enhancement of eCB signalling by URB597 administration (0.1 mg/kg) did not affect the adrenocortical and behavioural responses to this postnatal social challenge. The influence of gestational stress was also evaluated in the infant offspring of rats dams exposed to restraint stress (PRS, three episodes/day, on gestation days 14 till delivery); however, PRS did not modify neonate responses to this postnatal challenge. Present findings provide evidence for the participation of the eCB system in the acute response to a social challenge in infant male rats. However, the lack of evidences from the pharmacological study encourages the investigation of alternative and/or indirect mechanisms that may participate in the behavioural and endocrine response to stress in developing animals. Further experiments are still needed to clarify the interactions between the HPA axis and the eCB system in stress reactivity at early postnatal stages.

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1. Introduction

The notion that adult individual adaptive capacities emerge from a continuous crosstalk between genetic predisposition and life experience constitutes a tenet in basic and translational neuro-psychiatry (Bateson et al., 2004). Whilst the environment regulates individual adjustments throughout the entire lifespan (Gluckman et al., 2009), the early determinants of adult phenotype have received considerable attention in countless studies (Gluckman and Hanson, 2004). Short-term neurochemical and neuroendocrine responses to environmental stress have been proposed as the key mediator of offspring developmental adjustments (D'Amato et al., 1998; Meaney et al., 2000; Dudley et al., 2011). Specifically, in response to external stressors, both dams and pups show sudden bodily reactions including increments in plasma corticotrophin releasing hormone (CRH), adrenocorticotropin (ACTH) and corticosterone (Macri et al., 2011). Similarly, changes in neurotrophin levels, such as brain derived neurotrophic factor (BDNF), have also been described following stressful events (Smith et al., 1995). Accumulating evidence demonstrates that, while being orchestrated by hypothalamus–pituitary axis (HPA) corticosteroids, the individual response to stress is also regulated by the central endocannabinoid (eCB) system (see Refs. (Finn, 2010; Hill and Tasker, 2012) for a review).

The eCB system within the brain is mainly constituted by type-1 cannabinoid receptors (CB1Rs), their endogenous ligands (such as *N*-arachidonoylethanolamine (AEA or anandamide) and 2-arachidonoylglycerol (2-AG)), and the enzymes responsible for their synthesis and degradation (Di Marzo, 2009). Such a system is involved in a great variety of physiological functions (Maccarrone, 2010), including in stress responsiveness and emotional processing (Viveros et al., 2005b; Gorzalka et al., 2008). The eCB signalling has been shown to regulate the HPA axis both in the maintenance of basal and stress-induced responses (e.g. (Finn, 2010; Hill et al., 2010b)). The central distribution of CB1Rs in brain areas associated with emotion regulation and stress responsiveness, such as prefrontal cortex, hippocampus, amygdala and hypothalamus (Mackie, 2005) together with results from genetic and pharmacological blockade of CB1Rs provide consistent evidence for a role of the eCB system in emotional homeostasis (Gorzalka et al., 2008; Marco and Viveros, 2009; Finn, 2010; Hill et al., 2010b; Moreira and Wotjak, 2010). However, whilst the role of eCB signalling in stress response and emotional homeostasis in adult animals has been extensively studied, it has only been scarcely investigated in developing animals (Viveros et al., 2005a; D'Asti et al., 2010; Lee and Gorzalka, 2012; Marco and Laviola, 2012).

In rodent pups, a reliable index of emotional distress can be obtained by measuring ultrasonic vocalizations (USVs) which have an important function in mother–infant communication. Rat pups between 4 and 16 days of age emit vocalizations at a frequency range of 30–90 kHz when separated from their mothers. USVs emissions follow a clear ontogenetic profile with a peak around 10–12 days of age in rats (Portfors, 2007; Scattoni et al., 2009) that is significantly modulated by physical and social parameters [for review see (Hofer et al., 2002)]. In addition, isolation-induced USVs are susceptible of modulation by CBR agonists. Administration of high doses of CP55,940 markedly reduced the rate of USVs causing an almost complete inhibition of calls (McGregor et al., 1996); as did the blockade of AEA hydrolysis by URB597 administration and the inhibition of AEA transport by AM404, these two drugs enhance eCB signalling and decreased the number of USVs emitted by rat pups in isolation (Kathuria et al., 2003; Bortolato et al., 2006). Therefore, a role for eCB signalling in emotional control from early neonatal ages might be suggested, although it has not been directly tested yet.

Among a great variety of stressors, social stressors are selected given their biological relevance and adaptive value (Sachser et al., 2011). Herein, we aimed at directly investigating the participation of eCB signalling in stress responsiveness at infancy. For that purpose, we employed a paradigm consisting of repeated social isolation challenge at postnatal day 12. Firstly, to confirm the stressful nature of the social stressor protocol, we investigated emotional and neuroendocrine activation, by measuring: (i) ultrasound vocalizations (USV) emitted by pups during the separation periods, (ii) pups' general arousal condition, (iii) circulating corticosterone concentrations, brain eCB content in hippocampus and striatum and (iv) brain derived neurotrophic factor (BDNF) in the same brain areas. Furthermore, the variations of eCB content in brain regions critically involved in emotion and cognitive processing, i.e. hippocampus and striatum, were specifically investigated. In addition, an inhibitor of AEA hydrolysis by the fatty acid amide hydrolase (FAAH), URB597, was administered prior to the social challenge and behavioural and adrenocortical responses were evaluated.

Finally, in order to evaluate whether the behavioural and endocrine responses to the neonatal social challenge were also affected by previous stress exposure, we exposed half of pregnant dams to restraint stress (prenatal restraint stress, PRS). Stress exposure during gestation has been shown to induce physiological, neurochemical and behavioural alterations in the offspring (Bowman et al., 2004; Laviola et al., 2004; Darnaudery and Maccari, 2008). In this regard, we analysed whether PRS affected the basal set up as well as the capacity of the offspring, through changes in the eCB system, to face with stressful situations at early age of life.

2. Methods

2.1. Animals

All animal procedures were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) about animal use for experimental purposes, and protocols were approved by the Ministry of Health Committee (Italy). All efforts were made to minimize animal suffering and to reduce the number of animals used. Pregnant *Wistar* rats, purchased from Charles River®, were housed in an air-conditioned room (temperature 21 ± 1 °C, relative humidity 60 ± 10%) in a standard 12-h light–dark cycle (lights on at 0600 h), with free access to water and food (Mucedola®, Italy). On the day after birth (day of birth was considered postnatal day, pnd, 0), litters were sex balanced and culled to 9 ± 1 pups per dam. Litters were left

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