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21. Water samples were collected on 28 September 1978 with a Niskin bottle; the temperature and salinity were measured, and 84 ml of water was drained through a Tygon tube into a 125-ml serum bottle containing 1.0 ml of 160 m*M* $HgCl_2$. No differences were seen between $HgCl_2$-treated samples and untreated samples. The bottles were then capped with serum-bottle stoppers. The samples were brought to room temperature in the laboratory and then equilibrated by shaking by hand for 2 minutes. Duplicate 1.5-ml samples of the water-equilibrated gas phase were analyzed for N_2O as described by Seitzinger *et al.* (*12*). The water temperature was measured within 5 minutes after sampling. We calculated the total N_2O in the bottles and the N_2O concentrations of the water samples, using the N_2O solubility equation of R. Weiss and B. A. Price for the appropriate temperature and salinity [*Mar. Chem.* **8**, 347 (1980)]. We calculated the original percent saturation of the samples by assuming a dry-air mixing ratio for N_2O of 301 parts per billion (ppb). We based this on data (*5*) showing that the tropospheric N_2O concentration is increasing at a rate of ~ 0.2 percent per year and that the dry-air mole fraction as of 1 January 1978 in the Northern Hemisphere was 300.2 ppb. An N_2O mixing ratio of 301.25 ppb was calculated for 1 October 1979.
22. The Field's Point sample was taken directly above the outfall pipe, which was approximately 2 m below the water surface. The salinity of that sample was 18 per mil and the N_2O concentration was 295 n*M*. It is known that some seawater enters the Field's Point STP; although the salinity of the effluent was not known precisely, we estimated it to be 4 per mil on the basis of information supplied by the plant engineer. The effluent was assumed to mix with river water of 24 per mil and 25 n*M* N_2O, as measured in a surface-water sample taken about 200 m from the outfall pipe. Using this information, we calculated that the effluent concentration was ~ 925 n*M*.
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24. The STP discharge to the Providence River area includes Field's Point (11×10^3 m^3 $hour^{-1}$), Bucklin Point (4×10^3 m^3 $hour^{-1}$), and East Providence (1.2×10^3 m^3 $hour^{-1}$). The only STP discharge of any consequence to Narragansett Bay is at Newport with an input of 1.7×10^3 m^3 $hour^{-1}$ (Rhode Island Department of Environmental Management 1979 files). The calculated Field's Point N_2O concentration (925 n*M*) (*22*) was used as an estimate to calculate N_2O input from all treatment plants.
25. Area of Providence River is 2.4×10^7 m^2 (U.S. Department of Commerce, National Ocean Survey Chart 13224); the N_2O production for September, assuming that the Providence River station is typical of all of river is ~ 700 nmole m^{-2} $hour^{-1}$ (see Fig. 2); area of Narragansett Bay is 24.37×10^7 m^2 [J. N. Kremer and S. W. Nixon, *A Coastal Marine Ecosystem* (Springer, Berlin, 1978)]; the N_2O production for September is ~ 80 nmole m^{-2} $hour^{-1}$, assuming that the mid-Bay station is representative of the rest of the bay area.
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Ethanol Modulation of Opiate Receptors in Cultured Neural Cells

Abstract. *The mouse neuroblastoma–rat glioma hybrid cell line NG108-15 was used to study the acute and chronic interaction of ethanol with intact neural cells. In the short term, ethanol inhibited opiate receptor binding, but after long-term exposure the cells exhibited an apparent adaptive increase in the number of opiate binding sites; this was reversible when ethanol was withdrawn. High concentrations of ethanol (200 m*M*) increased opiate binding after 18 to 24 hours, whereas lower concentrations (25 to 50 m*M*) produced similar changes after 2 weeks. This model system has potential for exploring the cellular and molecular mechanisms underlying ethanol intoxication, tolerance, and withdrawal.*

Ethanol intoxication produces well-known behavioral and neurological effects. Tolerance develops to many actions of ethanol, and physical dependence follows chronic ethanol abuse. These phenomena may derive from the physical interaction of ethanol with neural membranes and the adaptation of these membranes to the continued presence of ethanol (*1–3*). Short-term ethanol exposure can disorder membrane lipids (*2*). After long-term ethanol administration, the membranes may exhibit several possibly adaptive alterations: (i) resistance to further disordering by ethanol; (ii) occasionally increased membrane order; and (iii) modifications in cholesterol, phospholipid, or fatty acid composition (*3*). Since these changes in membrane properties can alter the function of critical membrane proteins (*4*), many investigators have begun to characterize changes in neuroreceptors and related enzymes resulting from ethanol exposure (*5–9*). Biochemical studies in animals have been limited because of the heterogeneity of brain and the need to use disruptive tissue preparations. We have begun to use cells in culture as a rich source of intact and homogeneous neural tissue (*10*). We now report that in the mouse neuroblastoma–rat glioma hybrid cell line NG108-15, short- and long-term exposure to ethanol causes striking modifications in opiate binding in ways that may reflect the membrane events associated with intoxication, tolerance, and withdrawal.

Table 1. Response of opiate receptors to 4 days of ethanol treatment. The experiment was repeated four or five times, and means and standard errors (S.E.M.) were calculated. Long-term ethanol treatment did not significantly affect K_d ($F = 0.32$). The increase in B_{max} was tested by paired *t*-tests.

Ethanol concentration (m*M*)	B_{max} (Ethanol: control)	P	K_d (n*M*)
0			1.60 ± 0.09
100	1.40 ± 0.04	< 0.002	1.77 ± 0.09
200	1.85 ± 0.21	< 0.02	1.70 ± 0.21

The NG108-15 cells of passage 18 to 25 were cultured as previously described (*11*). When medium containing ethanol was added to cells, all flasks were kept tightly capped and the medium was changed daily. The concentration of ethanol in the medium, measured by gas-liquid chromatography, remained unchanged after 24 hours of incubation with confluent cells. Opiate receptor binding assays (*12*) were performed by incubating at 37°C a suspension of washed whole cells with tritiated methionine enkephalinamide ([^{3}H]MEA) (39.5 to 50.5 Ci/mm) (*11*). Specific binding, the difference between counts per minute in the presence or absence of 10^{-5} *M* naloxone, was normalized according to cell number (femtomoles per 10^6 cells). The standard deviation of triplicate samples ranged from 5 to 10 percent. The maximum number of binding sites (B_{max}) and receptor affinity (K_d) were calculated by linear regression analysis according to the method of Scatchard.

When ethanol was added to suspended whole cells immediately before the addi-

tion of [^{3}H]MEA, the specific binding of 2 n*M* MEA was inhibited (Fig. 1A). Isosmotic sucrose can reproduce some ethanol effects in cultured cells (*6*); sucrose (50 to 500 m*M*) had no effect on MEA binding.

Whereas short-term ethanol treatment reduced opiate binding, long-term exposure produced the opposite effect. After cells were cultured 4 days in 200 m*M* ethanol, the binding of 2 n*M* [^{3}H]MEA to washed cells increased 75 ± 4 percent [mean ± standard error (S.E.M.)] above pair-matched control values [$t(14) = 19.6$, $P < 0.001$]. This increase in opiate binding was due to an increased B_{max} of unchanged affinity (Table 1). Although opiate binding in the absence of ethanol was higher in these cells, short-term reexposure to ethanol generally inhibited MEA binding to the same degree as in control cells (Fig. 1A).

The time course for the increase in opiate receptor binding was determined by incubating confluent cells for 6 to 24 hours in the presence or absence of 200 m*M* ethanol. An increase in opiate binding was first apparent after 18 hours of ethanol exposure; after 24 hours, 5 n*M* MEA binding was 47 percent higher than in control cells (Fig. 1B). Binding also increased when cells were exposed to lower concentrations of ethanol (Table 2), but the time required to produce this effect was much longer.

The increase in opiate binding during long-term ethanol exposure was not due to changes in cell volume or cell growth. Cell volume did not differ significantly between cells cultured 24 hours in the presence and absence of 200 m*M* ethanol (*13*). Flasks cultured 24 hours with 200 m*M* ethanol contained 10 to 30 percent fewer cells than control flasks, but 13 days' exposure to 25 and 50 m*M* ethanol also produced large increases in opiate binding without reducing cell number.

Ethanol may exert its primary effects within specific domains of the hydrophobic membrane core, either by disordering membrane lipids (*1, 3*) or by interacting more directly with membrane proteins (*14*). Alcohols of higher chain length are more lipid-soluble than ethanol and reproduce many of ethanol's pharmacologic effects with greater potency (*15*). If long-term ethanol treatment increases opiate receptor binding as a consequence of ethanol's ability to dissolve in membrane lipids, alcohols of higher chain length might be expected to produce more striking responses. Accordingly, cells were cultured 48 hours in 25 m*M* ethanol (chain length, 2), 25 m*M* *n*-propanol (length, 3), 25 m*M* *n*-butanol (length, 4), or 200 m*M* ethanol (Fig. 1C).

Table 2. Opiate receptor binding in response to 13 days of exposure to low concentrations of ethanol. Binding of 5 n*M* [^{3}H]MEA was determined in cells washed free of ethanol. Data represent mean (± S.E.M.) specific binding from a representative experiment with quadruplicate assay samples. The experiment was performed a total of three times with similar results.

Ethanol concentration (m*M*)	[^{3}H]MEA specifically bound (count per minute per 10^6 cells)	Percent of control
0	1692 ± 59	
25	2542 ± 28	150
50	2640 ± 29	156

Culturing cells 48 hours in 200 m*M* ethanol increased opiate binding as usual, but 25 m*M* ethanol had the expected negligible effect. In contrast, 25 m*M* *n*-propanol and *n*-butanol each caused large increases in opiate receptor binding in proportion to the chain length of the alcohols ($r = 0.99$). Thus the increase in opiate binding found after long-term alcohol exposure is probably related to the lipid solubility of the alcohols and not to an osmotic effect of alcohols on intact cells (*6*).

The increase in opiate binding produced by ethanol was reversible. Cells cultured in 200 m*M* ethanol for 3 to 4 days were placed in fresh medium without ethanol. Specific MEA binding remained elevated during the first 12 hours after ethanol withdrawal and returned to control levels after 24 hours (Fig. 1D).

The interaction of ethanol with different opiate receptor subtypes has been studied in animals (*7–9*). Short-term in vitro ethanol exposure reduced the binding affinity of rat brain and neuroblastoma membranes for the selective δ receptor agonist D-Ala^2-D-Leu^5-enkephalin (DADL) (*7*) and long-term ethanol administration to rats increased the binding affinity of striatal homogenates for DADL (*8*).

Some evidence suggests that opiate systems play a role in the behavioral effects of ethanol in humans and animals (*16*). It is not clear whether the opiate receptor changes are specific or due to general effects of ethanol on cell membranes. Since the δ opiate receptor of NG108-15 also undergoes marked changes, it is now possible to investigate

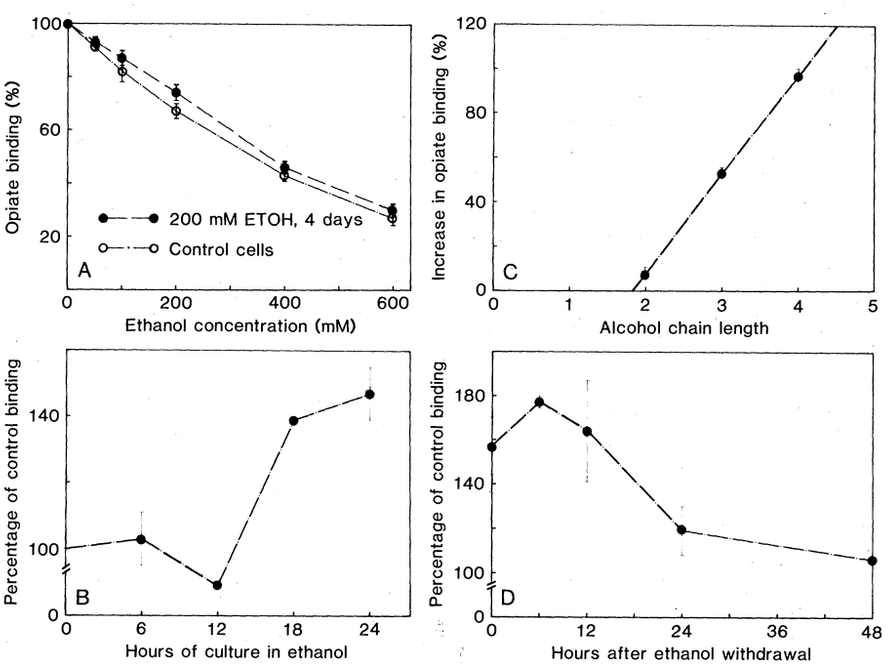

Fig. 1. (A) Opiate receptor binding in the presence or absence of ethanol. For each experiment specific binding in the absence of ethanol was normalized to 100 percent. Each point represents the mean [± standard error (S.E.M.), $N = 9$]. (B) Time course for the increase in opiate binding. The medium of control cells was changed at identical times, and specific binding in control cells was normalized to 100 percent at each time point. Data at 6 and 24 hours represent mean (± S.E.M.) percentage of control binding from three independent determinations. (C) Opiate receptor binding after 48 hours' exposure to 25 m*M* alcohols of different chain length. Each data point represents the mean (± S.E.M.) increase in opiate binding from four or five experiments compared with control flasks assayed at the same time. The line was fitted to the data by linear regression analysis, $r = 0.99$. (D) Time course for reversal of increased opiate receptor binding after ethanol withdrawal. Data points obtained from two or three experiments are mean (± S.E.M.) percentage increase in specific binding with respect to control cells harvested at the same time.

the molecular mechanisms underlying these adaptive responses at a cellular level. Long-term ethanol treatment may increase opiate binding in NG108-15 by increasing receptor synthesis, decreasing receptor degradation or processing, or increasing the availability of latent or hidden membrane receptors to bind opiate ligands (*17*).

The changes in receptor binding we describe may parallel the clinical phenomena of intoxication, tolerance, and withdrawal. For example, decreased binding produced by ethanol in the short-term could lead to depression of some receptor-mediated activity. During continued ethanol exposure the cell membrane may undergo adaptive changes, causing increased receptor density and restored cellular function. After abrupt ethanol withdrawal, the increased number of receptors would no longer be inhibited by ethanol, resulting in heightened receptor-mediated activity. With further passage of time, the number of receptors would return to control values, again restoring cellular homeostasis.

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The Human Gene for the β Subunit of Nerve Growth Factor Is Located on the Proximal Short Arm of Chromosome 1

Abstract. *Fragments of the recently cloned human gene for the β subunit of nerve growth factor (β-NGF) were used as hybridization probes in analyzing two sets of rodent-human somatic cell hybrids for the presence of human β-NGF sequences. Results from the first set of hybrids assigned the human β-NGF gene to chromosome 1 and ruled out the presence of sequences of comparable homology on any other chromosome. With the second set of hybrids, which contained seven different, but overlapping, regions of chromosome 1, the NGF locus was mapped to band 1p22.*

Nerve growth factor (NGF) is important in the development and function of peripheral adrenergic and sensory neurons and of adrenal chromaffin cells (*1*). As a polypeptide that binds to a specific receptor, NGF may serve as a model system for the action of hormones and growth factors. Furthermore, NGF appears to play a role in several human genetic disorders of the nervous system (*2*). In the mouse, the 118-amino-acid β chain, which is responsible for stimulating nerve growth, is synthesized as part of a larger precursor molecule (*3*). Oligonucleotide primers based on the known amino acid sequence of the gene (*4*) were synthesized and used to clone the mouse β-NGF gene (*5, 6*). Mouse β-NGF complementary DNA was then used to isolate the human β-NGF gene. Sequencing studies revealed considerable homology between human and mouse amino acid sequences (*6*). The β-NGF gene has not yet been assigned to a specific chromosome in any mammalian species.

The mapping of the human β-NGF locus is of particular interest for three reasons. First, β-NGF represents the active subunit of the NGF complex, which consists of three different polypeptide chains (α, β, and γ). The expression of these presumably independent genes appears to be synchronized during development and adulthood. Identification of the chromosomal locations of these genes will help to determine whether synchronized gene expression of the NGF subunit genes is due to regulation of one genetic locus containing a cluster of NGF subunit genes, or whether characteristics common to three physically separated genes control their synchronous expression.

Second, low levels of amino acid homology with insulin have suggested that β-NGF may be a member of the insulin gene family (*1*). The insulin locus is now well established on the distal short arm of human chromosome 11 (*7*), but other insulin-related genes have not yet been mapped. Knowledge about the chromosomal location of each member of a gene family can contribute to our understanding of the patterns and mechanisms of genome evolution (*8*). In this context, the insulin gene family represents an interesting case, in that its members (IGF-1, IGF-2, relaxin, insulin, and possibly β-NGF) have acquired different regulatory functions while retaining various amounts of structural homology.

Third, at least ten cellular proto-oncogenes (that is, sequences that are related to retroviral transforming genes or that have been identified in the activated state by transfection assays with human tumor cell DNA) have been assigned to specific human chromosomes or chromosome regions (*9*). A search for relations between proto-oncogenes and