Expression of the Corticotropin Releasing Factor (CRF) system of neuropeptides and receptors in mouse microglia cells.

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Summary

The CRF system is the key mediator of the stress response in the CNS and in the periphery. Expression of CRF neuropeptides and binding sites was examined in mouse microglial cells. Indirect immunofluorescence revealed the presence of immunoactive CRF and urocortin 1 in the cytoplasm, whereas both their receptors CRF1 and CRF2 were found in the cell membrane of the mouse microglial cell line BV-2. These results were confirmed by RT-PCR. The concomitant presence of the receptors with their neuropeptide ligands implies an autocrine role of the CRF system in microglial cells.

Introduction

The CRF system, consisting of the CRF and Urocortin (Ucn) neuropeptides, their receptors CRF1 και CRF2 and the CRF-Binding Protein (CRF-BP), is the key mediator of the endocrine, behavioral, autonomic and visceral responses to stress 1. CRF's principle role is the regulation of the hypothalamus-pituitary-adrenal axis. However, CRF and its homologue neuropeptides, the urocortins have been identified in several sites in the central nervous system (CNS), acting as neurotransmitters. It has been suggested that malfunction of the CRF system could contribute in the pathophysiology of depression, anxiety, neurodegenerative and other chronic diseases. The potential use of CRF antagonists in such disorders is currently under intense investigation 2; 3.

Microglial cells hold an active role in the defense (acting as macrophage cells) but also in the neuronal repair in the CNS. Thus, activation of microglia is related to progressive neuronal apoptosis in neurodegenerative human brain disorders, such as Parkinson's disease. CRF has been shown to induce apoptosis in mouse microglial cells 4.

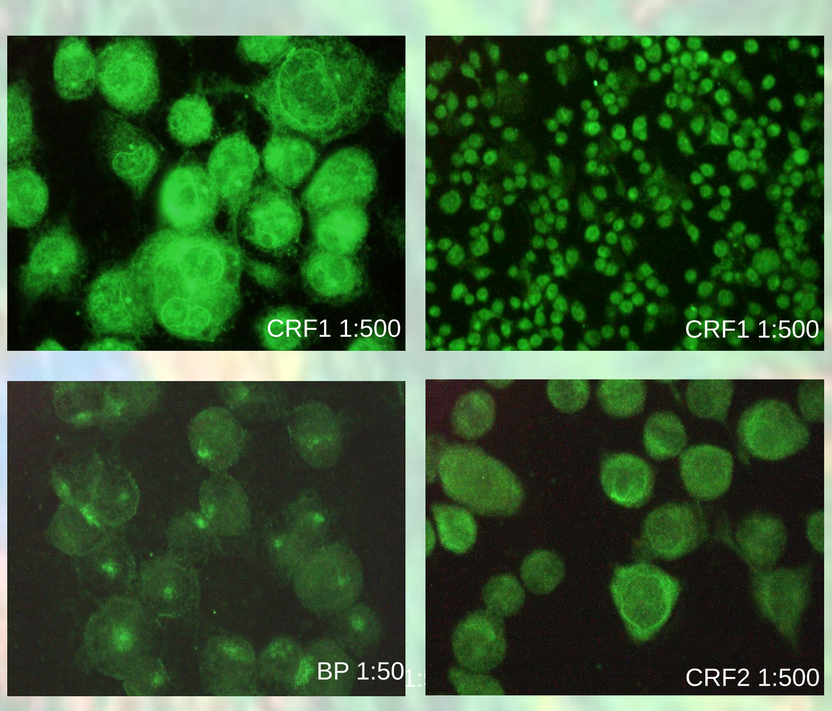
Aim of the present study was to investigate the expression of the members of the CRF system (i.e. neuropeptides, receptors and binding protein) in microglia, and in particular in the mouse microglial cell line BV-2.

Materials and Methods

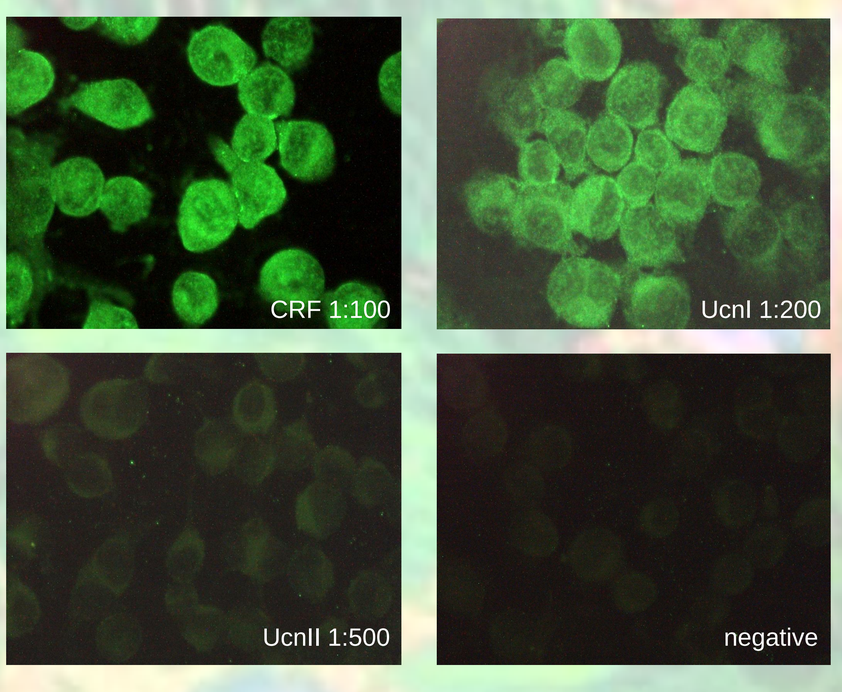
Cell Culture. The cell line BV-2 has derived from primary cell culture of mouse microglial cells after transfection with a J2 retrovirus carrying the *v-raf/v-myc* oncogene 5. Cells were grown in DMEM culture medium supplemented with 10%FCS in standard cell culture conditions.

RT-PCR. Reverse transcription PCR was performed in total RNA extracted from cells, using primers specific for CRF, Ucn, CRF1, CRF2(α), CRF2(β) and CRF-BP published gene sequences. Negative control samples where no RT enzyme was added (no RT) or without DNA template (no DNA) were included in every assay. RT-PCR for β-actin was also performed in order to assure RNA quality.

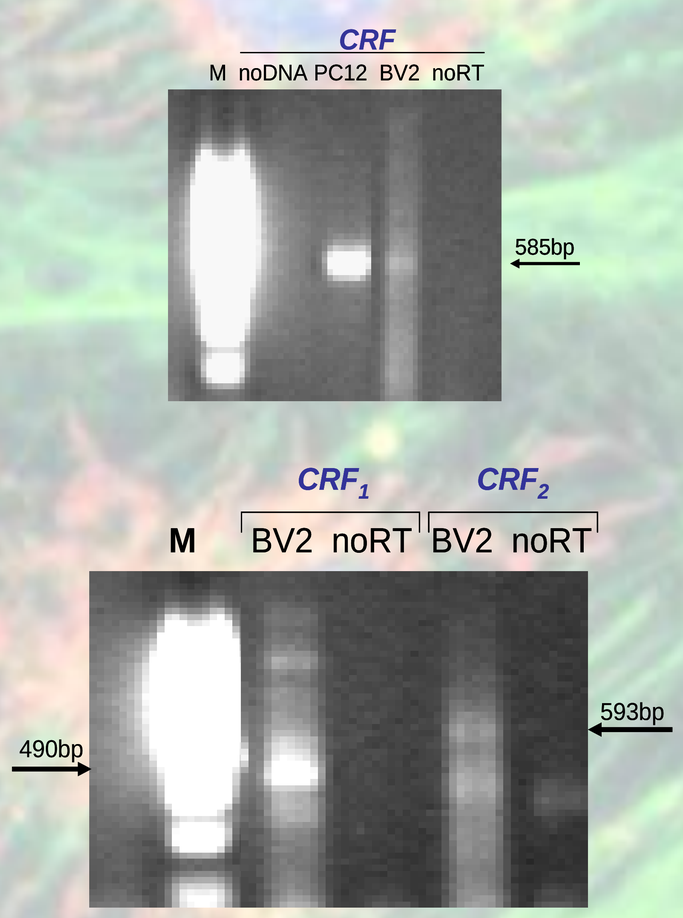
Immunofluorescence. The antisera used for CRF, Ucn and CRF-BP detection were obtained from Phoenix Pharmaceuticals and from Santa Cruz Biotechnology. The anti-CRF receptor antisera were the 4467a-CRF1 and 2064a-CRF2, 6; 7 and they were kindly donated by Neurocrine Bioscience Inc, San Diego, CA, USA. All antisera were raised in rabbit. Immunofluorescent staining of cells grown on multi-well chambers was conducted using standard methodology.



Immunofluorescent staining of BV-2 cells for **CRF receptors** 1 and 2 (CRF1,CRF2) and for CRF-BP.



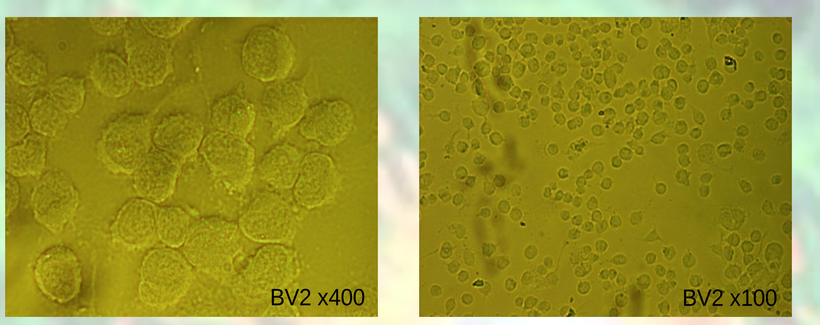
Immunofluorescent staining of BV-2 for **CRF neuropeptides** CRF, Ucn1 an Ucn2, in parallel with negative control.



RT-PCR for CRF and CRF receptors in total RNA isolated from BV-1 cells

Results

Immunofluorescence revealed specific staining of BV-2 cells when antibodies against CRF, urocortin 1, CRF1 and CRF2 receptors were used. Neuropeptides CRF and urocortin 1 were localized in the cytoplasm, whereas their receptors were found in the cell membrane. Immunofluoresence for urocortin 2 and CRF-BP revealed no specific staining in the BV-2. These results were confirmed by RT-PCR, showing the presence of gene transcripts in total RNA isolated from BV-2 cells.



BV-2 mouse microglia cells grown in culture, under light microscopy.

Discussion

Our findings show expression of the CRF system in the mouse microglial cells at gene and peptide levels, and in particular the neuropeptides CRF and urocortin I as well as both types of receptors CRF1 and CRF2. The concomitant presence of the receptors with their neuropeptide ligands implies a possible autocrine regulatory role of this system in microglial cells. More experiments are needed to elucidate the biological role of this system in the pathophysiology of the CNS.

References

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