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PSYCHIATRIC DISORDERS

Fear factors

The molecular events that underlie depression and anxiety are poorly understood but probably involve many factors. Corticotrophin-releasing factor (CRF) and serotonin (5-hydroxytryptamine (5-HT)) have been independently implicated in these disorders through their activation of CRF receptor 1 (CRFR1) and several 5-HT receptors, respectively. It was unknown whether these two systems interact, but now Magalhaes *et al*. show that CRFR1 activation increases the recruitment of 5-HT receptor 2A (5-HT_{2A}) to the cell surface, thereby modulating 5-HT_{2A} signalling.

CRFR1 and 5-HT_2 receptors are G protein-coupled receptors. Upon activation, 5-HT_2 receptors stimulate the phospholipase C signalling cascade, resulting in inositol phosphate production. The authors showed that CRF pretreatment of mouse cortical slices or cultured cells expressing both 5HT_{2A} and CRFR1 increased the 5-HT-induced formation of inositol phosphate. Inhibiting protein kinases downstream of either CRFR1 or 5-HT_{2A} activation did not change the CRF-mediated effect, indicating that it was not mediated by the signalling pathways associated with the receptors. The authors therefore examined whether CRFR1 activation directly influences 5-HT_{2A} receptors.

Immunofluorescence experiments showed that 5-HT_{2A} receptors were constitutively internalized, whereas CRFR1 only became internalized after CRF treatment. Blocking receptor internalization by expressing a dominant-negative endocytosis inhibitor eliminated the effect of CRF pretreatment on 5-HT_{2A} signalling. Thus, CRFR1 endocytosis is required for CRF-induced sensitization of the 5-HT_{2A} response to serotonin.

The authors further investigated the role of receptor trafficking in the interaction between CRF and 5-HT, and found that CRF pretreatment increased the cell surface expression of 5-HT_{2A}. Moreover, inhibiting receptor recycling by treating cells with monensin or by blocking fast recycling endosomes (by expressing a dominant-negative form of RAB4) respectively reduced and prevented the effect of CRF pretreatment on 5-HT_{2A} signalling. This suggests that CRF-induced sensitization of 5-HT_{2A} signalling involves receptor endocytosis and recycling, resulting in increased 5-HT_{2A} surface expression.

Receptor trafficking is regulated by PDZ domain-containing proteins, and both 5-HT_{2A} and CRFR1 contain interacting motifs for this domain. The authors showed that deleting these motifs reduced the effect of CRF pretreatment on 5-HT_{2A} surface expression and 5-HT_{2A} signalling.

The behavioural relevance of these findings was tested in mice in open field and elevated plus-maze tests for anxiety. The mice received a CRF infusion into the prefrontal cortex, followed by intraperitoneal administration of the 5-HT receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Although CRF or DOI treatment alone did not affect anxiety levels in these tests, the combined treatment increased anxiety-like behaviour. This synergistic effect was inhibited by pretreating mice intraperitoneally with a 5-HT_{2A} antagonist, indicating that an interaction between CRF and 5-HT_{2A} receptors increases anxiety-like behaviour.

Previous research had shown that CRF can stimulate the release of 5-HT. These new findings identify an additional way in which the two neurotransmitter systems interact to regulate anxiety-like behaviour, and hint at the potential of 5-HT_{2A} antagonists as anxiolytic therapeutics.

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ORIGINAL RESEARCH PAPER Magalhaes, A. C. *et al.* CRF receptor 1 regulates anxiety behavior via sensitization of 5-HT2 receptor signaling. *Nature Neurosci.* **13**, 622–629 (2010)
FURTHER READING Joëls, M. & Baram, T. Z. The neuro-symphony of stress. *Nature Rev. Neurosci.* **10**, 459–466 (2009)