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Alcohol-Related Electrophysiology

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SIGGINS, G. R. AND F. E. BLOOM. *Alcohol-related electrophysiology*. PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 203–211, 1980.—Considerable effort has been expended to determine the electrophysiological actions of ethanol (EtOH) on nervous tissue. However, the site and mechanisms of EtOH intoxication, tolerance and dependence remain largely unknown. We and our co-workers have used two central mammalian models to clarify these problems: the rat cerebellar Purkinje cell (CPC) and the rat hippocampal pyramidal cell (HPC). Direct application of EtOH to CPCs via microelectroosmosis or micropressure depresses spontaneous discharge weakly, while tetrahydropapaveroline (THP) depresses firing markedly. Single IP injections of EtOH (1–4 g/kg) increase the frequency of climbing-fiber (CF)-evoked bursts in CPCs but only occasionally increase simple-spike rates. However, CPCs of rats chronically treated with EtOH for 11–14 days and recorded 0–3 hrs after the last EtOH dose do not differ from controls. Rats chronically treated and then withdrawn from EtOH show significant decreases in CF activity and simple-spike rates 3–32 hrs after the last EtOH dose. These changes in CPC firing may be neuronal correlates of EtOH intoxication, tolerance and dependence. In the hippocampus, with direct application of EtOH to HPCs *in vivo* about half of the cells are excited and half inhibited. Salsolinol excites most HPCs, in parallel with opiate actions. THP is mostly inhibitory. EtOH and salsolinol excitatory effects are often antagonized by naloxone. With intracellular recording in the hippocampal slice, EtOH perfusion (10–400 mM) has mixed depolarizing/hyperpolarizing actions, depending on dose and time of application. Excitations may arise from depolarization, increase in membrane resistance, enhancement of evoked EPSPs or block of IPSPs, in contrast to opiate actions which are always associated with reduction in IPSPs. *In vivo*, systemic EtOH (3 g/kg, IP) significantly enhances both excitatory and inhibitory responses to stimulation of afferent pathways to HPCs. The excitatory EtOH actions on HPCs may relate to the hippocampal EEG spiking sometimes elicited by low doses of EtOH. However, further studies are needed to verify a link with opiate-like mechanisms.

Ethanol Opiates Electrophysiology Microiontophoresis Tetrahydroisoquinolines
Cerebellar Purkinje cells Hippocampal pyramidal cells Single units Intracellular recording

RATIONALE AND STRATEGY FOR NEUROPHARMACOLOGY IN ALCOHOL RESEARCH

NEUROPHARMACOLOGY is the scientific discipline which examines the actions of drugs on the nervous system. Such research is of obvious importance to the effects of ethanol and alcoholism as the central and peripheral nervous systems undoubtably must be the primary sites where ethanol works to produce intoxication, dependence and neurotoxicity. Although there have been major advances in the fundamental understanding of the nervous system and its relevance to human substance abuse problems [3,70–72], relatively little of this new knowledge has yet been able to impact upon ethanol research. Nevertheless, important research opportunities presently exist which hold great promise for human problems of ethanol action and alcoholism.

For example, it should be possible to determine the cellular sites and molecular mechanisms of ethanol action in the brain responsible for the behavioral syndrome of intoxication. Perhaps this knowledge may lead to methods by which acute intoxication may be reversible by drugs. Some researchers have likened the search for 'sobering-up" drugs, or amethystic agents as they are more properly termed, to a search for a mythical magical bullet akin to Ehrlich's magic bullet against syphilis (cf. [20]). Yet, the need for such a drug is considerable, given the extensive costs of treating alcoholic coma and stupor in hospital emergency wards. Far from being an excuse to drink with even less regard for short term consequences, it is probable that the availability of amethystic preparations could have considerable impact in lessening the death toll of intoxicated drivers. Recent efforts to apply opiate antagonists to the reversal of alcoholic stupor [5,67] have been one of the surprising offshoots of the attempt to explain all previously unknown drug actions through recently discovered brain chemicals, like the endorphins [63]. Even though there is no direct evidence to believe that intoxicating actions of ethanol are mediated through the endorphins, there is some circumstantial evidence to support this presumption. This is clearly a line of opportunity for future pharmacological research, if only to establish which patients in alcoholic stupor are aroused by opiate antagonists and how that effect occurs.

It is disappointing to realize how very little is established as to where and how the several phases of intoxication result from exposure to elevated blood alcohol levels. Many questions exist as to whether it is ethanol itself, something derived from ethanol, or some endogenous biochemical equilibrium upset by ethanol which leads to the cellular and molecular events which produce the CNS effects. We shall pursue this question in more detail below.

In the initial phase of biochemical research on ethanol, correlative changes in the brain concentrations of neurotransmitters or their metabolites were sought, with the rationale that if ethanol intoxication, or the withdrawal from chronic ethanol exposure, involved one of these transmitter systems, its concentration and metabolism should be

changed. In the next phase, the approach was shifted to probe for correlations with changes in the rates of synthesis of neurotransmitters, from which changes in neuronal activity could be inferred. These correlative changes in ethanol actions and transmitter metabolism did not provide clearcut data on the specific involvement of any transmitter system. This shortcoming is perhaps due to the fact that neuronal events operate on the time-scale of seconds or less, while behavioral actions and the time resolution of the chemical measurements require many minutes or hours. Thus, given the complexity of brain organization and the speed of intercellular events, any major effect of alcohol, even if it were initially restricted to one pathway or one transmitter, ultimately may affect most interconnected pathways and, therefore, most transmitters. As a result primary effects are difficult to distinguish from secondary effects (see ref. [22]).

An appraisal of the major directions which cellular analyses of alcohol action on the CNS might take, could start with the premise that ethanol's actions are either 'non-specific' or 'specific'. If the actions of ethanol are, in fact, so non-specific that every neuronal component of the CNS reacts identically, then only cellular testing is capable of demonstrating the alleged non-specificity. In contrast, if the effects of ethanol are generally propagated throughout the CNS through mediation of actions on a particular structural or functional substrate, then again cellular level research is the minimal resolution required to detect such specific general substrates. It is our view that research into interneuronal communication, as evidenced by structural, chemical or electrophysiological analyses, is the most incisive route to detecting these effects of ethanol.

Specific drug effects are usually construed as identifiable molecular mechanisms unique to specific cells with receptors for the drug. In the classic cases of most potent psychoactive drugs, a response or change in activity arises because the drug interacts with the receptors by which specific transmitters influence the specific target neurons. Conversely, non-specific drugs derive their effects from actions on many molecular mechanisms on many cell types. Such non-specific effects could represent general changes in membrane properties or cellular metabolism. In this view, ethanol has often been considered a general, or non-specific, depressant of the CNS based upon the lack of data incriminating any specific cellular or molecular event [36, 37, 51, 59] and upon considerable recent data supporting general membrane actions [28].

Important opportunities exist to understand the response of the brain to repeated chronic exposures to ethanol and to consider individual variation in susceptibility to ethanol addiction and to its toxic actions on the structure and funtion of the brain. Current efforts in neuropharmacology concentrate on the adaptive changes by which the nervous system reacts to drugs over a long time course of exposure. For many drugs it is now recognized that the initial effects of a drug on the brain are often lost and even reversed as drug exposure continues. This adaptive change can be recognized both in the behavioral actions of the drug (i.e., drug tolerance) and in the molecular properties of the receptors for some, but not all, neurotransmitters.

Elucidation of the cellular mechanisms underlying neuronal tolerance to ethanol is likely to contribute significantly to the eventual understanding of adaptive mechanisms in general, including those involved in learning and physiological habituation. In addition, neuropharmacological research on chronic ethanol actions holds promise for illuminating potential causes for toxic actions of ethanol on embryonic brain development in the fetal alcohol syndrome, including the relative role of nutritional factors [75] or other potential toxic co-habits such as cigarette [62] or tranquilizer [26] use.

THE ROLE OF ELECTROPHYSIOLOGY

We consider that several of the major alcohol-related questions posed above can be approached from an electrophysiological viewpoint. These questions can be more concisely enumerated as follows: (1) Are acute alcohol effects primarily exerted at specific brain areas and on specific neuron types? If so, how can these primary effects be detected and dissected from remote effects on other brain areas or neurons, given the wide interconnections between many, if not most, brain regions and neurons? (2) As a corollary to the latter question, are acute, intoxicating effects of alcohol on neurons mediated by ethanol or by some by-product of ethanol metabolism (e.g., acetaldehyde) or its condensation with some endogenous neurochemical (producing, e.g., tetrahydroisoquinolines (TIQs))? Could these products be elaborated in the brain or in the periphery (e.g., the liver or adrenals)? Similarly, could alcohol exert its acute effects by releasing a transmitter such as one of the opioid endorphin peptides, which are thought to have, like ethanol, dependence producing properties? (3) Does acute alcohol have a variety of time- and dose-dependent effects on central neurons? (4) Are the acute effects of alcohol specific to certain chemical types of neurons or to specific synaptic or receptor-transmitter interactions, or are all neuronal membranes affected equally in an unspecific manner? (5) What happens to the acute responses to alcohol when the organism is administered alcohol chronically? Do electrophysiological effects show tolerance and withdrawal phenomena?

We believe it is apparent that such a variety of questions, although often related or interlocking, cannot be answered by a single electrophysiological approach or model. Indeed, a variety of models, mostly invertebrate or peripheral vertebrate neuron systems, have been used to approach some of these questions. In our laboratory, we have utilized four different electrophysiological methodologies aimed at several different mammalian brain regions. Selected results of these experiments will be outlined following a brief background review of relevant literature reports.

A SELECTED LITERATURE SURVEY ON ETHANOL ACTIONS

Invertebrate and Peripheral Vertebrate Systems

While considerable effort has been expended to determine the acute electrophysiological actions of ethanol on nervous tissue [29,36], the site and mechanisms of ethanol intoxication, tolerance or dependence still remain unknown. However, since most studies have utilized a variety of invertebrate and vertebrate model systems, and the concentrations of ethanol employed have been larger than those associated with moderate intoxication or ataxia, it is not surprising that a myriad of actions have been ascribed to ethanol. Indeed, the majority of the electrophysiological studies have utilized peripheral vertebrate or isolated invertebrate preparations, and have revealed multiple cellular mechanisms of ethanol that generally fall into one of the following categories: (1) a direct reduction in neuronal excitability through alterations in the voltage dependent ionic conductances underlying action potential generation [2, 8, 49, 50]; (2) effects on excitability consequent to changes in passive

membrane permeabilities and resting membrane potentials [2, 8, 27, 41]; (3) pre- or post-synaptic changes in synaptic transmission. The latter effect includes alteration in transmitter release [14, 21, 48, 76] or in transmitter sensitivity of the post-synaptic membrane receptors [4, 7, 11, 24, 55]. While most studies describe a depressant effect of ethanol on neuronal excitability, it should also be stated that in some instances ethanol enhances excitability, as for example in muscle spindle afferent excitation [44] and in the activity of spinal Renshaw cells [46].

Barker (see [4]) has suggested that anesthetics specifically reduce all excitatory post-synaptic potentials (EPSPs). However, ethanol enhances cholinergic transmission both in the cat spinal cord [46] and at the frog neuromuscular junction [24,55]. Therefore, a generalized reduction in EPSPs appears somewhat over-simplified. However, Adams and co-workers have recently found [1] that ethanol reduces the duration of the excitatory postsynaptic current at a crustacean neuromuscular junction, an effect opposite to that at the frog neuromuscular junction [25].

An interesting series of studies has recently been reported by Faber and Klee [21,22], using the large Mauthner (M) cell of the goldfish medullary network. Their general conclusion is that synaptic transmission is more sensitive to ethanol than are either spike electrogenesis or passive membrane properties; this conclusion is based on the finding that synaptic transmission is depressed in this system by ethanol concentrations significantly lower than those which alter other processes. Thus, ethanol at brain concentrations which are threshold for behavioral effects (hyperexcitability) in goldfish, specifically blocks collateral inhibition of the M cell [21]. This effect is selective for the cholinergic excitatory synapse between M cell collaterals and inhibitory interneurons. Much higher concentrations are required to produce only minor effects on M cell excitability and resting membrane potential. Furthermore, the ethanol actions on the M cell are selective: not all post-synaptic potentials are blocked or reduced. It is likely, at least in this animal, that effects of ethanol on synaptic transmission are primarily responsible for the more specific behaviors such as hyperexcitability and ataxia, whereas direct effects on membrane properties would contribute to general depressant actions of ethanol.

A significant effect of ethanol recently reported by Traynor *et al.* [69] and Woodson *et al.* [76] is an acceleration of the decay of post-tetanic potentiation in an identified *Aplysia* neuron, R15. These workers suggest that the decay of PTP is related to membrane fluidity; the ethanol action is due to an increased membrane fluidity. The same laboratory has also demonstrated a sustained tolerance to this specific effect of ethanol [69]. However, these studies need to be repeated in a vertebrate brain preparation, especially since a high ethanol concentration (0.8–1 M) was used.

Mammalian Brain

A variety of types of electrophysiological measures have been used to assess the action of ethanol on mammalian brain, including EEG [30, 34, 40, 56, 68], evoked activity [6, 12, 13, 16, 17, 18, 56], multiple-unit activity [39,40] and single-unit discharge [20, 23, 30, 43, 47, 53, 58, 60, 65, 66, 74]. These methods have been used to study the excitability of neurons or groups of neurons in a number of brain regions including cerebellum [20, 47, 60, 65], lateral hypothalamus, thalamus, zona incerta, cerebral cortex [34, 43, 73, 74, 78], hippocampus [12, 13, 17, 18, 23, 30, 38, 40, 53, 57], septum, reticular formation, geniculate nucleus [38,39] and locus coeruleus [58]. A considerable degree of variability is seen in the response of these brain areas to ethanol, probably owing in part to the confusing array of doses, rates and routes of administration (usually systemic) and species used. However, a general trend does seem to emerge (see refs [29,36] for a more in-depth review): in many cases lower doses of ethanol tend to be excitatory, while higher concentrations depress nervous activity. This trend seems to fit with the conclusion drawn above with respect to the actions of ethanol on non-mammalian neurons.

However, to our knowledge no studies have been reported (other than those described below) using intracellular recording of mammalian brain neurons with ethanol administration. Therefore, no conclusions can be drawn as to whether synaptic transmission is specifically affected or if general membrane excitability is altered. Furthermore, *in vivo* brain studies have generally failed to demonstrate whether these effects are primary or arise from secondary actions on other central or peripheral neurons. Moreover, few reports on electrophysiological effects of *chronic* ethanol treatment, other than EEG studies (see, e.g., [56]), have appeared. Ethanol withdrawal presents still more complex problems. The withdrawal syndrome is thought to be associated with increased neuronal excitability [68], but even this simple observation remains uncorroborated at the cellular level.

CEREBELLUM

Single-Unit Recording and Systemic Ethanol

In an effort to clarify some of these problems we initiated our series of experiments on the cerebellar cortex of the rat. This structure was chosen as an experimental target for several reasons. Cerebellar function may be relevant to the known vestibular actions of intoxicating doses of ethanol (see [20]). The cerebellar Purkinje cell is a favorable primary model because it is readily identified by electrophysiological means, receives a well-defined and easily-activated synaptic circuitry [19], with several inputs of known neurotransmitter identity [9, 32, 33, 77], and it discharges spontaneously at uniform high rates [33]. Thus, we used computer-based methods of analysis of firing patterns of rat cerebellar Purkinje cells *in vivo* as an index for assessing electrophysiological effects of ethanol [60].

In this study, a single ethanol injection (2–4 g/kg, IP) had no immediate effect on Purkinje cell simple-spike firing. However, beginning about 10 min after injection (when blood alcohol levels were rapidly increasing) there was a progressive increase in climbing fiber (CF) burst activity with recovery beginning around 80 min after injection (a time when blood alcohol levels were still above 150 mg%). Even more extreme changes than this were occasionally encountered in the CF activity after ethanol: in a few cells, the pattern of firing changed from normal regular bursts of CF discharge at 1–2 sec intervals, to flurries of regularly-spaced CF bursts at 5–10/sec lasting for up to 12 sec. In one series of ten rats, CF activation was observed in 4 out of 5 Purkinje units after 4 g/kg ethanol and 2 out of 3 Purkinje cells after 2 g/kg ethanol. After lower doses, no significant CF changes were evident.

It could be argued that the slight increase in firing and the large increase in CF activity described above may be due to artifacts inherent in long-term recording from single cells.

For this reason we also recorded many different neurons for shorter times before and after ethanol (rather than following the same neuron through time-dependent changes), and used a specific computer program to compile interspike interval histograms (ISHs) of 65 different Purkinje neurons before ethanol; we then compare these properties of 'typical' Purkinje cells with average ISHs of 22 different neurons 10–60 min after 1–4 g/kg acute ethanol. Like the long-term records from individual neurons, these different cells showed significant mean increases in CF burst activity compared to pre-ethanol control cells. Simple spikes/sec did not differ significantly between control and acute ethanol cells.

Effects of Chronic Ethanol Treatment and Ethanol Withdrawal

We employed either a chronic vapor-inhalation method [61] or a chronic intragastric method to administer ethanol to rats over a 14–21 day treatment period [60]. Body growth in the vapor-exposed rats was comparable to littermate controls and blood alcohol levels averaged 150–200 mg%. In contrast to effects of acute ethanol, Purkinje neurons recorded after chronic treatment showed no significant alteration in firing patterns 0–3 hrs after the last administration of chronic ethanol [60]. After 12–32 hrs of withdrawal from chronic ethanol, however, marked differences in Purkinje cell firing appear. In general these changes are opposite to those observed after acute ethanol: CF bursts/sec are significantly decreased, and indeed all parameters of firing rate are significantly affected, including the increased percentage of long (greater than 75 msec) interspike intervals. Furthermore, the decrease in CF bursting can be viewed as a graded withdrawal response, significantly dependent on the time since the last chronic ethanol administration. By administering a 4 g/kg booster dose of ethanol, it is also possible transiently to restore cells of withdrawn rats to relatively normal firing patterns. In fact, at this very high dose, CF burst rates climb beyond normal levels and become comparable to those seen in naive rats given 4 g/kg acute ethanol [60].

These *in vivo* studies indicate that while ethanol may have few primary effects within the cerebellum—as reflected by changes in simple spike firing of Purkinje cells—it does exert interactive secondary effects at sites remote from the cerebellum, as exemplified by changes in CF-mediated burst activity. Furthermore, both primary and secondary ethanol effects on Purkinje cells exhibit dynamic changes relative to the duration of ethanol exposure: acute ethanol increases CF bursts and, occasionally, simple spike firing; chronic exposure results in an adaptation to the acute effects, and withdrawal from chronic exposure frequently reveals more clearly the underlying compensatory effect. These changes, therefore, appear to be cellular correlates of ethanol intoxication, tolerance and withdrawal, that can be inferred by analysis of firing patterns for this well-studied cell population.

The result of our initial studies are insufficient to determine the loci of neuronal action or the possible receptor mechanisms by which ethanol intoxication, tolerance or withdrawal syndromes are generated. They do indicate, however, that ethanol effects cannot be characterized either as 'generally depressant' or 'generally excitatory'. Rather, our results indicate that ethanol actions are probably specific to particular brain structures or neuronal systems. The short- and long-term changes in CF activity presumably reflect changes in activity in the inferior olive, which is the source of climbing fibers. The similarity between the stimulating effect of ethanol and harmaline warrants further investigation, since compounds similar to harmaline have been suggested to be produced in the brain after ethanol administration, and harmaline is known to act directly on the inferior olive, apparently through an interaction with 5-HT [15].

Iontophoresis and Micro-Pressure Studies [66]

One method for minimizing indirect or remote drug effects is to apply drugs directly to single neurons by iontophoresis. In the case of ethanol, which is poorly ionized, one would ordinarily use the method of electro-osmosis in which the drug is passed from the pipette tip via bulk flow within a sodium hydration shell. However, we have recently modified the pneumatic (micro-pressure) ejection technique [42, 45, 64] which facilitates the ejection from micropipettes of poorly soluble or poorly ionized substances. Moreover, with this technique it is more feasible to construct dose/response curves (using different concentrations of drug in the pipette barrel) than with the iontophoresis techniques.

In an effort to control for the numerous artifacts that apply to the electro-osmosis and micropressure methods, we have developed a method of ejecting alcohol onto single neurons (while recording their extracellular action potentials from another barrel of the same pipette assembly) by either method alternately or simultaneously [66]. By this means we have found that alcohol applied by either method usually depresses cerebellar Purkinje cells in an apparent concentration-dependent manner. Thus, with alcohol in a 1 M concentration (in normal saline, pH 7) in the pipette barrel, weak effects or no effects are noted; however, at concentrations of 2 M or above, the depressions appear to be non-specific, and local-anesthetic in nature, since they are often accompanied by reduction in spike size with broadening of spike duration. In accord with this interpretation, and in contrast to previous reports [15,16], we noted that the inhibitions of activity produced by either iontophoretic GABA or norepinephrine (NE) were antagonized non-specifically by direct application of alcohol to Purkinje cells [66].

Preliminary studies [66] on the neuronal effects of TIQs have been initiated in our laboratory with iontophoresis of tetrahydropapaveroline·HCl (THP), synthesized and kindly provided by Dr. W. T. Shier. As with ethanol, the predominant response to iontophoretic or pressure-ejected THP in Purkinje neurons is depression of spontaneous discharge. However, in contrast to the alcohol effects, little or no 'local anesthetic' action is seen with THP. Furthermore, at equivalent ejection currents or pressures, the THP depressions appear more pronounced than those with alcohol (1–2 M in the pipette), in spite of the much lower maximum solubility of THP (about 3 mM in the pipette). Given the 500 to 1000-fold lower effective concentration, THP would appear to be a much more potent neuronal depressant than ethanol. Comparison of ejection efficiency and tissue diffusibility for the two substances will be required to substantiate this premise.

Analysis of the ionic and/or receptor mechanisms of the neuronal depressions produced by THP and ethanol might provide data relevant to a possible role for these agents in alcohol tolerance and dependence. It may be only coincidental that catecholamines, like alcohol and THP, uniformly depress the spontaneous activity of cerebellar Purkinje cells [32]. Extensive pharmacological testing will be required to

verify a link between these alcohol, THP and catecholamine depressant actions. We have specifically looked for possible interactions between THP and catecholamines, but in studies to date THP does not appear either to potentiate or antagonize NE-evoked depressions in cerebellum or hippocampus, nor does it affect dopamine-evoked depressions in caudate. Thus, in spite of its structural similarity to papaverine, THP does not act like a phosphodiesterase inhibitor or an anti-adrenergic agent in these test systems.

HIPPOCAMPUS

The hippocampal formation also possesses special advantages for electrophysiological study of ethanol effects. Thus, it contains many identifiable neurons (the pyramidal neurons) arranged in a laminar formation like the cerebellum, whose synaptic inputs may be specifically activated. The transmitters have been well-defined in several of the pathways [10,52], and the identifiable pyramidal cells are spontaneously active in many cases [23, 30, 40, 53]. In addition, the hippocampal formation can be readily isolated, sliced in coronal sections and kept alive in a perfusion chamber for many hours. Many of the intrinsic synaptic connections between the cells remain intact and can be activated; drugs can be perfused over the slices in known concentrations. Since long-term intracellular recording can be performed in the slices without the confounding effects of animal or tissue movement, the slices are ideal for the study of drug effects on synaptic and membrane properties.

Furthermore, studies in other laboratories have shown that ethanol does have an effect, usually inhibitory, on the electrophysiology of the hippocampus. Changes have been reported in the EEG and in multi-unit activity [30, 38, 40], in spontaneous activity of single units [30], and in evoked population fields in hippocampal slices *in vitro* [12, 13, 17, 18].

Single Units, Fields and Afferent Stimulation In Vivo

The effects of ethanol on synaptic transmission in rat hippocampus were studied [53] by changes in the response of CA3 pyramidal cells to stimulation of two afferent pathways, the dentate-mossy fiber pathway, and the commissural pathway. Both sources produce an excitatory response, as measured either by single unit spiking or by population spike (field potentials), followed by a period of post-stimulus inhibition (PSI). After the injection of 3 g/kg ethanol (IP), no consistent change in firing rate was seen, although both the excitatory responses to afferent stimulation and the duration of PSI were significantly increased. Because these changes occurred in both afferent pathways, they were not pathway-specific, but may be the result of the local microcircuitry in area CA3. Although the change in excitatory and inhibitory responses could occur simultaneously, detailed statistical analyses showed that neither the magnitude nor onset time were correlated. Thus, the two responses were functionally separable. In addition, the increase in the duration of PSI was related to the rate of rise of blood ethanol level and showed short-term tolerance. The maximum change in the PSI occurred after blood ethanol levels plateaued, suggesting a secondary process is necessary before the neurophysiological effects are apparent.

Thus, the results of these experiments seem to reinforce the notion that ethanol can have specific actions on mammalian synaptic transmission at doses that exert little effect on membrane excitability.

Iontophoresis and Micro-Pressure Studies: Comparison to Opiate Actions

Iontophoresis of opioid peptides predominantly depresses the discharge of neurons of most brain areas, whereas hippocampal pyramidal cells are usually excited [54,79]. These actions are usually naloxone reversible. This spectrum of actions was used [23] as an initial comparative model to test two hypotheses: (1) that ethanol effects are mediated by an opiate-like mechanism, and; (2) that formation of acetaldehyde-catecholamine condensation products (TIQs) might contribute to some actions of ethanol.

All drugs were applied to single neurons of rat brain by electro-osmosis or micro-pressure, as described above for Purkinje cells. Ethanol (1–3 M in the pipette) weakly inhibited most neurons of the caudate nucleus and parietal cortex, while 55% of presumed pyramidal neurons in the hippocampus were excited (n=31 cells). THP (3 mM in the pipette) inhibited the spontaneous and glutamate or ACh-induced firing of most neurons in all these regions.

In contrast, salsolinol and 7-Methyl salsolinol (3 mM in the pipette) excited 73 and 56%, respectively, of pyramidal neurons, while depressing the activity of a majority of parietal cortical neurons [23]. Iontophoretic or systemic naloxone antagonized the excitatory actions of ethanol (75% of tested cells) and salsolinol (89% of tested cells) on hippocampal pyramidal cells, with little change in the basal firing rate. The doses of naloxone used also blocked excitatory responses of pyramidal cells to methionine-enkephalin; however, ACh-induced speeding was also antagonized in 1/3 of the tested cells. Conversely, the antimuscarinic agent scopolamine antagonized the excitatory action of salsolinol, but not those of met-enkephalin, in some pyramidal cells.

These results show that acutely applied ethanol or salsolinol elicits a spectrum of neuronal effects in brain similar to that for the opioid peptides; namely inhibition of activity in several tested brain areas (including cerebellum), but excitation in hippocampus. A fair percentage of these excitatory effects are also antagonized by naloxone. However, the occasional non-specific effects of naloxone and the puzzling antagonism of some salsolinol-induced excitations by scopolamine casts some doubt as to the opiate-like actions of ethanol and salsolinol. Also, the intracellular studies described below indicate that the ethanol mechanisms of action are more complex than are those for activation of opiate receptors.

Opiate Mechanisms

The atypical excitation by opiates and opioid peptides of hippocampal pyramidal cells *in vivo* can be antagonized not only by naloxone, but also by iontophoresis of the GABA antagonist bicuculline or of magnesium ions [79]. Double recording experiments show that the firing of presumed inhibitory interneurons is depressed by these opioid agonists at the same time that presumed pyramidal cells are excited. These results are taken to suggest that the opioids excite pyramidal cells indirectly by inhibiting neighboring inhibitory, probably GABAergic, interneurons [79].

This disinhibition hypothesis is supported by results of intracellular recording experiments performed in the hippocampal slice preparation *in vitro* (Siggins and Zieglgänsberger, in preparation). In this model, perfusion of morphine, met-enkephalin or beta-endorphin in concentrations of 1–20 μM has no effect on resting membrane potential or input resistance; however, IPSPs (and to a lesser extent,

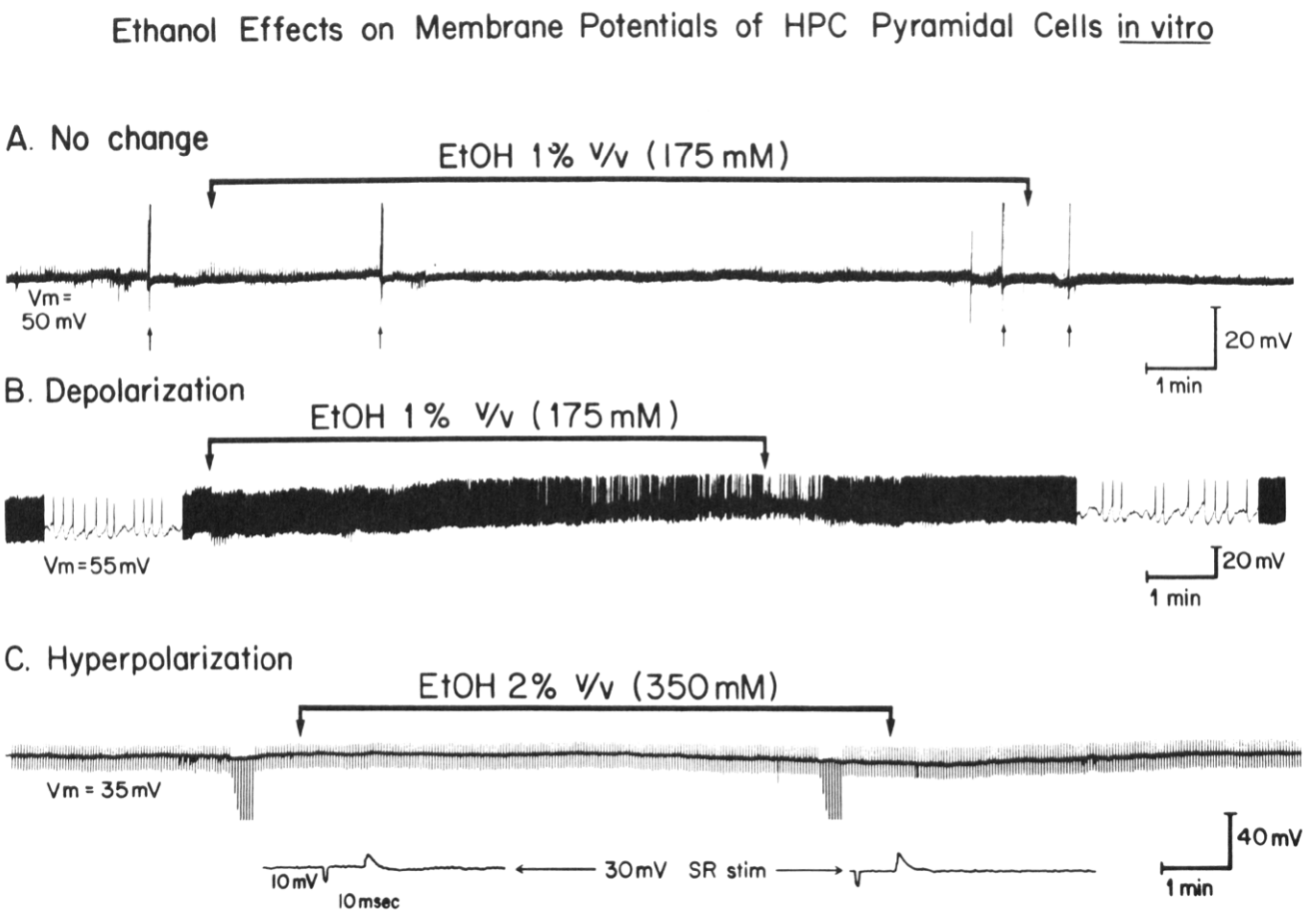

FIG. 1. Polygraph records showing the range of effects on membrane potentials of hippocampal pyramidal cells *in vitro*, produced by perfusion of high concentrations of ethanol. A. Many neurons exhibit no change in potential even at high ethanol concentrations. This quiescent neuron was occasionally activated via stimulation of the stratum radiatum-Schaeffer collateral system (arrows). B. Another neuron depolarized by 175 mM ethanol. Breaks in the continuity of the record occur when the polygraph was speeded up by 60× the calibrated speed to more clearly show the membrane potential and action potentials of the spontaneously firing cell (actual spike amplitude greatly diminished by the slow rise-time of the polygraph). Note that despite the depolarization, a reduction in firing occurred. C. Another quiescent neuron showing a weak late hyperpolarization (note, however, that the amplification of the trace is about half that of panel A). The hyperpolarization may be preceded by a slight depolarization. Light vertical lines throughout the record are a negative calibration pulse and an EPSP generated by SR stimulation, delivered every 3 sec (see samples taken at higher chart speed below the record; here the calibration pulse is 10 msec and 10 mV). Note that the EPSP gets larger during the hyperpolarization and persists throughout the recovery of membrane potential. The larger downward deflections before and during ethanol represent the current pulses applied through the recording electrode for determination of membrane resistance. Vm is resting membrane potential (Pittman and Siggins, unpublished).

EPSPs) are measurably reduced. Such a relatively selective reduction in the IPSPs would serve to remove the normal feedback inhibitory mechanisms (and perhaps the newly-described feedforward inhibition as well: R. Nicoll, personal communication) in this structure in favor of the feedforward excitatory circuits, thus perhaps explaining the epileptogenic action of beta-endorphin in the rat hippocampus [31].

Ethanol Mechanisms in the Hippocampal Slice

As suggested above, dose-response relationships of ethanol effects have not been forthcoming from *in vivo* research, and field potential studies in HPC slices [12, 13, 17, 18] have not revealed significant direct ethanol actions at doses much below those that would be fatal *in vivo*. To clarify this descrepancy, we [57] used intracellular recording to determine if bath-applied ethanol might selectively affect individual neurons in the HPC slice, at 'physiological' concentrations.

Hippocampi were quickly removed from rats (100 g body weight) and 400 μm transverse slices were cut and placed in a perfusion chamber where their lower surfaces were bathed in warmed (35°C) oxygenated artificial CSF solution and their upper surfaces exposed to warmed, humidified O_2/CO_2. After one hour of stabilization, the level of the medium was raised above the slices and oxygenated media was continuously perfused over the whole slice. The inflow system permitted introduction of drug-containing solutions without interrupting the flow of the perfusate.

Slices demonstrating high amplitude (3–10 mV), stable field potentials in response to stimulation of the stratum radiatum were selected for further investigation. Stable intracellular recordings of 1–4 hrs duration were obtained from 20 CA1 neurons; resting potentials ranged from 35 to 65

mV. In 10 of the 20 neurons, ethanol (10–400 mM) caused slight but repeatable hyperpolarizations (1–5 mV), whereas 4 other cells were weakly depolarized. The remaining 6 neurons did not respond directly to the ethanol, even at extremely high concentrations (Fig. 1). Although changes in input resistance (measured by current injection through the recording electrode) often accompanied these potential changes, no obvious relationship was seen between these two properties. Also, no clear relationship emerged between the concentration of ethanol and changes in membrane potential or resistance, although a few cells were noted in which an early excitatory, depolarizing action gave way to a later hyperpolarization.

Many pyramidal cells showed reproducible responses to low ethanol concentrations (10–40 mM). Nonetheless, many cells did not respond to ethanol at concentrations up to 200 mM. Many of the cells which did not respond directly to low doses of ethanol exhibited changes in their responses to synaptic activation. The amplitude of recurrent IPSPs evoked by stimulation of the stratum radiatum (SR) was decreased in 50% of testable cells and increased in 20%. SR-induced EPSPs increased in amplitude in 40% of tested cells and decreased in 25%. Of the cells showing an increase in EPSP amplitude, 40% were associated with an increased input resistance (thus accounting for the increase in EPSP size) and 30% were paradoxically associated with a decrease in membrane resistance.

Obviously, elucidation of the mechanisms underlying these changes in membrane and synaptic properties produced by ethanol will require further study. However, these findings do indicate that the individual hippocampal neurons display marked differences in their sensitivity to ethanol, possibly accounting for the apparent insensitivity to ethanol shown by field potential studies of the hippocampal slice. Moreover, the fact that 50% of the testable pyramidal cells responded to ethanol in the same fashion as to the opioid agonists, with a reduction in the size of the IPSPs evoked by afferent synaptic stimulation [57], may indicate some involvement of endogenous opioids or opiate receptors in selected hippocampal neurons. Interestingly, this is roughly the same percentage of hippocampal pyramidal neurons which were excited *in vivo* by iontophoretically-applied ethanol (see above and [23]).

The excitation of such a percentage of pyramidal neurons (whether by reduction of IPSPs and disinhibition, membrane depolarization, or increase in EPSP amplitude) could have a profound effect on the EEG of the hippocampus *in vivo*, similar perhaps to the epileptogenic effects of beta-endorphin in this structure [31]. It comes as little surprise, therefore, that low blood levels of ethanol can produce EEG spiking in the hippocampus under certain circumstances; significantly, this spiking action can occasionally be antagonized by low doses of naloxone (Henriksen and Bloom, in preparation).

SUMMARY AND CONCLUSIONS

The nature of research into the biological basis of alcohol and alcoholism demands by its very complexity that no single existing approach can yet be adopted exclusively. However, definite advantages can be obtained by comparing data from several different approaches. Thus, as exemplified by the recent results of studies from our laboratory and others, several of the major questions outlined in the introduction can be tentatively addressed.

The results of experiments on the cerebellum indicate that ethanol at an appropriate IP dose can have primary excitatory effects on a specific population of cell types, in this case the cells giving rise to the climbing fiber afferents to Purkinje cells. Such an effect remote to the Purkinje cell can be detected by the unusual bursting nature of the climbing fiber response, but in other brain areas a similar 'dissection' is more difficult. In hippocampus, stimulation experiments indicate that pyramidal cell responses to activation of specific pathways can be enhanced by parenteral ethanol. *In vitro* experiments (intracellular) also show that synaptic responses in a proportion of the pyramidal cells are altered by direct application of ethanol, although antagonism of synaptic potentials is more often the case. In contrast, iontophoretically or micro-pressure applied ethanol or salsolinol can alter the discharge of most hippocampal neurons. However, with these methods, the mechanism of action is not determined and the amount of drug reaching neuronal membranes is not known.

Iontophoretic and micro-pressure applied ethanol, and in some regions, salsolinol, appear to evoke a spectrum of effects on different brain regions similar to that seen with morphine and the opioid peptides. The possibility that release or generation of an opiate-like substance is responsible for the acute excitatory action in the hippocampus is further supported by the finding that naloxone can antagonize some of the excitatory actions of ethanol and salsolinol. Moreover, in some cells of the hippocampal slice the mechanism of ethanol action is strikingly similar to that for the opioids. However, the occasional non-selective effects of naloxone and the blockades of salsolinol effects by scopolamine suggest a cautious interpretation of these results at present.

The question of whether ethanol has specific effects on transmitter-related processes or only 'non-specifically' alters neuronal membranes can also be addressed by extracellular studies of pathway-activated responses in the *in vivo* hippocampus, and by intracellular studies of the type used in the hippocampal slice preparation. Such studies performed to data indicate that in some cells ethanol can indeed affect specific synaptic actions at doses that have little consistent direct effects on firing rate or membrane properties. Moreover, in the slice preparation, direct membrane actions of ethanol are often seen which cannot be ascribed to a 'local anesthetic' action on active or voltage-dependent membrane conductances. Generally, these 'non-specific' actions are seen only in concentrations that would be fatal *in vivo*. The fact that not all pyramidal neurons respond in the same way to ethanol *in vitro* also argues for a certain specificity of action.

Finally, the chronic effects of ethanol have been studied in the *in vivo* cerebellar preparation. From this research it appears that, with respect to the direct electrophysiological response, the single units can become tolerant to the excitatory effects of systemic ethanol after chronic ethanol administration; furthermore, this apparent compensatory mechanism is even more in evidence upon withdrawal from ethanol, when dramatic depression of firing is seen.

We believe these beginning investigations point up the utility of electrophysiological studies on mammalian brain neurons, using more recently-developed technologies, in the quest for understanding of alcohol effects in humans.

In the face of enormous uncertainty about the nature of the phenomena involved, there must also be equal uncertainty in selecting between various methodological approaches to the problem. Surely at this stage of the research endeavor, every effort must be made to encourage innova-

tive approaches and to support the most promising leads long enough to determine their value.

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