0026-895X/89/050744-05$02.00/0

MOLECULAR PHARMACOLOGY, 36:744–748

Adenosine Is Required for Ethanol-Induced Heterologous Desensitization

LAURA E. NAGY, IVAN DIAMOND, KATHLEEN COLLIER, LELEN LOPEZ, BUDDY ULLMAN, and ADRIENNE S. GORDON

Ernest Gallo Clinic and Research Center (L.E.N., I.D., K.C., L.L., A.S.G.) and Departments of Neurology (L.E.N., I.D., A.S.G.), Pharmacology (I.D., A.S.G.), and Pediatrics (I.D.), University of California, San Francisco, California, 94110 and Department of Biochemistry, University of Oregon, Portland, Oregon 97201 (B.U.)

Received May 9, 1989; Accepted July 26, 1989

SUMMARY

Recent evidence suggests that ethanol initially causes an increase in receptor-dependent cAMP levels, followed by heterologous desensitization of receptors coupled to G_s after chronic exposure. Here we investigated the role of adenosine in mediating these responses. We found that ethanol caused accumulation of extracellular adenosine in NG108-15 and S49 lymphoma cells. This adenosine activated adenosine receptors to increase intracellular cAMP levels. The addition of adenosine deaminase, to degrade accumulated extracellular adenosine, or isobutylmethylxanthine, an adenosine receptor antagonist, completely blocked ethanol-induced increases in cAMP levels in NG108-15 cells. Chronic exposure of NG108-15 and S49 wild type cells to ethanol resulted in heterologous desensitization of adenosine receptor- and prostaglandin E_1 receptor-dependent cAMP signal transduction. Coincubation of NG108-15 and S49 wild type cells with adenosine deaminase and ethanol for 48 hr prevented heterologous desensitization. Moreover, mutant S49 cells, which are unable to transport adenosine, did not accumulate extracellular adenosine after incubation with ethanol and did not develop ethanol-induced heterologous desensitization. Our results suggest that adenosine is an important mediator of both the acute and chronic effects of ethanol on cAMP signal transduction.

Increasing evidence suggests that ethanol-induced changes in cAMP signal transduction may play a critical role in the acute and chronic effects of ethanol (1–11). Although ethanol acutely increases hormone- and neurotransmitter-stimulated cAMP levels in many cellular preparations (1–11), a decrease in receptor-dependent cAMP levels develops after chronic exposure (5–11). This reduction appears to be significant in chronic alcoholism, because lymphocytes from alcoholics exhibit a 4-fold decrease in adenosine receptor-stimulated cAMP levels (11, 12). Membranes prepared from platelets of alcoholics also show decreased PGE_1 receptor-dependent cAMP levels (13).

Ethanol-induced decreases in cAMP levels appear to be due to heterologous desensitization of receptors coupled to the stimulatory guanine nucleotide regulatory protein G_s (5–10). We have recently shown that this desensitization is correlated with a decrease in messenger RNA for the α-subunit of G_s and a consequent decrease in α-subunit protein (6). Charness *et al.* (7) subsequently confirmed that the quantity of α_s protein is decreased in NG108-15 cells and reported changes in α_s and α_i in other cell lines. However, the mechanisms involved in this decrease in receptor-dependent cAMP levels after chronic exposure to ethanol are not known. Here we report that adenosine is required for ethanol-induced heterologous desensitization.

This work was supported by grants from the National Institute on Alcohol Abuse and Alcoholism, the Alcoholic Beverage Medical Research Foundation, and the National Institute of Diabetes, Digestive and Kidney Disease.

Experimental Procedures

Materials. ZK-62711 was a gift from Berlex Labs (Cedar Knolls, NJ). Chloroacetaldehyde was purchased from Fluka. Cell culture medium was purchased from Grand Island Biological Co. High pressure liquid chromatography-grade solvents were purchased from Burdick and Jackson. All other reagent grade chemicals and reagents were purchased from Sigma and Boehringer Mannheim.

Cell culture. NG108-15 neuroblastoma × glioma hybrid cells (passage number 18–25) were grown in defined media as described (5), in the absence or presence of 100 mM ethanol. S49 lymphoma wild type cells and S49 adenosine transport mutants (80-2A6 and 160-D4) were grown in defined media (11) (without phytohemagluttinin) with 1 unit/ml ADA, in the absence of ethanol, for at least 2 weeks. Cells were subcultured into fresh medium every 48 hr. Addition of ADA, a normal component of serum-containing medium, was necessary to maintain adenosine receptor response in S49 cells. S49 cells were then seeded at 3×10^5 cells/ml and cultured in media with or without 1.5 units/ml ADA for 48 hr at 37°, in humidified 5% CO_2, in the presence or absence of 100 mM ethanol.

cAMP levels. The effects of ethanol on cAMP levels in the absence

ABBREVIATIONS: PGE_1, prostaglandin E_1, ADA, adenosine deaminase, IBMX, isobutylmethylxanthine, PBS, phosphate-buffered saline, PIA, (−)-N^6-(*R*-phenylisopropyl)-adenosine.

of exogenously added agonist were determined in NG108-15 cells grown to a final density of 12–20 × 10^6 cells/175-cm^2 flask. Cell culture medium was removed and replaced with 9 ml of assay medium [defined cell culture medium without growth factors (5)] with or without 100 mM ethanol. Cells were incubated at 37° for 10 min with or without IBMX (10^{-5} M, a concentration of the adenosine receptor antagonist that does not inhibit phosphodiesterase activity) or ADA (1 unit/ml), and acetylated cAMP levels were determined by radioimmunoassay (5).

Receptor-stimulated cAMP levels were also measured after cells were grown 48 hr with or without ethanol in the presence or absence of ADA. ADA was active throughout the incubation period (data not shown). In this assay, cells were washed and incubated for 30 min, in the absence of ethanol, with 100 μM PIA or 1 μM PGE_1 in the presence of 10 μM ZK-62711, a phosphodiesterase inhibitor, and 1 unit/ml ADA. Adenosine receptor- and PGE_1 receptor-stimulated cAMP production were determined as previously described (5).

Extracellular adenosine. NG108-15 cells were subcultured in 4-well plates at a density of 3 × 10^5 cells/well and were grown for 5 days. Medium was removed and cells were washed once with 2 ml of autoclaved PBS containing 110 mM glucose and 25 mM HEPES, pH 7.4, and incubated with 2 ml of PBS, in the presence or absence of 200 mM ethanol, for 10 min. S49 wild type and 80-2A6 adenosine transport mutant cells were washed once and resuspended at 10^7 cells/ml in PBS. After a 5-min preincubation, ethanol was added to a final concentration of 200 mM. Cells were removed from the extracellular media by centrifugation through inert oil, after a total incubation time of 10 min (14). Fluorescent derivatives of the extracellular media were prepared with chloroacetaldehyde, for determination of extracellular adenosine concentration (15). Samples were injected onto a reverse phase high pressure liquid chromatography column that was equilibrated with 1.2 mM KH_2PO_4, pH 5, and were eluted with a 0–60% methanol gradient. Fluorescence of the eluted sample was monitored at an excitation wavelength of 280 nm. Peak areas were compared with those of known amounts of 1,N^6-ethenoadenosine. Identity of the adenosine was confirmed by disappearance of the adenosine peak upon pretreatment of the sample with ADA. Accumulation of adenosine during culture for 24 hr in the absence or presence of 100 mM ethanol was measured as described by Green (15).

Results and Discussion

Chronic exposure to ethanol results in heterologous desensitization of receptor-dependent cAMP levels (5–11). Because heterologous desensitization in other systems is characterized by an initial increase in cAMP levels (16), we first determined whether acute exposure to ethanol caused an increase in cAMP levels even in the absence of exogenously added agonist. When NG108-15 cells were incubated with 100 mM ethanol for 10 min, there was a 60% increase in intracellular cAMP levels (Fig. 1). Because ethanol does not activate adenylyl cyclase directly but stimulates only receptor-dependent cAMP production (17), we next explored the possibility that acute ethanol increases the extracellular concentration of a stimulatory agonist. Neural cells (15), lymphocytes (18, 19), and other cell types (20) release adenosine, and adenosine can cause both homologous and heterologous desensitization (21, 22). Moreover, adenosine has been implicated in the central nervous system effects of ethanol (23, 24). Therefore, adenosine concentrations in the media of control and ethanol-treated cells were measured using high pressure liquid chromatography. There was a significant increase in the concentration of extracellular adenosine after NG108-15 cells were incubated with 200 mM ethanol (Fig. 2). Within 10 min, adenosine concentrations reached 37 ± 1.2 nM/5 × 10^6 cells in ethanol-treated cells, while

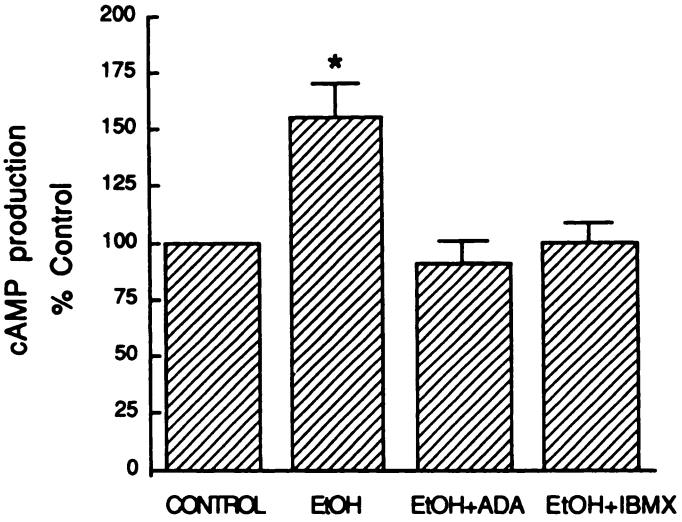

Fig. 1. Acute effect of ethanol (*EtOH*) on endogenous cAMP levels in NG108-15 neuroblastoma × glioma cells in the absence of added agonist. Cells were incubated with or without 100 mM ethanol in the presence or absence of 1 unit/ml ADA or 10 μM IBMX and cAMP levels were determined. cAMP levels in the absence of ethanol were 18.4 ± 2.4 pmol/10^6 cells (16 experiments). Values are expressed as the percentage of cAMP levels in cells not exposed to ethanol. Bars represent means ± standard errors (four to nine experiments). *Significant difference from cells not treated with ethanol ($p < 0.002$, Student's *t* test).

control cultures had 18.2 ± 3.7 nM adenosine (four experiments, $p < 0.005$).

Adenosine modulates the production of cAMP via A_1 and A_2 adenosine receptors (25). NG108-15 cells have only the A_2 adenosine receptor,[1] which is positively coupled to adenylyl cyclase (25). Therefore, the increase in intracellular cAMP levels produced by acute ethanol (Fig. 1) could be due to an ethanol-induced increase in extracellular adenosine concentration. If this were the case, then degradation of extracellular adenosine should prevent stimulation of cAMP production by ethanol. We used ADA to deaminate adenosine to inosine, a nucleoside with low affinity for the adenosine receptor (25). When NG108-15 cells were incubated with ADA, stimulation of cAMP production by ethanol was completely abolished (Fig. 1). Moreover, treatment of the cells with an adenosine receptor antagonist, IBMX, also completely blocked ethanol-induced increases in cAMP levels (Fig. 1). These data suggest that acute exposure to ethanol caused an increase in extracellular adenosine. This extracellular adenosine then activated the A_2 receptor to stimulate cAMP production.

In contrast to acute stimulation of cAMP levels by ethanol, chronic exposure to ethanol causes a decrease or desensitization of adenosine receptor- and PGE_1 receptor-dependent cAMP production (Fig. 3) (5, 6). If adenosine were responsible for ethanol-induced heterologous desensitization, ADA should prevent this response. Fig. 3 shows that, when NG108-15 cells are coincubated for 48 hr with ethanol and 1 unit/ml ADA, a concentration sufficient to block the acute increase in cAMP (Fig. 1), chronic ethanol-induced desensitization of adenosine receptor-stimulated cAMP levels is substantially blocked and desensitization of the PGE_1 receptor is completely prevented. These results suggest that an ethanol-induced increase in extracellular adenosine concentration is required for ethanol to

[1] Unpublished observations.

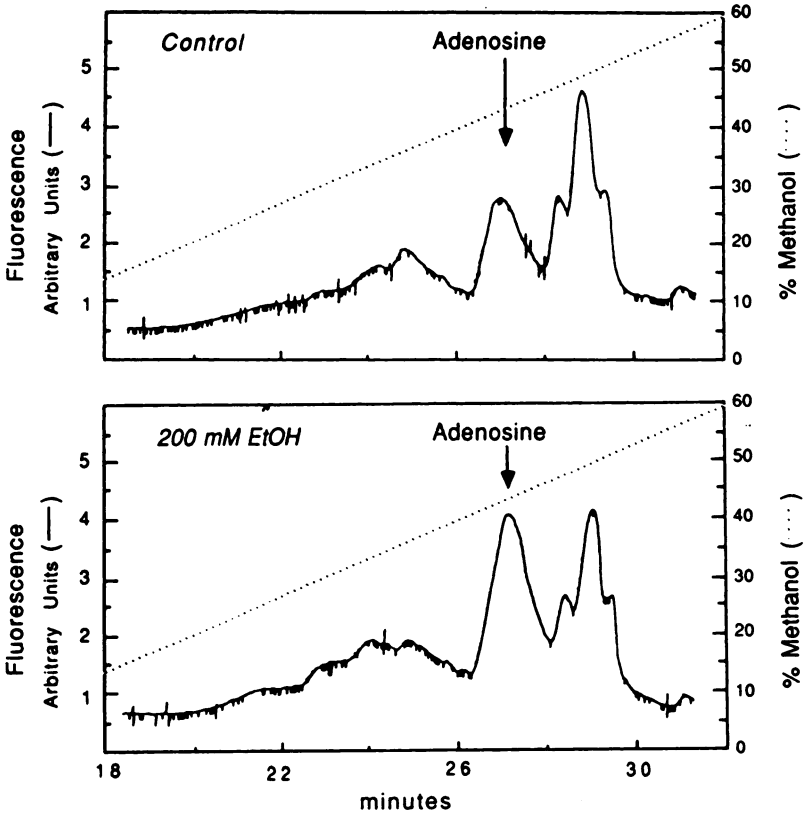

Fig. 2. Acute effect of ethanol (*EtOH*) on extracellular adenosine concentration in NG108-15 cells. Cells were incubated with or without 200 mM ethanol for 10 min. The extracellular adenosine concentration was determined by high pressure liquid chromatography as described in Experimental Procedures. Representative chromatograms of control and ethanol-treated cells are shown.

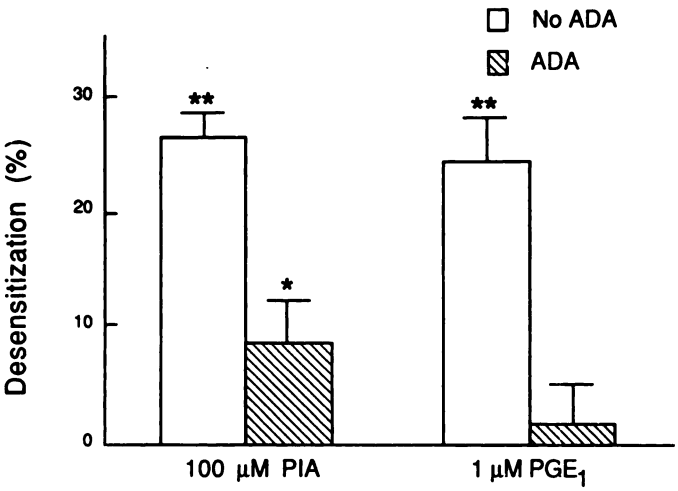

Fig. 3. Chronic effects of ethanol on desensitization of receptor-stimulated cAMP production in NG108-15 cells. Cells were maintained for 48 hr with or without 100 mM ethanol in the presence or absence of 1 unit/ml ADA. Cells were washed and incubated with 100 μM PIA or 1 μM PGE_1 for 30 min in the absence of ethanol (5). cAMP levels were measured by radioimmunoassay (5). Desensitization is expressed as the percentage decrease in ethanol-treated cells, compared with cells never exposed to ethanol; cells treated with ethanol and ADA are compared with cells treated with ADA alone. *Asterisks* indicate a significant difference from cells not treated with ethanol (*$p < 0.025$, **$p < 0.001$, Student's *t* test).

produce heterologous desensitization. In other preparations, desensitization by adenosine is dependent on the concentration of agonist and time of exposure (21). Consistent with our results, Green (15) has found, in C1300 neuroblastoma cells, that 10–20 nM endogenously released adenosine is sufficient to desensitize receptor-dependent cAMP production during an overnight culture. Moreover, we find that, after exposure to 70 nM PIA for 48 hr, adenosine receptor-dependent cAMP levels were decreased by 26% (data not shown), a reduction similar to that induced by 100 mM ethanol for 48 hr.

The requirement for extracellular adenosine in ethanol-induced heterologous desensitization was not specific for neural cells. S49 wild type lymphoma cells showed a significant increase in the concentration of extracellular adenosine when exposed to ethanol for 5 min or 24 hr (Table 1). When S49 wild type cells were treated with 100 mM ethanol for 48 hr, adenosine receptor- and PGE_1 receptor-stimulated cAMP levels were reduced to 65 ± 7 and 36 ± 6% of control, respectively (Table 1). As in NG108-15 cells, addition of ADA to S49 wild type cells prevented ethanol-induced heterologous desensitization (Table 1).

If accumulation of extracellular adenosine is required for ethanol-induced heterologous desensitization, then cells that do not release adenosine should not desensitize after chronic exposure to ethanol. Adenosine uptake and release are mediated via a single bidirectional transporter, which can be blocked by agents such as dipyridamole (26–28). However, we have found that concentrations of dipyridamole necessary to inhibit transport are toxic to S49 cells over a 48-hr period.[1] Therefore, the nucleoside transport-deficient mutants 80-2A6 and 160-D4 of the S49 lymphoma cell line (28, 29) have been utilized to determine whether adenosine transport is required for ethanol-induced heterologous desensitization. When the 80-2A6 mutant cell line was treated with 200 mM ethanol for 5 min or 100 mM ethanol for 24 hr, extracellular adenosine was not detectable (Table 1). There was also no desensitization of adenosine

TABLE 1

Adenosine transport is required for ethanol-induced heterologous desensitization in S49 cells

Ethanol-induced accumulation of extracellular adenosine and heterologous desensitization in S49 wild type and adenosine-transport mutants (80-2A6 and 160-D4). Extracellular adenosine concentrations were determined by high pressure liquid chromatography, as described in Experimental Procedures, after exposure of 1.2×10^7 cells in a total volume of 20 ml to no ethanol or 100 mM ethanol for 24 hr and 10^7 cells in a total volume of 1 ml to no ethanol or 200 mM ethanol for 5 min. Receptor-dependent cAMP levels were determined as described in the legend to Fig. 3 and Experimental Procedures. S49 wild type cells were grown in the absence (wild type) or presence of 1.5 units/ml ADA (wild type + chronic ADA). Basal levels of cAMP were 2.41 ± 0.28 pmol/10^6 cells for all cell types and did not change with chronic exposure to ethanol. PIA-stimulated cAMP levels were 3.54 ± 0.70, 3.58 ± 0.41, and 3.43 ± 1.11 pmol of cAMP/10^6 cells and PGE_1-stimulated cAMP levels were 25.9 ± 7.2, 62.0 ± 25.0, and 34.0 ± 10.2 pmol of cAMP/10^6 cells for S49 wild type, 80-2A6, and 160-D4, respectively. cAMP levels of ethanol-treated cells are expressed as a percentage of cAMP in cells never exposed to ethanol. Values represent means ± standard errors when $n > 2$, mean ± range when $n = 2$. Number of determinations (n) is indicated in parentheses.

Cell type	Extracellular adenosine		cAMP levels (100 mM ethanol, 48 hr)	
	No ethanol	Ethanol	100 μM PIA	1 μM PGE
	nM		% of control	
Wild type	4.4 ± 0.9 (5)[a] 21.9 ± 4.1 (2)[c]	17.6 ± 3.6 (5)[a,b] 56.7 ± 17.6 (2)[b,c]	65 ± 7[b] (27)	36 ± 6[b] (6)
Wild type + chronic ADA	ND	ND	118 ± 31 (4)	96 ± 17 (5)
80-2A6	0 (2)[a]	0 (2)[a]	115 ± 11 (6)	82 ± 7 (4)
160-D4	ND	ND	94 ± 9 (4)	98 ± 7 (4)

[a] Cells incubated for 5 min with or without 200 mM ethanol.
[b] Significant difference from cells not exposed to ethanol ($p < 0.001$).
[c] Cells incubated for 24 hr with or without 100 mM ethanol.

receptor- or PGE_1 receptor-stimulated cAMP levels when the adenosine transport-deficient cells were exposed to 100 mM ethanol for 48 hr (Table 1). Thus, the adenosine transporter is required for ethanol-induced heterologous desensitization of receptor-dependent cAMP production in S49 cells.

The lack of desensitization after chronic exposure to ethanol in the adenosine transport-deficient cells was not due to altered stimulation of cAMP by agonist. Incubation of S49 wild type cells with maximally effective concentrations of PIA or PGE_1 increased cAMP levels 1.92 ± 0.41-fold (18 experiments) and 13.5 ± 3.5-fold (six experiments) over basal, respectively. Similar results were obtained in the adenosine transport-deficient cells (see legend to Table 1), indicating that the coupling of the adenosine receptor and PGE_1 receptor to adenylyl cyclase is normal in the mutant cells. Although the adenosine transporter is a nonspecific nucleoside carrier (28), ethanol-induced desensitization does not appear to be due to other nucleosides, because they have very low affinities for the adenosine receptor and do not stimulate cAMP production (25). Moreover, exposure of S49 wild type cells to inosine for 48 hr did not desensitize the adenosine receptor (data not shown). Taken together, our results suggest that accumulation of extracellular adenosine is required for ethanol-induced heterologous desensitization.

In summary, we have shown that acute exposure to ethanol increases the concentration of extracellular adenosine, which then activates adenosine A_2 receptors resulting in an increase in intracellular cAMP levels. Accumulation of extracellular adenosine is also required for the subsequent ethanol-induced heterologous desensitization of receptor-stimulated cAMP production. By analogy to other systems that undergo heterologous desensitization (16), it seems likely that the acute ethanol-induced increase in cAMP caused by the accumulation of extracellular adenosine is responsible for the adaptive decrease in receptor-dependent cAMP levels after chronic exposure to ethanol. Goldstein and Goldstein (30) proposed that dependency develops as a cell or organism makes homeostatic adjustments to compensate for the primary effect of a drug. Our results in NG108-15 cells (5), S49 cells, and lymphocytes from alcoholics (11, 12) are consistent with this pathophysiologic mechanism and suggest that adenosine-dependent responses may be important in the development of alcoholism in humans. We can now test whether manipulations that either augment or inhibit adenosine-dependent responses exacerbate or prevent the development of ethanol dependence.

Acknowledgments

We would like to thank Dr. Daria Mochly-Rosen for helpful suggestions during the course of this study and critical reading of this manuscript.

References

1. Rabin, R. A., and P. B. Molinoff. Activation of adenylate cyclase by ethanol in mouse striatal tissue. *J. Pharmacol. Exp. Ther.* **216**:129–134 (1981).
2. Bode, D. C., and P. B. Molinoff. Effects of ethanol in vitro on the beta adrenergic receptor-coupled adenylate cyclase system. *J. Pharmacol. Exp. Ther.* **246**:1040–1047 (1983).
3. Stenstrom, S., and E. Richelson. Acute effect of ethanol on prostaglandin E_1-mediated cyclic AMP formation by a murine neuroblastoma clone. *J. Pharmacol. Exp. Ther.* **221**:334–341 (1982).
4. Luthin, G. R., and B. Tabakoff. Activation of adenylate cyclase by alcohols requires the nucleotide-binding protein. *J. Pharmacol. Exp. Ther.* **228**:579–587 (1984).
5. Gordon, A. S., K. Collier, and I. Diamond. Ethanol regulation of adenosine receptor-stimulated cAMP levels in a clonal neural cell line: An in vitro model of cellular tolerance to ethanol. *Proc. Natl. Acad. Sci. USA* **83**:2105–2108 (1986).
6. Mochly-Rosen, D., F.-H. Chang, L. Cheever, M. Kim, I. Diamond, and A. S. Gordon. Chronic ethanol causes heterologous desensitization of receptors by reducing alpha$_s$ messenger RNA. *Nature* (*Lond.*) **333**:848–850 (1988).
7. Charness, M. E., L. A. Querimit, and M. Henteleff. Ethanol differentially regulates G proteins in neural cells. *Biochem. Biophys. Res. Commun.* **155**:138–143 (1988).
8. Valverius, P., P. L. Hoffman, and B. Tabakoff. Effect of ethanol on mouse cerebral cortical β-adrenergic receptors. *Mol. Pharmacol.* **32**:217–222 (1987).
9. Saito, T., J. M. Lee, P. L. Hoffman, and B. Tabakoff. Effects of chronic ethanol treatment on the beta-adrenergic receptor-coupled adenylate cyclase system of mouse cerebral cortex. *J. Neurosci.* **48**:1817–1822 (1987).
10. Richelson, E., S. Stenstrom, C. Forray, L. Enloe, and M. Pfennig. Effects of chronic exposure to ethanol on the prostaglandin E_1 receptor-mediated response and binding in a murine neuroblastoma clone (NIE-115). *J. Pharmacol. Exp. Ther.* **239**:687–692 (1986).
11. Nagy, L. E., I. Diamond, and A. Gordon. Cultured lymphocytes from alcoholic subjects have altered cAMP signal transduction. *Proc. Natl. Acad. Sci. USA* **85**:6973–6976 (1988).
12. Diamond, I., B. Wrubel, W. Estrin, and A. Gordon. Basal and adenosine receptor-stimulated levels of cAMP are reduced in lymphocytes from alcoholic patients. *Proc. Natl. Acad. Sci. USA* **84**:1413–1416 (1987).
13. Tabakoff, B., P. L. Hoffman, J. M. Lee, T. Saito, B. Willard, and F. D. Leon-Jones. Differences in platelet enzyme activity between alcoholics and nonalcoholics. *N. Engl. J. Med.* **318**:134–139 (1988).
14. Aranow, B., K. Allen, J. Patrick, and B. Ullman. Altered nucleoside transporters in mammalian cells selected for resistance to the physiological effects of inhibitors of nucleoside transport. *J. Biol. Chem.* **260**:6226–6233 (1985).
15. Green, R. D. Release of adenosine by C1300 neuroblastoma cells in tissue culture. *J. Supramol. Struct.* **13**:175–182 (1980).
16. Sibley, D. R., and R. J. Lefkowitz. Molecular mechanisms of receptor desensitization using the beta-adrenergic receptor-coupled adenylate cyclase system as a model. *Nature* (*Lond.*) **317**:124–129 (1985).

17. Rabin, R. A., and P. B. Molinoff. Multiple sites of action of ethanol on adenylate cyclase. *J. Pharmacol. Exp. Ther.* **227**:551–556 (1983).
18. Newby, A. C., and C. A. Holmquist. Adenosine production inside rat polymorphonuclear leucocytes. *Biochem. J.* **200**:399–403 (1981).
19. Fredholm, B. B., G. Sandberg, and U. Ernstrom. Cyclic AMP in freshly prepared thymocyte suspensions. Evidence for stimulation by endogenous adenosine. *Biochem. Pharmacol.* **27**:2675–2682 (1978).
20. Stone, T. W., ed. *Purines: Pharmacology and Physiological Roles.* London, Macmillan Press, 1985.
21. Kenimer, J. G., and M. Nirenberg. Desensitization of adenylate cyclase to prostaglandin E_1 or 2-chloroadenosine. *Mol. Pharmacol.* **20**:585–591 (1981).
22. Newman, M. E., and A. Levitzki. Desensitization of normal rat kidney cells to adenosine. *Biochem. Pharmacol.* **32**:137–140 (1983).
23. Proctor, W. R., and T. V. Dunwiddie. Behavioral sensitivity to purinergic drugs parallels ethanol sensitivity in selectively bred mice. *Science* (*Wash. D.C.*) **224**:519–521 (1984).
24. Dar, M. S., S. J. Mustafa, and W. R. Wooles. Possible role of adenosine in the CNS effects of ethanol. *Life Sci.* **33**:1363–1374 (1983).
25. Daly, J. W. Adenosine receptors. *Adv. Cyclic Nucleotides Protein Phosphorylation Res.* **19**:29–46 (1985).
26. Taube, R. A., and R. D. Berlin. Membrane transport of nucleoside in rabbit polymorphonuclear leukocytes. *Biochim. Biophys. Acta* **255**:6–18 (1972).
27. Oliver, J. M., and A. R. P. Paterson. Nucleoside transport. I. A mediated process in human erythrocytes. *Can. J. Biochem.* **49**:262–270 (1971).
28. Cohen, A., B. Ullman, and D. W. Martin, Jr. Characterization of a mutant mouse lymphoma cell with deficient transport of purine and pyrimidine nucleosides. *J. Biol. Chem.* **254**:112–116 (1979).
29. Ullman, B., K. Kaur, and T. Watts. Genetic studies on the role of the nucleoside transport function in nucleoside efflux, the inosine cycle, and purine biosynthesis. *Mol. Cell. Biol.* **3**:1187–1196 (1983).
30. Goldstein, D. B., and A. Goldstein. Possible role of enzyme inhibition and repression in drug tolerance and addiction. *Biochem. Pharmacol.* **8**:48 (1961).

Send reprint requests to: Adrienne S. Gordon, Dept. of Neurology, The Gallo Center, Bldg. 1, Rm. 101, San Francisco General Hospital, San Francisco, CA 94110.