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NEUROCHEMICAL BASIS OF DISRUPTION OF HIPPOCAMPAL LONG TERM POTENTIATION BY CHRONIC ALCOHOL

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1. ABSTRACT

The aim of this review is to summarize the possible mechanisms underlying the long-term impairment of learning and memory resulting from chronic ethanol treatment (CET) especially that involving decrements in long-term potentiation (LTP) in hippocampus. CET for a 28-week duration affects the rat hippocampal formation in such a way as to decrease the magnitude of LTP; an effect that can last as long as 7 months after ethanol withdrawal. It appears that NMDA receptor number in hippocampus is unchanged after CET whereas the data suggest a more pronounced role for changes in GABAergic and cholinergic synaptic transmission in determining how CET influences the induction of LTP in hippocampus. In particular, changes in presynaptic modulation of neurotransmitter release in hippocampus may be one mechanism by which CET inhibits LTP. Thus, the mechanisms underlying the effect of CET on LTP are a result of changes in a number of neurotransmitter systems in hippocampus (GABAergic and cholinergic) rather than based solely on changes in glutamate transmission.

2. CET-INDUCED CHANGES IN MEMORY AND HIPPOCAMPAL FUNCTION

Long-term, excessive exposure to ethanol disrupts cognitive function as measured by a broad spectrum of techniques (1-4). The behavioral dysfunction can range from relatively mild cognitive deficits (4, 5) to Korsakoff's syndrome or alcoholic dementia characterized by a profound anterograde amnesia (1, 4). The relative contribution of nutritional deficiency and ethanol neurotoxicity to the mnemonic deficit has not been established (2, 6). Further, a relationship between neuropathological alterations and specific memory dysfunction has not been adequately substantiated.

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Numerous animal studies have confirmed that CET produces abnormal morphology and function in the rodent hippocampus (7). CET produces a progressive learning and memory deficit across a variety of behavioral tests including active avoidance (8-11), complex maze learning (12, 13), and tests of temporal (14, 15) and spatial (15-19) memory. The memory deficits grow progressively more severe with increasing exposure to CET and persist for prolonged periods following abstinence (8). Morphological changes in hippocampus associated with CET include a 10-40% loss of principal cells (16, 20-26) and interneurons (27, 28). The extent of the cell loss depends upon the duration of CET, the magnitude of exposure, genetic susceptibility to ethanol and the length of ethanol abstinence. For the purposes of this review, CET will be defined as continuous ethanol exposure for a sufficiently long period (e.g., 6 months) to result in lasting (or in some cases, permanent) structural and functional alterations of the hippocampus or its neural connections (7). At least 48 hrs withdrawal from ethanol is required so that the lasting functional disturbances associated with structural changes can be separated from the transient disturbances associated with ethanol tolerance and withdrawal (7). Periods of ethanol exposure that are not sufficient to cause persistent behavioral changes after withdrawal will be referred to as subchronic.

Neurons which survive CET also exhibit structural abnormalities (7). However, despite profound and often region-specific morphological changes (29-32), CET produces surprisingly subtle changes in the function of the hippocampus as directly assayed by electrophysiological methods. These functional changes include a reduction in intrinsic inhibitory processes (33-35) and a modification in the distribution of synaptic connections using current-source density analysis (36, 37).

3. CET-INDUCED CHANGES IN LONG-TERM POTENTIATION (LTP)

A more profound effect of CET on hippocampal function is that CET appears to alter the capacity for synaptic

plasticity such as LTP. LTP is defined as a long-term increase in synaptic efficacy induced by exposure of neurons to high-frequency stimulation of excitatory afferent pathways (38, 39). It is generally considered to be a synaptic model or substrate for learning and memory (40, 41). This enduring change, which can last from hours to days (40, 42, 43), is manifested by an increase in the amplitude and the slope of the extracellular recorded EPSP. LTP also results in an increased amplitude and decreased latency of the population spike. LTP is ordinarily divided into three separate processes: 1) induction, 2) maintenance and 3) expression. At the physiological level, the induction of LTP requires cooperativity of synaptic inputs resulting in a threshold depolarization of the postsynaptic membrane.

CET reduces the percentage of hippocampal slices exhibiting LTP of the population spike in hippocampal area CA1 (44). Similar *in vivo* studies reveal a diminished population spike amplitude after LTP conditioning trains in the dentate gyrus (17). CET reduces the synaptic component of LTP in addition to its effects on the population spike (45). While CET slices exhibit a progressive increase in LTP with successive conditioning trains, the magnitude of the LTP is substantially reduced relative to control slices. This decrement occurs regardless of whether a 48 hr or 5-7 month withdrawal period is given. When hippocampal tissue from CET and sucrose-treated animals was exposed to the $GABA_A$ antagonist, bicuculline methiodide, the CET-produced disruption of LTP is no longer observed (45). These data indicate that the mechanism for the CET-induced decrement in LTP involves activation of $GABA_A$ receptors.

4. CET-INDUCED CHANGES IN GLUTAMATE TRANSMISSION IN HIPPOCAMPUS

There is now more known about the molecular mechanisms important in the induction of the synaptic component of LTP in the CA1 region although there is still much controversy as to whether presynaptic (46) or postsynaptic (47) mechanisms are involved. Both activation of NMDA receptors (see 48) as well as inactivation of GABA receptors (49) appear to be important in the generation and maintenance of LTP although the interplay between these two processes has not been well-defined. The trigger for the induction of LTP is at the NMDA receptor/ion channel complex (see 50). The NMDA receptor can be composed of a mix of 7 splice variants of the NR1 protein subunit with one of four NR2 subunits thereby providing the possibility for functionally distinct receptor subtypes (see 51). Mice lacking a particular subtype of one of the protein subunits that comprise the NMDA receptor exhibit reduced LTP (52). The NMDA receptor is coupled to a nonspecific cation channel which can allow significant transmembrane calcium ion (Ca^{2+}) flux. This transient Ca^{2+} may trigger one or more Ca^{2+}-dependent enzymes such as Ca^{2+}/CAM kinase II, protein kinase C or proteases. One or all of these enzymes may play a critical role in the mechanisms underlying the enhancement of synaptic transmission.

Acute ethanol treatment has been shown to completely block LTP via a direct effect on NMDA-receptor mediated currents (53). Acute ethanol quite potently inhibits NMDA receptor function thereby decreasing Ca^{2+} influx in hippocampus as measured by electrophysiology, Ca^{2+} fluorescence and $^{45}Ca^{2+}$ uptake (54-56). The sensitivity of NMDA receptors to ethanol depends on receptor composition (57, 58). Subchronic repeated exposure to ethanol increases glutamate (59, 60) and NMDA binding sites (61-63) as well as NR1 receptor subunit immunoreactivity in hippocampus (64). Functionally, NMDA-stimulated Ca^{2+} uptake is increased after subchronic repeated ethanol treatment (65, 66) but there is no change in ethanol inhibition of NMDA receptor function (67). Thus, following subchronic ethanol exposure, there is an up-regulation of NMDA receptor number but no change in receptor function. It is important to distinguish that these studies measured the effects of fairly short-term ethanol exposure (days to weeks) and that after withdrawal from ethanol, NMDA receptors usually returned to normal.

It is not clear whether similar changes in NMDA receptor number and function would occur in hippocampus following withdrawal after long-term CET (e.g., 6 months). There is no change in the number or affinity of [^{3}H]MK-801 binding sites, nor is enhancement of this specific binding by glutamate affected by CET (68). These data support the hypothesis that the CET-induced decrease in LTP is not due to a change in NMDA receptor number. In agreement with these data, Northern blot analysis of NR1 mRNA levels in hippocampus indicate no effect of CET (69). These results suggest that although NMDA receptor number and mRNA levels may be altered after short-term exposure to ethanol, these changes do not occur after withdrawal from long-term CET. It will also be important to determine whether the functional status of the NMDA receptor is not altered after CET as well as whether presynaptic indices of glutamate transmission are altered by CET.

5. CET-INDUCED CHANGES IN GABA TRANSMISSION IN HIPPOCAMPUS

While the NMDA receptor serves as a critical trigger in the induction of LTP, alterations in NMDA receptor function appear to play little role in the expression of established LTP. Considerable controversy exists over the mechanism and site involved in the expression of LTP (70-72). Presynaptic $GABA_B$ receptors are also proposed as a mechanism affecting LTP, since a decrease in GABA release may contribute to an increased NMDA response (73-75). During induction of LTP, $GABA_A$-mediated inhibition is decreased thereby allowing NMDA-mediated excitation to increase (75). Blockade of $GABA_B$ receptors prevents the reduction in GABA inhibition, the increase in NMDA excitation and the induction of LTP (75).

It is possible that CET produces an enduring increase in pre- and/or postsynaptic elements of GABAergic synaptic transmission. This increase in GABA transmission could counteract the depolarizing effects of the LTP-induced NMDA receptor activation. This hypothesis is supported by the fact that the difference in LTP between CET and sucrose-control groups is abolished by bicuculline blockade of GABAergic synaptic transmission (45). These data indicate

that an increase in postsynaptic $GABA_A$ receptor activation is involved in CET inhibition of LTP.

The $GABA_A$ receptor/chloride (Cl^-) ionophore is a hetero-oligomer composed of a total of 4-5 polypeptide subunits of at least thirteen different types ($alpha_{1-6}$, $beta_{1-3}$, $gamma_{1-3}$ and delta), each displaying a unique regional expression even within hippocampal subregions (76, 77). The $gamma_2$ subunit is necessary for benzodiazepine modulation of function (78) and the alternatively spliced $gamma_{2L}$ form of this subunit must be appropriately phosphorylated by protein kinase C before ethanol sensitivity is conferred (79-81). In receptors that are sensitive to ethanol, there is an enhancement of GABA-stimulated Cl^- conductance (see 82). Subchronic ethanol exposure (5-10 days) results in a loss of *in vitro* enhancement of channel function by ethanol (83, 84). Although there has not been any consistent evidence for a change in the number or affinity of the $GABA_A$ receptor as a whole (85, 86), subchronic ethanol exposure decreases both mRNA and peptide levels for $alpha_1$, $alpha_2$ and $alpha_3$ subunits in cortex, increases $alpha_6$ in cerebellum (87-92) and causes long-term increases in both mRNA and peptides for the $beta_2$ and $beta_3$ subunits in both regions (93). Thus, subchronic ethanol exposure changes the subunit composition and very likely, the functional status of the receptor. However, most of the changes described above are transient in nature, returning to control levels within 48 hrs after ethanol withdrawal.

CET appears to increase the number of [^{3}H]bicuculline binding sites in hippocampus (94) which may indicate an increase in $GABA_A$ receptor number or an alteration in receptor subunit composition. However, CET does not change the efficacy or potency of muscimol to activate postsynaptic $GABA_A$-controlled Cl^- ionophores nor is there a difference in the efficacy or potency of bicuculline to block this agonist stimulation (95). However, changes in the number of binding sites may not always be accompanied by a functional change if spare receptors are present. Therefore, the functional significance of the increase in [^{3}H]bicuculline binding sites in hippocampal subregions of CET rats remains to be determined.

On the other hand, CET significantly increases electrically-stimulated [^{3}H]GABA release from superfused hippocampal slices (95) which could also explain why bicuculline could abolish the effects of CET on LTP. It is not likely that the increase in GABA release is due to hyper innervation of pyramidal cells by GABAergic interneurons since previous studies have found that CET reduces functional inhibition of CA1 pyramidal cells as well as the number of GABAergic interneurons in CA1 (33-35). Instead the mechanism for an increase in GABA release seems to involve changes in $GABA_B$ receptor-mediated presynaptic inhibition of GABA release (94). The effects of maximally effective doses of $GABA_B$ receptor agents on [^{3}H]GABA release are significantly decreased in CET rats (94). Results from binding experiments in similarly-treated rats suggest that CET has no overt effect on the number of $GABA_B$ receptors in hippocampus which are predominantly postsynaptic in nature (94). In support of this, CET decreases the presynaptically-mediated hyperpolarizing responses of CA1 pyramidal cells to bath-applied baclofen (96) without affecting the postsynaptic effect of baclofen to inhibit EPSPs. Similar effects of CET on baclofen-induced inhibition of GABA release have been found in cortex (97) although there is no effect of CET on baclofen-induced inhibition of isoproterenol-stimulated cAMP formation (98).

$GABA_B$ autoreceptors are capable of regulating induction of LTP via a decrease in GABA release thereby permitting sufficient NMDA receptor activation (99). On the other hand, postsynaptic $GABA_B$ receptors can cause hyperpolarization of the postsynaptic membrane resulting in an enhanced blockade of NMDA channels by Mg^{2+} thereby inhibiting NMDA-mediated EPSPs (100). Thus, the involvement of $GABA_B$ receptors in LTP may be quite complex since presynaptic disinhibition of GABA neurons would enhance LTP but postsynaptic activation of $GABA_B$ receptors could diminish NMDA-mediated EPSPs and decrease LTP.

6. CET-INDUCED CHANGES IN CHOLINERGIC TRANSMISSION IN HIPPOCAMPUS

Cholinergic innervation of hippocampus is also important for mediation of events leading to LTP. The balance between cholinergic and GABAergic systems may determine hippocampal theta activity (101) which has been shown to modulate LTP *in vivo* (75). The dentate gyrus is innervated by GABAergic and cholinergic fibers from the medial septum and the nucleus of the diagonal band (see 102) which can influence dentate gyrus excitability. There is a strong cholinergic influence on GABAergic and other neurons in hippocampus (103, 104). A vast majority of the GABA neurons in CA1, CA3 and dentate gyrus of hippocampus express muscarinic acetylcholine receptors (101). These muscarinic receptors may presynaptically influence GABA release in hippocampus and affect LTP.

Cholinergic influences also appear to be involved in the effects of CET on LTP in hippocampus. There are numerous reports that suggest that CET may induce a permanent loss in cholinergic function in hippocampus. CET decreases high affinity choline uptake in hippocampus (105) as well as ACh levels, choline acetyl transferase and acetylcholinesterase activity (19, 106, 107). Lesions of the septohippocampal nucleus block the effects of acute ethanol on LTP (108). Whether this effect is due to a loss of altered cholinergic influences on GABA neurons remains to be answered. CET decreases the effects of ACh on population spike amplitude but has no effects on carbachol-inhibition of EPSPs (109). Additionally, both cholinergic disinhibition and recurrent inhibition are decreased by CET. Similar results were found in response to application of carbachol (110). These data suggest that ACh response properties in CA1 exhibit differential sensitivity to CET and may reflect a distinct susceptibility of muscarinic receptor subtypes to the neurotoxic effects of ethanol. However, the reductions in cholinergic function produced by CET do not appear to be due to receptor loss since muscarinic receptor subtype densities were not found to be altered as determined by either immunoprecipitation of m_{1-5} subtypes (111) or by maximal [^{3}H]QNB binding and carbachol displacement of specific binding (112). Thus it is possible that the effects of CET on

cholinergic transmission involve changes in muscarinic second messenger systems (e.g., PI metabolism) or very select populations of muscarinic receptors. The effect of carbachol on the EPSP is thought to be due to the presence of presynaptic muscarinic cholinergic receptors on the terminals of the stratum radiatum afferents. When these receptors are activated by carbachol, neurotransmitter release is reduced. Thus, the effects of CET to decrease the activation of muscarinic cholinergic receptors seem to be selective for postsynaptic rather than presynaptic cholinergic receptors.

In terms of presynaptic receptors, it appears that separate populations of presynaptic muscarinic cholinergic receptors may also be regulated differently by CET. The cholinergic agonist carbachol increases [^{3}H]GABA release from superfused hippocampal slices and this effect is blocked by atropine (113). In CET rats, there is an increase in carbachol enhancement of [^{3}H]GABA release compared to that measured in sucrose-treated rats (113). In contrast, the effect of atropine, a muscarinic antagonist, is significantly decreased (113). Thus, in addition to a CET-induced down-regulation of $GABA_B$ autoreceptor function, presynaptic regulation of GABA release by ACh receptors in hippocampus is affected in a manner so as to increase GABA release. However, muscarinic receptors have been previously reported to decrease, not increase, GABA release in hippocampus or other brain regions. In contrast to these data, there is no effect of CET on the cholinergic control of presynaptic release of ACh. CET does not produce any reliable change in basal or stimulus-dependent [^{3}H]ACh release in hippocampal slices (113). In addition, exposure of synaptosomes to the muscarinic cholinergic agonist oxotremorine causes an equivalent attenuation of potassium-evoked [^{3}H]ACh release in both control and CET rats (113). The effects of the specific muscarinic cholinergic antagonist atropine are also similar (113). Therefore, in view of these findings, it appears that CET may be extremely selective in its effects on hippocampal cholinergic transmission.

7. Summary

LTP has been considered a significant physiological model of memory formation. In light of converging evidence of a critical role for the hippocampus in the formation of memory, LTP may be more than a model of memory. LTP may actually be a significant mechanism utilized by the hippocampus for encoding or indexing experiential representations of activity originating in cortical association areas (114, 115). Chronic alcohol abuse disrupts long-term memory formation in a manner that has not been adequately linked to gross neuropathological abnormalities. CET produces significant changes in the structural and functional properties of the hippocampus in rodents including a profound reduction in LTP at synapses in CA1. In addition to the NMDA receptor/ion channel complex, both GABA and acetylcholine receptors appear especially sensitive to the acute and chronic actions of ethanol. When coupled with the growing recognition of the role of the hippocampus and LTP in normal memory formation; collectively, this evidence provides a compelling rationale for studying CET and LTP. A complete understanding of how CET affects LTP in a persistent fashion will more than likely require an understanding of the probable interactions of these three neurotransmitter systems in hippocampus. A similar "multi-transmitter" approach has recently been successfully applied to the understanding of ethanol tolerance and the mechanism by which repeated exposure to ethanol may affect multiple receptor classes via changes in protein kinase C synthesis and activity (see 116). Thus, the mechanisms underlying the effect of CET on LTP may very likely be a result of changes in a number of neurotransmitter systems in hippocampus including GABAergic, glutamatergic and cholinergic. Even though CET may induce a number of changes in NMDA, GABA and cholinergic receptor number and function in hippocampus, we do not know whether these changes are the mechanism by which CET decreases LTP or to what extent these changes can account for CET effects on LTP. Thus, it is imperative that we test the relationship of the functional status of receptors affected by CET and the induction, maintenance and expression of LTP in CET rats.

8. ACKNOWLEDGMENTS

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