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Control of hormonal stress reactivity by the endogenous opioid system

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Summary
Regulations of hormonal stress responses entail the initiation, amplitude and termination of the reaction, as well as its integration with other stress response systems. This study investigates the role of endogenous opioids in the regulation and integration of behavioral, thermal and hormonal stress responses, as these neuromodulators and their receptors are expressed in limbic structures responsible for stress responses. For this purpose, we subjected mice with selective deletion of β-endorphin, enkephalin or dynorphin to the zero-maze test, a mildly stressful situation, and registered behaviors and stress hormone levels. Behavioral stress reactivity was assessed using zero-maze, light–dark and startle-reactivity paradigms. Animals lacking enkephalin displayed increased anxiety-related behavioral responses in each three, dynorphin knockouts in two models, whereas the responses of β-endorphin knockouts indicated lower anxiety level in the zero-maze test. All knockout strains showed marked changes in hormonal stress reactivity. Increase in ACTH level after zero-maze test situation, unlike in wild type animals, failed to reach the level of significance in Penk1$^{-/-}$ and Pdyn$^{-/-}$ mice. Corticosterone plasma levels rapidly increased in all strains, with a lower peak response in knockouts. In wild-type and β-endorphin-deficient mice, corticosterone levels returned to baseline within 60 min after stress exposure. In contrast, mice lacking dynorphin and enkephalin showed longer-lasting elevated corticosterone levels, indicating a delayed termination of the stress reaction. Importantly, the behavioral and hormonal responses correlated in wild-type but not in knockout mice. Hyperthermia elicited by stress was reduced in animals lacking dynorphin and absent in Penk1$^{-/-}$ mice, despite of the heightened behavioral anxiety level of these strains. These results demonstrate an important role on the endogenous opioid system in the integration of behavioral and hormonal stress responses.

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1. Introduction

An ancient part of the mammalian brain, the limbic system, organizes and initiates stress responses. It is not an anatomical entity, but rather consists of separate brain areas forming a functional unit. It receives somatosensory information from the thalamus and sensory cortex, efferent signals from the vegetative system, and it has access to the stored information from the hippocampus (Sah et al., 2003). The amygdaloid complex in strong cooperation with other elements of the limbic system integrates and evaluates incoming information, organizes and initiates stress responses (Akmaev et al., 2004). Cortical afferents from the amygdala contribute to behavioral stress responses, while multiple descending pathways influence vegetative responses and reflexes. The limbic system continuously evaluates a broad range of sensory stimuli, and triggers a stress response if the stimulus is perceived as being dangerous (Sah et al., 2003). The hypothalamic paraventricular nucleus (PVN), itself part of the limbic system, regulates the hormonal stress responses under the control of other limbic elements.

A dense capillary plexus provides a rapid access of steroid hormones to the PVN and enables the negative-feed back regulation of the corticotropin-releasing hormone (CRH) secreting parvocellular neurons through activation of neuronal nuclear steroid receptors. Local synaptic circuits (Boudaba et al., 1996) and inputs from the zone immediately surrounding the PVN provide the majority of inhibitory inputs (Herman et al., 2002). The PVN receives both excitatory and inhibitory inputs from limbic and extralimbic structures, either directly or indirectly through interneurons (Herman et al., 2003). The complex control of the activity of the PVN is necessary for the appropriate initiation, amplitude and termination of the hormonal stress response as well as for the integration of the hormonal stress reactivity with other executive stress reactivity pathways.

Neuromodulators are thought to play an important role in the regulation of hormonal stress responses. Modulatory peptidergic interneurons expressing β-endorphin, enkephalin and dynorphin are present in the PVN and in limbic areas that modulate PVN activity (Drolet et al., 2001; Herman et al., 2002). Endogenous opioids can thus modulate PVN activity directly and indirectly. The expression of endogenous opioids in the PVN is increased after stress exposure unrelated to the nature of the stressor (Palkovits, 2000; Reyes et al., 2003) suggesting that they participate in the regulation of stress reactivity. Pharmacological and genetic studies supported the potential role of endogenous opioids in the regulation of stress reactivity. Treatment with delta receptor antagonist (Saitoh et al., 2005), or genetic deletion of delta opioid receptors or its ligand enkephalin (Filliol et al., 2000; Bilkei-Gorzo et al., 2004) led to an increased emotionality, while the disruption of dynorphin/kappa opioid receptor signaling resulted in blunted stress responses (McLaughlin et al., 2003). Animal studies suggested a modulatory role of β-endorphin-μ-opioid receptor system in endocrine responses to stress (Vaanholt et al., 2003; Contet et al., 2006). Clinical studies also revealed an association between polymorphism in the μ-opioid receptor and hormonal stress response (Chong et al., 2006).

Expression of the c-Fos proto-oncogene is a marker of neuronal activation in response to various stimuli (Kovacs, 1998) and has been widely used to correlate behavioral phenotypes with neuronal activity (Wersinger et al., 2002; Matys et al., 2004; Bruening et al., 2006). Neuronal circuitries involved in stress reactivity showed a marked increase in c-Fos expression in different stress models. These methods have therefore been used to map out brain regions responding to stress (Duncan et al., 1996). The intensity of stress-induced c-Fos expression can be modulated by anxiolytic and anxiogenic drugs and is thus thought to reflect the animals' stress reactivity (de Medeiros et al., 2005; Bilkei-Gorzo et al., 2007).

In this study, we investigated the contributions of individual opioid peptides to the regulation of hormonal stress response with regard to the initiation, amplitude, termination of the hormonal reactivity and its integration with the behavioral responses. For this purpose, we challenged control animals and mice with a genetic deletion of the enkephalin- (Konig et al., 1996), dynorphin- (Zimmer et al., 2001), and β-endorphin- (Rubinstein et al., 1996) encoding genes (Penk1, Pdyn and POMC) in the zero-maze test paradigm, followed by measurements of stress-hormone levels and c-Fos expression in the PVN and in the basolateral amygdala.

2. Methods and materials

2.1. Animals

Mice with a deletion of the Penk1 and Pdyn genes have been generated in our laboratory (Konig et al., 1996; Zimmer et al., 2001), while β-endorphin-deficient mice were generated by Rubinstein et al. (1996) and obtained from the Jackson Laboratory. It is important to note that β-endorphin-deficient mice were generated by introducing a stop codon just upstream of the β-endorphin peptide sequences, thus leaving all other peptides that are also generated from the POMC precursor intact. All animals have been crossed more than 10 generations to C57BL/6J mice, and were therefore congenic for this genetic background. Control C57BL/6J animals were originally obtained from a commercial breeder (Janvier, France). Male mice (3–5 months old) were kept in a reversed light–dark cycle at least 2 weeks before testing and housed in groups of 3–5, except animals used for the measurement of plasma stress hormone (ACTH and corticosterone) levels and hyperthermia, which were housed individually. We isolated the animals 1 week before the hormone level measurements to eliminate the alterations in stress reactivity between cage-mates having different social status (Ferrari et al., 1998). Isolation, which is a stress factor for rats (Serra et al., 2007), rather decreases the anxiety level in mice (Hilakivi et al., 1989; Rodgers and Cole, 1993). Feral mice colonies usually consist of one dominant male, several females and the offspring, therefore individual housing of the males is closer to the natural requirement than group housing (Capanna et al., 1984). Each animal was used only once and was naïve to the model. Experiments were carried out in the active (dark) phase between 10:00 and 16:00 h. The experimenter was blind to knockout phenotype. Animal