ELSEVIER

Comparative Biochemistry and Physiology Part C 131 (2002) 395–404

CBP

www.elsevier.com/locate/cbpc

The effects of ethanol on pre-synaptic components of synaptic transmission in a model glutamatergic synapse: the crayfish neuromuscular junction

Jeffery R. Strawn, Robin L. Cooper*

101 T.H. Morgan School of Biological Sciences, University of Kentucky, Lexington, KY 40506-0225, USA

Received 24 October 2001; received in revised form 21 January 2002; accepted 22 January 2002

Abstract

We have elucidated some of the mechanisms by which ethanol (EtOH) reduces synaptic efficacy at model glutamatergic synapses. The crayfish phasic and tonic neuromuscular junctions are superb models for directly assessing the effects of EtOH on pre-synaptic components of synaptic transmission. The ability to perform quantal analysis of synaptic transmission has allowed us to assess pre-synaptic alterations of release. Using this system, we report that the application of EtOH, within a range observed in intoxicated humans (44 and 88 mM), resulted in a diminution of excitatory post-synaptic potentials (EPSP) amplitudes. Additionally, using focal macro-patch recordings, quantal synaptic currents were recorded to assess the pre-synaptic component as potential target sites for EtOH's action. At the tonic neuromuscular junctions, EtOH (88 mM) reduced the probability of release (p), and in some cases, reduced the number of the release sites (n), but did not alter facilitation index nor did it affect the latency of vesicular release. At the phasic neuromuscular junction, a reduction in synaptic charge occurred during the presence of EtOH. Thus, the observed decrease in synaptic strength is at least partially attributable to a pre-synaptic alteration, specifically the release of fewer vesicles.

Keywords: EtOH; Glutamate (GLU); Crayfish; Neuromuscular junction (NMJ); Synapse; Quantal

1. Introduction

The behavioral effects of ethanol (EtOH) intoxication are well documented in both humans and lower animals. Investigations of the neuronal and synaptic basis of these behavioral effects have focused on both pre- and post-synaptic mechanisms (Peoples et al., 1996). Still, many pre-synaptic components of synaptic transmission are inaccessible in previously studied neural systems. The crayfish neuromuscular junction (NMJ) allows direct assessment of the effects of EtOH on these pre-synaptic components by counting the number quantal events and measuring the kinetics of vesicular release. Although a number of studies have used crustaceans to address the actions of EtOH at NMJs (Adams et al., 1977; Barker, 1975; Blundon and Bittner, 1992; Finger and Stettmeier, 1984; Friedman et al., 1988; Wachtel, 1984), these studies did not assess directly the release of neurotransmitter from the nerve terminal, but rather did so indirectly by measuring changes in membrane potential of the muscle. The purpose of our study was to examine the actions of EtOH directly at the synaptic sites of pre-synaptic motor neurons so as to quantify the effect of EtOH on the number of release sites and to examine the influence of EtOH on the probability of vesicular release at each site. Thus, by recording synaptic

*Corresponding author. Tel.: +1-859-257-5050; fax: +1-859-257-1717.

E-mail address: RLCOOP1@pop.uky.edu (R.L. Cooper).

1532-0456/02/$ - see front matter
PII: S1532-0456(02)00026-1

currents followed by quantal analysis, we have determined the mean quantal content (m), the number of release sites (n), and the probability of release (p) at each of the release sites as well as the latency of vesicular release in the absence and presence of EtOH at tonic neuromuscular junctions. Similarly, at phasic neuromuscular junctions, we have measured changes in overall synaptic charge. Since the mechanisms underlying differences in synaptic efficacy among various types of neurons are wide ranging from the regulation in the extent of synaptic contacts to the amount of release at a given synapse, investigating both high- and low-output pre-synaptic terminals may hold promise in understanding some of the aspects to account for differential modulation of EtOH among synaptic sites.

Due to the relative simplicity of crustacean neuromuscular junctions, they serve as good model systems for experimentation of neuromodulators actions on synaptic plasticity since pre-and post-synaptic components can readily be differentiated. In addition, single vesicular events can be readily measured directly at the synaptic sites. Also, crustacean motor neurons are distinct as either tonic or phasic in nature. The opener muscle in the walking legs of crayfish is an interesting preparation since the entire muscle is innervated by a single excitatory tonic motor neuron (Atwood and Cooper, 1996). For comparison, the leg extensor muscle in the walking legs of the crayfish is a favorable preparation, since a phasic motor neuron can be selectively stimulated and only one phasic motor neuron innervates the entire muscle (Bradacs et al., 1997). Structurally, the phasic terminals are, overall, thinner with slight swellings along the terminals, whereas the tonic terminals contain larger swellings (varicosities). The synaptic structures are also different within the tonic and phasic terminals. Generally, the phasic terminals contain synapses with multiple active zones (complex synapses) whereas synapses in tonic terminals have one or a few active zones per synapse (King et al., 1996; Msghina et al., 1998, 1999; Govind et al., 1994; Cooper et al., 1996a). These structural differences between phasic and tonic terminals along with the correlated differences in the amount of synaptic output provide an opportunity to compare the effects of ETOH between high- and low-output terminals.

The results of this study have appeared previously in abstract form (Strawn and Cooper, 2000).

2. Materials and methods

2.1. Animals and dissection

All experiments were performed using the freshwater crayfish, *Procambarus clarkii*, measuring 6–10 cm in body length (Atchafalaya Biological Supply Co., Raceland, LA, USA). Animals were housed in an aquatic facility and fed dried fish food and carrots weekly.

2.2. Dissections

2.2.1. Leg opener muscle

The opener muscle of the first walking leg was prepared via standard dissection (Dudel and Kuffler, 1961; Cooper et al., 1995a). Additionally, all recordings were made from central fibers and dissected preparations were maintained in crayfish saline, a modified Van Harreveld's solution (Cooper et al., 1995a).

2.2.2. Leg extensor muscle

The leg extensor muscle of the first walking leg was exposed by removing the cuticle on the lateral aspect of the meropodite along with the entire flexor muscle and the main leg nerve. The motor nerve of the extensor muscle separates from the main leg nerve as it enters the meropodite and is left in place on the muscle after removal of the main nerve (Bradacs et al., 1997).

2.3. Electrophysiological recordings

2.3.1. Excitatory post-synaptic potentials (EPSPs)

Intracellular recordings were performed with standard intracellular microelectrodes (30–60 MΩ, 3M KCl) and responses were recorded as previously described (Strawn et al., 2000). The amplitudes of the EPSPs were obtained by the difference in the peak value to the baseline preceding the train of stimulation (Crider and Cooper, 2000). To determine the facilitation index for the train facilitation, the amplitude of the 10th EPSP pulse was divided by the amplitude of the fifth EPSP and the resulting number had 1 subtracted from it. All events were appropriately scaled to known test pulses applied through the electrode and directly measured on an oscilloscope to the signals recorded with the MacLab Scope software (version 3.5.4). Nerve stimulation was performed as previously described (Cooper et al., 1995b) and

excitatory post-synaptic potentials (EPSPs) were recorded in the central fibers of the opener muscle (Cooper et al., 1995b; Dudel and Kuffler, 1961). The excitatory axon was stimulated with trains of 10 stimuli at 30 Hz with a train interval of 10 s. Averages of 10–20 trains were used for measurement. EPSPs and excitatory post-synaptic currents (EPSCs) were obtained by selectively stimulating the phasic excitatory motor neuron at various frequencies with the use of a focal electrode with an inner diameter of 15-20 μm placed directly on the axon (Bradacs et al., 1997). The axon type is easily identified after staining with the vital dye 4-Di-2-ASP which labels the tonic axon more brightly than the phasic axon due to its greater mitochondrial density (Atwood and Cooper, 1996).

2.3.2. Excitatory post-synaptic currents (EPSCs)

Synaptic currents were measured with focal macropatch electrodes, thus allowing for a determination of the effect of EtOH on vesicular release. The synaptic currents were obtained using the loose patch technique by lightly placing a 10–20-μm fire-polished glass electrode directly over a spatially isolated varicosity along the nerve terminal. The nerve terminal which had been visualized after exposure to the vital florescent dye 4-Di-2-ASP (Molecular Probes; Magrassi et al., 1987; Cooper et al., 1995a). The seal resistance was in the range of 100 KΩ to 1 MΩ. Because the seal could have easily been lost had the muscle twitched, stimulation was restricted to 1 Hz. Evoked excitatory post-synaptic currents (EPSCs) and miniature excitatory post-synaptic currents (mEPSCs) were recorded and analyzed to determine the mean quantal content (m), the number of release sites (n), and the probability of release at the sites (p) (Cooper et al., 1995b, 1996b; del Castillo and Katz, 1954). Mean quantal content was determined by direct counts (m):

$$\text{Direct counts } (m) = \frac{\sum (\#\ \text{failures})(0) + (\#\ \text{singles})(1) + (\#\ \text{doubles})(2)\ldots}{\text{total number of sweeps}}$$

By direct counts of the evoked quantal events, n and p were estimated. In some cases, there were no evoked events that followed the nerve terminal spikes; such a failure in evoked release was counted as a zero. If only one single event occurred after the spike, it was counted as one; when double events occurred, they were referred to as two (Table 1). A common method of obtaining the quantal parameters (n) and (p) is based on direct counts and estimating (n) and (p) by fitting various discrete distributions such as a Poisson, uniform binomial, or non-uniform binomial to the observed data, thus one can predict the distribution of the number of events resulting from each stimulus (Pred, Table 1). Once the type of distribution was determined, a maximum likelihood estimate was used by a bootstrapping approach to determine n and p (Smith et al., 1991; Cooper et al., 1995b). The integral of quantal release (current×time or charge) was determined by measuring the total area of the evoked current. The time of peak-evoked events varied due to latency fluctuations, so that when multiple events occurred, the measurements of peak amplitude were not as reliable as the area measurement (Cooper et al., 1995b). To monitor charge over time, the area of the evoked current was measured for each event. Additionally, the latency of evoked release was determined for each event as previously described (Southard et al., 2000). Since exposure to EtOH produced gradual changes in all of the quantal parameters, each 1000 evoked trials of data were divided into bins of 200 events. The subsets of 200 trails were found to be sufficient to obtain quantal predictions.

3. Results

3.1. Excitatory post-synaptic potentials (EPSPs)

To determine if EtOH altered synaptic transmission within the concentration range clinically observed in intoxicated humans (44–88 mM, respectively), we first examined the post-synaptic potentials induced by high output phasic motor nerves of the crayfish in the leg extensor muscle preparation. These high output terminals produced relatively large amplitude EPSPs, usually in the range of 10–20 mV between preparations from various sized crayfish (Bradacs et al., 1997; Shearer et al., 2000). For the size range of animals used in this study, the average amplitudes of the EPSPs recorded in saline was 15 mV (Fig. 1a). The decrease in the EPSP amplitude was rapid and observed in all preparations. A representative response is illustrated in Fig. 1b. Mean amplitude was determined after the maximum response occurred during the exposure to EtOH (Fig. 1c).

Table 1
Influence of EtOH on quantal release parameters: mean quantal content (m), number of sites releasing (n), and probability of release at the sites (p)

	All Trials			Trials 1–200		Trials 201–400		Trials 401–600	
	Events	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred
Prep. 1									
Saline	0	309	323	103	107	111	111	95	96
	1	248	216	73	64	81	79	94	98
	2	13	36	3	10	6	9	4	4
	3	5	0	2	0	1	1	2	0
	m	**0.503**		**0.47**		**0.482**		**0.554**	
	n	**3**		**3**		**3**		**3**	
	p	**0.168**		**0.16**		**0.16**		**0.18**	
EtOH	0	358	367	128	128	124	120	106	106
	1	216	178	55	55	75	72	86	86
	2	6	44	2	2	0	8	4	4
	3	1	3	0	0	1	0	0	0
	m	**0.398**		**0.319**		**0.390**		**0.480**	
	n	**3**		**2**		**3**		**2**	
	p	**0.144**		**0.16**		**0.13**		**0.24**	
Prep. 2									
Saline	0	515	515	172	172	173	173	170	172
	1	81	81	27	27	26	26	28	28
	2	3	3	1	1	0	0	2	0
	m	**0.145**		**0.145**		**0.140**		**0.155**	
	n	**2**		**2**		**1**		**1**	
	p	**0.073**		**0.073**		**0.13**		**0.144**	
EtOH	0	582	583	197	197	194	195	191	191
	1	17	17	3	3	5	5	9	9
	2	1	0	0	0	1	0	0	0
	m	**0.0317**		**0.015**		**0.03**		**0.045**	
	n	**1**		**1**		**1**		**1**	
	p	**0.0284**		**0.015**		**0.0251**		**0.045**	
Prep. 3									
Saline	0	554	559	183	184	183	185	188	190
	1	41	41	16	16	15	16	10	10
	2	5	0	1	0	2	0	2	0
	m	**0.085**		**0.09**		**0.095**		**0.070**	
	n	**1**		**1**		**1**		**1**	
	p	**0.069**		**0.084**		**0.076**		**0.051**	
EtOH	0	571	574	188	189	194	195	189	190
	1	26	26	11	11	5	5	10	10
	2	3	0	1	0	1	0	1	0
	m	**0.053**		**0.065**		**0.035**		**0.06**	
	n	**1**		**1**		**1**		**1**	
	p	**0.044**		**0.055**		**0.025**		**0.0502**	
Prep. 4									
Saline	0	458	458	175	176	178	179	105	105
	1	59	59	24	24	21	21	14	14
	2	2	2	1	0	1	0	0	0
	m	**0.105**		**0.130**		**0.115**		**0.118**	
	n	**2**		**2**		**1**		**1**	
	p	**0.061**		**0.065**		**0.106**		**0.118**	
EtOH	0	588	568	185	186	191	191	190	191
	1	32	32	14	14	9	1	9	9
	2	2	0	1	0	0	0	1	0
	m	**0.06**		**0.08**		**0.045**		**0.055**	

Table 1 *(Continued)*

	All Trials			Trials 1–200		Trials 201–400		Trials 401–600	
	Events	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred
	n	**1**		**1**		**1**		**1**	
	p	**0.054**		**0.070**		**0.045**		**0.045**	
Prep. 5									
Saline	0	481	474	158	153	160	159	163	167
	1	100	112	44	41	35	36	32	44
	2	18	13	8	6	5	4	5	5
	3	1	1	1	1	0	0	0	0
	m	**0.232**		**0.26**		**0.225**		**0.21**	
	n	**Poisson**		**Poisson**		**Poisson**		**1**	
	p	–		–		–		**0.1641**	
EtOH	0	518	527	170	173	175	179	173	176
	1	72	73	27	27	21	21	24	24
	2	10	0	3	0	4	0	3	0
	m	**0.1544**		**0.165**		**0.145**		**0.15**	
	n	**Poisson**		**Poisson**		**Poisson**		**Poisson**	
	p	–		–		–		–	

Direct quantal counts obtained at the neuromuscular junction of the opener muscle are listed as the number of events (Events) for the observed distributions (Obs) and in determining the best fit models for a predicted distribution (Pred) to calculate the number of sites releasing (n), and probability of release at the sites (p). The mean quantal content (m) is shown and the distributions that were Poisson in nature, in which n and p could not be determined, are listed.

Application of EtOH (44 mM) to extensor NMJs decreased the EPSP amplitudes by $13 \pm 5\%$ ($n = 5$, $P < 0.05$ Wilcoxon Rank sum test) while at 88 mM, amplitudes were reduced by $17 \pm 7\%$ ($n = 5$, $P < 0.05$ Wilcoxon Rank sum test).

3.2. Excitatory post-synaptic currents (EPSCs)

Because EPSP amplitudes were reduced in the presence of EtOH, it was important to determine if the effect was due, in part, to a pre-synaptic mechanism. A pre-synaptic change would be supported if the number of quanta released per evoked event decreased in the presence of EtOH. These changes were readily assessed by recording the excitatory post-synaptic currents (EPSCs) and counting the number of evoked quanta over time (Fig. 2). The post-synaptic effects have been investigated in early works on crustacean muscles (Blundon and Bittner, 1992; Blundon, 1992).

Exposure of the phasic NMJ to EtOH resulted in a reduction in the amount of synaptic charge over time. The profile of the effect during a 1-Hz stimulation paradigm is illustrated for a representative preparation in Fig. 3a. In previous studies (Cooper et al., 1995b), it was shown that, in measuring traces deemed as failures, there are small deviations from zero, which is a measure of noise. The noise of the currents is centered at zero with negative and positive deviations as seen in Fig. 3a,c. Upon grouping, the average response for each 200 bins (i.e. 200 s) before and during EtOH exposure the depression of synaptic transmission was apparent (Fig. 3b). Histograms of the occurrences in the ranges of synaptic charge revealed a reduction in the occurrences of the larger events and an increase of the frequency of single quanta. These changes were also accompanied by an increase in the number of evoked trials that failed to elicit an EPSC (Fig. 3c).

The reduction in EPSCs in the phasic nerve terminals warranted further investigation. However, because a large number of vesicles fuse to the membrane during an evoked event at the phasic synapse, the opener muscle preparation, that is innervated by a single tonic motor neuron, was used to investigate the effects of EtOH on vesicular dynamics and quantal currents. The area of the evoked synaptic current is a measure of charge (current $\times$ time). Because of fluctuations in latency, measurements of charge are more accurate in determining quantal current than are determinations using peak current amplitude (Cooper et al., 1995b). Additionally, at the opener NMJ, direct quantal counts can be made which permit the determination of mean quantal content (m). In five out of five experiments, m decreased initially (Fig. 4a). To assess the rate of EtOHs modulatory effect

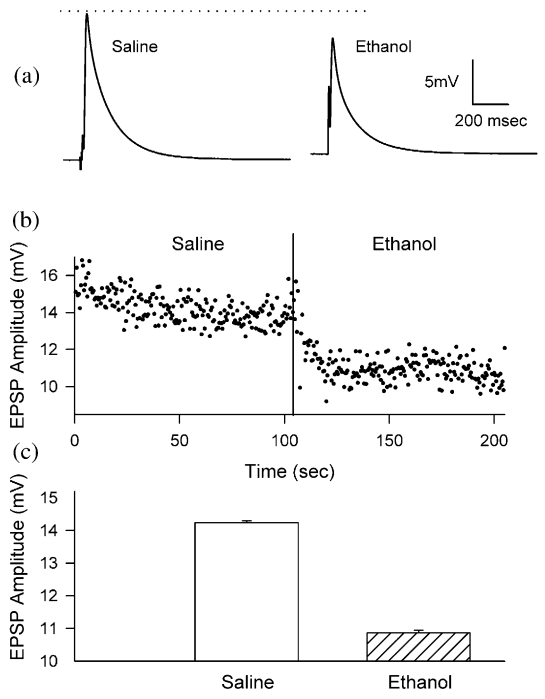

Fig. 1. (a) Excitatory post-synaptic potentials can be recorded from the extensor muscle fiber while exposing the fiber to EtOH. Using this technique, the size of average evoked post-synaptic potentials can be compared for saline (left) and EtOH (right). Additionally, one can see the EtOH (88 mM) has a depressive effect on synaptic transmission by observing the changes in individual EPSPs while the nerve is stimulated at 1 HZ. (b) Note that this graph illustrates the rapid depression upon exposure to EtOH. (c) Histograms show the mean EPSP amplitudes before and after EtOH treatment in each (mean amplitudes $\pm$ S.E.M.). Application of EtOH (44 mM) to extensor NMJs decreased the EPSP amplitudes by $13 \pm 5.0\%$ ($n = 5$, $P < 0.05$ Wilcoxon Rank sum test) while at 88 mM, amplitudes were reduced by $17 \pm 7.1\%$ ($n = 5$, $P < 0.05$ Wilcoxon Rank sum test).

on the quantal parameters, every 200 s were grouped for quantal analysis. The percentage change induced by EtOH in m for each of the five preparations is depicted in Fig. 4b. The mean reduction in m was $-49.6 \pm 11.7\%$; however, it should be noted that percentage change varied concomitantly with the initial amount of synaptic strength of the preparation (Fig. 4b).

Since m decreased as a result of EtOH exposure, a more thorough analysis of the probability of vesicular release (p) and the number of release sites (n) was performed. This approach allowed us to determine the site of EtOHs action in the reducing transmitter release from the pre-synaptic terminals. To obtain estimates of the quantal parameters (n and p), the statistical distribution for each set of 1000 trials and the 200 trial subsets was used (Table 1). Prior to exposure to EtOH, some preparations exhibited a baseline $n = 1$, in which case n could not be reduced. In addition, when distributions were best fit by a Poisson distribution, n and p could not be reliably estimated. Nonetheless, direct counts revealed an increase in the number failures and attenuation in the frequency of multiple events during exposure to EtOH. The main contribution to the reduction in mean quantal content was attributable to a reduction in the p (Table 1).

3.3. Latency of evoked release

Since exposure to EtOH decreased p, it was likely that it might also alter the rate of evoked release. To assess the kinetics of vesicular events, the latency from the time of the arrival of the action potential at the nerve terminal to the initial post-synaptic current is commonly used (Southard et al., 2000). The latency period encompasses the

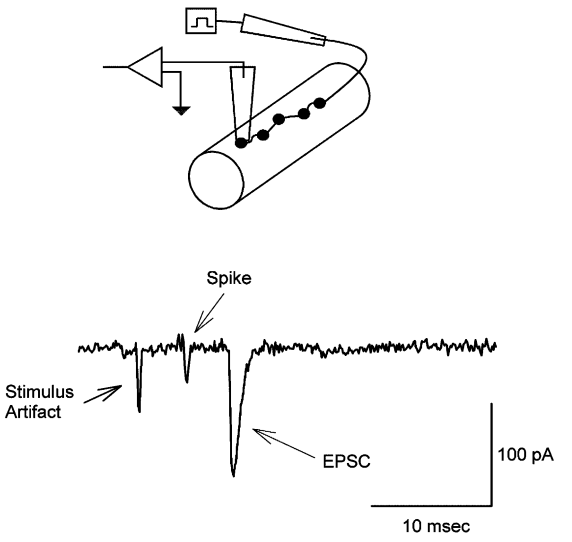

Fig. 2. Quantal recordings allow for an assessment of pre-synaptic modulation by 88 mM EtOH. (a) A focal macro-patch electrode is placed over a single, spatially isolated varicosity along the nerve terminal, which allows for the recording of synaptic currents. (b) Each time the nerve is stimulated, a stimulus artifact and spike are recorded as shown. Additionally, an excitatory post-synaptic current (EPSC) may occur. The areas of these EPSCs were determined by integration of the trace, which allows for an assessment of charge before and during exposure to EtOH. Direct counts of individual evoked quantal events are also obtained in this manner.

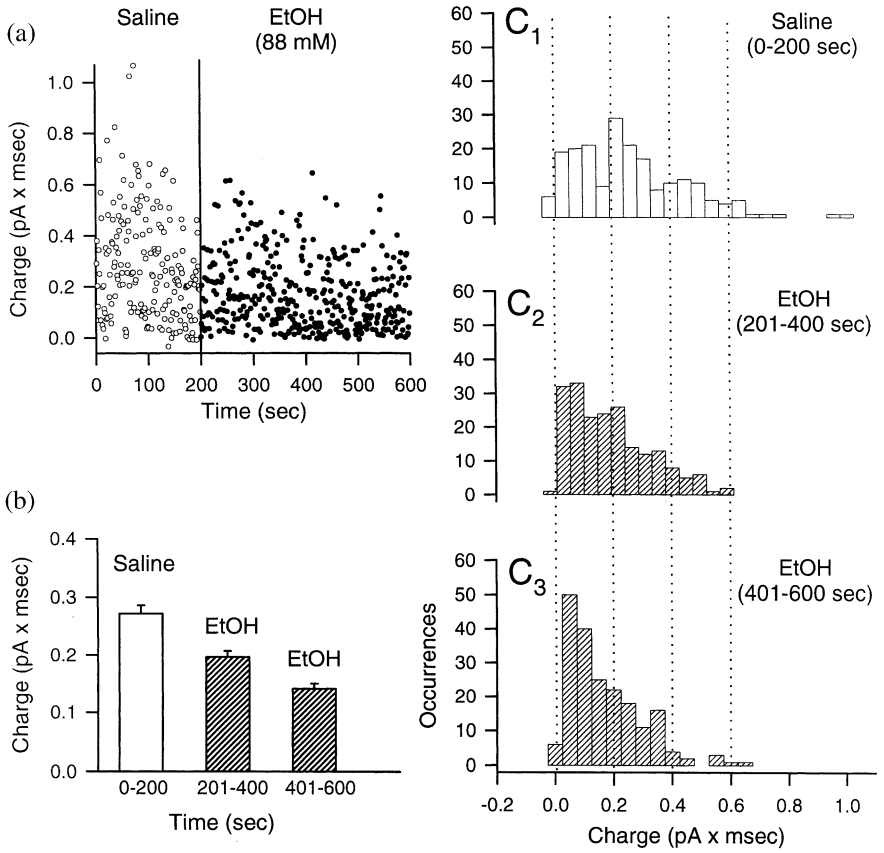

Fig. 3. In the high output phasic nerve terminals EtOH rapidly depresses the synaptic currents. Currents over time become reduced in size (a). Upon averaging the responses in 200-s blocks of time the overall responses clearly show a continued decline in the amount of synaptic charge. Mean charges (±S.E.M.) are shown (b). Bar charts of quantal charge were plotted in groups of 200 s, which allows for an examination of shifts in distribution associated with the application of EtOH. Initially, the charge ranged from 0.0 to 1.1 (C_1). However, with exposure to 88 mM EtOH (C_1 and C_3), there is a leftward shift in the histogram indicating smaller evoked responses, likely due to the release of fewer vesicles. The vertical dotted lines portray the horizontal scales for each histogram.

time of the action potential reaching the nerve terminal, the inward calcium flux, primed vesicle fusing to the membrane, transmitter release and diffusion to the post-synaptic ligand-gated receptors, and finally, the sodium current influx into the muscle cell. Since there is a distribution in the normal latency of release in the absence of EtOH, we determined whether EtOH shifted the whole distribution of latencies or particular subsets of latencies. To quantify these changes, latencies for each evoked event were plotted in a rank sum order from lowest to highest before and after exposure to EtOH. No net shift was observed in the latency distributions of any preparation when exposed to EtOH (88 mM) (data not shown).

4. Discussion

The results of this study reveal that EtOH exposure, within a pharmacological range for humans (44–88 mM), reduces the number of evoked quantal events and subsequently depresses the amplitudes of EPSPs at the glutamatergic NMJ of the crayfish (Shinozaki and Shibuya, 1974). Moreover, our results suggest a direct action of EtOH at the pre-synaptic terminal. The observed decreases in mean quantal content, mostly a result of a decreased probability of vesicular release, suggest that EtOH altered properties of the pre-synaptic terminal.

Although the exact pre-synaptic mechanisms by which EtOH acts have not been fully elucidated

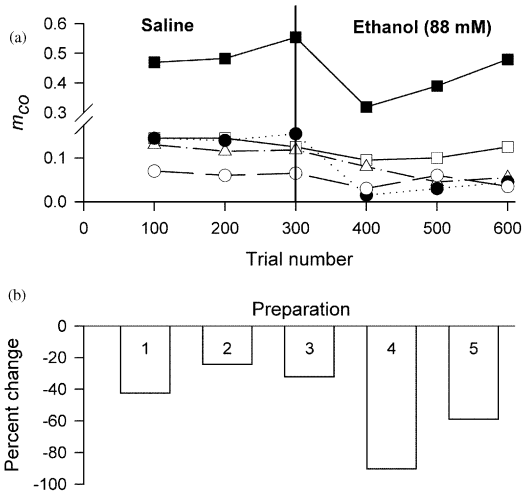

Fig. 4. Mean quantal content (m) determined for the tonic opener preparation indicates a depression induced by EtOH. (a) The m is plotted during saline exposure (0–300) and during exposure to 88 mM EtOH (400–600). Notice the decrease in m observed in all five preparations. The values within each 100 trials are averaged. (b) Percentage changes from the preparations shown in (a) are shown for the 200 stimulations before EtOH exposure and the first 200 stimulations during EtOH treatment.

in this study, there are several possibilities. First, EtOH could have directly affected ion channels. Alteration of Na^+ or Ca^{+2} channels would diminish net depolarization or Ca^{+2} entry. Second, EtOH could interfere with protein–protein interactions involved in the vesicular docking and the fusion. Possibly with calcium sensitive indicators and imaging of the crayfish motor nerve terminals (Cooper et al., 1995a), one could determine if EtOH alters Ca^{+2} loading at the terminal. In addition, biochemical studies of isolated proteins could detect differences in known protein–protein interactions that occur within the nerve terminals for vesicular related events (He et al., 1999).

Earlier studies of pre-synaptic effect of EtOH at the vertebrate NMJ suggested that EtOH diminished acetylcholine release by blocking Na^+ conductance, and that nerve terminals releasing ACh are most sensitive to EtOH (Carmichael and Israel, 1975). However, these effects were observed at high concentrations of EtOH (100 mM). Within a pharmacologic range (10–100 mM), a later study using fetal spinal cord neurons, revealed that the frequency of excitatory post-synaptic potentials decreased in the presence of EtOH (Groul, 1982). This also suggests that EtOH alters synaptic release.

A more speculative possibility is that EtOH exposure at the crayfish NMJ may have an action on pre-synaptic autoreceptors. It is known that, in rats, EtOH blocks adenosine triphosphate-induced currents (Li et al., 2000), and recently it was shown that the crayfish NMJ, as used in this study, have pre-synaptic adenosine receptors (Schramm and Dudel, 2001). However, at the crayfish NMJ, adenosine depresses synaptic transmission; therefore, the results of our study indicate that EtOH is not blocking adenosine receptors in the crayfish system.

A number of crustacean NMJ preparations have been used to examine the effect of EtOH on synaptic potentials (Adams et al., 1977; Barker, 1975; Blundon and Bittner, 1992; Finger and Stettmeier, 1984; Friedman et al., 1988; Wachtel, 1984). Wachtel (1984) investigated in the crayfish (*Cherax destructor*) opener muscle the effects of different chain lengths of aliphatic alcohols on synaptic transmission. In addition, Finger and Stettmeier (1984) had shown, in the crayfish opener muscle, that the single channel current, activated by quisqualate, was reduced by EtOH in a concentration-dependent fashion, but that the mean open time of the channels was unaffected. Also, in contrast to Blundon and Bittner (1992), Finger and Stettmeier (1984) showed that the size of the quantal post-synaptic inhibitory currents did not decrease, but that ethanol selectively depressed EPSCs. In addition, Adams et al. (1977) had shown in the opener muscle of the crab (*Scylla serrata*) that *m*EPSCs have faster decay rate in the presence of EtOH (0.25 M). Likewise, the miniature excitatory post-synaptic currents (*m*EPSCs) at NMJs in a toad are shortened by the alcohol, octanol (Gage et al., 1974, 1978). The membrane resistance of crustacean muscle fibers also decreases with prolonged exposure to high concentrations of EtOH (Blundon and Bittner, 1992). The results of their studies support the notion that the fluidity of the membrane, in the post-synaptic sites, is increased which has an effect on the receptors. With respect to pre-synaptic actions, Friedman et al. (1988) noted concentration-dependent, biphasic responses. In their study, concentrations of EtOH in the range of 10–100 mM increased the occurrences of spontaneous events, whereas higher concentrations of EtOH from 300 to 600 mM decreased the evoked EPSP amplitudes in facili-

tated responses. Unfortunately though, the findings of these past studies are difficult to interpret, as they have not been conducted within the same pharmacologic range. Since we report a decrease in overall mean quantal content, mostly contributed to a decrease in the probability of vesicular release, it appears that EtOH substantially alters the properties of the pre-synaptic terminal by reducing overall synaptic transmission. The actions appear to reduce overall synaptic transmission; however, in five experiments in which synaptic facilitation was measured, we did not observe any change in the facilitation indices. Thus, our results suggest that the cooperativity of calcium binding is not affected by EtOH within the range (44–88 mM) used in this investigation. Further analysis of the latency in evoked release was conducted in three preparations by measuring the time from the spike induced in the nerve terminal until the time of discerning the initial phase of the synaptic current from baseline (Southard et al., 2000). The distribution of the observed latencies were analyzed for shifts in the distribution of latency prior and during EtOH exposure. No significant shifts in the synaptic latency could be observed for the quantal currents measured at the NMJs on the opener muscle.

We have focused on pre-synaptic actions in this study, but there is evidence that EtOH has direct effects on post-synaptic receptivity to neurotransmitters. However, many of these studies have used high concentrations of EtOH. For thorough reviews on both pre-synaptic and post-synaptic actions that emphasize the responses related to the experimental concentrations of EtOH, consult Deitrich et al. (1989), Weight (1992) and Blundon (1992).

Acknowledgments

Funding was provided, in part, by NSF grants IBN-9808631 (RLC), NSF-ILI-DUE 9850907 (R.L. Cooper) as well as an undergraduate training fellowship from HHMI and a NSF-REU to (J.R. Strawn). Appreciation is given to Dr Jeffrey G. Netzeband (Dept of Neuropharmacol., The Scripps Research Institute, CA, USA) for critical comments on experimentation design and on the manuscript content.

References

Adams, D.J., Gage, P.W., Hamill, O.P., 1977. Ethanol reduces excitatory postsynaptic current duration at a crustacean neuromuscular junction. Nature 288, 739–741.

Atwood, H.L., Cooper, R.L., 1996. Synaptic diversity and differentiation, Crustacean neuromuscular junctions. Invert. Neurosci. 1, 291–307.

Barker, J.L., 1975. CNS depressants, effects on post-synaptic pharmacology. Brain Res. 92, 35–55.

Blundon, J.A., 1992. Effects of ethanol on crayfish behavior and synaptic transmission. In: Watson, R.R. (Ed.), Alcohol and Neurobiology: Receptors, Membranes, and Channels, CRC Press, Boca Raton, FL pp. 215–238.

Blundon, J.A., Bittner, G.D., 1992. Effects of ethanol and other drugs on excitatory and inhibitory neurotransmission in the crayfish. J. Neurophysiol. 67, 576–587.

Bradacs, H., Cooper, R.L., Msghina, M., Atwood, H.L., 1997. Differential physiology and morphology of phasic and tonic motor axons in a crayfish limb extensor muscle. J. Exp. Biol. 200, 677–691.

Carmichael, F.J., Israel, Y., 1975. Effects of ethanol on neurotransmitter release by rat brain cortical slices. J. Pharmacol. Exp. Ther. 193, 824–834.

Cooper, R.L., Marin, L., Atwood, H.L., 1995a. Synaptic differentiation of a single motor neuron, conjoint definition of transmitter release, presynaptic calcium signals, and ultrastructure. J. Neurosci. 15, 4209–4222.

Cooper, R.L., Stewart, B.A., Wojtowicz, J.M., Wang, S., Atwood, H.L., 1995b. Quantal measurement and analysis methods compared for crayfish and Drosophila neuromuscular junctions and rat hippocampus. J. Neurosci. Meth. 61, 67–78.

Cooper, R.L., Harrington, C., Marin, L., Atwood, H.L., 1996a. Quantal release at visualized terminals of crayfish motor axon, Intraterminal and regional differences. J. Comp. Neurol. 375, 583–600.

Cooper, R.L., Winslow, J., Govind, C.K., Atwood, H.L., 1996b. Synaptic structural complexity as a factor enhancing probability of calcium-mediated transmitter release. J. Neurophysiol. 75, 2451–2488.

Crider, M.E., Cooper, R.L., 2000. Differentially facilitation of high- and low-output nerve terminals from a single motor neuron. J. Appl. Physiol. 88, 987–996.

Deitrich, R.A., Dunwiddie, T.V., Harris, R.A., Erwin, V.G., 1989. Mechanism of action of ethanol, initial central nervous system actions. Pharmacol. Rev. 41, 489–537.

del Castillo, J., Katz, B., 1954. Quantal components of the end-plate potential. J. Physiol. (Lond) 124, 560–573.

Dudel, J., Kuffler, S.W., 1961. The quantal nature of transmission and spontaneous miniature potentials at the crayfish neuromuscular junction. J. Physiol. (Lond.) 155, 514–529.

Finger, W., Stettmeier, H., 1984. Postsynaptic actions of ethanol and methanol in crayfish neuromuscular junctions. Eur. J. Physiol. 400, 113–120.

Friedman, R.N., Bittner, G.D., Blundon, J.A., 1988. Electrophysiological and behavioral effects of ethanol on crayfish. J. Pharmacol. Exp. Ther. 246, 125–131.

Gage, P.W., McBurney, R.N., Van Helden, D., 1974. Endplate currents are shortened by octanol, possible role of membrane lipid. Life Sci. 14, 2277–2283.

Gage, P.W., McBurney, R.N., Van Helden, D., 1978. Octanol reduces end-plate channel lifetime. J. Physiol. 274, 279–298.

Govind, C.K., Pearce, J., Wojtowicz, J.M., Atwood, H.L., 1994. ‘Strong’ and ‘weak’ synaptic differentiation in the

crayfish opener muscle: structural correlates. Synapse 16, 45–58.

Groul, D.L., 1982. Ethanol alters synaptic activity in cultured spinal cord neurons. Brain Res. 243, 25–44.

He, P., Southard, R.C., Whiteheart, S.W., Cooper, R.L., 1999. Role of a-SNAP in promoting efficient neurotransmission at the crayfish neuromuscular junction. J. Neurophysiol. 82, 3406–3416.

King, M.J., Atwood, H.L., Govind, C.K., 1996. Structural features of crayfish phasic and tonic neuromuscular terminals. J Comp Neurol. 372, 618–626.

Li, C., Xiong, K., Weight, F.F., 2000. Ethanol inhibition of adenosine 5′-triphosphate-activated current in freshly isolated adult rat hippocampal CA1 neurons. Neurosci. Lett. 295, 77–80.

Magrassi, L., Purves, D., Lichtman, J.W., 1987. Fluorescent probes that stain living nerve terminals. J. Neurosci. 7, 1207–1214.

Msghina, M., Govind, C.K., Atwood, H.L., 1998. Synaptic structure and transmitter release in crustacean phasic and tonic motor neurons. J Neurosci. 18, 1374–1382.

Msghina, M., Millar, A.G., Charlton, M.P., Govind, C.K., Atwood, H.L., 1999. Calcium entry related to active zones and differences in transmitter release at phasic and tonic synapses. J Neurosci. 19, 8419–8434.

Peoples, R.W., Li, C., Weight, F.F., 1996. Lipid vs protein theories of alcohol action in the nervous system. Annu. Rev. Pharmacol. Toxicol. 36, 185–201.

Schramm, M., Dudel, J., 2001. Pertussis toxin does not affect the time course of quantal release in crayfish and mouse muscle, but has other post- and presynaptic effects, especially on adenosine autoreceptors. Neurosci. Lett. 299, 193–196.

Shearer, J., Zolman, J.F., Cooper, R.L., 2000. The differential effects of 5-HT on tonic and phasic motor nerve terminals. Abst. Soc. Neurosci. 26, 441.17.

Shinozaki, H., Shibuya, I., 1974. A new potent excitant, quisqualic acid, effects on crayfish neuromuscular junction. Neuropharmacology 13, 665–672.

Smith, B.R., Wojtowicz, J.M., Atwood, H.L., 1991. Maximum likelihood estimation of nonuniform transmitter release propabilities at the crayfish neuromuscular junction. J. Theor. Biol. 150, 457–472.

Southard, R.C., Haggard, J., Crider, M.E., Whiteheart, S.W., Cooper, R.L., 2000. Influence of serotonin on the kinetics of vesicular release. Brain Res. 871, 16–28.

Strawn, J.R., Neckameyer, W.S., Cooper, R.L., 2000. The effects of 5-HT on sensory neurons, CNS command, and neuromuscular junctions of central and motor neurons driving the crayfish abdominal superficial flexor muscle in the crayfish. Comp. Biochem. Physiol. B 127, 544–550.

Strawn, J.R., Cooper, R.L., 2000. Ethanol induced changes in synaptic transmission: roles of pre- and post-synaptic mechanisms. Abst. Soc. Neurosci. 26, 441.1.

Wachtel, R.E., 1984. Aliphatic alcohols increase the decay rate of glutamate-activated currents at the crayfish neuromuscular junction. Br. J. Pharmacol. 83, 393–397.

Weight, F.F., 1992. Cellular and molecular physiology of alcohol actions in the nervous system. Intl. Rev. Neurobiol. 44, 289–348.