CURRENT LITERATURE

NEUROSTEROIDS AS ENDOGENOUS REGULATORS OF SEIZURE SUSCEPTIBILITY

Stress-induced Deoxycorticosterone-derived Neurosteroids Modulate $GABA_A$ Receptor Function and Seizure Susceptibility

Reddy DS, Rogawski MA

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Stress affects seizure susceptibility in animals and humans, but the underlying mechanisms are obscure. Here we provide evidence that γ-aminobutyric acid subunit A ($GABA_A$) receptor–modulating neurosteroids derived from deoxycorticosterone (DOC) play a role in stress-related changes in seizure control. DOC, an adrenal steroid, the synthesis of which is enhanced during stress, undergoes sequential metabolic reduction by 5α-reductase and 3α-hydroxysteroid oxidoreductase to form 5α-dihydrodeoxycorticosterone (DHDOC) and allotetrahydrodeoxycorticosterone (THDOC), a $GABA_A$ receptor–modulating neurosteroid with anticonvulsant properties. Acute swim stress in rats significantly elevated plasma THDOC concentrations and raised the pentylenetetrazol (PTZ) seizure threshold. Small systemic doses of DOC produced comparable increases in THDOC and PTZ seizure thresholds. Pretreatment with finasteride, a 5α-reductase inhibitor that blocks the conversion of DOC to DHDOC, reversed the antiseizure effects of stress. DOC also elevated plasma THDOC levels and protected mice against PTZ, methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate, picrotoxin, and amygdala-kindled seizures in mice (ED_{50} values, 84–97 mg/kg). Finasteride reversed the antiseizure activity of DOC (ED_{50}, 7.2 mg/kg); partial antagonism also was obtained with indomethacin (100 mg/kg), an inhibitor of 3α-hydroxysteroid oxidoreductase. Finasteride had no effect on seizure protection by DHDOC and THDOC, whereas indomethacin partially reversed DHDOC but not THDOC. DHDOC, like THDOC, potentiated GABA-activated Cl^- currents in cultured hippocampal neurons (≤1 μ*M*) and directly activated $GABA_A$ receptor currents (≥1 μ*M*), compatible with a role for DHDOC in the antiseizure activity of DOC. DOC is a mediator of the physiological effects of acute stress that could contribute to stress-induced changes in seizure susceptibility through its conversion to neurosteroids with modulatory actions on $GABA_A$ receptors including THDOC and possibly also DHDOC.

Enhanced Neurosteroid Potentiation of Ternary $GABA_A$ Receptors Containing the δ Subunit

Wohlfarth KM, Bianchi MT, Macdonald RL

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Attenuated behavioral sensitivity to neurosteroids has been reported for mice deficient in the γ-aminobutyric acid subunit A [GABA(A)]-receptor δ subunit. We therefore investigated potential subunit-specific neurosteroid pharmacology of the following $GABA_A$-receptor isoforms in a transient expression system: α1β3γ2L, α1β3δ, α6β3γ2L, and α6β3δ. Potentiation of submaximal $GABA_A$-receptor currents by the neurosteroid tetrahydrodeoxycorticosterone (THDOC) was greatest for the α1β3δ isoform. Whole-cell GABA concentration–response curves performed with and without low concentrations (30 n*M*) of THDOC revealed enhanced peak $GABA_A$-receptor currents for isoforms tested without affecting the GABA EC_{50}. The α1β3δ currents were enhanced the most (>150%), whereas the other isoform currents were enhanced 15–50%. At a higher concentration (1 μ*M*), THDOC decreased peak α1β3δ2L-receptor current amplitude evoked by GABA (1 m*M*) concentration jumps and prolonged deactivation but had little effect on the rate or extent of apparent desensitization. Thus the polarity of THDOC modulation depended on GABA concentration for α1β3δ2L $GABA_A$ receptors. However, the same protocol applied to α1β3δ receptors resulted in peak current enhancement by THDOC of >800% and prolonged deactivation. Interestingly, THDOC induced pronounced

desensitization in the minimally desensitizing $\alpha1\beta3\delta$ receptors. Single-channel recordings obtained from $\alpha1\beta3\delta$ receptors indicated that THDOC increased the channel-opening duration, including the introduction of an additional longer duration open state. Our results suggest that the $GABA_A$-receptor delta subunit confers increased sensitivity to neurosteroid modulation and that the intrinsic gating and desensitization kinetics of $\alpha1\beta3\delta$ $GABA_A$ receptors are altered by THDOC.

COMMENTARY

These two articles explore the actions of a group of compounds, classified as neurosteroids, that have been shown to be powerful endogenous positive modulators of $GABA_A$ receptors, similar to the benzodiazepines (BZDs) and barbiturates. Neurosteroids also are capable of interacting directly with $GABA_A$ receptors, albeit at high concentration. Of clinical significance, neurosteroids have been demonstrated to have anxiolytic, hypnotic, anesthetic, and anticonvulsant effects, and have been implicated as having a role in memory enhancement, behavioral actions, and neuroprotection.

The Reddy and Rogawski article explores the role of deoxycorticosterone (DOC) and its metabolites 5-dihydrodeoxycorticosterone (DHDOC) and allotetrahydrodeoxycorticosterone (THDOC), formed by reduction by 5-reductase and 3-hydroxysteroid oxidoreductase, respectively, in the regulation of seizure susceptibility by stress. The Wohlfarth, Bianchi, and Macdonald study takes a more reductionist approach, looking at the allosteric modulation of $GABA_A$ receptors currents by the neurosteroid THDOC in mammalian cells transiently transfected by recombinant $GABA_A$ receptors containing different subunits. Taken together, these two articles define how neurosteroids act at the single-channel level and how these physiologic changes can mediate the effects of stress on seizure susceptibility.

In an elegantly designed set of experiments, Reddy and Rogawski showed that the stress-induced neurosteroid DOC is capable of positively modulating $GABA_A$ receptors through its metabolites DHDOC and THDOC. These results are well articulated in the abstract. In addition, DOC was shown to have protective activity against seizures induced by $GABA_A$-receptor antagonists such as PTZ and picrotoxin, but not against seizures induced by *N*-methyl-d-aspartate (NMDA) or kainate, or the glycine antagonist strychnine. Although the results with these latter agents are not surprising, it is curious that the neurosteroids protect against the $GABA_A$-receptor blockers, unless we are to assume that $GABA_A$ receptors are only partially blocked. If neurosteroids modulate $GABA_A$ responses, then under conditions of complete blockade of $GABA_A$ receptors, neurosteroids should have no effect. However, if these receptors are only partially blocked, as might occur with intraperitoneal delivery of the drugs, and incomplete penetration into the CNS, then neurosteroids could still exercise their augmenting effects on $GABA_A$ inhibition, and thereby mediate a protective action.

A relation between emotional factors and seizure control has been recognized for quite some time. Most studies have found that high stress levels and stressful events are indeed associated with more frequent epileptiform EEG abnormalities and seizures, although there are a few exceptions that the authors have identified in the text. If stress triggers seizures, as is generally accepted, the authors ask how the present observations can be reconciled. They argue that there are many neural and endocrine pathways through which stress can alter brain function and thereby affect seizure susceptibility. The likelihood of seizures represents a balance between these pathways, some that bias toward seizures [e.g., glucocorticoids, hypercarbia (from hyperventilation)] and others (e.g., neurosteroids) that protect against seizures. Stress-induced seizures would thus occur when the balance is shifted toward proconvulsant factors. This scheme plus the known relation that stress generally predisposes to EEG abnormalities and seizures, leads to the conclusion that in the presence of stress, proconvulsant factors will assuredly dominate over protective ones, including neurosteroids. This would imply that stress-induced neurosteroids are generally ineffective in preventing seizures, although one could rationalize that more potent similar compounds could be useful anticonvulsants.

The results of the second article under consideration are also well described in the abstract. What is not made clear in the abstract is that the preparation used in the experiments is human embryonic kidney cells (HEK293T). Whether the results obtained have relevance for neurons is an important issue, which the authors recognize. This subject is thoughtfully considered in the discussion of the results of the present report, which showed neurosteroid potentiation of $GABA_A$ receptors, and those of relevant previous studies that present opposing arguments. In this context, important considerations in neuronal preparations versus recombinant systems would include the possibility that a neuronal environment may alter the sensitivity of $GABA_A$ receptors, and that posttranslational receptor modifications (e.g., phosphorylation dependence) would be impossible in recombinant systems.

It is believed that neurosteroids, like barbiturates, exert their effect on $GABA_A$ receptors through two separate binding sites. Low (nanomolar) concentrations of neurosteroids allosterically enhance GABA-mediated currents, whereas higher (micromolar) concentrations directly activate $GABA_A$ receptors. In addition, a rebound current was seen on washout for

concentrations ≥10 μ*M* in all tested $GABA_A$-receptor isoforms. This result was suggested to indicate that a third binding site, presumably within the channel pore, produces a low-affinity open-channel block similar to that observed for barbiturates. The action of THDOC on channel-gating kinetics appears analogous to the action of barbiturates, which have been shown to prolong native (suggested by the authors to be) $GABA_A$-receptor single-channel mean open time by shifting the relative distribution of existing open durations. Whether barbiturates or other modulators can alter the gating behavior of $GABA_A$ receptors in a manner similar to that of THDOC is unknown.

Based on their results as well as those of others, the authors suggest that neurosteroids modulate inhibitory postsynaptic currents in two ways: (a) by altering the peak current (either positively or negatively), depending on the concentration of both GABA and the neurosteroid; and (b) by lengthening the duration of the inhibitory postsynaptic current (by slowing deactivation), independent of the GABA concentration. For extrasynaptic isoforms, neurosteroids may increase basal inhibition by enhancing the response to ambient GABA levels. Further, it is possible that modulation of neuronal circuits necessary for neurosteroid effects depends (either directly or indirectly) on $GABA_A$ receptors containing the subunit.

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