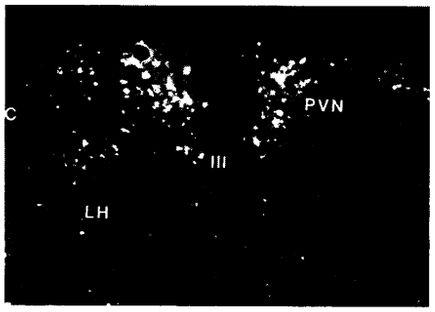

Fig. 3. In situ hybridization histochemistry (dark-field photomicrograph) of pre-proTRH mRNA in a 20-μm coronal section through the hypothalamus of rat brain fixed with 4 percent paraformaldehyde. A Pst I–Eco RI fragment (1241 bp) of pLW 4-2 was inserted into the expression vector pSp65 in reverse orientation and used to generate a [^{32}P]guanosine 5′-triphosphate–labeled, antisense, single-stranded RNA probe. The tissue was hybridized for 16 hours at 43°C in a buffer containing 2× SSC, 250 m*M* tris (*p*H 7.5), 0.5 percent sodium pyrophosphate, 0.5 percent SDS, 10 percent dextran sulfate, 0.25 percent PVP 360, 50 percent formamide, 0.25 percent bovine serum albumin, 0.25 percent Ficoll 400, denatured salmon sperm DNA (250 μg/ml), and the radiolabeled (5 × 10^5 count/min per 10 μl) antisense RNA probe (*21*). Silver grains are seen in the autoradiogram after a 6-day exposure over neurons in the paraventricular nucleus (PVN) and in the lateral hypothalamus (LH). Original magnification ×40.

arise from the amino terminal portion of the molecule after cleavage of an Arg-Arg sequnce 22 amino acids upstream from the first TRH sequence (Fig. 2, A and B). Some of these non-TRH peptides may be secreted. This possibility is supported by the observation that an epitope that we have immunologically identified in dense core vesicles in axon terminals in the median eminence (*14*) also appears to be present in the fusion protein produced by the proTRH immunopositive bacteriophage (*15*). We previously speculated that this epitope may represent part of the TRH precursor molecule (*14*). In addition, the presence of immunoreactive proTRH (*16*) and TRH mRNA (*17*) in regions of the central nervous system in which the tripeptide TRH has not been identified (*12, 14*) suggests that processing of the TRH prohormone to peptides other than TRH may occur in certain brain regions.

These findings establish that the mode of TRH biosynthesis in the mammalian brain is by posttranslational cleavage of a larger precursor protein. Like the enkephalin precursor (*18*), processing of proTRH could yield several copies of the biologically active peptide but may also generate other peptides of potential importance. Only the repeated TRH coding units dispersed throughout the precursor, however, are maintained between the amphibian (*6*) and mammalian prohormones. Conservation of this pattern throughout evolution suggests that the ability of a precursor to generate multiple bioactive peptides may be an important mechanism in the amplification of hormone production.

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Systemic Ethanol: Selective Enhancement of Responses to Acetylcholine and Somatostatin in Hippocampus

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In rat hippocampal pyramidal cells tested in situ by iontophoresis of several neurotransmitters, ethanol significantly enhanced excitatory responses to acetylcholine and inhibitory responses to somatostatin-14 but had no statistically significant effect on excitatory responses to glutamate or inhibitory responses to γ-aminobutyric acid or, in preliminary tests, to norepinephrine or serotonin. The effects of ethanol on responses to acetylcholine and somatostatin-14 may provide insight into synaptic mechanisms underlying the behavioral consequences of ethanol intoxication.

ETHANOL HAS PRONOUNCED EFfects on human behavior and a wide variety of effects on neuronal activity (*1, 2*). However, in spite of considerable research, the basic neuronal mechanisms underlying ethanol intoxication, tolerance, and dependence remain to be elucidated. Previously, we (*3*) evaluated the effects of ethanol on synaptic transmission in the rat hippocampus, because this aspect of neuronal activity is highly sensitive to the action of psychoactive drugs (*4*), including ethanol (*2, 5*). Indeed, systemic application of ethanol at doses associated with behavioral intoxication increased the excitatory and in-

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hibitory responses of pyramidal cells to stimulation of afferent pathways (*3*). To identify the transmitters involved in this action of ethanol, we have now tested its effects on the responses of pyramidal cells to iontophoretically applied transmitters. We report that systemic doses of ethanol selectively potentiate inhibitory responses to somatostatin-14 (SS-14) and excitatory responses to acetylcholine (ACh), but do not affect responses to other putative transmitters in the hippocampus.

Male Sprague-Dawley rats (n = 47, 250 to 350 g) were anesthetized with halothane (2 percent during surgery and 0.75 to 1 percent during the experimental procedures) through a tracheal cannula and mounted in a stereotaxic apparatus. After craniotomy, recording pipettes were inserted into areas CA1 and CA3 of the dorsal hippocampus (*6, 7*). After each experiment the recording site was verified by histological examination (*7*). Five-barreled micropipette assemblies, with overall tip diameters of 4 to 10 μm, were used for recording from cells and for microiontophoretic drug delivery (*7*).

Systemic ethanol markedly enhanced the excitatory responses to ACh (Fig. 1) in ten rats, decreased them in two, and had no statistically significant effect in one (*8*). Such ethanol-induced increases in responses to ACh were observed in all six CA1 cells. The effects of ethanol were less consistent in CA3 cells: responses to ACh increased in four cells, decreased in two, and did not change in one. Since ethanol per se frequently reduces spontaneous firing of hippocampal cells (*3, 9*), we evaluated responses to ACh both in absolute number of spikes, number of spikes per second, and as a percentage of the baseline rate of firing. Enchancement of ACh excitation was the predominant effect, regardless of the method used to evaluate the magnitude of the excitation (*8*).

Significant ACh-facilitatory effects were evident by 15 minutes after injection of ethanol (Fig. 1B) and reached a peak at about 30 minutes. Recovery from the actions of ethanol usually occurred about 60 minutes after injection, although in some cases recovery was not complete until after 2 hours (Fig. 1D) (*10*). Enhancement of ACh excitation by ethanol appears to be selective, since no comparable effect was observed on glutamate-induced excitation in three cells (20 trials) tested alternately with both transmitters (Fig. 1). This effect of ethanol appears not to have been due to the specific experimental conditions, complications of iontophoresis, or anesthesia, since in the hippocampal slice preparation low concentrations of ethanol also potentiated responses to superfused ACh (*11*).

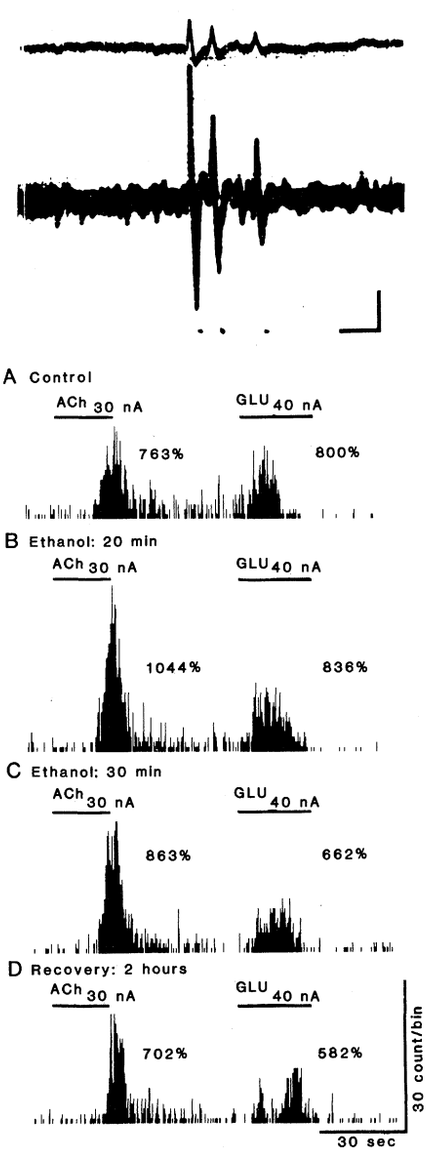

Fig. 1. Selective enhancement by ethanol of excitatory responses to ACh. The two oscillographic traces at the top show the "raw" (d.c.) and filtered (a.c.) spontaneous spikes recorded from a single CA1 hippocampal pyramidal cell. These cells fire bursts of several spikes with decreasing amplitudes after the first spike. [Calibration bars: 100 μV (vertical) and 5 msec (horizontal).] (A to D) Peri-drug interval histograms, each representing two sweeps of two drug pulses each. (A) Control responses to brief iontophoretic pulses of ACh and glutamate (GLU). The subscripts indicate the current used to eject the drug; the bar, the period of application. ACh caused a 763 percent increase in firing rate over baseline, and glutamate, an increase of 800 percent. (B) Twenty minutes after injection of ethanol (1.5 g/kg), ACh caused a 1044 percent increase in firing (a response 37 percent larger than in the control), whereas responses to glutamate were not significantly different. (C) Thirty minutes after ethanol injection, responses to ACh averaged 13 percent larger than control (863 percent increase above baseline), while those of glutamate were 17 percent smaller than control (662 percent above baseline). (D) Recovery from the effects of ethanol was complete after 2 hours. ACh responses were 8 percent smaller than control (702 percent above baseline), whereas those of glutamate were 27 percent smaller (582 percent). The effect of ethanol on ACh excitations was statistically significant (matched pairs sign test, $P < 0.05$).

Systemic ethanol also significantly increased the amplitude and duration of postsynaptic inhibitory responses to SS-14 (24 tests in five CA3 cells and one CA1 cell) (Fig. 2). Significant increases in the magnitude and duration of SS-14–induced inhibitions were evident in all cells (*12*) 10 to 15 minutes after ethanol injection (Fig. 2B), with recovery (Fig. 2C) occurring at 60 to 80 minutes. The total duration of the ethanol–SS-14 interaction was usually less variable than that for ethanol and ACh. In preliminary studies, ethanol had no statistically significant effect on inhibitory responses to serotonin or norepinephrine [21 tests in three cells (two CA3 and one CA1) for each transmitter]. Because the effects of γ-aminobutyric acid (GABA) are enhanced by very low doses of ethanol (*13*), we also examined the actions of this transmitter. Ethanol caused a small (on average, 25 percent) but consistent potentiation of inhibitory responses to GABA in ten rats (eight CA1 and two CA3 cells), a reduction in one rat (one CA3 cell), and no change in four rats (two CA1 and two CA3 cells). Most of these potentiations, however, had not been reversed within 3 hours of ethanol administration. Furthermore, a similar increased responsiveness to GABA was observed in five of six saline-injected control subjects in which individual hippocampal neurons were tested repeatedly for comparable periods. Experiments on seven other hippocampal cells with GABA applied from two different barrels (*14*) suggests that the apparent potentiation of GABA was an artifact of the repetitive iontophoretic application under these experimental conditions rather than a true pharmacological interaction. In contrast, changes in responses to ACh and SS-14 were observed only after ethanol injections and were reversed within 1 to 2 hours.

Evidence from a wide variety of vertebrate and invertebrate preparations indicates that ethanol can alter neuronal function by specific modulation of synaptic transmission (*1, 2, 5*). At vertebrate neuromuscular junctions, where ACh is known to be the transmitter, alcohol facilitates excitatory synaptic transmission (*15*). In frog sympathetic ganglia, ethanol has a dual effect, with low doses facilitating and high doses depressing ACh-mediated synaptic transmission (*16*). In the Mauthner cell of the goldfish, low doses of ethanol depress ACh-mediated collateral transmission (*17*). This is, to our knowl-

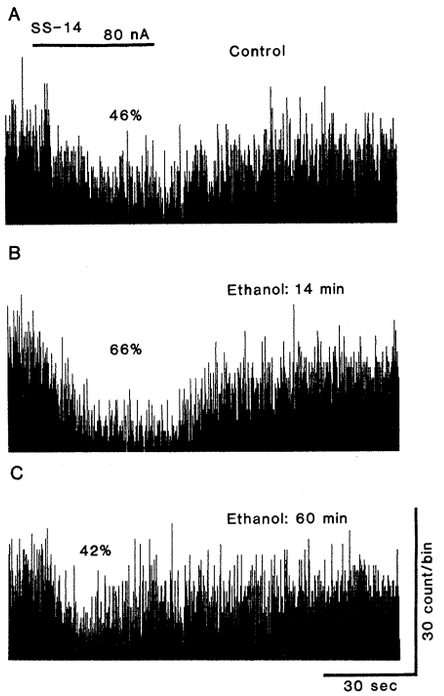

Fig. 2. (A to C) Ethanol-induced increases in the amplitude and duration of inhibitory responses to SS-14. Peri-drug interval histograms display the average responses to repeated applications [two, four, and seven sweeps for (A), (B), and (C), respectively] of SS-14 before and after ethanol. (A) SS-14 reduced the firing rate of a CA3 pyramidal cell by 46 percent. The period of significant inhibition (± 1 standard deviation) lasted 53 seconds. (B) Fourteen minutes after ethanol (1.5 g/kg), SS-14 reduced the firing rate 66 percent, a response 43 percent larger than that of the control. The period of significant inhibition increased to 85 seconds. (C) Recovery was complete 60 minutes after ethanol, with the magnitude of inhibition back to 42 percent and lasting 38 seconds. The enhancement of SS-14 was statistically significant (matched pairs sign test, $P < 0.016$).

edge, the first report of an effect of systemic ethanol on responses to somatostatin. However, we were not able to confirm the previously reported enhancement of presumed GABA-mediated transmission in rat cortical neurons (*13*). That enhancement may reflect the colocalization (and presumed corelease) of SS and GABA detected immunocytochemically in interneurons (*18*). Inhibitory effects of such coreleased SS, rather than GABA, might then have been potentiated, as we observed in the hippocampus.

We prefer not to generalize from observations of specific actions of ethanol on a given transmitter, brain region, or neuronal type, since ethanol has diverse effects on more than one aspect of neuronal activity (*1–5, 9, 15–17*) and can alter responses to more than one transmitter (*1–3*). Furthermore, the postsynaptic actions of a transmitter can be altered by several factors, including interactions with other transmitters and the state of the postsynaptic membrane (*19*). Any of the factors that can change postsynaptic responses to neurotransmitters under study could be the actual primary target of ethanol. In fact, during this study we observed that SS-14 potentiated responses to ACh in the hippocampus (*14*). It is possible, then, that ethanol-induced enhancement of responses to ACh may be secondary to an enhancement of the effects of endogenously released somatostatin (*20*), which in turn "enables" or enhances postsynaptic responses to iontophoretically applied ACh.

Ethanol-induced alteration of neuronal responses to ACh and SS-14 may help to explain the enhancement by ethanol of inhibitory and excitatory synaptic transmission in the hippocampus (*3*). Furthermore, the findings associating intoxicating doses of ethanol with alteration of a well-defined component of neuronal activity may be valuable for understanding cellular and molecular mechanisms of alcohol intoxication, particularly those involving limbic or hippocampal functions.

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8. Changes in ACh responses were evaluated with the matched-pairs sign test and found to be statistically significant ($P < 0.05$). When the magnitude of ACh responses was evaluated as the percentage of the increase in firing rate over baseline, the responses of ten cells were 380, 182, 110, 106, 104, 97, 75, 32, 28, and 2 percent higher (mean, 112 percent) at the peak of ethanol. Ethanol decreased the responses of two cells 87 and 56 percent and had no effect on one. When responses were assessed as the number of spikes generated during the period of significant excitation, as defined in (*7*), ACh responses were significantly enhanced in eight cells (88, 63, 61, 57, 29, 25, 15, and 12 percent more spikes after ethanol; mean, 44 percent), reduced in three (94, 57, and 47 percent fewer spikes; mean, 66 percent), and there was no significant effect in two. Variations in the duration of ACh responses were not statistically significant.
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