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GABA$_A$ Receptor Subtypes Regulate Stress-Induced Colon Inflammation in Mice

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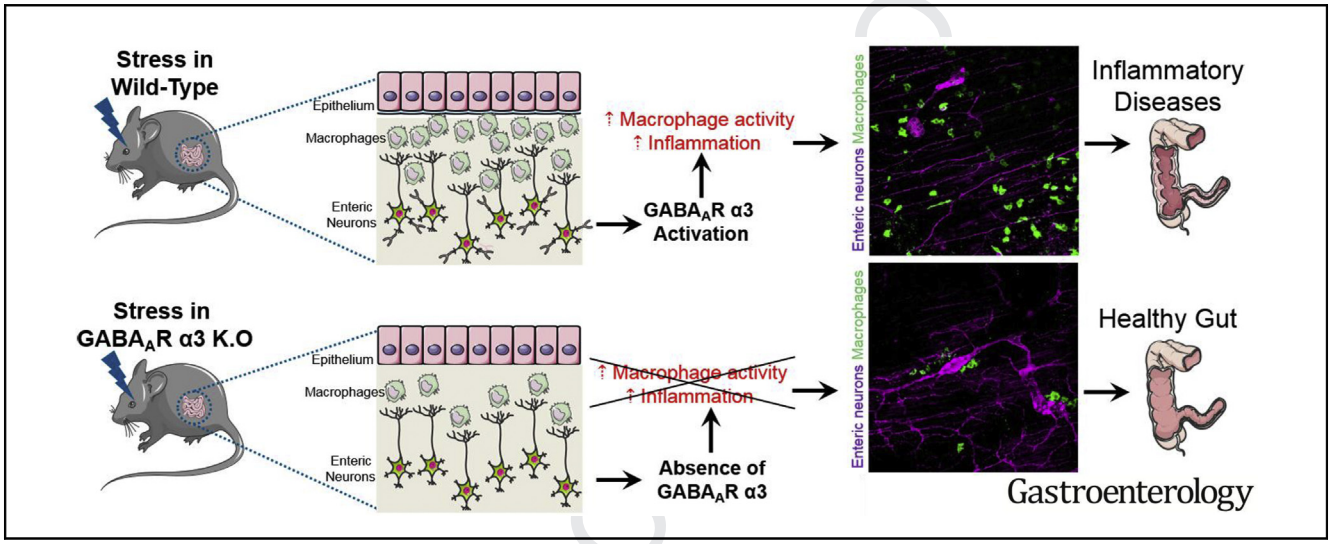

BACKGROUND & AIMS: Psychological stress, in early life or adulthood, is a significant risk factor for inflammatory disorders, including inflammatory bowel diseases. However, little is known about the mechanisms by which emotional factors affect the immune system. γ-Aminobutyric acid type A receptors (GABA$_A$Rs) regulate stress and inflammation, but it is not clear whether specific subtypes of GABA$_A$Rs mediate stress-induced gastrointestinal inflammation. We investigated the roles of different GABA$_A$R subtypes in mouse colon inflammation induced by 2 different forms of psychological stress. **METHODS:** C57BL/6J mice were exposed to early-life stress, and adult mice were exposed to acute-restraint stress; control mice were not exposed to either form of stress. We collected colon tissues and measured contractility using isometric tension recordings; colon inflammation, based on levels of cluster of differentiation 163 and tumor necrosis factor messenger RNA (mRNA) and on protein and myeloperoxidase activity; and permeability, based on levels of tight junction protein 1 and occludin mRNA and protein. Mice were given fluorescently labeled dextran orally and systemic absorption was measured. We also performed studies of mice with disruption of the GABA$_A$R subunit α3 gene (*Gabra3*$^{-/-}$ mice). **RESULTS:** Mice exposed to early-life stress had significantly altered GABA$_A$R-mediated colonic contractility and impaired barrier function, and their colon tissue had increased levels of *Gabra3* mRNA compared with control mice. Restraint stress led to colon inflammation in C57/BL6J mice but not *Gabra3*$^{-/-}$ mice. Colonic inflammation was induced in vitro by an α3-GABA$_A$R agonist, showing a proinflammatory role for this receptor subtype. In contrast, α1/4/5-GABA$_A$R ligands decreased the expression of colonic inflammatory markers. **CONCLUSIONS:** We found stress to increase expression of *Gabra3* and induce inflammation in mouse colon, together with impaired barrier function. The in vitro pharmacologic activation of α3-GABA$_A$Rs recapitulated colonic inflammation, whereas α1/4/5-GABA$_A$R ligands were anti-inflammatory. These proteins might serve as therapeutic targets for treatment of colon inflammation or inflammatory bowel diseases.

Keywords: Alprazolam; IBD; Inflammatory Response; THIP.

Abbreviations used in this paper: **α3$^{-/-}$, γ-aminobutyric acid subtype A receptor α3 gene–deleted mice; CD163, cluster of differentiation 163; ELS, early-life stress; ENS, enteric nervous system; FITC, fluorescein isothiocyanate; GABA, γ-aminobutyric acid; GABA$_A$R, γ-aminobutyric acid subtype A receptor; GI, gastrointestinal; IBD, inflammatory bowel disease; MPO, myeloperoxidase; mRNA, messenger RNA; PND, postnatal day; qPCR, quantitative real-time polymerase chain reaction; RMA, repeated measures analysis of variance; RST, restraint stress; TNF-α, tumor necrosis factor α; WT, wild type.**

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WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

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Psychological stress is a risk factor for gastrointestinal (GI) inflammation[1,2] and psychiatric disorders that are often comorbid with inflammatory bowel diseases (IBDs), such as anxiety and depression.[3] Therefore, identifying the molecular machinery that translates such emotional triggers into GI inflammation is a prerequisite for developing effective treatments for stress-associated GI inflammation. Diverse neural pathways cooperate with the body's organ systems to bring about a coordinated stress response using an array of chemical messengers capable of bridging the respective neuroimmune systems.[4] However, the stress response varies according to the duration of the stimulus and the age of the individual. Indeed, stress experienced during one's childhood appears to have the most profound impact on the immune system later in life.[5] Therefore, given the complexity of stressors encountered through life and the variability of the ensuing stress response, we must identify common biological mechanisms for such diverse processes if we are to address the associated disorders. One such emerging molecular integrator of the stress, nervous, and immune systems is the γ-aminobutyric acid (GABA)–GABA subtype A receptor ($GABA_AR$) system.[6]

$GABA_ARs$ are integral membrane ion channel complexes composed of 5 subunits. Up to 19 different subunits have been identified within the mammalian nervous system, and these are termed α1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–3.[7] Over several decades, this subunit diversity has been shown to result in multiple receptor subtypes, which vary according to their anatomic expression, physiological characteristics, and pharmacologic profiles within the brain.[8] These $GABA_AR$ expression and functional phenotypes within the brain have been shown to be susceptible to stress-induced plasticity.[9,10] Emerging evidence now points to various $GABA_AR$ subtypes directly associated with neuroinflammation within the brain[11] and inflammatory disorders in peripheral organs, such as asthma.[12] Furthermore, the anti-epileptic drug topiramate, which has $GABA_AR$ agonist properties, has been shown to ameliorate the macroscopic and microscopic GI inflammation score in an animal model of IBD.[13] Despite this convincing evidence for a role of $GABA_ARs$ in contributing to stress and inflammatory responses, their roles in IBD are relatively poorly understood, primarily because of our limited understanding of $GABA_AR$ function in the GI immune system.

We have recently shown the expression of various $GABA_AR$ subunits by neurochemically diverse cell types of the mouse enteric nervous system (ENS).[14] Furthermore, we showed that different $GABA_AR$ subtypes had contrasting effects on the spontaneous contractility of the mouse colon.[14] Importantly, acute stress altered not only native colonic contractility but also $GABA_AR$-mediated contractility.[14] This indicates that stress robustly engages the ENS and alters its influence on GI functions. Because the ENS also plays an important role in regulating the local GI immune system, it is reasonable to speculate that this stress-induced change in $GABA_AR$-mediated ENS function could alter ENS-mediated immune function as well. In the current study, we show that exposing mice to various forms of stress robustly induces GI inflammation via α3-$GABA_ARs$, whereas α1/4/5-$GABA_ARs$ have an anti-inflammatory role within mouse colon. Thus, this study positions GI $GABA_ARs$ as dynamic bidirectional regulators of intestinal inflammation.

Materials and Methods

All procedures involving animal experiments were approved by the Animal Welfare and Ethical Review Board of the University of Portsmouth and were performed by a personal license holder, under a Home Office–issued project license, in accordance with the Animals (Scientific Procedures) Act, 1986 (UK) and associated procedures.

See Supplementary Material for a detailed description of Materials and Methods.

Animals

For wild-type (WT) mice, the C57BL/6J strain obtained from the University of Portsmouth Bioresource center was used. In some experiments, $GABA_AR$ α3 subunit gene–deleted (*Gabra3*$^{-/-}$) mice and their WT littermates, raised against the C57BL/6J background, were also used.[15] Animals were bred in-house in a temperature- and humidity-controled environment under a 12-hour light/dark cycle, with free access to standard chow and water. Only male mice were used to preclude any confounding from sex hormones or the estrous cycle.

Early-Life Stress

A validated animal model of ELS, which is based on a fragmented mother-pup interaction during the first week of life,[16] was used. Briefly, pregnant dams were housed together with male partners and monitored every 12 hours for the birth of pups. The day of birth was termed postnatal day 0 (PND 0).