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Activation of CRF neurons by phosphorylation of the cAMP response element binding protein in the hypothalamic paraventricular nucleus during a morphine-conditioned place preference paradigm.

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Introduction

Corticotropin-releasing factor (CRF) is an important regulator of stress response that exerts its actions through activation of two different types of G-protein-coupled receptors: CRF1 (expressed throughout the entire central nervous system) and CRF2. CRF1 binding sites have been demonstrated in several key brain areas involved in the addictive processes [e.g., paraventricular nucleus of the hypothalamus (PVN), nucleus of tractus solitarius (NTS) and nucleus accumbens (NAc)] that are involved in reward, reinforcement and withdrawal.

Drugs of abuse activate CRF neurons in the PVN and the hypothalamic-pituitary-adrenal (HPA)-axis, which results in an elevation of plasmatic glucocorticoids levels. The transcription factor cAMP response element (CRE) binding protein (CREB) has been implicated in neural plasticity, including the changes that occur during stress and drug addiction. Various classes of drugs can alter CREB levels. CREB is activated by phosphorylation at serine residue 133 to become phosphorylated CREB (pCREB), and then, it regulates transcription of several genes, like gene of CRF.

Here, we investigated changes in activation of CRF neurons expressing pCREB in the PVN and their efferent fibbers to NTS and NAc. Moreover, we measured the response of the hypothalamic-pituitary-adrenocortical (HPA) axis after a place preference conditioning paradigm (CPP).

Methods

Swiss mice (4-5 animals/group) were rendered dependent on morphine and conditioned by the CPP. Mice received morphine (6 mg/kg, i.p.) on days 1, 3, and 5 and saline on days 2, 4, and 6. Animals were confined in one room for 20 min immediately after morphine or saline injections. In addition, we use a group of animals treated equally but not conditionated, in order to investigate the effects of environment in addiction. For pCREB and CRF double-label immunohistochemistry, tissue sections from each mouse in each treatment group were processed as follows: pCREB was revealed with DAB intensified with nickel in the first position, and CRF was revealed with DAB in the second position. pCREB and CRF immunohistochemistry was performed using a rabbit anti-pCREB antibody (1:750) and a rabbit anti-CRF antibody (1:1000). Finally, we measured plasma levels of corticosterone and adrenocorticotropin (ACTH) by commercially available kits for mice radioimmunoassay (RIA). Data were analyzed by 2-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test.

Results and conclusions

Our results demonstrate that morphine treatment produces an increment in the number of neurons expressing CRF and pCREBser133 versus saline groups in the PVN and an increase in the density of CRF efferent fibbers in the NTS but not in the NAc. Moreover, we observed an elevation of ACTH levels but no changes were found in corticosterone levels. In addition, these results were more evident in the groups conditioned in the CPP apparatus. In conclusion, our results suggest that CRF, pCREBser133 and environment play a role in the memory during drug addiction.