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ACUTE EFFECTS OF ETHANOL ON HIPPOCAMPAL LONG-TERM POTENTIATION AND LONG-TERM DEPRESSION ARE MEDIATED BY DIFFERENT MECHANISMS

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Abstract—To determine potential mechanisms contributing to ethanol-induced cognitive impairment, we examined acute effects of ethanol on hippocampal *N*-methyl-D-aspartate receptors and forms of synaptic plasticity thought to underlie memory processing. In the CA1 region of rat hippocampal slices, ethanol partially inhibited *N*-methyl-D-aspartate receptor-mediated synaptic responses at concentrations up to 180 mM. The block of synaptic *N*-methyl-D-aspartate receptors by 60 mM ethanol occluded the effects of 10 μM ifenprodil, an agent that has relative selectivity for *N*-methyl-D-aspartate receptors expressing NR1 and NR2B subunits. Ethanol did not occlude the effects of a low concentration of 2-amino-5-phosphonovalerate, an antagonist with less *N*-methyl-D-aspartate receptor subtype selectivity. Recent studies indicate that ifenprodil and other NR2B-selective antagonists inhibit *N*-methyl-D-aspartate receptor-dependent long-term depression but not long-term potentiation. We found that ethanol reversibly inhibited long-term depression in a manner consistent with its effects on synaptic *N*-methyl-D-aspartate receptors. Ethanol also inhibited the induction of *N*-methyl-D-aspartate receptor-dependent long-term potentiation, but the actions on long-term potentiation were complex and largely irreversible over the time course of our experiments. Furthermore, ethanol inhibited a form of long-term potentiation induced by very high frequency stimulation that does not depend on *N*-methyl-D-aspartate receptor activation. The effects of ethanol on both forms of long-term potentiation, but not on long-term depression, were at least partially reversed by block of GABA type A receptors with picrotoxin. These results indicate that pharmacologically relevant concentrations of ethanol exert preferential effects on a subtype of synaptic *N*-methyl-D-aspartate receptors in the CA1 hippocampal region. Inhibition of synaptic *N*-methyl-D-aspartate receptors appears to contribute strongly to ethanol-mediated long-term depression inhibition, but effects on long-term potentiation are complex, involving, at least partially, changes in GABAergic transmission. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: ethanol, synaptic plasticity, hippocampus, ifenprodil, blackouts.

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Abbreviations: ACSF, artificial cerebrospinal fluid; APV, D,L-2-amino-5-phosphonovalerate; EPSPs, excitatory postsynaptic potentials; LTD, long-term depression; LTP, long-term potentiation; NMDARs, *N*-methyl-D-aspartate receptors; P, postnatal day; PS, population spike; PTXN, picrotoxin.

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An intriguing feature of ethanol is its ability to induce an acute amnestic state (White, 2003). During ethanol intoxication, difficulties with short-term memory formation are often observed and some individuals exhibit memory "blackouts." The latter are periods in which individuals perform complex activities, but for which there is no subsequent recollection of the behavior. The ability to perform complex tasks during a blackout suggests that working memory and short-term processing is intact, but the ability to transfer short-term registration into longer-term memory storage is impaired.

Although the mechanisms underlying learning and memory are not completely understood, there is evidence that certain forms of use-dependent synaptic plasticity are likely to be important (Martin et al., 2000; Lynch, 2004). In particular, studies in rodents suggest the importance of processes akin to hippocampal long-term potentiation (LTP) and long-term depression (LTD). LTP and LTD represent forms of lasting use-dependent synaptic change that require, in most cases, activation of the *N*-methyl-D-aspartate class of glutamate receptors (NMDARs). Treatments that alter the function or expression of NMDARs (Sheng and Kim, 2002) or that disrupt the NMDAR protein complex (Grant and O'Dell, 2001) impair these forms of synaptic plasticity and disrupt learning and memory in rodents. Similarly, drugs with inhibitory actions at NMDARs are known to impair memory formation in humans.

Ethanol is known to inhibit NMDARs (see Allgaier, 2002; Dodd et al., 2000 for review) and appears to act at several NMDAR subtypes, with greatest effects against receptors expressing NR1 and NR2A or NR1 and NR2B subunits (Allgaier, 2002). Some studies indicate significant overlap in the actions of ethanol and ifenprodil, an antagonist with selectivity for NMDARs expressing NR1 and NR2B subunits. This suggests that effects on a specific subtype of NMDARs may be important in determining the effects of ethanol (Dodd et al., 2000; Yaka et al., 2003; Ron, 2004).

Consistent with the ability of ethanol to inhibit NMDARs, there is evidence that ethanol blocks NMDAR-dependent forms of LTP in the hippocampus (Chandler, 2003). The concentrations of ethanol required to inhibit LTP, however, vary from study to study. Some studies indicate that concentrations as low as 5 mM can at least partially block LTP (Blitzer et al., 1990), although concentrations above 50 mM have been required in many experiments. In some cases, even concentrations of ethanol as high as 50–60 mM have failed to block LTP (Sinclair and Lo, 1986; Swartzwelder et al., 1995; Schummers et al.,

1997). In clinical terms, ethanol concentrations of ~20 mM (~0.1%) represent legal intoxication in most communities, while 50 mM (~0.2%) is a substantially intoxicating level in most humans (White, 2003). Additionally, there is evidence that the effects of ethanol on NMDARs alone may be insufficient to explain effects on LTP (Schummers et al., 1997; Schummers and Browning, 2001, but see Morrisett and Swartzwelder, 1993). The effects of acute ethanol on hippocampal LTD have not been investigated in detail to date.

In the present study, we examined the effects of acute ethanol administration on synaptic NMDARs and the induction of LTP and LTD in the CA1 region of hippocampal slices prepared from adolescent rats (postnatal days (P) 30–32). Our interest in revisiting this issue is driven by recent findings indicating that the induction of CA1 hippocampal LTP and LTD may involve different subtypes of NMDARs (Liu et al., 2004; Massey et al., 2004) and the finding that certain NMDAR subtypes may be more sensitive to the effects of ethanol (Allgaier, 2002). Our results suggest that the effects of ethanol on LTD induction are likely explained by actions at a specific class of synaptic NMDARs, but that effects on LTP are complex and involve mechanisms in addition to, or instead of, NMDAR block.

EXPERIMENTAL PROCEDURES

Hippocampal slice physiology

Hippocampal slices were prepared from P30–32 Sprague–Dawley rats using standard methods (Zorumski et al., 1996). Rats were anesthetized with halothane and decapitated. Hippocampi were rapidly dissected and placed in artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 5 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 22 $NaHCO_3$, 10 glucose, bubbled with 95% O_2–5% CO_2 at 4–6 °C, and cut transversely into 450 μm slices using a vibrotome. Acutely prepared slices were placed in an incubation chamber containing gassed ACSF for 1 h at 30 °C.

At the time of study, slices were transferred individually to a submersion-recording chamber. Experiments were done at 30 °C with continuous bath perfusion of ACSF at 2 ml/min. Extracellular recordings were obtained from the apical dendritic layer of the CA1 region for analysis of population excitatory postsynaptic potentials (EPSPs) and from the cell body layer for analysis of population spikes. NMDAR synaptic potentials were recorded in ACSF containing 0.1 mM Mg^{2+}, 2.5 mM Ca^{2+} and 30 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX).

For most experiments, evoked synaptic responses were elicited with 0.2 ms constant current pulses through a bipolar stimulating electrode (Rhodes Medical Instruments, Tujunga, CA, USA) placed in the Schaffer collateral–commissural pathway. Synaptic responses in CA1 were monitored by applying single stimuli to the Schaffer collateral pathway every 60 s at intensity sufficient to elicit a half maximal response. After establishing a stable baseline for at least 10 min and a control input–output curve, LTP was induced in most experiments by applying a single 100 Hz×1 s tetanus using the same intensity stimulus. In some experiments, LTP was induced using a 200 Hz×1 s tetanus. This latter stimulus can produce a form of LTP that does not require NMDARs but does involve L-type voltage-activated calcium channels (Grover and Teyler, 1990). LTD was induced using the same intensity stimulus and applying single pulses at 1 Hz for 15 min. Input–output curves were repeated 20 min and 60 min following tetanic or 1 Hz stimulation. In studies of synaptic plasticity, values reported in the text represent percent changes in EPSP slopes measured 60 min following tetanic or 1 Hz stimulation relative to baseline responses in the same slice with positive values reflecting potentiation and negative values reflecting depression.

Statistical analysis

Data in the text and figures are expressed as mean±S.E.M. Student's *t*-test was used for comparisons between groups. Statistical comparisons in studies of synaptic plasticity were based on analysis of input–output curves at baseline and 60 minutes following tetanic or 1 Hz stimulation. *P* values of less than 0.05 were considered statistically significant.

RESULTS

Acute effects of ethanol on synaptic NMDARs

In initial experiments, we examined the effects of acute ethanol treatment on synaptic NMDARs in the CA1 region using low frequency activation of the Schaffer collateral pathway. When perfused for 10–15 min, ethanol inhibited synaptic NMDARs in a concentration-dependent fashion (Fig. 1A). At concentrations as high as 180 mM, the block of NMDARs remained incomplete (67±10% inhibition, $N=3$). The effects of ethanol at concentrations up to 60 mM were readily reversible following ethanol washout. At 180 mM, recovery was slower and less complete.

Because some studies suggest significant overlap in the effects of ethanol and the NR2B-selective NMDAR antagonist, ifenprodil (see Dodd et al., 2000 and Allgaier, 2002 for review), we examined interactions between these agents. At concentrations up to 10 μM, ifenprodil is a relatively selective inhibitor of NMDARs expressing NR1+NR2B subunits (Williams, 1993; Priestley et al., 1995). We found that 10 μM ifenprodil inhibits synaptic NMDARs by 42.8±5.6% ($N=6$). At 60 mM, ethanol depressed NMDAR EPSPs by 49.6±7.6% ($N=5$). In the presence of 60 mM ethanol, 10 μM ifenprodil had little effect on NMDAR EPSPs, suggesting significant overlap in the actions of these agents (Fig. 1B–D). Similarly, 60 mM ethanol produced little further inhibition of NMDAR EPSPs when administered in the presence of 10 μM ifenprodil (Fig. 1D). In contrast, the competitive NMDAR antagonist, D,L-2-amino-5-phosphonovalerate (APV) inhibited synaptic NMDARs by 66.1±4.3% ($N=6$) at 10 μM when administered alone (Fig. 1D), but nearly completely abolished NMDAR synaptic responses in the presence of ethanol (95.8±0.6% overall inhibition, Fig. 1B–D). Lower concentrations of APV depressed residual NMDAR EPSPs by 57.9±10.1% at 1 μM and 68.2±4.0% at 3 μM in the presence of 60 mM ethanol. Similar to effects observed in the presence of ethanol, 10 μM APV nearly completely blocked synaptic NMDARs when administered in the presence of 10 μM ifenprodil (−93.3±0.7% change, $N=4$, Fig. 1D).

Ethanol reversibly inhibits LTD

Recent studies indicate that homosynaptic LTD in the CA1 region is inhibited by NMDAR antagonists with preference for receptors expressing NR1+NR2B subunits and that NR2B-selective antagonists are ineffective against LTP (Liu et al., 2004; Massey et al., 2004; Mallon et al., 2005).