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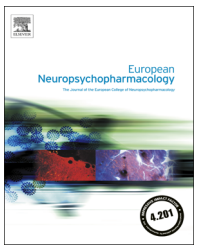

β-endorphin degradation and the individual reactivity to traumatic stress

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Abstract
Reactivity to traumatic stress varies between individuals and only a minority of those exposed to trauma develops stress-induced psychopathologies. Currently extensive effort is made to unravel the specific mechanisms predisposing to vulnerability vs. resilience to stress. We investigated in rats the role of β-endorphin metabolism in vulnerability to acute traumatic stress. Responders (showing extreme anxiety; *n*=7) and resilient non-responders (not differing from the non-stressed individuals; *n*=8) to traumatic foot-shock stress were compared for their blood levels of stress hormones as well as brain levels and activity of two opioid-degrading enzymes. β-endorphin is a substrate to insulin degrading enzyme, which also degrades insulin. Therefore, the effects of insulin application on behavioral and hormonal responses and on β-endorphin degradation were tested. Pre- and post-stress levels of serum corticosterone, and post-stress plasma β-endorphin concentration differentiated between the responders and the non-responders. In brain, responders showed enhanced degradation rates of β-endorphin, assessed by Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS), in hippocampal and amygdalar slices as compared to non-responders. Application of insulin to the amygdala, prior to exposure to traumatic stress, reduced post-stress anxiety and serum corticosterone levels only in the responders. In parallel, amygdalar β-endorphin degradation rate was also reduced by insulin. These results suggest that slowing down β-endorphin degradation rate may constitute an integral part of the normal stress-response, upon a failure of which an extreme anxiety develops. Modulation of opioid degradation may thus present a potential novel target for interference with extreme anxiety.

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1. Introduction

In a population exposed to severe stressors about 20–30% will develop post-traumatic stress disorder (PTSD), suggesting an individual conductance of stress-response mechanisms (Yehuda and LeDoux, 2007). Many physiological systems have been studied in an attempt to delineate factors predisposing certain individuals to vulnerability vs. resilience to trauma. For example, the magnitude of serum surge of cortisol (in rodents-corticosterone; CORT), which is suggested as one of the major indexes of stress response intensity, has been shown to differentiate between responders and non-responders to trauma (Charmandari et al., 2005). Another key-factor of stress response is the endogenous opioids system (EOS), extensively implicated in analgesia, reward and anxiety (Bodnar, 2009), which reciprocally interacts with other components of stress response, such as CORT surge (Blalock and Smith, 1985; Charmandari et al., 2005; Bodnar, 2009; Wittmann et al., 2009). The major representative of the endorphin subclass of this system is β-endorphin, a 31 amino-acid peptide (Bodnar, 2009). Studies in animals and in humans demonstrated blood and cerebrospinal fluid levels of β-endorphin as differentiating between the vulnerable and resilient individuals at rest or in stress-related situations (Hoffman et al., 1989; Darko et al., 1992; Hamner and Hitri, 1992; Kocijan-Hercigonja et al., 1996; Baker et al., 1997; Fontana et al., 1997; Grisel et al., 2008).

One of the factors determining the functioning of EOS is the activity of proteases that degrade endogenous opioids. β-endorphin is a substrate for a soluble enzyme Insulin Degrading Enzyme (IDE) (Safavi et al., 1996; Reed et al., 2008). Hormones of HPA-axis, specifically CORT, influence levels and activity of these proteases (Jaskowski et al., 1989; Safavi et al., 1996; Kulstad et al., 2005; Reed et al., 2008; Osmanovic et al., 2010) and it was suggested that slowing down their activity may reduce anxiety (Jutkiewicz, 2007). Despite ample evidence on EOS involvement in stress response, its specific functional modifications that underlie the individual reactivity to traumatic stress and the role of the enzymes that degrade endogenous opioids are still unclear. We hypothesized that functioning of opioid-degrading enzymes in traumatic stress contributes to the vulnerability/resilience of the individual.

The present study investigated in the rat the degradation rate of β-endorphin in dorsal hippocampus and the basolateral amygdala (BLA) in individual response to acute traumatic stress. These two brain structures are extensively implicated in stress and anxiety and contain high concentrations of both opioid receptors and IDE (Bremner, 2006; Bodnar, 2009). Our previous results showed these regions to be modified by the behavioral stressors used in this study (Kavushansky and Richter-Levin, 2006; Kavushansky et al., 2009). Insulin was applied to the amygdala in an attempt to interfere with β-endorphin degradation, as both are substrates to the same protease. Insulin effects on behavioral and hormonal responses, as well as on degradation rate of β-endorphin were studied.

2. Experimental procedures

2.1. Animals

Male Sprague-Dawley rats (Harlan, Israel) weighing 250–275 g were used. Naïve rats were handled similarly to the stress group, but did not undergo the stress exposure. The subjects were housed in Plexiglas cages (five per cage) in a temperature-controlled (23 ± 1 °C) animal quarters and maintained on a free-feeding regimen with a 12:12 h light/dark schedule. Before starting the experiments, animals were allowed to acclimate to their new environment for five days and then given three more days of handling once a day. Between treatments, the animals were returned to their home cages. Every apparatus was cleaned with ethanol (70%), dried with clothing and ventilated for 3 min after each animal. Testing order of animals was counterbalanced between the groups. All the behavioral procedures were performed between 8:30 and 13:00. Immediately after the last behavioral test, rats were sacrificed and their trunk blood and brains were quickly collected, and stored at −80 °C for further analyses. No anesthetic was used in order to preserve hormonal and molecular profiles. All efforts were made to minimize the suffering and the number of the animals used and to utilize alternatives to in-vivo techniques, if available.

2.2. Ethical approval

Experiments were approved by the institutional Animal Care and Use Committee and were carried out in accordance with the Guide for the care and use of laboratory animals as adopted and promulgated by the National Institute of Health.

2.3. Experimental outline (see also Supplementary material (S-1))

In the first part (two replicating experiments, $N=44$ in each), rats ($n=35$) were subjected to acute foot-shock stress (FS), or left undisturbed (naïve, $n=9$), and 24 h later were analyzed by Open field (OF) and Elevated Plus-Maze (EPM) tests, according to which FS rats were categorized as responders or non-responders ($n=7$ and $n=8$ respectively in each experiment). Post-stress plasma concentration of β-endorphin and pre- and post-stress blood levels of CORT were measured. Brain levels of IDE and an additional enzyme, Puromycin-Sensitive Aminopeptidase (PSA; degrading enkephalins), as well as β-endorphin brain degradation rates were assessed.

In the second part of the study, rats ($N=62$) were bilaterally implanted intra-BLA (to bregma: 3 mm posterior, 5 mm lateral and 7.4 mm ventral) with guide cannulas as previously described (Maroun and Akirav, 2008). Following one week of recovery animals were subjected to elevated platform stress (EP; $n=50$) or left undisturbed (naïve; $n=12$). Forty-eight hours later they underwent the first OF test, according to which the EP rats were categorized as responders ($n=8$) or non-responders ($n=10$). Forty-eight hours later each group (naïve, responders and non-responders) was divided into two sub-groups: one group was intra-amygdala injected with insulin (Sigma; 4 mUI/0.5 μL/hemisphere) delivered over 1 min, and the other group with saline (0.5 μL/hemisphere). The injection cannula was left in place for 1 min after the injection to prevent dragging of the injection fluids. Two minutes after the injection, the EP rats were subjected to FS, naïve rats were returned to their home cages. The animals were not exposed twice to FS for two reasons: (1) to reduce suffering of exposure to two painful stressors (FS+FS); and (2) to prevent the effects of acclimation and remembrance of the experimental setup; the EP and the FS were performed in different rooms. Pilot experiments showed a significant correlation of the responses of the rats to the two stressors. Twenty-four hours post-FS, all the rats underwent a second OF and EPM tests and were sacrificed. Their serum CORT concentrations and effects of insulin on β-endorphin catabolism in amygdala slices were assessed. The histological verification of the cannulas' placement was performed, each animal showed a correct placement (see also: Maroun and Akirav, 2008).