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ENDOGENOUS OPIOIDS REGULATE GLUCOCORTICOID-DEPENDENT STRESS-COPING STRATEGIES IN MICE

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Abstract—Coping skills are essential in determining the outcomes of aversive life events. Our research was aimed to elucidate the molecular underpinnings of different coping styles in two inbred mouse strains, C57BL/6J and SWR/J. We compared the influence of a preceding stressor (0.5 h of restraint) on behavioral and gene expression profiles between these two strains. The C57BL/6J strain exhibited increased conditioned fear and high immobility (passive coping). Oppositely, the SWR/J mice demonstrated low freezing and immobility, low post-restraint anxiety and considerable struggling during the forced swim test (active coping). Gene profiling in the amygdala revealed transcriptional patterns that were related to the differential stress reactivity, such as the activation of glucocorticoid-dependent genes specifically in the C57BL/6J mice. Post-restraint blood sampling for corticosterone levels confirmed the association of hypothalamic–pituitary–adrenal (HPA) activation with a passive coping style. Pharmacological tools were used to modulate the stress-coping strategies. The blockade of opioid receptors (ORs) before the aversive event caused transcriptional and neuroendocrine changes in the SWR/J mice that were characteristic of the passive coping strategy. We found that treatment with a glucocorticoid receptor (GR) agonist (dexamethasone (DEX), 4 mg/kg) impaired the consolidation of fear memory in the C57BL/6J mice and that this effect was reversed by OR blockade (naltrexone (NTX), 2 mg/kg). In parallel, a glucocorticoid receptor antagonist (mifepristone (MIF), 20 mg/kg) reversed the effect of morphine (20 mg/kg) on conditioned fear in the C57BL/6J mice. Our results suggest that in mice, stress-coping strategies are determined by opioid-dependent mechanisms that modulate activity of the HPA axis.

Key words: coping, stress, gene expression, glucocorticoids, opioids, mice.

INTRODUCTION

The impact of stressful life events on physical and psychological well-being is highly variable. In general, individuals under these specific conditions react differently to stressful stimuli depending on how well they cope with the experience. Coping refers to alternative response patterns that occur in reaction to a challenging environment and depends on many factors including genotype, development, early-life experience and social support. Coping can be divided into two broad categories, active (fight or flight) or passive (decreased responsiveness to the environment, immobility), of behavioral strategies based on the presence or absence of attempts to act upon the stressor (Koolhaas et al., 2010). Task-oriented active strategies are thought to be associated with a lower level of emotional distress (Holton et al., 2015), whereas passive coping may be related to posttraumatic stress disorder and depression (Tiet et al., 2006).

The different strategies are characterized by distinct patterns of physiological and neuroendocrine changes, but little is currently known about the biological mechanisms that control coping strategies. It is suggested that active coping is associated with high sympathetic reactivity to stressors, whereas the passive style is generally associated with higher hypothalamic–pituitary–adrenal (HPA) axis reactivity (Koolhaas, 2008). Glucocorticoids (GCs) are steroid hormones released from the HPA axis that regulate crucial homeostatic functions, including metabolism, cell growth and development (Ayroldi et al., 2014). GCs have been proposed to play either a protective or harmful role, depending on genetic makeup or early-life experiences (Daskalakis et al., 2013). Physiological factors that modulate the responsiveness of the HPA axis play a significant role in the etiology of stress-related psychiatric disorders.

The endogenous opioid peptide system is mostly recognized for its role in modulating pain and pleasure; however, more recent studies have demonstrated opioid involvement in emotional learning and the regulation of stress as well (Przewlocki, 2009; Bowers et al., 2012; Arico and McNally, 2014; Valentino and Van Bockstaele, 2015). Opioid neuromodulators, together with their receptors, are expressed in limbic structures and are functionally connected with the HPA axis (Bilkei-Gorzo

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Abbreviations: CTX, conditioned context re-exposure; DEX, dexamethasone; ELISA, enzyme-linked immunosorbent assay; FC, fear conditioning; GCs, Glucocorticoids; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal; MIF, mifepristone; NTX, naltrexone; OR, opioid receptor; PVN, paraventricular nucleus of the hypothalamus; SAL, short attack latency.

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et al., 2008; Pascoe et al., 2008). Opioid-dependent individuals under naltrexone (NTX) treatment experience higher levels of stress and report less frequent use of adaptive coping strategies compared with controls (Hyman et al., 2009). Cortisol/corticosterone secretion and its feedback effects are tied to the endogenous opioid system (Drolet et al., 2001; Lovallo et al., 2015), but the effects of this specific interaction on coping remain elusive.

Complex interactions between an individual's predispositions and environmental stressors determine the initial response to negative events as well as the capacity for coping. Our studies of inbred mouse strains indicated that C57BL/6J and SWR/J mice represent diverse behavioral phenotypes, that corresponds to distinct traits observed in humans, including response to stressors and sensitivity to opioids. C57BL/6J strain is prone to develop higher freezing/immobility and stronger morphine preference in comparison to SWR/J strain which is considered to be less anxious and more aggressive. We also found inter-strain differences in the expression of markers for the glucocorticoid and opioid systems that are related to stress responsiveness (Korostynski et al., 2007; Solecki et al., 2009; Gieryk et al., 2010; Szklarczyk et al., 2012, 2015). Those two endophenotypes may predispose to separate stress-coping strategies.

The current study aimed to assess restraint-induced coping abilities in C57BL/6J and SWR/J mice, which would mimic the diverse behavioral strategies to deal with trauma found in human populations. Our next goal was to examine the neuroendocrine and neurobiological changes occurring after the preceding stressor (restraint) and potentially underlying the subsequent behavioral consequences. Moreover, we attempt to modify the expression of behavioral strategies with pharmacological compounds. Exploring the biological foundation of individual stress reactivity will provide knowledge about the processes that determine adaptability or vulnerability to stress-related disorders. Identifying effective coping styles and understanding the underpinnings of disease susceptibility/resilience constitutes a basis for efficient pharmaco- and psychotherapeutic interventions.

EXPERIMENTAL PROCEDURES

Animals

All tests were performed on two inbred mouse strains, C57BL/6J and SWR/J. The mice were male, 21–30 g, and 8–10 weeks old (Jackson Laboratories, Bar Harbor, ME, USA). The mice were housed in groups of five per cage (aspen litter MIDI LTE E-002, AnimaLab, Poznań, Poland) in a temperature- and humidity-controlled room under a 12:12-h light/dark cycle (lights on from 08:00 to 20:00) with free access to water and food (standard diet, Special Food Services, Essex, England). All experimental procedures were approved by the local Bioethics Commission (Krakow, Poland).

Experimental schedule and groups

The first experiment was conducted to assess the behavioral, neuroendocrine and transcriptional correlates of different responses to stress in the C57BL/6J and SWR/J strains. All the animals went through the preceding aversive event – restraint (R) procedure (n = 25–30 per strain) or received compensatory handling (C – control animals, n = 26–28 per strain). In order to assess the behavioral consequences of restraint, the mice were randomly divided into four groups that underwent four separate behavioral procedures six days after the stress exposure: fear conditioning (FC), open field, tail suspension or forced swimming (Fig. 1A, n = 6–9 per treatment: R or C per strain in each behavioral procedure). Independent groups of animals were used for gene expression profiling (n = 4–9 per group per strain) and blood sampling for plasma corticosterone levels (n = 5 per group per strain). The amygdala tissue and blood samples were collected 2 h after one of the treatments to examine relatively early transcriptional and neuroendocrine alterations that could determine the subsequent behavioral profile. Moreover, we evaluated the effects on gene expression of varying intensity of aversive stimuli. Thus, the amygdala samples were also taken 2 h after fear conditioning (FC: 5 × 1 mA, 2 s, 1-min interval) and 2 h after a re-exposure to the stressful context (CTX: 3-min re-exposure, 2 weeks after FC). These two procedures were preceded 6 days earlier by the restraint (R + FC, R + CTX) or by the compensatory handling (C + FC, C + CTX).

In the second experiment, the effects of opioid receptor (OR) blockade on stress-induced glucocorticoid activity were analyzed in the SWR/J strain. Previously, we showed that NTX restored symptoms of trauma in SWR/J (Szklarczyk et al., 2015). Here, plasma corticosterone levels and the expression of selected glucocorticoid-dependent genes (n = 4–7) in response to restraint and/or NTX treatment were examined in this strain.

In the third experiment, the influence of opioid and synthetic glucocorticoid compounds on the passive coping strategy (measurement of freezing in a fear-conditioning chamber) was analyzed in C57BL/6J mice. The tested drugs (dexamethasone (DEX), NTX, morphine and mifepristone (MIF)) were administered twice: on the training day and upon the animal's first re-exposure to the context (n = 8–10 per drug). This treatment scheme was used to assess both the consolidation and reconsolidation of fear memory.

Restraint stress test

The procedure (Hill et al., 2011) was used to induce an uncontrollable aversive situation that produces both physical and psychological acute stress, and in consequence leads to neuronal and behavioral alterations (Patel et al., 2005; Borghans and Homberg, 2015). The animals were put into a plastic cylindrical tube (diameter 28 mm and length 115 mm) with breathing holes and an aperture in the cap for the tail for 30 min. The tubes were long