

Anxiolytic drug targets: beyond the usual suspects

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Commentary

Individuals suffering from anxiety experience an unpleasant emotional state defined by psychological and physiological responses to the anticipation of real or imagined danger. Physical manifestations include increased heart rate, altered respiration, sweating, trembling and fatigue, while psychological concomitants include feelings of powerlessness, apprehension, and potential danger. Many effective treatments for anxiety target the primary inhibitory neurotransmitter, γ -aminobutyrate (GABA). Within the brain, there are two principal subtypes of GABA receptor complexes: GABAA and GABAB. The subunits of the pentameric GABAA receptor form a tight group enclosing a chloride channel whereby GABA activation induces channel opening and subsequent influx of chloride ions (Cl^-) into the intracellular medium. The increase in negative charge causes neuronal membrane hyperpolarization and a resultant inhibition of neurotransmitter release. In this way, GABA agonists can induce anxiolysis, sleep, and anesthesia. Treatment of anxiety disorders: modulating GABAA receptors Allosteric modulators of GABAA receptors have anxiolytic, sedative/hypnotic, and anesthetic properties, presumably derived from this ability to enhance inhibitory neurotransmission through facilitation of receptor function. Benzodiazepines and barbiturates have been utilized with great success as anxiolytics in humans, but their use is limited due to their addictive potential and sedating side effects. An additional class of GABAA receptor modulators, the neurosteroids, have seen limited use as anesthetics, and have been proposed as potential therapeutic agents for anxiety disorders. However, the poor [...]

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Individuals suffering from anxiety experience an unpleasant emotional state defined by psychological and physiological responses to the anticipation of real or imagined danger. Physical manifestations include increased heart rate, altered respiration, sweating, trembling and fatigue, while psychological concomitants include feelings of powerlessness, apprehension, and potential danger. Many effective treatments for anxiety target the primary inhibitory neurotransmitter, γ -aminobutyrate (GABA). Within the brain, there are two principal subtypes of GABA receptor complexes: GABA_A and GABA_B. The subunits of the pentameric GABA_A receptor form a tight group enclosing a chloride channel whereby GABA activation induces channel opening and subsequent influx of chloride ions (Cl⁻) into the intracellular medium. The increase in negative charge causes neuronal membrane hyperpolarization and a resultant inhibition of neurotransmitter release. In this way, GABA agonists can induce anxiolysis, sleep, and anesthesia.

Treatment of anxiety disorders: modulating GABA_A receptors

Allosteric modulators of GABA_A receptors have anxiolytic, sedative/hypnotic, and anesthetic properties, presumably derived from this ability to

enhance inhibitory neurotransmission through facilitation of receptor function. Benzodiazepines and barbiturates have been utilized with great success as anxiolytics in humans, but their use is limited due to their addictive potential and sedating side effects. An additional class of GABA_A receptor modulators, the neurosteroids, have seen limited use as anesthetics, and have been proposed as potential therapeutic agents for anxiety disorders. However, the poor bioavailability, solubility, and side effect profiles of these compounds has limited their application in humans. The search for more practical anxiolytics relies on the identification of novel targets impinging upon this GABA_A receptor-mediated pathway.

In this issue of the *JCI*, Hodge and colleagues suggest such a target (1). Combining genetic, behavioral, and pharmacological levels of analysis, the

authors demonstrate that the GABA_A modulators themselves have a modulator: the ϵ isozyme of protein kinase C (PKC ϵ). They present an intriguing link between PKC ϵ , anxiety, and endogenous allosteric enhancers of GABA_A function, suggesting that the clinical benefits of modulating GABA_A receptor activity might be realized by targeting this kinase.

PKC ϵ -deficient mice, neurosteroids and anxiety

In the current article (1), Hodge et al. present further characterization of a line of knockout mice lacking PKC ϵ . As reported previously, these mice are characterized by enhanced sensitivity to GABA_A receptor allosteric modulators, including ethanol, benzodiazepines, and barbiturates (2). The authors now extend these observations to include the endogenous

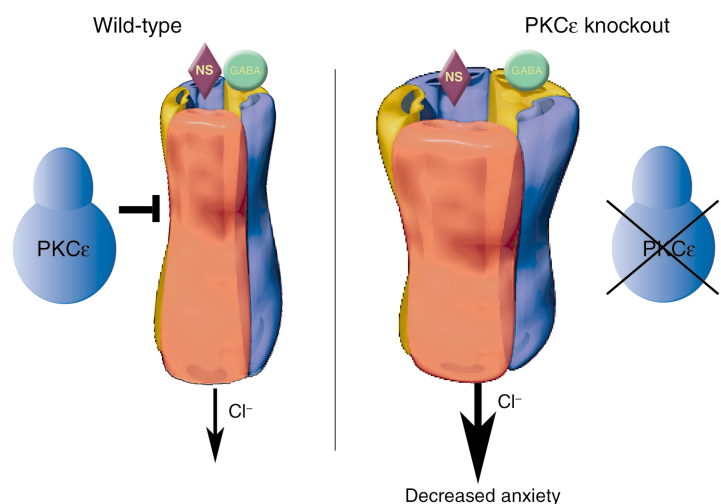


Figure 1

In PKC ϵ knockouts, the GABA_A receptor has enhanced sensitivity to neurosteroids, resulting in increased Cl⁻ conductance when activated by both GABA and endogenous neurosteroids. PKC ϵ knockouts also have reduced anxiety-like behavior, suggesting that PKC ϵ deficiency leads to decreased anxiety by making the GABA_A receptor more sensitive to the anxiolytic action of endogenous compounds such as neurosteroids.

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Nonstandard abbreviations used: γ -aminobutyrate (GABA); chloride ions (Cl⁻); protein kinase C ϵ (PKC ϵ).

neurosteroids, allopregnanolone and pregnanolone, which routinely enhance GABA_A receptor activity and are synthesized by both neurons and glia (3). In PKC ϵ knockout mice, these compounds increased muscimol-stimulated Cl⁻ uptake from cortical microsacs to a greater degree than in wild-type mice (Figure 1). Behaviorally, pregnanolone induced a more prolonged loss of the righting reflex in the PKC ϵ mutants, demonstrating greater sensitivity to the anesthetic effect in these animals (1).

The PKC ϵ knockout mice also had reduced levels of anxiety-like behavior in the elevated plus maze and open field test, two well-characterized rodent models of anxiety. Intriguingly, doses of the GABA_A receptor antagonist bicuculline, that had no effect in wild-type mice, restored anxiety-like behavior in the PKC ϵ mutants, suggesting that increased GABA_A receptor activity, as opposed to another, uncharacterized effect of the gene deletion, is responsible for the behavioral phenotype (1). A parsimonious, though hardly conclusive, explanation of these results is that increased sensitivity to an endogenous allosteric modulator results in increased GABA_A receptor activity, thereby decreasing anxiety-like behaviors.

Neurosteroids are attractive candidates for the role of endogenous modulators. Available data suggest their effect on GABA_A receptor function is most likely responsible for the significant behavioral traits observed following their application. Neurosteroids that enhance GABA_A receptor function have anxiolytic-like properties in animal models, including the elevated plus maze (4). Neurosteroids that inhibit GABA_A receptor function act in an anxiogenic fashion (3, 5). Picrotoxin, which blocks GABA_A receptors, generally prevents the anxiety-related effects of the neurosteroids (3). Recently, abnormal neurosteroid levels have been found in patients suffering from anxiety and depressive disorders, suggesting direct relevance to human psychiatric illness (6, 7). Unfortunately, attempts to capitalize on these effects by developing clinically useful synthetic neurosteroids have met with minimal success (8).

PKC ϵ and GABA_A receptor sensitivity

Given that allosteric enhancers of GABA_A receptor activity have proven

clinically problematic, the finding that PKC ϵ deficiency yields anxiolytic-like results is promising. Nonetheless, caution must be exercised in the interpretation of results from constitutive knockout experiments. These mice lack the protein kinase throughout development, leaving open the possibility that PKC ϵ deficiency causes an increase in GABA_A receptor sensitivity to allosteric modulators through a developmental or compensatory process. Application of PKC ϵ antagonists in the adult, therefore, might not yield the same result. Indeed, direct activation of PKC by phorbol esters has been shown to increase GABA_A receptor sensitivity to neurosteroids (9). This result is the opposite of what would be expected given the data presented by Hodge et al. (1), and would suggest that PKC deficiency should decrease, not increase, GABA_A receptor sensitivity to these compounds.

Fortunately, existing data suggests an alternative hypothesis for the discrepancy between the effects of the PKC ϵ knockout and the effects of phorbol ester-induced activation. The authors have previously shown that a PKC ϵ -specific translocation inhibitor peptide (10) induces a similar increase in GABA_A receptor sensitivity to allosteric modulation in wild-type but not PKC ϵ knockout mice (2). Similar effects of acute, specific inhibition of PKC ϵ on GABA_A receptor activity have also been demonstrated in cardiac myocytes (11). Eliminating the kinase throughout development appears, therefore, to have the same effects as acutely antagonizing PKC ϵ function. The data argue that the phorbol ester experiment, by inducing activation of all PKC family members, obscures the specific role of the PKC ϵ isozyme.

Identifying the mechanism

How might PKC ϵ deficiency enhance allosteric modulation of the GABA_A receptor? A quite general mechanism must be required, as the sites of action of neurosteroids, barbiturates, and benzodiazepines on the GABA_A receptor are distinct (8). Although it appears that neurosteroids are capable of modulating GABA_A receptors with a variety of subunit compositions (8), a recent report suggests that the δ -subunit may be required for their anxiolytic and physiological effects (12). In contrast, the benzodiazepine binding site, for

example, involves the α and γ subunits (13). In order to facilitate the actions of different modulators acting at disparate sites, PKC ϵ must interact with diverse subunits. The most obvious candidate mechanism for such an interaction is phosphorylation – perhaps the kinase phosphorylates numerous sites across the various receptor subunits. The authors point out, however, that phosphorylation at known PKC-mediated sites is not altered in the PKC ϵ knockouts (2). Further research into the precise mechanisms by which PKC ϵ and neurosteroids modulate GABA_A receptors is clearly needed to further understand the nature of the interaction.

PKC ϵ : Possible target for anxiolytic therapy?

The exciting take-home message from Hodge et al., is the idea that PKC ϵ should be considered as a potential target for anxiolytic therapy (1). All classes of allosteric modulators of GABA_A receptor activity have been demonstrated to be effective anxiolytics, in humans and/or animal models (14). The current work demonstrates that: (a) PKC ϵ deficiency results in enhanced GABA_A receptor sensitivity to members of each of these classes of allosteric modulators, (b) this enhanced sensitivity is accompanied by decreased levels of anxiety-like behaviors, and (c) these behaviors can be normalized by mild GABA_A receptor blockade. Further work is necessary to prove a causal relationship between enhanced modulator sensitivity (as opposed to some other, yet unidentified, alteration in GABAergic neurotransmission) and the behavioral effect. It is important to identify which endogenous modulators, if any, are required for the behavioral effect. These caveats notwithstanding, it is attractive to imagine that PKC ϵ blockade would result in anxiolysis by making our brains more sensitive to our own endogenous anxiolytics.

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