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A Site of Alcohol Action at the NMDA Receptor M3-M4 Domain Interface

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0717

A NOVEL ALCOHOL-SENSITIVE SITE IN THE M3 DOMAIN OF THE NMDA RECEPTOR GLUN2A SUBUNIT

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Accumulating studies have demonstrated that the N-methyl-D-aspartate receptor is one of the most important targets of ethanol in the central nervous system. Previous studies from this laboratory have found that one position in the third (F637) and two positions in the fourth (M823 and A825) membrane-associated (M) domains of the N-methyl-D-aspartate receptor GluN2A subunit modulate alcohol action and ion channel gating. Using site-directed mutagenesis and whole-cell patch-clamp recording, we have found an additional position in M3 of the GluN2A subunit, F636, which significantly influences ethanol sensitivity and functionally interacts with F637. Tryptophan substitution at F636 significantly decreased the ethanol IC_{50} , decreased both peak and steady-state glutamate EC_{50} , and altered agonist deactivation and apparent desensitization. There was a significant correlation between steady-state:peak current ratio, a measure of desensitization, and ethanol IC_{50} values for a series of mutants at this site, raising the possibility that changes in ethanol sensitivity may be secondary to changes in desensitization. Mutant cycle analysis revealed a significant interaction between F636 and F637 in regulating ethanol sensitivity. Our results suggest that F636 in the M3 domain of the GluN2A subunit not only influences channel gating and agonist potency, but also plays an important role in mediating the action of ethanol. These studies were supported by grants R01 AA015203-01A1 and AA015203-06A1 from the NIAAA to R.W.P.

0718

A SITE OF ALCOHOL ACTION AT THE NMDA RECEPTOR M3-M4 DOMAIN INTERFACE

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The N-methyl-D-aspartate (NMDA) glutamate receptor is a major target of ethanol in the brain. Previous studies have identified positions in the third and fourth membrane-associated (M) domains of the NMDA receptor GluN1 and GluN2A subunits that influence alcohol sensitivity. The structural model of the NMDA receptor, predicted from the structure of the related GluA2 subunit, indicates a close apposition of the alcohol-sensitive positions in M3 and M4 between the two subunit types. We investigated possible interactions between the M3 and M4 domain positions of the two subunit types affecting the ethanol sensitivity of the receptor by using dual substitution mutants. In an initial screen of single-substitution mutants, we found that a position in both subunits adjacent to one previously identified, GluN1(G638) and GluN2A(F636), can strongly regulate ethanol sensitivity. Significant interactions affecting ethanol inhibition were observed at four pairs of positions in GluN1/GluN2A: G638/M823, F639/L824, M818/F636, and L819/F637. Two of these interactions involve a position in M4 of both subunits, GluN1(M818) and GluN2A(L824), that does not by itself alter ethanol sensitivity, and one of the previously identified positions affecting ethanol sensitivity, GluN2A(A825), did not appear to interact with any other position tested. These results also indicate a shift by one position of the predicted alignment of the GluN1 M4 domain. These findings have allowed for the refinement of the NMDA receptor M domain structure, and support the existence of four sites of alcohol action on the NMDA receptor at the M3-M4 domain intersubunit interfaces. These studies were supported by grants R01 AA015203-01A1 and AA015203-06A1 from the NIAAA to R.W.P.

0719

A ROLE FOR TLR4 AND IL-1RI IN ETHANOL EFFECTS ON GABAergic TRANSMISSION AND ETHANOL DRINKING IN THE MOUSE

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A growing body of evidence indicates that neuroinflammation is involved in enhanced alcohol consumption and contributes to the progression to alcoholism. Several inflammatory signaling pathways have been shown to play a role in increased ethanol drinking and dependence. We have focused on two such pathways, TLR4 (toll-like receptor 4) and IL-1 (interleukin-1), and examined effects of their activations on GABAergic transmission in the central amygdala (CeA). GABAergic transmission is augmented by ethanol in CeA and is hypothesized to be a crucial mediator of ethanol drinking. Therefore, we superfused lipopolysaccharide (LPS) or IL-1 β to activate TLR4 and IL-1RI receptors, respectively, and performed intracellular recording of GABAergic IPSPs with sharp electrodes in murine brain slices. Our previous studies showed that acute superfusion of LPS (25 mg/ml) increased (by 42%), whereas IL-1 β (50 ng/ml) superfusion decreased (by 22%), IPSP amplitudes in CeA neurons. The ethanol augmentation of GABA $_A$ -IPSPs is markedly diminished by knocking out CD14 (an essential accessory protein for TLR4 activation by LPS). Conversely, acute pre-application of LPS potentiates ethanol effects (by 25%) on CeA GABAergic transmission in control mice, and restores ethanol effects in CD14KO mice. Acute activation of the IL-1 pathway diminished IPSPs and prevented ethanol potentiation (93% of control) of GABAergic transmission in CeA. The opposite effects of IL-1 β on GABAergic transmission in the CeA compared to LPS and ethanol may suggest a negative feedback of acute IL-1 β in the CeA. In support of this construct, our preliminary data from behavioral studies indicate that IL-1RI plays a role in negative regulation of EtOH drinking in mice, suggesting that increased activation of IL-1R decreases EtOH drinking. Thus, our data support the hypothesis of immune mechanisms underlying ethanol effects and drinking.

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0720

ETHANOL-MEDIATED FACILITATION OF AMPA RECEPTOR FUNCTION IN THE DORSOMEDIAL STRIATUM: IMPLICATIONS FOR ALCOHOL DRINKING BEHAVIOR

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We previously found that acute *ex vivo* or repeated cycles of *in vivo* ethanol exposure and withdrawal produces a long-lasting increase in the activity of NR2B-containing NMDA receptors (NMDARs) in the dorsomedial striatum (DMS) of rats^{1,2}. Activation of NMDARs is required for the induction of long-term potentiation (LTP) of AMPA receptor (AMPA)-mediated synaptic response (AMPA-LTP) in the DMS³. We therefore examined whether the ethanol-mediated upregulation of NMDAR activity alters the induction of AMPAR-LTP. We found that *ex vivo* acute exposure of striatal slices to, and withdrawal from, ethanol facilitates the induction of AMPAR-LTP in DMS neurons, which is abolished by inhibition of NR2B-containing NMDARs. We also found that repeated systemic administration of ethanol causes an NR2B-NMDAR-dependent facilitation of AMPAR-LTP in the DMS. LTP triggers the insertion of AMPAR subunits to the synaptic membrane⁴. In line with this concept, we found that repeated systemic administration of ethanol as well as excessive ethanol consumption produce a long-lasting increase in synaptic localization of the GluR1 and GluR2 subunits of AMPARs in the DMS. Importantly, we report that inhibition of AMPARs in the DMS attenuates operant self-administration of ethanol, but not of sucrose. Together, our data suggest that aberrant synaptic plasticity in the DMS induced by repeated cycles of ethanol exposure and withdrawal contributes to the molecular mechanisms underlying the development and/or maintenance of excessive ethanol consumption.

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3. Partridge et al. 2000 J Neurophysiol.
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