Differential Effects of Ethanol on the Striatal and Cortical Adenylate Cyclase System

Toshikazu SAITO, Gary R. LUTHIN*, John M. LEE**, Paula L. HOFFMAN*** and Boris TABAKOFF***

Department of Neuropsychiatry, Sapporo Medical College, Sapporo, Hokkaido 060, Japan
*Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.
**Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL 60680, U.S.A.
***National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20205, U.S.A.

Accepted October 24, 1986

Abstract—In the present study, effects of ethanol (EtOH) on C57/BL mouse cortical β-adrenergic receptor coupled adenylate cyclase (AC) were shown to be different from the effects of EtOH on striatal dopaminergic-stimulated AC activity. The addition of EtOH (500 mM) increased the AC activity by 60% in cortical membrane and by less than 10% in striatal membrane preparations in the absence of guanine nucleotide. The dose-response relationship for EtOH stimulation of cortical AC activity in the presence of guanylylimidodiphosphate (Gpp(NH)p) was biphasic, whereas, in the striatum, a linear dose-response relationship for EtOH was found for stimulation of AC in the presence of Gpp(NH)p. Activation of AC by Gpp(NH)p occurred as an apparent pseudo-first order process. EtOH increased the pseudo-first order rate constant for activation of AC by Gpp(NH)p in the cortex, but not in the striatum. Following 10 min preincubation with Gpp(NH)p, catecholamines and Gpp(NH)p were not able to stimulate further the AC activities in either tissue. Nevertheless, EtOH increased AC activity in both cortex and striatum following the preincubation with Gpp(NH)p. These data suggest that one effect of EtOH in striatal tissue is to promote the interaction of an activated guanine nucleotide-binding regulatory protein (G-protein) with the catalytic unit of AC. In cortical tissue, the effects of EtOH may be attributable to direct actions on the catalytic activity of the enzyme, effects on the rate of activation of the G-protein, and an altered interaction of G-protein with the catalytic unit.

Recent research on the mechanism of ethanol’s effects on the central nervous system (CNS) has generated the “membrane hypothesis” of ethanol’s action (1). Ethanol is one of a series of compounds (e.g., chloroform and halothane) which can cause general anesthesia, but does not possess specific neuronal receptors for its actions. A number of recent studies concerned with the mechanism of ethanol’s actions have focussed on the lipid-perturbing effects of ethanol. It is generally thought that in the presence of ethanol, cellular membrane lipid properties in the CNS are altered, and this affects the ability of neuronal cells to properly conduct or transmit information. Although the membrane lipids contribute to the structural matrix of cellular membranes, membrane proteins are also functional entities which mediate the conduction and transmission of information. It is conceivable that ethanol could exert its effects on CNS neurons by altering the lipid characteristics and thus cause secondary changes in protein function. It is also conceivable that ethanol could directly influence the function of membrane proteins.

Adenylate cyclase (AC) is a membrane-
bound protein that is believed to reside on the inner leaflet of the CNS neuronal membrane as it does in the plasma membrane of cells of peripheral tissues (2, 3). AC plays a major role in regulating intracellular levels of cyclic-AMP, thereby generating intracellular messages in response to extracellular stimuli. It has been reported that ethanol increases adenylate cyclase activity in membranes of a number of peripheral organs (4–6). Although certain studies (4, 5) have provided evidence for a lipid locus for ethanol’s effects on AC activity, these early studies of ethanol’s effects on hormone-stimulated AC in peripheral organs paid little attention to the various factors (i.e., guanine nucleotides and magnesium) now known to regulate AC activity.

Recent studies indicate that hormone-regulated AC activity is a product of interactions between subunits of a multicomponent system. At least three classes of interacting protein components, located within the cellular plasma membrane, contribute to the expression of AC activity (3, 7–9). The first class of components consists of hormone or neurotransmitter receptors which act as recognition sites for the agonists and antagonists modulating AC activity (3). Other components of adenylate cyclase that transduce the extracellular to the intracellular message are the family of guanine nucleotide-binding regulatory proteins (G-proteins) (7, 10, 11). These G-proteins are composed of at least two, and possibly three, subunits (9, 12–14). The interactions of the subunits of G-proteins allows for the activation or inhibition of AC. A third component of the system is the catalyst of AC. We have previously described a guanine nucleotide-dependent requirement for some effects of ethanol in the striatal dopamine-coupled AC system (15, 16), and multiple sites of action for ethanol in the cerebral cortical β-receptor coupled AC system (17).

We designed the current study to compare, in greater detail, the sites of action of ethanol on cortical and striatal AC systems.

**Materials and Methods**

Tris (hydroxymethyl) aminomethane (Tris), disodium-adenosine 5′-triphosphate (ATP), sodium-cyclic adenosine 3′,5′-monophosphate (cyclic-AMP), guanylylimidodiphosphate [Gpp(NH)p], phosphocreatine (di-Tris salt), creatine phosphokinase, ethylene-glycol-bis-(β-aminoethyl)ether)-N,N′-tetraacetic acid (EGTA), Dowex 50 W×4 (200–400 mesh, hydrogen form), (−)-isoproterenol hydrochloride, digitonin, and neutral alumina were obtained from Sigma Chemical Co. (St. Louis, MO). [α-32P]ATP (10–30 Ci/mmol) was obtained from New England Nuclear (Boston, MA) and was purified on Dowex 50 W×4 prior to use. [8-3H]cyclic AMP (10–20 Ci/mmol) was obtained from Research Products International (Mt. Prospect, IL) and was purified on Dowex 50 W×4 prior to use. Scintillation cocktail (3a70b) was also obtained from Research Products International. All other reagents were of the purest grade commercially available.

**Tissue preparation:** Male mice (C57B1/6) were sacrificed by decapitation. The brains were removed, and the cerebral cortex and corpus striatum were dissected on ice. Each brain area was separately homogenized in 20 volumes of 2 mM Tris-maleate buffer (pH 7.4) which contained 2 mM EGTA. The homogenate was centrifuged at 600 × g for 10 min at 4°C. The resulting supernatant was recentrifuged at 48,000×g for 20 min. The resulting pellet was washed with 2 mM Tris-maleate (pH 7.4) containing 2 mM EGTA, and the washed pellet was resuspended in the 2 mM Tris-maleate (pH 7.4) buffer and used for assays of adenylate cyclase activity (16). The protein concentration of the final suspensions ranged from 2.0 to 2.4 mg/ml.

**AC assay:** AC activity of cortical membranes was measured in a volume of 0.5 ml, as previously described (15–17). The reaction mixture contained 25 mM Tris-maleate (pH 7.4), 10 mM theophylline, 2 mM MgSO₄, 0.4 mM EGTA, 0.5 mM ATP, and approximately 4×10⁶ cpm/tube of [32P]-ATP. The effects of dopamine (DA) and isoproterenol (ISP), guanine nucleotides, magnesium and ethanol were measured by including these compounds, at concentrations described in the figures and tables, in the incubation mixture. The reaction mixture was preincubated at 30°C for 3 min;
the reactions were initiated by the addition of cortical membranes (50 µl, 110–130 µg protein). Incubations were continued at 30°C for 5 min. After the incubation, tubes were placed in a boiling water bath for 3 min and were then placed on ice with 0.5 ml of a solution containing 8 mM ATP and 0.28 mM cyclic-AMP (20,000 cpm [3H]cyclic-AMP) being added to each tube prior to chromatography on Dowex and alumina as described by Salomon (18). Ten milliliters of scintillation fluid was added to eluates from the chromatographic columns, and radioactivity was quantitated using a Beckman liquid scintillation counter. Reported values have been corrected for recovery based on the recovery of [3H]-cAMP.

For measurement of the time course of activation of cortical AC activity by Gpp(NH)p, reactions were carried out in a final volume (0.5 ml) containing 1 µM Gpp(NH)p, 10 mM creatine phosphate, and 0.1 mg/ml creatine phosphokinase in addition to the above assay components. The reactions were initiated by the addition of a prewarmed solution of ATP and Gpp(NH)p and were carried out at 30°C.

For measurement of the effects of magnesium and manganese, free magnesium and manganese concentrations in the assay were calculated according to Ohanian et al. (19).

Solubilization of AC activity: Pelleted cortical or striatal membranes, prepared as described above, were resuspended in 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM EGTA and 1% digitonin, such that the final suspension contained 2.2–2.6 mg of membrane protein per milliliter. The mixture was allowed to stand in ice for 25–30 min and then was centrifuged at 105,000 xg for 1 hr at 4°C. The clear supernatant constituted the solubilized preparation of AC. The final protein concentration in this preparation was 1.1–1.3 mg/ml.

Pretreatment of cortical membranes with Gpp(NH)p: Cortical membranes, prepared as described above, were incubated in 25 mM Tris-maleate buffer (pH 7.4) containing 0.4 mM EGTA, 10 mM theophylline, 0.5 mM ATP, 2 mM MgSO4, 1 µM ISP or 1 µM DA, and 10 µM Gpp(NH)p for 8 min at 30°C. No radiolabelled ATP was present during this preincubation. Following preincubation, the reaction mixture was placed on ice, centrifuged at 48,000 xg for 20 min, and then resuspended in 20 volumes of Tris-maleate buffer and used for assays of AC, as described above.

Protein: Protein determinations were based on the methods of Lowry et al. (20) and, for solubilized AC preparations, the method of Bradford (21). Bovine serum albumin was utilized as a standard.

Statistics: Statistical analysis of data was performed using a two-tailed Student's t-test: P values of <0.05 were taken to indicate a significant difference in the mean values being compared.

Results

Effects of ethanol and Gpp(NH)p on AC activity: Ethanol increased adenylate cyclase activity in the cortical membranes but not in the striatal membranes in the absence of added guanine nucleotides. In the cerebral cortex, 50 mM (230 mg%) ethanol increased AC activity from 16.9±0.70 pmol/min/mg protein (mean±S.E.M., n=3) to 18.7±0.4 pmol/min/mg protein (n=3), and 500 mM ethanol increased AC activity by 60% in the absence of added guanine nucleotides. In the presence of the guanine nucleotide, Gpp(NH)p, ethanol caused a further significant increase in AC activity in both cortical and striatal membranes. The dose-response curves for the effect of ethanol on cortical AC activity in the presence of Gpp(NH)p were consistently biphasic (Fig. 1). Thus the increase in activity produced by the addition of concentrations of ethanol between 0 and 50 mM deviated from the apparently linear response observed at higher EtOH concentrations. In cortical membranes, 50 mM ethanol increased AC activity by 88% and striatal AC activity by 100% under the
Fig. 1. Activation of AC by ethanol in the presence of 10 μM Gpp(NH)p. AC activity was measured as described in Materials and Methods in the presence of varying concentrations of EtOH and with the addition of 10 μM Gpp(NH)p. Data are representative of results obtained in six separate experiments. •, cortex, ▲, striatum.

Table 1. Effect of ethanol on EC50 and Vmax values for Gpp(NH)p

| Tissue  | Additions      | EC50 (μM)a | Vmax b (pmol cAMP formed/min/mg protein) |
|---------|----------------|------------|------------------------------------------|
| Cortex  | None            | 1.9 ±0.18  | 72.2±5.89 (6)                            |
|         | EtOH (500 mM)   | 1.2 ±0.16* | 151.8±19.5* (4)                          |
| Striatum| None            | 0.21±0.05  | 354.1±17.91 (3)                          |
|         | EtOH (500 mM)   | 0.19±0.01  | 712.9±29.87* (3)                         |

The assay constituents were membrane protein (about 130 μg), Tris-maleate (25 mM, pH 7.4), 10 mM theophylline, 2 mM MgSO4, 0.4 mM EGTA and 0.5 mM ATP. Gpp(NH)p concentrations in the assays were varied from 0.15 μM to 10 μM in the cortex and from 0.01 μM to 2.5 μM in the striatum.

aEC50 was expressed as the mean±S.E.M. calculated from Hane’s plots of the experimental data.
bVmax was expressed as the mean±S.E.M. pmole cAMP formed/min/mg protein calculated from Hane’s plots of the experimental data. Number in parentheses refers to the number of separate determinations.

Since guanine nucleotides appeared to enhance the stimulation of adenylate cyclase activity produced by ethanol in both cortical and striatal membranes, we further characterized the regulation of AC activity by guanine nucleotides both in the presence and absence of ethanol. AC activity was determined at various concentrations of Gpp(NH)p, and the apparent Vmax values obtained from replots of the data were 72.2±5.89 pmol/min/mg protein (n=6) in the cortex and 354.1±17.91 pmol/min/mg protein (n=3) in the striatum, respectively. The addition of ethanol (500 mM) increased the apparent Vmax values in both cortical and striatal membranes (Table 1). The concentration of Gpp(NH)p required for the half-maximal activation of adenylate cyclase (apparent EC50) under our experimental conditions in cortical membrane was 1.9±0.18 μM (n=6), and in striatal mem-
branes, it was $0.21 \pm 0.051 \mu M$ ($n=3$). In cortical membranes, ethanol decreased the apparent EC50 for Gpp(NH)p to $1.2 \pm 0.16 \mu M$ ($n=4$). In contrast, ethanol did not alter the apparent EC50 for Gpp(NH)p in striatal membranes (Table 1).

**Effects of ethanol on AC in the presence of activated G-protein:** After an eight-minute preincubation of membranes with Gpp(NH)p ($10 \mu M$) and catecholamines (DA or ISP) (CA), and after washing the membranes (see Materials and Methods), adenylate cyclase activity was unresponsive to further stimulation by Gpp(NH)p and CAs in both cerebral cortex and striatum. However, ethanol was still capable of increasing adenylate cyclase activity under these conditions in either tissue (Fig. 2). The dose-response curve for ethanol-induced stimulation of AC activity in the pretreated membranes was linear (Fig. 2).

After preincubation of striatal membranes with Gpp(NH)p plus DA, the increase in AC activity due to ethanol no longer required the addition of exogenous guanine nucleotides. The percent increase in AC activity produced by 500 mM ethanol was about 100% in both preincubated or untreated striatal membrane (Figs. 1 and 2). However in Gpp(NH)p plus ISP pretreated cortical membranes, the percent increase in AC activity produced by 500 mM ethanol was lower than that seen with untreated membranes incubated in the presence of Gpp(NH)p (53% vs. 88%, respectively).

**Changes in the lag time of activation of AC:** Activation of AC by Gpp(NH)p generally occurs after a time-lag which is characteristic of the rate-limiting steps involved in activation of AC (22). The pattern of enzyme activation by Gpp(NH)p can be described mathematically by the following equation:

$$\ln \frac{V_m - V_t}{V_m - V_i} = -kt$$

where $V_i$ is the catalytic activity determined in the absence of Gpp(NH)p, $V_m$ is the maximal catalytic activity obtained at infinite time, and $V_t$ is the activity attained at any given time ($t$); $k$ is an apparent pseudo-first-order rate constant.

Fig. 2. The effects of ethanol on AC activity in membranes preloaded with Gpp(NH)p. Membranes were preincubated for 8 min with Gpp(NH)p as described in Materials and Methods. AC activity was measured as described in Materials and Methods in the presence of varying concentrations of EtOH. AC activity (pmole/minute/mg protein), shown for a typical experiment, is the average of triplicate determinations at each point. Similar results were obtained in two separate experiments. ○, cortex, ▲, striatum.
The assay constituents were membrane protein (about 130 μg), Tris-maleate (25 mM, pH 7.4), 1 μM Gpp(NH)p, 10 mM theophylline, 0.4 mM EGTA, 2 mM MgCl2, 0.5 mM ATP, 10 mM creatine phosphate and 0.1 mg/ml creatine phosphokinase.

The pseudo-first order rate constant (K) is expressed as the mean±S.E.M. and calculated as described in the text.

Maximal AC activity is expressed as the mean±S.E.M. of pmole cAMP formed/min/mg protein. Number in parentheses refer to the number of separate determinations.

*Significantly different (P<0.05) when compared to no additions.

### Table 2. Kinetic constants for hysterectic activation of cortical and striatal adenylate cyclase activity

| Tissue | Additions | K(sec⁻¹)ᵃ | Maximalᵇ activity |
|--------|-----------|-----------|-------------------|
| Cortex | None      | 5.5±0.82 (3) | 133±0.8 (3) |
|        | EtOH (50 mM) | 7.0±0.20 (3)* | 141±4.6 (3) |
|        | EtOH (250 mM) | 8.7±0.75 (3)* | 152±4.8 (3)* |
| Striatum | None      | 5.9±0.51 (5) | 192±7.5 (5) |
|         | DA (10 μM) | 13.1±0.64 (3)* | 226±3.6 (3)* |
|         | EtOH (250 mM) | 6.3±1.00 (3) | 307±18.5 (3)* |

The assay constituents were membrane protein (about 130 μg). Tris-maleate (25 mM, pH 7.4), 1 μM Gpp(NH)p, 10 mM theophylline, 0.4 mM EGTA, 2 mM MgCl2, 0.5 mM ATP, 10 mM creatine phosphate and 0.1 mg/ml creatine phosphokinase.

ᵃThe pseudo-first order rate constant (K) is expressed as the mean±S.E.M. and calculated as described in the text.

ᵇMaximal AC activity is expressed as the mean±S.E.M. of pmole cAMP formed/min/mg protein. Number in parentheses refer to the number of separate determinations.

*Significantly different (P<0.05) when compared to no additions.

order rate constant characterizing the transition of Vt to Vm (22) under these experimental conditions. In the cortical membranes, when ethanol (50 mM) was added to the reaction mixtures, the apparent pseudo-first order rate constant for the ability of Gpp(NH)p to increase adenylate cyclase activity was increased from 5.5±0.82 sec⁻¹ (n=3) to 7.0±0.20 sec⁻¹ (n=3). Ethanol (250 nm) increased maximal AC activity in the presence of 1 μM Gpp(NH)p from 133±0.8 pmol/min/mg protein (n=3) to 152±4.8 pmol/min/mg protein (n=3) and also increased the apparent pseudo-first order rate constant for activation of AC to 8.7±0.75 sec⁻¹ (n=3) (Table 2). In striatal membranes, the addition of 10 μM dopamine significantly increased the maximal AC activity and increased the apparent pseudo-first order rate constant for activation of AC by Gpp(NH)p. However, in contrast to cortical membranes, ethanol did not increase the rate constant for activation of AC by Gpp(NH)p in striatal membranes (Table 2).

**Effect of ethanol on soluble AC activity:** AC activity measured in a preparation of the enzyme solubilized from cortical membrane using 1% digitonin was 75.6±2.21 pmol cyclic AMP produced/min/mg protein. Ethanol increased the soluble AC activity in a linear, dose-dependent manner. Ethanol (500 mM) resulted in a 44% increase in AC activity (Fig. 3). However, 10 μM Gpp(NH)p did not produce any measurable change in the activity of solubilized AC (Fig. 3). The percentage increase in solubilized AC activity produced by EtOH was similar (45%) in the presence of 2 mM MnCl₂ and 2 mM MgCl₂ (not shown). However, in contrast with the data obtained using AC activity solubilized from cortical membranes, AC activity solubilized from striatal membranes was not altered by the addition of 500 mM EtOH or 10 μM Gpp(NH)p.

**Discussion**

The results shown in the present study confirm earlier observations (15–17, 23, 24) of ethanol-induced increases in the activity of AC from mouse brain. When striatal (16) and cortical (17) AC activity was assayed in the absence of neurotransmitters or guanine nucleotides, ethanol produced significant effects on cortical but not striatal AC activity. Under comparable assay conditions, cortical AC activity, in the absence of guanine nucleotide, was increased approximately 60% by the addition of 500 mM ethanol, but striatal AC activity was increased less than 20% at this concentration of ethanol. To examine further the effects of ethanol on the catalytic moiety of cortical AC and to attempt to reduce or eliminate the actions of ethanol on or through the G-proteins, we solubilized...
Fig. 3. The effect of ethanol on digitonin solubilized AC activity. A. Cortical membranes were solubilized and AC activity was determined as described in Materials and Methods, in the presence of the indicated concentrations of ethanol. B. Cortical and striatal membranes were prepared and enzyme activity assayed as in 7A. Effect of ethanol and Gpp(NH)p on solubilized AC activity were determined. O, no additions; TT, 500 mM ethanol; M, 10 μM Gpp(NH)p. Results shown are the mean of three determinations. Similar results were obtained in two separate experiments. *Significantly different compared to no additions.

Ethanol's Effects on AC System in Brain

AC activity from membranes. The solubilized AC activity was no longer affected by the addition of Gpp(NH)p to assay mixtures. Ethanol produced a significant enhancement of the solubilized AC activity in the cortex, but not in the striatum (Fig. 3). It should be emphasized that under the condition chosen in this study, digitonin, in contrast to other detergents, has been shown to disrupt interactions between receptor and G-protein and G-protein-catalytic unit (25). The demonstration that Gpp(NH)p failed to alter solubilized AC activity support these observations.

In the presence of Gpp(NH)p, the dose-response relationship for the stimulation of cortical AC activity produced by ethanol was not linear. The rate of increase in activity produced by the addition of concentrations of ethanol between 0 and 50 mM was much sharper than the rate of increase in activity produced by concentrations in excess of 50 mM (Fig. 1). This was in contrast to striatum, where an apparently linear dose-response relationship for ethanol was found for stimulation of AC in the presence of Gpp(NH)p over a wide range of EtOH concentrations (Fig. 1).

When cortical membranes were incubated with Gpp(NH)p prior to measurement of AC activity, the apparent biphasic nature of ethanol's effects on AC activity was eliminated. Ethanol under these conditions produced similar effects to those seen in the striatum, that is, the dose-response to ethanol was linear (Fig. 2). This suggests that the binding of Gpp(NH)p by the G-protein may, under normal circumstances, contribute to the biphasic dose-response to ethanol seen when using cortical AC (Fig. 2). A comparison of the extent of ethanol-induced activation of AC in cortical membranes preincubated with Gpp(NH)p (about 60%) and ethanol's effect in assays containing solubilized, Gpp(NH)p-insensitive, AC activity (44%) may indicate the presence of another component of ethanol's action on AC activity. This may not be explainable by direct effects of ethanol on the catalytic unit or by an effect of ethanol on the G-protein activation process. This potential third component of ethanol's actions may reflect the apparent ability of ethanol to shift the equilibrium involving the interaction of the subunit of Gs and the catalytic unit of AC to favor the formation of the activated Gs-catalytic unit complex (16).

In the striatum, ethanol increased AC activity only when guanine nucleotides were included in the assay. This observation suggested that the action of ethanol may be relatively specific in mouse striatum and could potentially occur at the level of nucleo-
tide interaction with the G-protein or at the level of G-protein interaction with the catalytic unit of AC. In addition, ethanol stimulated AC activity in striatal membranes that had been preincubated with Gpp(NH)p. We suggest that this may reflect an altered interaction of the G-protein with the AC catalytic unit produced by ethanol (16).

Activation of AC by Gpp(NH)p occurs after a lag (26, 27), indicative of one or more rate-limiting steps in activation of the enzyme (22, 28). Addition of ethanol increased the rate of enzyme activation by Gpp(NH)p only in cortical membrane (Table 2). In cortical membranes, ethanol also decreased the apparent EC50 value for the guanine nucleotide (Table 1). Since exchange of GTP for GDP on the G-protein has been suggested as one mechanism for enzyme activation, one can postulate in this case that ethanol may increase the rate of enzyme activation by increasing the affinity of the G-protein for GTP or by reducing its affinity for GDP.

Our studies which compared the actions of a series of aliphatic alcohols and other lipid perturbing agents support the concept that a major portion of ethanol's actions can be explained by ethanol's lipid perturbing properties (16, 17). This is in contrast to results obtained recently by other investigators (29). An important point with regard to the apparent regional specificities of ethanol's action in the brain is that ethanol may have rather selective effects on different receptor-coupled AC systems, depending on the cellular membrane environment in which the receptor-AC systems are localized.

References
1 Lyon, R.C., McComb, J.A., Schreurs, J. and Goldstein, D.B.: A relationship between alcohol intoxication and the disordering of brain membranes by a series of short-chain alcohols. J. Pharmacol. Exp. Ther. 218, 669–675 (1981)
2 Emmelot, P. and van Hoeven, R.P.: Phospholipid unsaturation and plasma membrane organization. Chem. Phys. Lipids 14, 236–246 (1975)
3 Stadel, J.M., DeLean, A. and Lefkowitz, R.J.: Molecular mechanisms of coupling in hormone receptor-adenylate cyclase systems. Adv. Enzymol. 53, 1–43 (1982)
4 Gorman, R.E. and Bitensky, M.W.: Selective activation by short-chain alcohols of glucagon responsive adensyl cyclase in liver. Endocrinology 87, 1075–1081 (1970)
5 Uhlemann, E.R., Robberecht, P. and Gardner, J.D.: Effects of ethanol on the actions of VIP and secretin on acinar cells from guinea pig pancreas. Gastroenterology 76, 917–925 (1979)
6 Whetton, A.D., Needham, L., Dodd, N.J.F., Heyworth, C.M. and Houslay, M.D.: Forskolin and ethanol both perturb the structure of liver plasma membranes and activate adenylyl cyclase activity. Biochem. Pharmacol. 32, 1601–1608 (1983)
7 Rodbell, M.: The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature 284, 17–22 (1980)
8 Cooper, D.M.F.: Bimodal regulation of adenylyl cyclase. FEBS Lett. 138, 157–163 (1982)
9 Katada, T., Bokoch, G.M., Northup, J.K., Ui, M. and Gilman, A.G.: The inhibitory guanine nucleotide-binding regulatory component of adenylyl cyclase: Properties and function of the purified protein. J. Biol. Chem. 259, 3568–3577 (1984)
10 Pfeffer, T. and Helrich, E.J.M.: Activation of pigeon erythrocyte membrane adenylyl cyclase by guanylnucleotide analogues and separation of a nucleotide binding protein. J. Biol. Chem. 250, 867–876 (1975)
11 Ross, E.M. and Gilman, A.G.: Reconstitution of catecholamine-sensitive adenylyl cyclase activity: interaction of solubilized components with receptor-replete membranes. Proc. Natl. Acad. Sci. U.S.A. 74, 3715–3719 (1977)
12 Northup, J.K., Smigel, M.D., Sterweis, P.C. and Gilman, A.G.: The subunits of the stimulatory regulatory component of adenylyl cyclase: resolution of the activated 45,000-dalton (alpha) subunit. J. Biol. Chem. 258, 11369–11376 (1983)
13 Northup, J.K., Sterweis, P.C. and Gilman, A.G.: The subunits of the stimulatory regulatory component of adenylyl cyclase: resolution, activity, and properties of the 35,000-dalton (beta) subunit. J. Biol. Chem. 258, 11361–11368 (1983)
14 Katada, T., Bokoch, G.M., Smigel, M.D., Ui, M. and Gilman, A.G.: The inhibitory guanine nucleotide-binding regulatory component of adenylyl cyclase. Subunit dissociation and the inhibition of adenylyl cyclase in S49 Lymnoma cyt* and wild type membranes. J. Biol. Chem. 259, 3586–3595 (1984)
15 Tabakoff, B. and Hoffman, P.L.: Development of
functional dependence on ethanol in dopaminergic systems. J. Pharmacol. Exp. Ther. 208, 216–222 (1979)

16 Luthin, G.R. and Tabakoff, B.: Activation of adenylate cyclase by alcohols requires the nucleotide-binding protein. J. Pharmacol. Exp. Ther. 288, 579–587 (1984)

17 Saito, T., Lee, J.M. and Tabakoff, B.: Ethanol’s effects on cortical adenylate cyclase activity. J. Neurochem. 44, 1037–1044 (1985)

18 Salomon, Y.: Adenylate cyclase assay. In Advances in Cyclic Nucleotide Research. Edited by Brooker, G., Greengard, P. and Robison, G.A., p. 35–54, Raven Press, New York (1979)

19 Ohanian, H., Borhanian, K., de Farias, S. and Bennun, A.: A model for the regulation of brain adenylate cyclase by ionic equilibria. J. Bioenerg. Biomembr. 13, 317–355 (1981)

20 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)

21 Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254 (1976)

22 Frieden, C.: Slow transitions and hysteretic behavior in enzymes. Annu. Rev. Biochem. 48, 471–489 (1979)

23 Rabin, R.A. and Molinoff, P.B.: Activation of adenylate cyclase by ethanol in mouse striatal tissue. J. Pharmacol. Exp. Ther. 216, 129–134 (1981)

24 Rabin, R.A. and Molinoff, P.B.: Multiple sites of action of ethanol on adenylate cyclase. J. Pharmacol. Exp. Ther. 227, 551–556 (1983)

25 Kilpatrick, B. and Caron, M.G.: Dopamine receptor of porcine anterior putitary gland: Solubilization and characterization. Biochem. Pharmacol. 33, 1981–1988 (1984)

26 Londos, C., Salomon, Y., Lin, M.C., Harwood, J.P., Schramm, M., Wolff, J. and Robdell, M.: 5’-Guanylylimidodiphosphate, a potent activator of adenylate cyclase systems in eukaryotic cell. Proc. Natl. Acad. Sci. U.S.A. 3087–3090 (1974)

27 Schramm, M. and Robdell, M.: A persistent active state of the adenylate cyclase system produced by the combined actions of isoproterenol and guanylylimidodiphosphate in frog erythrocyte membranes. J. Biol. Chem. 250, 2232–2237 (1975)

28 Neet, K.E. and Ainslie, G.R.: Hysteretic enzymes. Methods Enzymol. 64, 192–225 (1980)

29 Rabin, R.A., Bode, D.C. and Molinoff, P.B.: Relationship between ethanol-induced alterations in fluorescence anisotropy and adenylate cyclase activity. Biochem. Pharmacol. 35, 2331–2336 (1986)