0022-3565/02/3013-938–944$7.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
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JPET 301:938–944, 2002
Vol. 301, No. 3
4734/986096
Printed in U.S.A.

Ifenprodil and Ethanol Enhance NMDA Receptor-Dependent Long-Term Depression

ADAM W. HENDRICSON, C. L. ALEK MIAO, MELANIE J. LIPPMANN, and RICHARD A. MORRISETT

The Waggoner Center for Alcohol and Addiction Research and the Division of Pharmacology and Toxicology, College of Pharmacy, University of Texas, Austin, Texas

Received November 8, 2001; accepted February 14, 2002 This article is available online at http://jpet.aspetjournals.org

ABSTRACT

Long-term alterations in synaptic transmission are thought to underlie various types of alcohol-related brain disorders. While ethanol effects on synaptic potentiation are well documented, ethanol effects on synaptic depression have not been addressed. Herein, we performed experiments to assess the role of ethanol on long-term depression (LTD) formation. In rat hippocampal slices, prolonged low-frequency stimulation (LFS) of CA1 Schaffer collaterals (1 Hz for 7 min) induced saturable, long-lasting, reversible *N*-methyl-D-aspartate (NMDA) receptor-dependent LTD of stimulus-evoked dendritic population excitatory postsynaptic potentials. This depression (−26% LTD amplitude) was observed in young rats (12–20 days old), but not adult rats (28–35 days old). Induction of LTD was blocked (−3% LTD amplitude) when the LFS was delivered in the presence of the NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid. When the conditioning LFS was delivered in the presence of ethanol, there was a significant enhancement in the induction of NMDA receptor-dependent LTD versus control LTD (−36% LTD amplitude). Ifenprodil, an *N*-methyl-D-aspartate receptor subunit 2B (NR2B)-selective antagonist, also significantly facilitated the induction of LTD (−40% LTD amplitude). Consistent with this result, ifenprodil did not affect the NMDA receptor-dependent component of the baseline synaptic response, whereas D-2-amino-5-phosphonovaleric acid caused significant depression of the NMDA component. These data indicate that whereas ethanol is known to *inhibit* NMDA receptor function in a variety of systems, it significantly *enhances* the induction of NMDA receptor-dependent LTD. Furthermore, since ifenprodil is known to select for ethanol-sensitive subtypes of NR2B-NMDA receptors, these data also suggest that NR2B-containing NMDA receptor subpopulations do not contribute to LTD, but instead may actually play inhibitory roles in LTD induction.

Long-term depression (LTD) of synaptic transmission is defined as a long-lasting decrease in synaptic strength induced by the application of certain types of conditioned stimulation, and is considered as an important form of synaptic plasticity especially during neuronal development (Staubli and Scafidi, 1997). In area CA1 of the hippocampus, prolonged low-frequency stimulation (LFS; 1–3 Hz) is known to elicit a form of LTD requiring activation of NMDA receptors (Bear and Malenka, 1994). Current data indicate that low to moderate activation of NMDA receptors by LFS results in a moderate influx of Ca^{2+}, which in turn leads to activation of protein phosphatase 1/2A (Lee et al., 2000). Protein phosphatase 1/2A then dephosphorylates the protein kinase A site, Ser845 of AMPA receptors, with ensuing attenuation of fast synaptic transmission. Increased AMPA receptor endocytosis related to PDZ-domain-regulated processes may also contribute to this depression of synaptic transmission (Carroll et al., 1999; Scannevin and Huganir, 2000; Xia et al., 2000). LTD may be induced by the activation of specific NMDA receptor subpopulations, especially NR2C/D subunits (Hrabetova et al., 2000), which are considered "low-conductance" NMDA receptors requiring only modest depolarization to overcome Mg^{2+} blockade (Monyer et al., 1992; Wyllie et al., 1996).

Convincing evidence has shown that NMDA receptors constitute an important site of action of ethanol. Pharmacologically relevant concentrations of ethanol antagonize native and recombinant NMDA receptor currents (Lovinger et al., 1989; Morrisett and Swartzwelder, 1993). Noncompetitive inhibition of NMDA receptors by ethanol is mediated via a molecular site distinct from the various regulatory sites associated with NMDA receptors (Chu et al., 1995). Finally, the ethanol sensitivity of native NMDA receptors is determined, at least in part, by the subunit composition of the receptor, whereby NMDA receptor subpopulations containing NR2B

This work was supported by Grants R01AA11845 and R01AA09230 from the National Institute of Alcohol Abuse and Alcoholism (to R.A.M.).

ABBREVIATIONS: LTD, long-term depression; ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; D-APV, D-2-amino-5-phosphonovaleric acid; pEPSP, population excitatory postsynaptic potential; LFS, low-frequency stimulation; mGlu, metabotropic glutamate; NMDA, *N*-methyl-D-aspartate; LTP, long-term potentiation; NR2B and NR2C/D, *N*-methyl-D-aspartate receptor subunits 2B and C/D.

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subunits appear to be more sensitive to ethanol inhibition (Lovinger, 1995).

Our laboratory and a number of other laboratories have demonstrated that acutely administered ethanol can inhibit NMDA receptor-dependent synaptic potentiation (Durand and Carlen, 1984; Sinclair and Lo, 1986; Mulkeen et al., 1987; Blitzer et al., 1990; Morrisett and Swartzwelder, 1993). Furthermore, acute ingestion of alcohol can produce amnesic effects in both human and animal studies (Lister et al., 1987). We have also demonstrated alterations in NMDA receptor-dependent synaptic transmission directly linked with hyperexcitability and neurotoxicity following withdrawal from prolonged exposure to ethanol (Thomas et al., 1998; Thomas and Morrisett, 2000). Coincidentally, new reports indicate that alterations in glutamatergic transmission and NMDA receptor-dependent plasticity may underlie synaptic alterations related to cocaine reinforcement (Ungless et al., 2001; Vorel et al., 2001). Such reports indicate that alterations in NMDA receptor-dependent plasticity may underlie ethanol reinforcement and related alterations in neural function. Therefore, we sought to identify the direct effects of ethanol on NMDA receptor-dependent synaptic depression mediated by low level NMDA receptor activation.

Materials and Methods

Slice Preparation. Slices used in this study were prepared from 12- to 20-day-old Sprague-Dawley rats of both sexes except for the older animal group (28–35 days old). After decapitation, the brains were rapidly removed and placed in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) for 3 to 4 min. The hippocampi were then removed bilaterally, and 500-μm transverse sections were cut and transferred to a holding chamber containing ACSF (120 mM NaCl, 25 mM $NaHCO_3$, 3.3 mM KCl, 1.2 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 2.4 mM $MgSO_4$, 10 mM dextrose) bubbled with 95% O_2/5% CO_2 (carbogen) and maintained at 32–35°C. After incubation in ACSF at 32–35°C for 1 to 2 h, slices were transferred to the recording chamber, continuously perfused with carbogen-saturated ACSF at a flow rate of approximately 1.5 ml/min via peristaltic pump (Rabbit-Plus; Rainin Instruments, Woburn, MA). The recording ACSF was identical to that used for slice preparation except that the concentrations of $CaCl_2$ and $MgSO_4$ were 2.0 mM and 0.9 mM, respectively. Drugs were dissolved in ACSF and applied to the slice by switching solution reservoirs. All drugs were obtained from Sigma-Aldrich (St. Louis, MO) except ethanol (95%), obtained from Aldrich Chemical Co. (Milwaukee, WI).

Extracellular Recording. The extracellular field potential (pEPSP) recordings were performed in normal recording ACSF containing 0.9 mM $MgSO_4$ and 2.0 mM $CaCl_2$. Population field potentials were recorded at 32°C from the CA1 pyramidal cell layer with glass microelectrodes made from thin-walled borosilicate glass (TW150F-4; WPI, Sarasota, FL; 1–3 MΩ) and filled with a 150 mM NaCl solution. Recordings were made using DC differential amplifier (WPI), filtered at 1 kHz, and digitized at 10 to 20 kHz using a Digidata interface (Axon instruments, Union City, CA). Synaptic responses were evoked by stimulation of Schaffer collateral fibers with constant-current pulses (100-μs duration, 15- to 35-μA amplitude) applied through a stimulus isolation unit driven by a digital stimulator (Master-8; A.M.P.I., Jerusalem, Israel).

LTD Induction and Drug Application. The low-frequency conditioning stimulation was a 100-μs train of 15 to 35 μA, constant current pulses delivered at 1 Hz for 7 min. In general, baseline pEPSPs were evoked every 45 s and observed for 10 to 20 min prior to delivery of the LFS, and then pEPSPs were observed for at least an additional 30 min. For drug application, the ACSF solution was switched to the drug solution for at least 10 to 15 min before any additional treatments. The concentrations of the agents used in the experiments were as follows: 25 μM D-APV, 75 mM ethanol, and 10 μM ifenprodil.

Data Acquisition and Analysis. The baseline of pEPSP slope was obtained by averaging the slopes over 200 to 500 μs of the linear section of the pEPSP down-stroke. The average pEPSP slope after the LFS train was obtained by averaging the pEPSP slopes recorded during the second 10 min after the train. The average post-train pEPSP slope was then compared with the baseline pEPSP slope to obtain the percentage of pEPSP depression as an indication of the extent of long-term depression. Measures were expressed as mean ± SEM for all experiments. Drug effects were evaluated using Student's t test with Bonferroni correction in comparison with the control group.

Results

Changes in synaptic strength were assessed by monitoring the slope of pEPSPs. As depicted in Fig. 1, application of a 1-Hz, 7-min LFS train to 12- to 20-day-old rat CA1 Schaffer collaterals reliably induced a statistically significant long-term depression of synaptic transmission which saturated after repeated LFS train administration (max. suppression: 35.6 ± 1.5%, $n = 3$, $P < 0.001$) and persisted for an extended period of time (mean suppression, measured at time points between 40 and 60 min post-LFS: 29.7 ± 3.2%, $n = 3$, $p < 0.001$). Furthermore, after one or more 100 Hz, 1 s high frequency stimulation (HFS) trains, this suppression was reversed to a statistically significant degree (suppression after HFS: 14.3 ± 2.7%, $n = 3$, $p < 0.001$). These findings indicate that the LFS-induced depression observed herein was of sufficient duration to be termed true LTD, and was caused by synaptic reorganization, as opposed to stimulus-induced tissue damage.

Figure 2 shows typical responses of a slice conditioned in the presence of D-APV, followed by conditioning under control conditions (no D-APV). When the LFS was delivered during bath application of D-APV, only a minimal change in the pEPSP slope was observed (Fig. 6; −3.6 ± 3.4%, $n = 5$, $p > 0.5$). Following D-APV washout, the application of a 1-Hz/7-min train of low-frequency stimulation strongly and significantly depressed the synaptic response by approximately 26% (Fig. 6; $n = 13$, $p < 0.001$). This reduction lasted for the duration of the recording following the LFS (>30 min). To further verify the relationship between animal age and LTD induction, we also tested the effects of LFS conditioning on slices from 28- to 35-day-old animals. As shown in Fig. 3, LFS did not induce LTD as seen in the younger group. The average depression of the pEPSP slope in older animals was insignificant (Fig. 6, −4.2 ± 3.8%, $n = 9$, $p > 0.3$).

Since our laboratory and others have reported ethanol inhibition of a variety of NMDA responses, we sought to determine whether NMDA receptor-dependent LTD was ethanol sensitive, as well. Typical effects of ethanol (75 mM) delivered prior to and during LFS are shown in Fig. 4. Surprisingly, we observed a substantial degree of LTD when the conditioning LFS was delivered in ethanol. As shown in Fig. 6, the extent of depression of synaptic transmission was −38.1 ± 4.0% ($n = 15$ slices from 9 rats, $p < 0.001$ for post- versus pre-LFS). The degree of LTD observed when LFS was delivered in ethanol was significantly greater than that observed when LFS was delivered under control conditions

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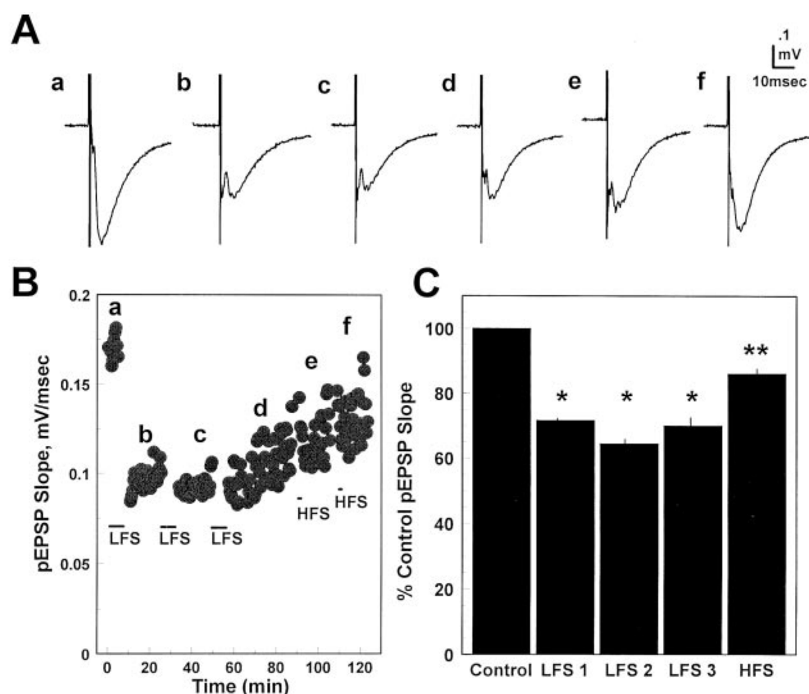

Fig. 1. The decrease in synaptic strength induced by LFS is saturable, persistent, and reversible by high-frequency stimulation (HFS). A, evoked pEPSPs recorded at 30-s intervals from CA1 dendrites of a 14-day-old rat hippocampal slice. Trace a: average of 10 control pEPSPs. Trace b: average pEPSP recorded 10 min after a 7-min, 1-Hz LFS train. Trace c: average pEPSP recorded 10 min after a second LFS train. Trace d: average pEPSP recorded 25 min after a third LFS train. Trace e: average pEPSP recorded 10 min after a 1-s, 100-Hz HFS train. Trace f: average pEPSP recorded 10 min after a second HFS train. B, complete time course of experiment depicted in A. ●, individual pEPSP slope values, recorded at 30-s intervals throughout (except LFS and HFS periods). a to f correspond to pEPSPs shown in A. C, cumulative pEPSP slope data, normalized to control. LFS 1 and LFS 2: mean suppression of pEPSP slopes after each of two LFS trains, administered 15 min apart (in all experiments, LTD saturation was attained after two LFS trains). Both LFS 1 and LSF 2 differed significantly from control ($n = 3$; $p < 0.001$). LFS 3: statistically significant suppression of mean pEPSP slopes, measured at time points between 40 and 60 min post-LFS ($n = 3$; $p < 0.001$). HFS: statistically significant reversal of peak mean pEPSP slope suppression after one or two episodes of HFS ($n = 3$; $p < 0.001$). Bars indicate S.E.M.

(−25.8% for control LTD versus −38.1% for ethanol, $p < 0.02$).

Since ethanol-NR2B selectivity has been reported, and the agent ifenprodil also displays an NR2B subunit selectivity (Williams, 1993; Lovinger, 1995), we elected to examine the effect of this agent on NMDA-LTD (Fig. 5). Similar to our observations with ethanol, when ifenprodil was bath-applied prior to and during LFS, we found that LTD could be robustly induced, with a significantly stronger degree of depression than that observed when LFS was delivered under control conditions. As shown in Fig. 6, the depression of synaptic transmission was −40.1 ± 3.4% ($n = 13$ slices from 7 rats; $p < 0.001$ for pre- versus post-LFS), which is significantly greater than LTD observed in the control group ($p < 0.003$).

Because the NMDA antagonists D-APV and ifenprodil displayed opposite effects on NMDA-LTD, we assessed the actions of these compounds on the NMDA component of the pEPSP itself. As shown in Fig. 7, the NMDA component is recognized as the late, slow component of the pEPSP. The amplitude of the NMDA response was measured at 20 ms poststimulus, as indicated in Fig. 7A. After bath application of ifenprodil, the amplitude of the NMDA response showed no significant change (in three slices tested, the average amplitude of NMDA response during ifenprodil application was 93 ± 8.9% of the baseline amplitude). However, when D-APV was then applied, the NMDA component displayed a significant decrease of amplitude (29 ± 3.1% of baseline). After wash-off of D-APV, the amplitude of the NMDA response recovered to the baseline level (92 ± 12.4% of baseline).

Discussion

Different types of LTD have been described in various central synapses and in the hippocampus as well; therefore, it is important to qualify our present findings relative to the various types of synaptic depression extant. In neonatal animals, induction of LTD is dependent on metabotropic gluta-

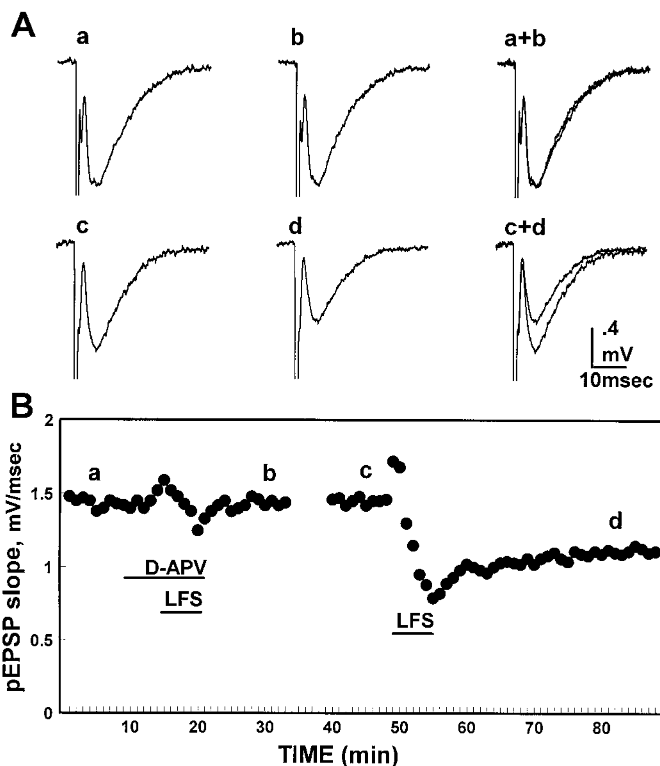

Fig. 2. D-APV blocks the induction of LTD in the CA1 region of hippocampal slices. A, evoked pEPSPs recorded from a hippocampal slice from a 15-day-old rat. Traces a to d correspond to pEPSPs denoted in B at bottom. Trace a was elicited before D-APV application as baseline; trace b was recorded 15 min after LFS. In (a + b), trace a and trace b are superimposed for comparison. Trace c was obtained 25 min after LFS and wash-off of D-APV. Trace d was obtained 25 min after a second LFS. In (c + d), traces c and d are superimposed. B, time course of a D-APV and LTD experiment. Baseline pEPSPs were evoked every 45 s and observed for more than10 min; then D-APV was applied, followed immediately by a 1-Hz, 7-min train (LFS). At the end of the train, the bath flow was switched back to normal ACSF and another LFS train was delivered, 25 min after D-APV wash-off and significant depression of pEPSP occurred.

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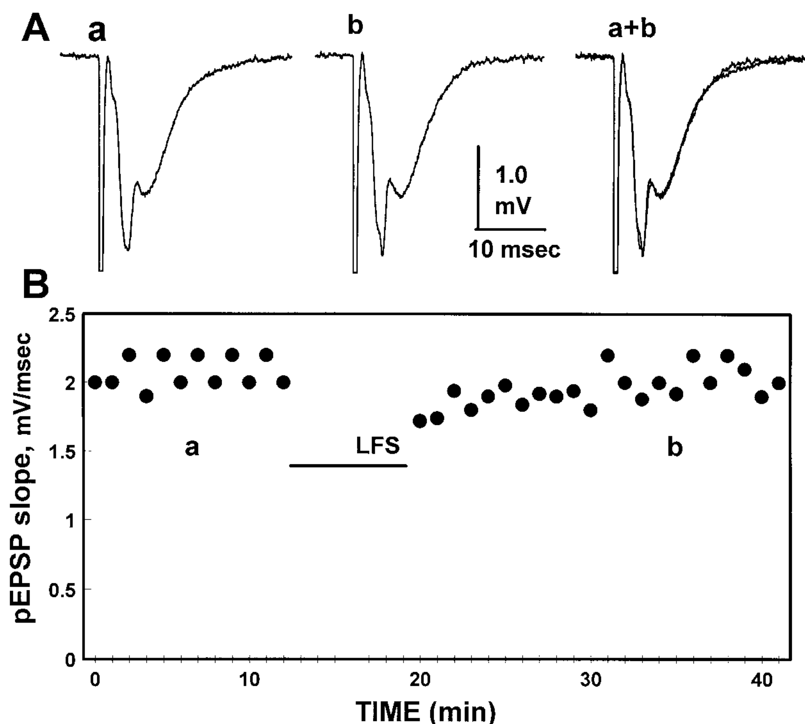

Fig. 3. Hippocampal slices from older rats are resistant to LTD induction. A, evoked pEPSPs recorded from a hippocampal slice prepared from a rat aged 33 days. Traces a and b correspond to pEPSPs elicited as denoted in panel B. Trace a was recorded before LFS as the baseline pEPSP. Trace b was recorded 15 min after LFS. In (a + b), traces a and b were superimposed for comparison. B, baseline pEPSPs were observed for more than10 min, and then the LFS train was delivered with observation continued for 30 min; the pEPSP remained stable following LFS, indicating no LTD occurred.

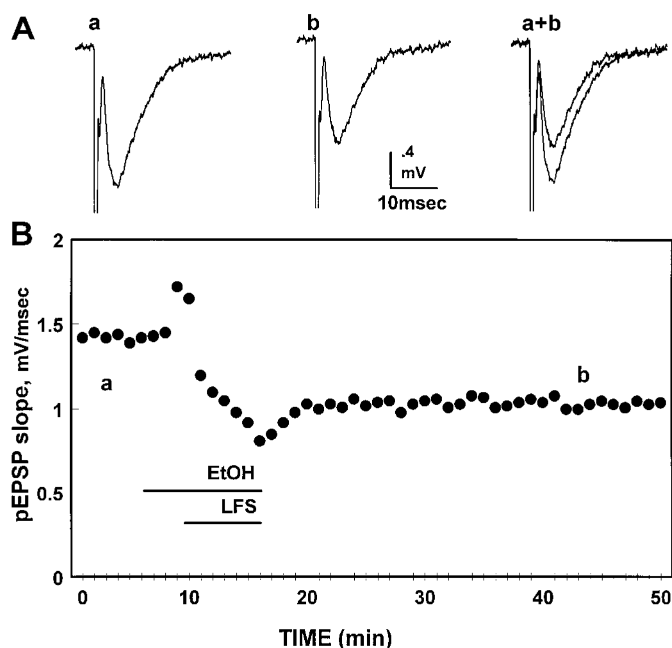

Fig. 4. Ethanol (EtOH) enhances LTD in the CA1 region of hippocampal slices from young rats. A, trace a was recorded before ethanol application as baseline and correspond to pEPSPs elicited as denoted in panel B. Trace b was recorded 15 min after LFS. In (a + b), traces a and trace b are superimposed. B, time course of this experiment. Baseline pEPSPs were observed for 5 min; then 75 mM ethanol was applied. Five minutes later, an LFS train was delivered, and ethanol was immediately washed out. The observation continued for 30 min and the depressed pEPSP remained stable.

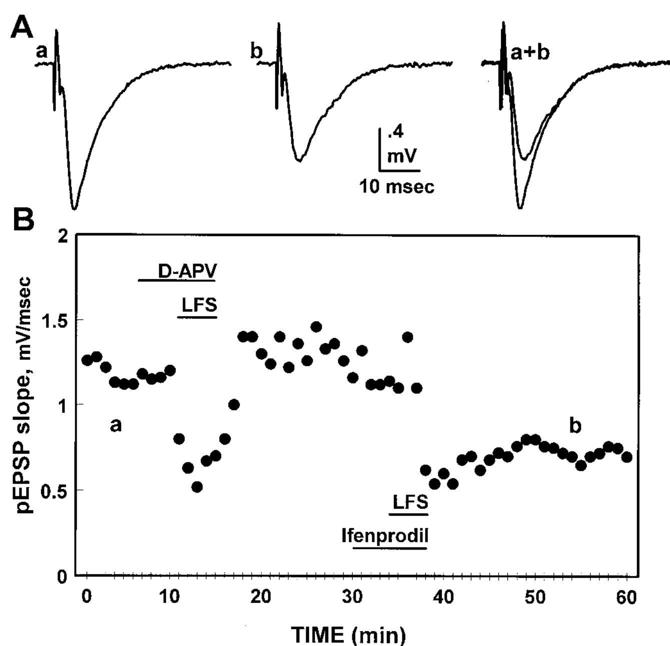

Fig. 5. Ifenprodil enhances D-APV-sensitive LTD in the CA1 region of hippocampal slices from young rats. A, trace a was recorded as baseline and corresponds to pEPSPs elicited as denoted in panel B, which depicts the time course of an experiment to demonstrate the effect of ifenprodil on NMDA-LTD induction. An LFS was then delivered in D-APV as previously demonstrated (Fig. 1), and no LTD was observed. Another LFS was delivered in ifenprodil, and pEPSPs were monitored for an additional 25 min. Trace b was recorded 15 min after LFS and superimposed in (a + b).

mate (mGlu) receptor and voltage-gated calcium channel activation (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997). In young animals, LTD induction relies on NMDA receptor activation (Dudek and Bear, 1992; Mulkey and Malenka, 1992). In adult animals, induction of LTD is dependent on activation of AMPA/kainate or mGlu receptors (Kemp and Bashir, 1999; Kemp et al., 2000).

Our results confirm that in young rats (12–20 days old), LFS reliably induces NMDA receptor-dependent LTD in the CA1 region of the hippocampus. Furthermore, in agreement with other studies (Errington et al., 1995; Wagner and Alger,

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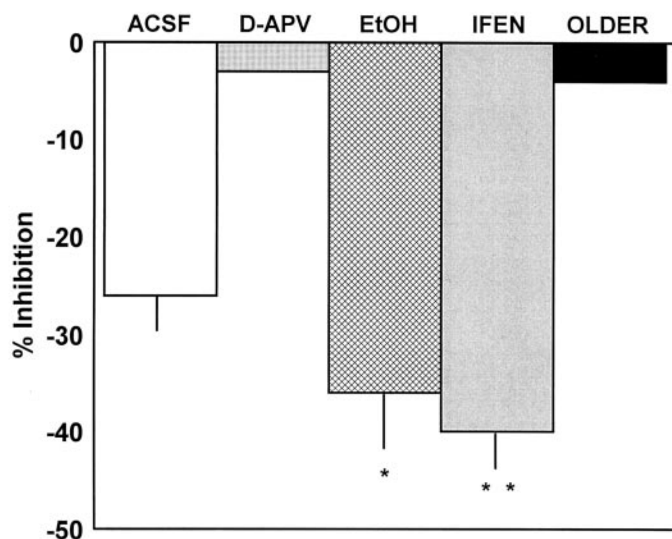

Fig. 6. Cumulative statistical analysis. Data from all five experimental groups are presented. ACSF depicts standard LTD in slices from 12- to 20-day-old rats. D-APV, EtOH, and IFEN depicts LTD in a group identical to ACSF except LFS was delivered in D-APV, ethanol, or ifenprodil, respectively. The p values for the EtOH and IFEN groups were compared with the ACSF group via Student's t test; ⋆, $p < 0.02$; ⋆⋆, $p < 0.003$. Responses following LFS delivered in D-APV and OLDER groups did not differ significantly from baseline pre-LFS responses.

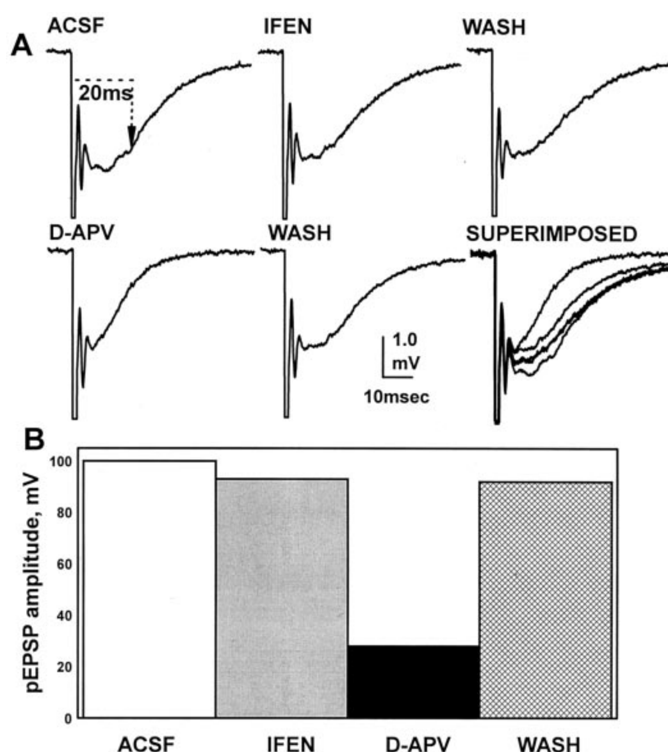

Fig. 7. Ifenprodil does not affect the NMDA receptor-dependent slow component of synaptic transmission. A, effects of ifenprodil and D-APV on maximal pEPSPs recorded form a slice from a younger animal (17 days old). ACSF depicts the baseline response; the arrow indicates the point at which the pEPSP slow component amplitude was measured (at 20 ms after the stimulus artifact). IFEN was recorded 10 min after ifenprodil application; WASH was recorded 15 min after wash-off of ifenprodil; D-APV was recorded 10 min after D-APV application; and the second WASH shows the response following D-APV washout. B, amplitude of NMDA pEPSPs during ifenprodil application was not significantly different from ACSF, whereas during D-APV application, amplitude of the NMDA component of the pEPSP was markedly reduced ($p < 0.001$; $n = 3$).

1995; Norris, 1996), LFS does not induce LTD in adult rats. It has been shown that paired-pulse LFS induces LTD in adult animals through activation of AMPA/kainate and mGlu receptors (Thiels et al., 1996; Kemp and Bashir, 1999), whereas, in young animals, the same stimulation protocol induces an LTD that is NMDA receptor-dependent (Kemp et al., 1998). These data strongly suggest that the participation of NMDA receptors in the induction of LTD is developmentally regulated.

A recent study shows that induction of NMDA receptor-dependent LTD relies chiefly upon the activation of NMDA receptor subpopulations containing NR2C/D subunits (Hrabetova et al., 2000). The expression level of NR2D is significant, although weaker than NR2B, in the hippocampus of young animals (Monyer et al., 1994; Kirson et al., 1999), during which period LFS can most efficiently induce LTD. Given that NR2D subunit expression decreases abruptly during the transition to adulthood (Monyer et al., 1994), such processes may account for the drastic developmental changes in efficiency in LTD induction by LFS in area CA1 of the hippocampus.

Acute ethanol exposure is known to inhibit both NMDA receptor function and NMDA receptor-dependent LTP. Indeed, inhibition of NMDA receptor function by ethanol appears to be required for ethanol inhibition of LTP (Morrisett and Swartzwelder, 1993; Schummers et al., 1997). Yet, there is no published literature on the effect of ethanol on NMDA receptor-dependent LTD. The present experiments indicate that both ethanol and ifenprodil, a noncompetitive NMDA receptor antagonist that selectively inhibits NMDA receptors containing NR2B subunits via a polyamine-sensitive site (Carter et al., 1997), significantly facilitate the induction of LTD in young rats. Consistent with our observations on LTD, our data reveal that in hippocampal slices of young rats, ifenprodil also has little effect on the NMDA receptor-mediated component of pEPSPs.

Taken together, these results prompt two major conclusions. First, because NMDA receptor-dependent LTD is not inhibited but, rather, is facilitated by ethanol, this phenomenon must involve NMDA receptor-dependent processes distinct from those required for LTP (which, as mentioned, is inhibited by ethanol). Second, similarities between the effects of ethanol and ifenprodil on NMDA receptor-dependent LTD suggest that LTD may not be dependent upon NR2B function and that, indeed, this subunit (or other receptor components sensitive to ethanol and ifenprodil) may have inhibitory effects on LTD.

We propose several hypotheses to explain the differential effect of ethanol on these two different forms of NMDA receptor-dependent synaptic plasticity. Our first hypothesis is related to the selective inhibition of NMDA receptor subtypes by ethanol, and the preferential involvement of NMDA receptor subpopulations in synaptic plasticity. In cortical neurons, native NR2B-containing receptors are more sensitive to ethanol inhibition than receptors containing other NR2 subtypes (Lovinger, 1995). Indeed, our previous in situ hybridization studies suggest that the expression of NR2B mRNA in hippocampal explants is enhanced by chronic ethanol exposure (Thomas et al., 1998). Such selective up-regulation of NR2B expression may reflect a chronic selective inhibition of NR2B function by ethanol. If such selective inhibition in fact

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occurs, then the present findings indicate that the NR2B subunit contributes little to induction of LTD but, rather, may subserve a regulatory function. Hrabetova et al. (2000) have demonstrated that distinct subpopulations of NMDA receptors characterized by different NR2 subunits contribute to the induction mechanisms of LTP and LTD, respectively. Because ethanol appears to selectively inhibit NR2B subunit function, it is not surprising that ethanol did not block the induction of LTD in the present study, if NR2B receptors do not contribute to LTD.

Although our results are in good accordance with the observations of Hrabetova et al. (2000), there are opposing data. Studies using transgenic mice over-expressing NR2D showed a shift from LTD to LTP in juvenile mice when a higher-frequency conditioning stimulation was used (Okabe et al., 1998). In combination with immunoblot analysis of NR2 subunits, these investigators concluded that NR2B subpopulations may indeed be more important for the induction of LTD in juvenile animals. However, since these studies employed a more LTP-like conditioning protocol, the link between the loss of LTD and the relative abundance ratio of NR2D to NR2B is difficult to extrapolate to the findings described herein.

The second possible explanation concerns specific sites of ethanol action which may exert differential effects on synaptic plasticity. LTD and LTP have quite different mechanisms of expression (Lee et al., 2000) and, therefore, ethanol actions on such distal components of LTP expression may not have a large effect on LTD. Ethanol is a highly soluble molecule and can easily permeate the cell and act on molecular components required for LTP which may not be involved in LTD expression.

A third explanation is that LTD induction may have a higher "safety factor" than LTP. Induction of LTD indeed appears to require a relatively small amount of Ca^{2+} influx through NMDA receptors. Our laboratory has previously reported that ethanol maximally inhibits 40% of NMDA receptor-mediated currents (Morrisett and Swartzwelder, 1993). Thus, ethanol inhibition of NMDA receptors may not be sufficient to completely block the low level Ca^{2+} influx required to support induction of LTD. On the other hand, LTP induction requires much stronger activation of NMDA receptors and an accordingly strong Ca^{2+} influx. The 40% inhibition of NMDA receptor currents by ethanol seen in our laboratory in the slice preparation is sufficient to block LTP induction (Morrisett and Swartzwelder, 1993).

Increasing evidence suggests that drug reinforcement mechanisms may rely upon certain mechanisms akin to synaptic plasticity. The molecular and cellular components implicated in LTP and LTD in the hippocampus might also be relevant in addiction in medial forebrain systems (Nestler, 2001). Alterations in glutamate receptor levels and in glutamate-mediated transmission have been reported in the ventral tegmental area and the nucleus accumbens after repeated exposure to a drug of abuse, and have been shown to modify drug responsiveness. Very recently, reports indicating contributions of glutamatergic transmission and related plasticity mechanisms in cocaine addiction have been published (Ungless et al., 2001; Vorel et al., 2001). Accordingly, long-term alterations in synaptic function are thought to play a major role in the development of tolerance and dependence to ethanol (Chandler et al., 1998; Thomas et al., 1998; Thomas and Morrisett, 2000). Additionally, the blockade of LTP and the slight facilitation of LTD by ethanol could also relate to the ability of excessive ethanol consumption to impair new memory formation, and to facilitate memory saturation and forgetting.

Our results also suggest that NR2B containing NMDA receptor subpopulations may play an inhibitory role in the induction of NMDA receptor-dependent LTD, and confirm that induction of NMDA receptor-dependent LTD is strongly developmentally regulated. A caveat to this hypothesis which bears mention is that, if NR2B expression inhibits LTD formation, then LTD might be expected to increase, rather than decrease, in aged animals, given that NR2B expression appears to decrease with age (Lovinger, 1995; Okabe et al., 1998). One explanation is that concurrent age-dependent reductions in the expression of NR2C/D subunits (Monyer et al., 1994; Hrabetova et al., 2000), necessary for LTD formation, might predominate. Quite possibly, other age-dependent mechanisms which have yet to be characterized may play a role in this system, as well. Nonetheless, when taken together, the present findings indicate differential actions of ethanol on related yet different forms of NMDA receptor-dependent synaptic plasticity. The facilitation of LTD formation by ethanol, possibly via actions on NR2B-containing NMDA receptors, is a previously unreported depressant effect of ethanol on synaptic function.

Acknowledgments

We thank Dr. Pat Randall for assistance with statistical data analysis.

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Address correspondence to: Dr. Richard Morrisett, College of Pharmacy, Division of Pharmacology and Toxicology, The Institute for Neuroscience, University of Texas, Austin, TX 78712-1074. E-mail: ramorris@mail.utexas.edu

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