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Research report

Alteration of kappa-opioid receptor system expression in distinct brain regions of a genetic model of enhanced ethanol withdrawal severity

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Abstract

Abrupt withdrawal from chronic alcohol exposure can produce convulsions that are likely due to ethanol (EtOH) neuroadaptations. While significant efforts have focused on elucidating dependence mechanisms, the alterations contributing to EtOH withdrawal severity are less well characterized. The present studies examined the kappa-opioid receptor (KOP-R) system in Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mice, selected lines that display severe and mild convulsions upon removal from chronic EtOH exposure. Previous data demonstrated significant increases in whole brain prodynorphin (*Pdyn*) mRNA in WSP mice only during EtOH withdrawal. No significant effects of EtOH exposure or withdrawal were observed in WSR mice. The present study characterized *Pdyn* mRNA and the KOP-R in WSP and WSR mice during EtOH withdrawal using in situ hybridization (ISH) and KOP-R autoradiography. Analyses were performed in brain regions that express *Pdyn* mRNA and/or KOP-R and that might participate in seizure circuitry: the piriform cortex, olfactory tubercle, nucleus accumbens, caudate–putamen, claustrum, dorsal endopiriform nucleus, and cingulate cortex. ISH analyses confirmed previous findings; EtOH withdrawal increased *Pdyn* mRNA in multiple brain regions of WSP mice, but not WSR. Basal KOP-R binding was higher in WSR mice than in WSP mice, suggesting an anti-convulsant role for receptor activation. Finally, increased KOP-R density was present during EtOH withdrawal in WSP mice. These data suggest that differences in the KOP-R system among the lines might contribute to their selected difference in EtOH withdrawal severity.
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1. Introduction

Chronic alcohol (ethanol, EtOH) exposure produces physical dependence as a result of central nervous system (CNS) adaptations that enable an organism to function in the presence of this CNS depressant and thus re-establish internal homeostasis [22,23]. A great deal of effort has focused on elucidating the neuroadaptive changes that occur to produce EtOH dependence [27], however, we know much less about the mechanisms that contribute to EtOH withdrawal. Unlike withdrawal from some drugs of abuse, severe EtOH withdrawal can be a life-threatening event. Thus, it is important to understand the neurochemical alterations that contribute to the physical and affective components of EtOH withdrawal to develop better methods for treatment.

Abbreviations: EtOH, ethanol; WSP, Withdrawal Seizure-Prone; WSR, Withdrawal Seizure-Resistant; HIC(s), handling induced convulsion(s); AR, autoradiography; Pyr, pyrazole; D2, DBA/2J

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The complex polygenic nature of alcoholism makes dissecting the genetic contributions to this disease a challenge. However, animal models that exhibit differences in one or more alcohol-related phenotypes exist. Selective breeding is one way these models are developed, and the ability to select for a phenotype is proof of principle that the trait of interest is at least partially controlled by genetic factors [6]. For example, Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mice are lines that have been selected in replicate from genetically heterogeneous mice for mild and severe EtOH withdrawal seizure severity [8,9]. To index withdrawal severity in these lines, handling induced convulsions (HICs) are measured following 72 h of EtOH vapor inhalation. Selection pressure has fixed many of the genes important for EtOH withdrawal seizure severity or resistance in a homozygous state in WSP and WSR mice. Thus, these selected lines represent a rich resource for identifying candidate genes that may participate in EtOH withdrawal seizures.

The WSP and WSR selected lines can be used to determine whether there is a genetic correlation between the selection phenotype (HICs) and other traits of interest (discussed in [11]). When a pair of selected lines is found to differ significantly on a trait other than the one for which they were selected, this significant genetic correlation between two traits implies the action of a common set of genes on the two phenotypes [11]. The strongest evidence for genetic codetermination of EtOH withdrawal severity and another trait is seen when both pairs of reciprocally selected lines differ in the correlated response (e.g., WSP-1 > WSR-1 and WSP-2 > WSR-2).

Because little is known about the mechanisms contributing to EtOH withdrawal HICs, one approach to examining their origin is to identify systems known to participate in other seizure types. An interesting candidate for investigation in WSP and WSR mice is the kappa-opioid receptor (KOP-R) system, which consists of the prodynorphin gene (*Pdyn*), the ligand dynorphin, and the KOP-R. Differences in dynorphin and KOP-R receptor abundance between a variety of seizure-sensitive and seizure-resistant animals models have been observed, as well as alterations in the KOP-R system both pre- and post-seizure induction [1,12,16,35,36,38]. However, both pro- and anti-convulsant roles for KOP-R activity have been suggested depending upon the animal and seizure models used.

Previous analyses of whole brain levels of *Pdyn* mRNA in WSP and WSR mice following chronic EtOH and withdrawal in our laboratory revealed that *Pdyn* abundance was significantly increased in WSP mice during EtOH withdrawal [2]. No significant changes were observed in WSR mice. These data suggest that *Pdyn* mRNA expression is selectively increased during EtOH withdrawal in a mouse line that exhibits severe EtOH withdrawal HICs and that alterations in the KOP-R system might contribute to the generation or severity of EtOH withdrawal seizures.

However, analyzing whole brain *Pdyn* mRNA abundance did not allow us to identify changes in discrete brain regions that might help elucidate the specific role of the KOP-R system in EtOH withdrawal severity. Thus, the purpose of the present study was to further characterize the response of the KOP-R system to EtOH exposure and withdrawal in WSP and WSR mice. Using in situ hybridization analysis and KOP-R autoradiography (AR), we examined the effects of chronic EtOH exposure and subsequent withdrawal on *Pdyn* expression and KOP-R density. Chronic ethanol vapor exposure was used to temporally separate ethanol withdrawal from ethanol exposure in order to determine whether ethanol exposure, withdrawal, or both alter the KOP-R system in these lines. We focused on brain regions that have previously been identified as participants in seizure activity: the piriform cortex, olfactory tubercle, nucleus accumbens, caudate–putamen, cingulate cortex, claustrum, and dorsal endopiriform nucleus. Changes in *Pdyn* message and KOP-R receptor density were compared to differences in withdrawal severity between the two lines to examine the hypothesis that altered KOP-R system function in the WSP and WSR selected lines is a correlated response to selection that contributes to their divergent EtOH withdrawal severity.

2. Materials and methods

All animal procedures and animal care were approved by the Portland Oregon VA Medical Center Institutional Animal Care and Use Committee and met NIH guidelines for appropriate care and use of animals in research.

2.1. Animal subjects

WSP and WSR mice were bred and generously provided by the laboratory of Dr. John Crabbe in Portland, OR. Drug-naive adult male mice from selected generation 26 (filial generations G_{77}–G_{90}) were used. The WSP and WSR selective breeding protocol was replicated, thus there are two independently derived replicate WSP and WSR lines [5,7–10]. Male mice of both replicates of the WSP (WSP-1 and WSP-2) and WSR (WSR-1 and WSR-2) lines were tested in these studies. Ages of the animals at the onset of the experiments ranged from 55–93 days, mean age 77 days; body weights ranged from 21.6–34.6 g, with a mean body weight of 27.6 g. Mice were maintained under a light/dark cycle of 06:00–18:00 light with water and Purina Lab Diet chow available ad libitum. Room temperatures were maintained at 22 ± 1 °C. EtOH exposure was initiated between 07:30–09:30 h.

2.2. Drug sources, reagents, and preparation

Pyrazole HCl (Pyr) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). EtOH (ethyl alcohol,