Journal of Cellular Biochemistry 66:450–456 (1997)

Regulation of Adenylyl Cyclase Isoforms by *N*-Alkanols

Toshiaki Ebina, Yoshiyuki Toya, Jun-ichi Kawabe, and Yoshihiro Ishikawa*

Department of Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115

Abstract We examined the effect of *n*-alkanols on adenylyl cyclase isoforms (types II and V) overexpressed in insect cells. Ethanol stimulated the type II isoform but not the type V isoform. Ethanol stimulated type II adenylyl cyclase greater than GTPγS, and the treatment of the membrane with GDPβS or cholera toxin did not affect this stimulation. Other *n*-alkanols inhibited type V adenylyl cyclase activity in proportion to their lipophilic potency. In contrast, type II adenylyl cyclase was stimulated by weakly lipophilic *n*-alkanols and inhibited by strongly lipophilic *n*-alkanols. When solubilized membranes and purified preparations were used, all the *n*-alkanols inhibited type II adenylyl cyclase. Our data suggest that *n*-alkanols regulated adenylyl cyclase isoform-dependently. Stimulation of the type II isoform was independent from the interaction with Gsα but required the presence of an intact membrane structure. Our study may provide another step to understanding how membrane protein subtypes are differentially regulated by *n*-alkanols. J. Cell. Biochem. 66:450–456, 1997.

Key words: type II adenylyl cyclase; type V adenylyl cyclase; insect cells; Gsα; solubilization

Adenylyl cyclase is a membrane-bound enzyme that catalyzes the conversion of ATP to cAMP, an intracellular second messenger. Numerous reports have indicated that cAMP signaling through G protein–adenylyl cyclase plays a key role in a variety of signal transduction pathways [for review see Ishikawa and Homcy, 1997; Iyengar, 1993]. Many hormone receptors are coupled to G protein–adenylyl cyclase, producing cAMP and thus initiating cascaded phosphorylation reactions to regulate cellular function. Recent molecular cloning studies of adenylyl cyclase have elucidated the presence of multiple isoforms within this enzyme family, which are distributed among distinct tissues and cell types. They all share the same membrane topology (i.e., a module of six transmembrane spans linked to a large cytoplasmic domain that was tandemly repeated) which is rather similar to the membrane topology of transporters or ion channels [Greenberger and Ishikawa, 1994].

Ethanol and other *n*-alkanols are known to regulate the function of various enzymes, including ion channels, transporters, and other effector enzymes [for review see Deitrich et al., 1989]. Ethanol also regulates cAMP signaling in cells, although the exact mechanism of this regulation has been difficult to illustrate [for review see Hoffman and Tabakoff, 1990]. Ethanol may affect the membrane fluidity and thus alter agonist-receptor, receptor–G protein, or G protein–adenylyl cyclase coupling. Alternatively, it may directly affect these protein molecules.

The availability of the potent baculovirus expression system has allowed investigators to study the regulation of each adenylyl cyclase isoform as the dominant positive adenylyl cyclase isoform overexpressed in insect cells. Using this insect cell expression system, investigators have found that the biochemical properties of each isoform are strikingly distinctive [Ishikawa and Homcy, 1997; Iyengar, 1993]. Direct regulators of adenylyl cyclase isoforms vary from simple cation concentration [Pieroni et al., 1995] to various kinases [Jacobowitz and Iyengar, 1994; Kawabe et al., 1994b] and, of course, G protein subunits [Taussig et al., 1993a, 1994].

Abbreviations: ATP, adenosine 5′-triphosphate; cAMP, adenosine 3′:5′-cyclic monophosphate; GDPβS, guanosine 5′-O-(2-thiodiphosphate); GTP, guanine 5′-triphosphate; GTPγS, guanosine 5′-O-(3-thiotriphosphate).

Contract grant sponsor: United States Public Health Service, contract grant number HL38070; contract grant sponsor: American Heart Association, contract grant number 13-533-945.

*Correspondence to: Yoshihiro Ishikawa, Cardiovascular & Pulmonary Research Institute, Allegheny University, 320 East North Ave., Pittsburgh, PA 15212.

Received 7 April 1997; accepted 7 May 1997

In the present study, we intended to study the mechanism of *n*-alkanol action on adenylyl cyclase using the isoforms (types II and V) overexpressed as the dominant positive adenylyl cyclase in insect cells. This study addresses the following questions. First, do *n*-alkanols including ethanol regulate the catalytic activity of adenylyl cyclase? Second, do the effects of *n*-alkanols differ among adenylyl cyclase isoforms?

MATERIALS AND METHODS

Membrane Preparations

Type II (from Dr. R. Reed, Johns Hopkins University) and type V adenylyl cyclase isoforms as well as mammalian Gsα were overexpressed in High Five (H5) insect cells (Invitrogen, La Jolla, CA) as previously described [Iwami et al., 1995; Kawabe et al., 1994a,b]. Sixty hours after infection, cells were washed twice with ice-cold phosphate-buffered saline and homogenized in a buffer containing 50 mM Tris/HCl (pH 8.0), 1 mM EGTA, 1 mM EDTA, 1 mM dithiothreitol, 200 mM sucrose, and a protease inhibitor mixture. They were disrupted with a sonicator and centrifuged at 500*g* for 10 min at 4°C. The supernatants were further centrifuged at 100,000*g* for 30 min at 4°C. The resultant pellets were resuspended in the same buffer without EGTA. The crude membrane preparations were further incubated with 0.8–1.6% n-dodecyl β-D-maltoside for 1 h at 4°C and centrifuged at 100,000*g* for 30 min at 4°C. The supernatant was used as a solubilized membrane preparation.

Purification of Adenylyl Cyclase

Purification of the two isoforms overexpressed in insect cells was performed by affinity chromatography as previously described by Taussig et al. [1993b] and modified by us [Iwami et al., 1995; Kawabe et al., 1994a,b].

Cholera Toxin Treatment

Membranes were treated with cholera toxin as previously described [Gill and Woolkalis, 1991].

Adenylyl Cyclase Assay

Adenylyl cyclase activity was measured as previously described [Iwami et al., 1995; Kawabe et al., 1994a,b]. Briefly, reaction mixtures were incubated for 20 min at 30°C in the presence of 20 mM Hepes (pH 8.0), 0.2 mM EDTA, 0.2 mM dithiothreitol, 0.1 mM ATP, 0.1 mM cAMP, 1 mM creatine phosphate, 8 units/ml creatine phosphokinase, and ^{32}P-αATP (about 1 μCi/assay tube). Five millimolar $MgCl_2$ was included in the reaction mixture unless otherwise specified.

RESULTS

Ethanol Stimulates the Type II Isoform But Not the Type V Isoform

We examined the effects of ethanol, a common *n*-alkanol, on two adenylyl cyclase isoforms (types II and V) in the presence and absence of forskolin. Ethanol stimulated type II catalytic activity in both basal and forskolin-stimulated conditions (Fig. 1A). Stimulation was ethanol concentration–dependent and was significant at all the concentrations examined (86–865 mM). In clear contrast, ethanol did not influence type V catalytic activity. At higher concentrations, ethanol weakly inhibited this isoform.

As shown in the Lineweaver-Burk plot analysis in Figure 1B,C, kinetic studies showed that ethanol changed the Vmax but not the Km in both isoforms; ethanol increased the Vmax value of type II while decreasing that of type V. These findings suggest that ethanol does not affect the interaction of the enzyme with the substrate ATP binding but changes the maximal velocity of the two isoforms in opposite directions.

Ethanol-Induced Stimulation Does Not Involve Gsα

It was previously proposed that ethanol stimulates adenylyl cyclase by enhancing the association between Gsα and adenylyl cyclase, leading to the stimulation of adenylyl cyclase by Gsα in mammalian cells (8). Thus, ethanol may increase the association between putative insect Gsα and type II adenylyl cyclase in our system, thereby increasing catalytic activity. So we examined the effect of ethanol in the presence of various activators and an inhibitor of Gsα.

The maximum activation of type II adenylyl cyclase by insect Gsα was ~30% (Fig. 2A) as determined in the presence of an increasing

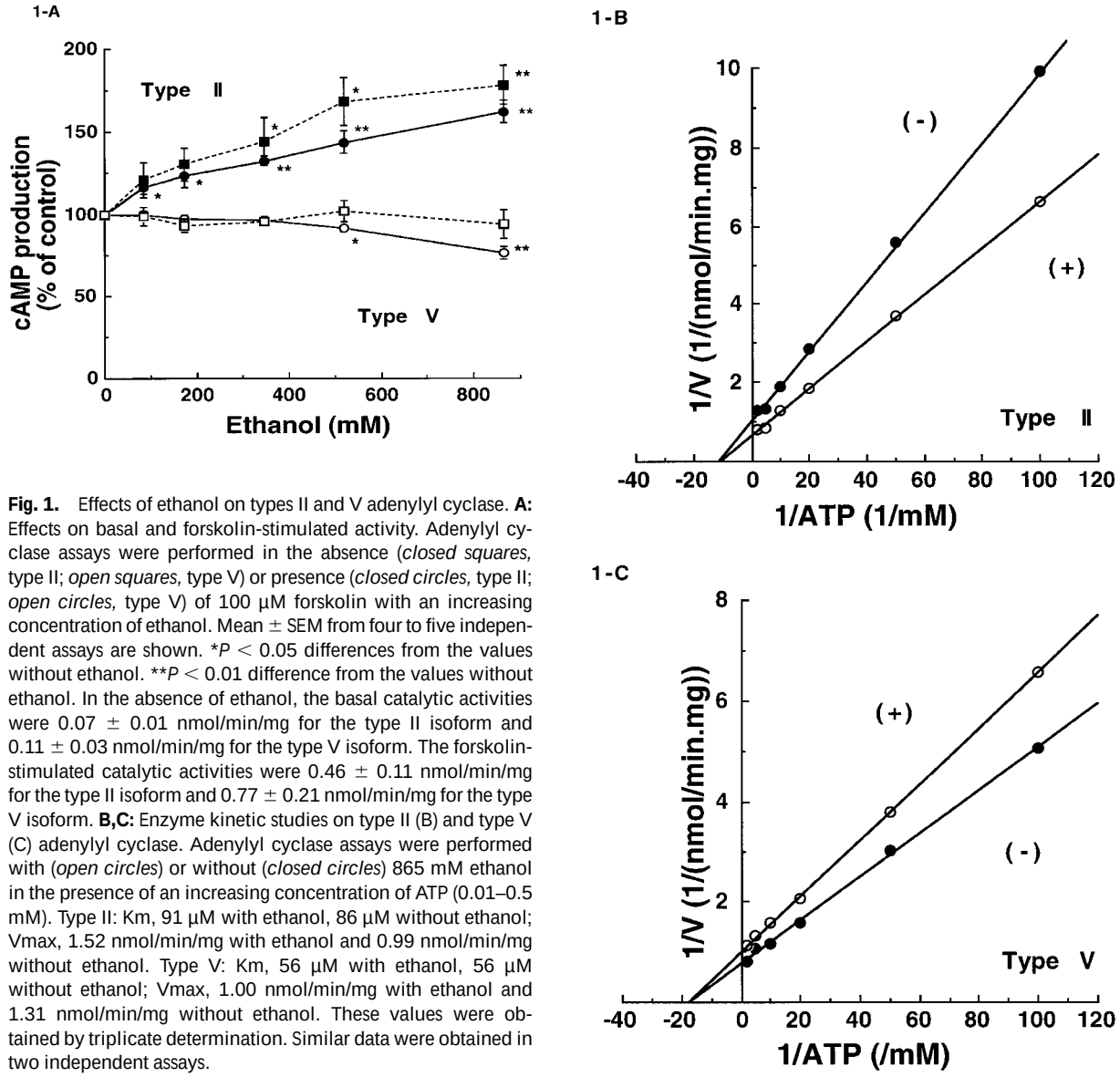

Fig. 1. Effects of ethanol on types II and V adenylyl cyclase. **A:** Effects on basal and forskolin-stimulated activity. Adenylyl cyclase assays were performed in the absence (*closed squares,* type II; *open squares,* type V) or presence (*closed circles,* type II; *open circles,* type V) of 100 μM forskolin with an increasing concentration of ethanol. Mean ± SEM from four to five independent assays are shown. *$P < 0.05$ differences from the values without ethanol. **$P < 0.01$ difference from the values without ethanol. In the absence of ethanol, the basal catalytic activities were 0.07 ± 0.01 nmol/min/mg for the type II isoform and 0.11 ± 0.03 nmol/min/mg for the type V isoform. The forskolin-stimulated catalytic activities were 0.46 ± 0.11 nmol/min/mg for the type II isoform and 0.77 ± 0.21 nmol/min/mg for the type V isoform. **B,C:** Enzyme kinetic studies on type II (B) and type V (C) adenylyl cyclase. Adenylyl cyclase assays were performed with (*open circles*) or without (*closed circles*) 865 mM ethanol in the presence of an increasing concentration of ATP (0.01–0.5 mM). Type II: Km, 91 μM with ethanol, 86 μM without ethanol; Vmax, 1.52 nmol/min/mg with ethanol and 0.99 nmol/min/mg without ethanol. Type V: Km, 56 μM with ethanol, 56 μM without ethanol; Vmax, 1.00 nmol/min/mg with ethanol and 1.31 nmol/min/mg without ethanol. These values were obtained by triplicate determination. Similar data were obtained in two independent assays.

concentration of GTPγS. Further activation was achieved only in the presence of coexpressed mammalian Gsα. In contrast to the weak stimulation by insect Gsα, the activation of type II adenylyl cyclase by ethanol was ~80% (Fig. 1A). Thus, ethanol stimulated adenylyl cyclase more than insect Gsα did. Similarly, cholera toxin treatment increased the catalytic activity of type II adenylyl cyclase by 30%. Ethanol further stimulated type II adenylyl cyclase in a concentration-dependent manner (Fig. 2B). In the presence of 100 μM GDPβS, ethanol still stimulated type II adenylyl cyclase (Fig. 2C). Thus, neither activation nor inactivation of Gsα altered the profile of ethanol-mediated stimulation of type II adenylyl cyclase, suggesting that ethanol did not exert its stimulatory effects through potentiation of the insect Gsα/adenylyl cyclase association in our system.

Effects of *N*-Alkanols

We also examined the effect of different *n*-alkanols on the catalytic activity of adenylyl cyclase isoforms. As shown in Figure 3A, all *n*-alkanols inhibited the catalytic activity of type V adenylyl cyclase. The degree of inhibition correlated directly with the number of carbon atoms (i.e., the lipophilic potency) and the concentration of *n*-alkanols. This result seemed reasonable since the number of carbon atoms in an *n*-alkanol correlates directly with its ability to perturb membrane and to bind hydrophobic

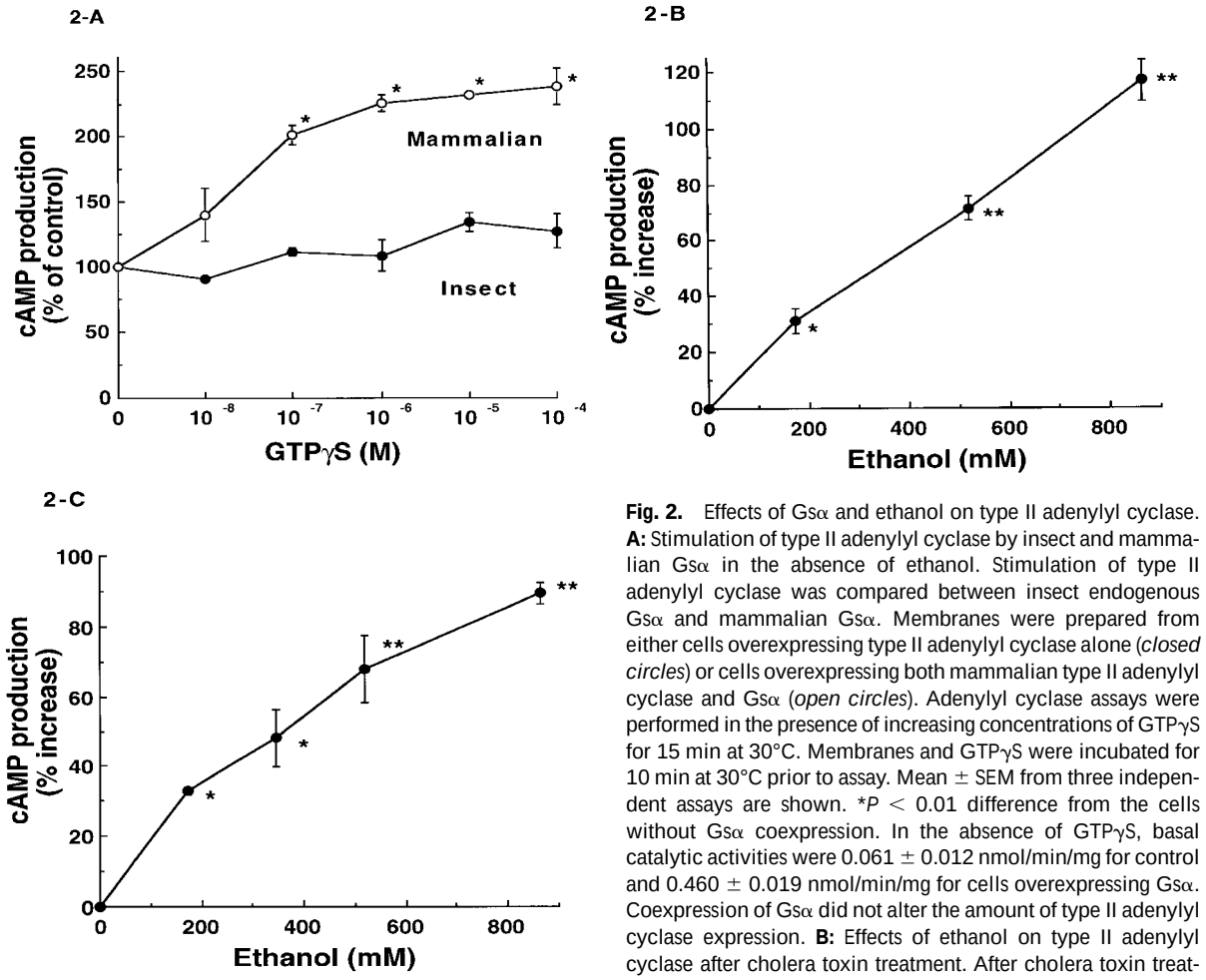

Fig. 2. Effects of Gsα and ethanol on type II adenylyl cyclase. **A:** Stimulation of type II adenylyl cyclase by insect and mammalian Gsα in the absence of ethanol. Stimulation of type II adenylyl cyclase was compared between insect endogenous Gsα and mammalian Gsα. Membranes were prepared from either cells overexpressing type II adenylyl cyclase alone (*closed circles*) or cells overexpressing both mammalian type II adenylyl cyclase and Gsα (*open circles*). Adenylyl cyclase assays were performed in the presence of increasing concentrations of GTPγS for 15 min at 30°C. Membranes and GTPγS were incubated for 10 min at 30°C prior to assay. Mean ± SEM from three independent assays are shown. $*P < 0.01$ difference from the cells without Gsα coexpression. In the absence of GTPγS, basal catalytic activities were 0.061 ± 0.012 nmol/min/mg for control and 0.460 ± 0.019 nmol/min/mg for cells overexpressing Gsα. Coexpression of Gsα did not alter the amount of type II adenylyl cyclase expression. **B:** Effects of ethanol on type II adenylyl cyclase after cholera toxin treatment. After cholera toxin treatment, adenylyl cyclase assays of the membranes overexpressing type II adenylyl cyclase were performed in the presence of increasing concentrations of ethanol (0–865 mM). Mean ± SEM from four independent assays are shown. $*P < 0.05$ difference from the values without ethanol. $**P < 0.01$ difference from the values without ethanol. Catalytic activity of type II adenylyl cyclase in the absence of ethanol was 0.074 ± 0.015 nmol/min/mg. **C:** Effects of ethanol on type II adenylyl cyclase after inactivation of insect Gsα. Adenylyl cyclase assays of the membranes overexpressing type II adenylyl cyclase were performed with 100 μM GDPβS and 100 μM forskolin in the presence of increasing concentrations of ethanol (0–865 mM) for 15 min at 30°C. Membranes and GDPβS were incubated for 10 min at 30°C prior to assay. Mean ± SEM from three independent assays are shown. $*P < 0.05$ difference from the values without ethanol. $**P < 0.01$ difference from the values without ethanol. Forskolin-stimulated catalytic activity in the absence of ethanol was 0.435 ± 0.102 nmol/min/mg.

sites of proteins [Deitrich et al., 1989; Franks and Lieb, 1994].

However, each *n*-alkanol regulated type II adenylyl cyclase differently (Fig. 3B). Weakly lipophilic *n*-alkanols (methanol and ethanol) stimulated this isoform, while strongly lipophilic *n*-alkanols (propanol and butanol) inhibited this isoform in a concentration-dependent manner. Thus, *n*-alkanols had dual effects according to the number of carbon atoms and its concentration.

Thus, the effects of *n*-alkanols were clearly different for the two adenylyl cyclase isoforms. In particular, the dual effects of *n*-alkanols on type II adenylyl cyclase were unexpected and suggested that mixed mechanisms exist for *n*-alkanols to regulate this isoform (i.e., perturbation of the lipid membrane and/or binding to hydrophobic sites of proteins) [Deitrich et al., 1989; Franks and Lieb, 1994]. If either stimulation or inhibition of type II adenylyl cyclase by *n*-alkanols was due to perturbation of the membrane by *n*-alkanols, solubilization of the membrane and thus a loss of an intact membrane structure should abolish that effect. In addition, the potency of *n*-alkanols should become also correlate directly with the number of carbon atoms and concentration.

Effects of *N*-Alkanols in Solubilized Membranes

When solubilized membrane preparations were used, *n*-alkanols became all inhibitory to both isoforms (Fig. 4A,B); stimulation of the

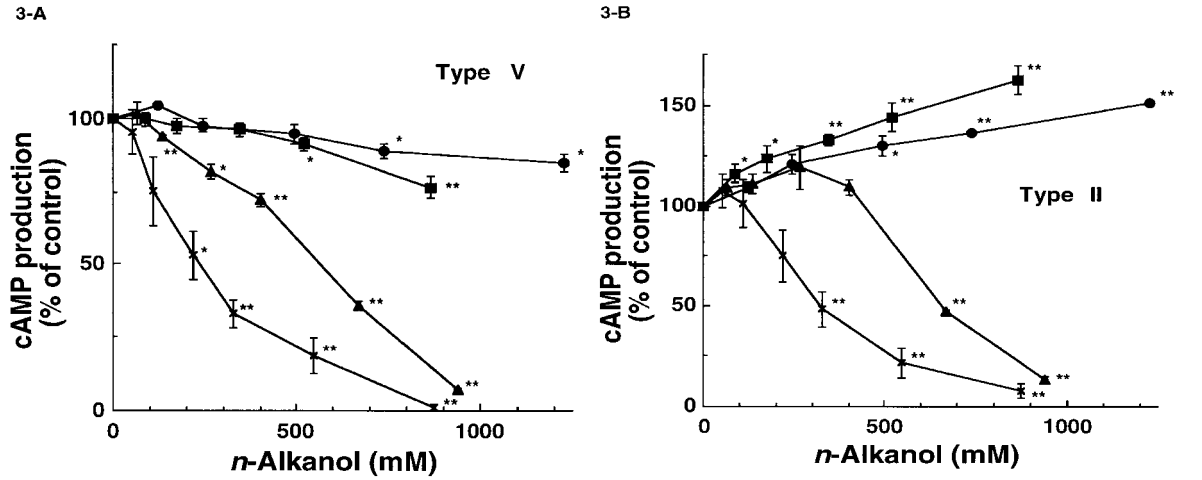

Fig. 3. Effect of *n*-alkanols on adenylyl cyclase catalytic activity in crude membrane preparations. **A,B:** Effect on the type V (A) and type II (B) isoforms. Adenylyl cyclase assays were performed in the presence of increasing concentrations of methanol (*circles*), ethanol (*squares*), 1-propanol (*triangles*), or 1-butanol (*crosses*). Mean ± SEM from three to five independent assays are shown. *$P < 0.05$ difference from the values without *n*-alkanols. **$P < 0.01$ difference from the values without *n*-alkanols.

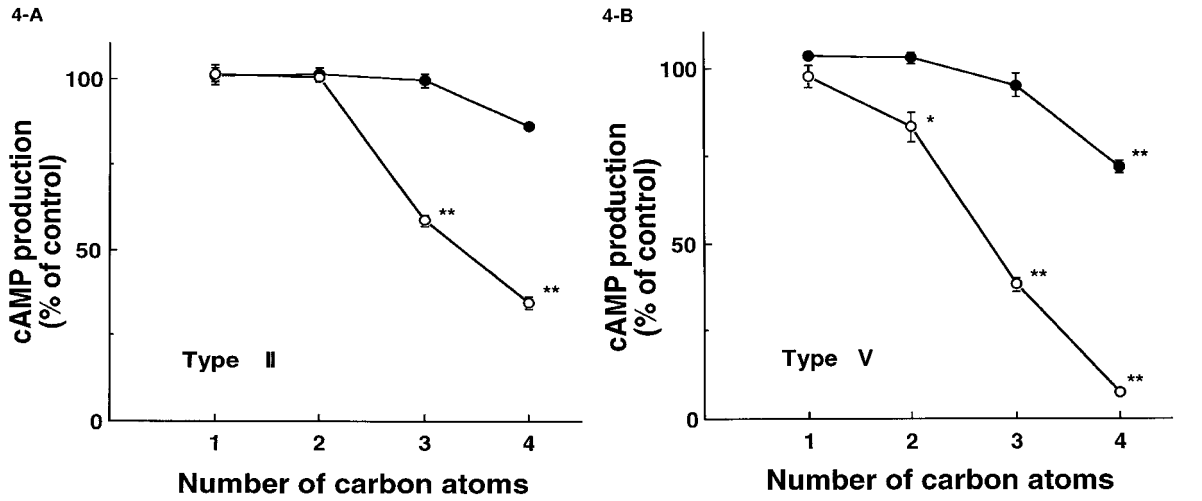

Fig. 4. Effect of *n*-alkanols on adenylyl cyclase catalytic activity in solubilized membranes. **A,B:** Effect on the type II (A) and type V (B) isoforms. Adenylyl cyclase assays were performed in the presence of 100 mM (*closed circles*) or 500 mM (*open circles*) of methanol, ethanol, 1-propanol, or 1-butanol. Mean ± SEM from four to five independent assays are shown. *$P < 0.05$ difference from the values without *n*-alkanols. **$P < 0.01$ difference from the values without *n*-alkanols.

type II isoform by *n*-alkanols disappeared. The degree of inhibition on both isoforms now correlated directly with the number of carbon atoms and the concentrations of the *n*-alkanols. Therefore, the mechanism of stimulation of the type II isoform differed from that of inhibition and depended upon the presence of an intact membrane structure.

Similarly, the stimulatory effect of ethanol on the type II isoform was abolished in solubilized membranes at all concentrations (Fig. 5), and ethanol inhibited this isoform. In contrast, the inhibitory effect on the type V isoforms was unchanged: it continued to be dependent on the ethanol concentration. Furthermore, ethanol inhibited both isoforms of purified adenylyl cyclase in a lipid-free solution (Fig. 6). These data suggest that ethanol has a direct inhibitory effect on both isoforms.

DISCUSSION

The regulation of adenylyl cyclase by *n*-alkanols occurred in both adenylyl cyclase isoform– and conformation–dependent manners, which most likely resulted from distinct mechanisms of action of *n*-alkanols on adenylyl cy-

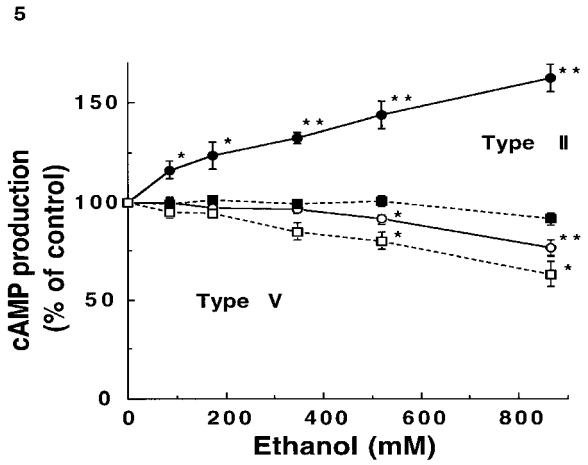

Fig. 5. Effects of ethanol on adenylyl cyclase catalytic activities in solubilized membrane preparations. Catalytic activity of the type II (*closed symbols*) and the type V (*open symbols*) isoforms was measured in either crude (*circles*) or solubilized membrane preparations (*squares*). Mean ± SEM from four independent assays are shown. $*P < 0.05$ differences from the values without ethanol. $**P < 0.01$ difference from the values without ethanol. The catalytic activity of crude membrane preparations was 0.46 ± 0.11 nmol/min/mg (type II) and 0.77 ± 0.21 nmol/min/mg (type V). The catalytic activities of solubilized membrane preparations were 0.28 ± 0.06 nmol/min/mg (type II) and 1.86 ± 0.32 nmol/min/mg (type V).

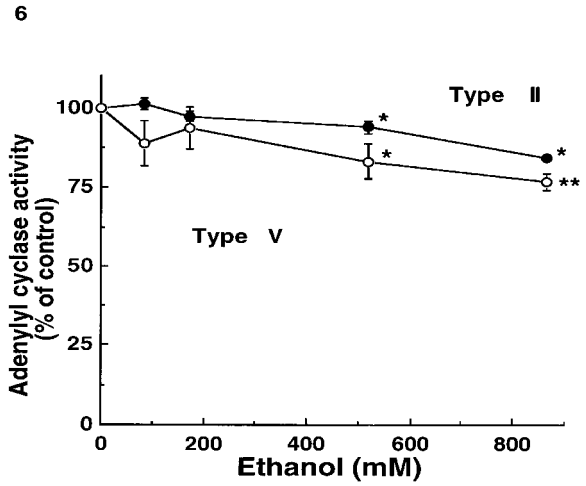

Fig. 6. Effect of ethanol on the purified adenylyl cyclase. Catalytic activity of the purified enzyme (*closed circles,* type II; *open circles,* type V) was measured in the presence of increasing concentrations of ethanol. Assays were performed without an ATP regeneration system. ATP concentration in the assay was 0.2 mM. Specific activities of the purified enzymes were 37.4 ± 2.2 nmol/min/mg (type V) and 41.5 ± 1.8 nmol/min/mg (type II). Mean ± SEM from triplicate determinations are shown. Similar results were obtained in two separate experiments. $*P < 0.05$ difference from the values without ethanol. $**P < 0.01$ difference from the values without ethanol.

clase isoforms. It is interesting to compare our findings with those for protein kinase C, another key effector enzyme in cellular signaling [Slater et al., 1993]: various *n*-alkanols also regulated protein kinase C at concentrations similar to those used in our study.

Importantly, the stimulation of type II by ethanol was independent from the interaction with Gsα. The type II isoform was sensitive to ethanol, while the type V isoform was not. Ethanol stimulated type II adenylyl cyclase at all the concentrations examined (86–865 mM). The regulatory mechanism of cAMP signaling by ethanol has been extensively investigated for many years [for review see Hoffman and Tabakoff, 1990]; however, the exact target of ethanol action has been difficult to specify using the conventional experimental system. Ethanol may directly affect the protein components: this mechanism has been difficult to examine because the three major components within the cAMP signal (receptor, G protein, and adenylyl cyclase) are ubiquitously expressed in mammalian cell types. It is technically difficult to examine a single component without the influence of each on the others because ethanol may influence the interaction among the components by altering the lipid membrane fluidity. The usage of the H5 insect cell expression system in our study had a certain advantage over the mammalian cell expression system in this regard. The endogenous insect Gsα could no longer efficiently activate adenylyl cyclase in H5 cells (Fig. 2). Therefore, in our expression system, we could examine the effects of ethanol on adenylyl cyclase with little interference by Gsα.

The diversity in the regulation of adenylyl cyclase isoforms may correspond to the difference in the amino acid sequence between the two isoforms in our study. The amino acid sequence within the transmembrane domains is poorly conserved; thus, *n*-alkanols may exert their effects by changing the membrane properties and thus conformation of this enzyme. It is noteworthy that we and others have recently reported that the activation of type II adenylyl cyclase by kinase is modified by the detergent-induced conformational changes of the adenylyl cyclase [Ebina et al., 1997; Zimmermann and Taussig, 1996]. The amino acid sequence in the cytoplasmic catalytic domains, including clusters of hydrophobic pockets, is well conserved between type II and type V adenylyl cyclase. *N*-alkanols may bind directly to these areas and inhibit the enzyme. However, at present, we do not know the exact site(s) of interaction of *n*-alkanols within this enzyme.

Adenylyl cyclase has the same membrane topology as transporters and ion channels [Greenberger and Ishikawa, 1994]. Many of these molecules are composed of two domains: the transmembrane domain with a six transmembrane spanning structure that forms a tunnel for molecular transportation and the cytoplasmic domain with ATP binding for catalysis. Over the years, studies targeting these molecules have argued whether *n*-alkanols affect the molecules by changing the membrane properties [Harris and Bruno, 1985] or by binding directly to hydrophobic pockets of the proteins [Covarrubias et al., 1995; Li et al., 1994]. Our data suggest that the two mechanisms coexist for adenylyl cyclase and operate in an isoform-dependent manner. Ion channels and transporters are composed of multiple subtypes, among which the effects of *n*-alkanols may differ. In support of this concept, subtype-specific regulation by *n*-alkanols has been shown in glucose transporter [Krauss et al., 1994] and nucleoside transporter [Krauss et al., 1993].

A recent study using adenylyl cyclase isoforms overexpressed in mammalian cells demonstrated that ethanol activated the type VII isoform via enhancing the interaction between Gsα and adenylyl cyclase. Our findings and theirs suggest that ethanol influences the lipid membrane fluidity and, either by changing the conformation of the enzyme or by enhancing the association with Gsα, activates adenylyl cyclase [Yoshimura and Tabakoff, 1995]. It is noteworthy that type VII adenylyl cyclase belongs to the same subgroup as the type II isoform. Thus, multiple mechanisms may coexist to increase the intracellular cAMP concentration via a subset of adenylyl cyclase isoforms by ethanol.

ACKNOWLEDGMENT

We thank Ms. J. Olivo for editorial assistance.

REFERENCES

Covarrubias M, Vyst TB, Escobar L, Wei, A (1995): Alcohols inhibit a cloned potassium channel at a discrete saturable site. J Biol Chem 270:19408–19416.

Deitrich RA, Dunwiddie TV, Harris RA, Erwin VG (1989): Mechanism of action of ethanol: Initial central nervous system actions. Pharmacol Rev 41:489–537.

Ebina T, Kawabe J, Katada T, Ohno S, Homcy CJ, Ishikawa Y (1997): Conformation-dependent activation of type II adenylyl cyclase by protein kinase C. J Cell Biochem 64:492–498.

Franks NP, Lieb WR (1994): Molecular and cellular mechanisms of general anesthesia. Nature 367:607–614.

Gill DM, Woolkalis MJ (1991): Cholera toxin–catalyzed ^{32}P-ADP-ribosylation of proteins. Methods Enzymol 195: 267–280.

Greenberger L, Ishikawa Y (1994): ATP-binding cassette proteins: Common denominators between ion channels, transporters and enzymes. Trends Cardiovasc Med 4:193–198.

Harris RA, Bruno P (1985): Membrane disordering by anesthetic drugs: Relationship to synaptosomal sodium and calcium fluxes. J Neurochem 44:1274–1281.

Hoffman PL, Tabakoff B (1990): Ethanol and guanine nucleotide binding proteins: A selective interaction. FASEB J 4:2612–2622.

Ishikawa Y, Homcy CJ (1997): The adenylyl cyclases as integrators of transmembrane signal transduction. Circ Res 80:297–304.

Iwami G, Kawabe J, Ebina T, Cannon PJ, Homcy CJ, Ishikawa Y (1995): Regulation of adenylyl cyclase by protein kinase A. J Biol Chem 270:12481–12484.

Iyengar R (1993): Molecular and functional diversity of mammalian Gs-stimulated adenylyl cyclases. FASEB J 7:768–775.

Jacobowitz O, Iyengar R (1994): Phorbol ester–induced stimulation and phosphorylation of adenylyl cyclase 2. Proc Natl Acad Sci U S A 91:10630–10634.

Kawabe J, Ebina T, Ismail S, Kitchen D, Homcy CJ, Ishikawa Y (1994a): A novel peptide inhibitor of adenylyl cyclase (AC): A peptide from type V AC directly inhibits AC catalytic activity. J Biol Chem 269:24906–24911.

Kawabe J, Iwami G, Ebina T, Ohno S, Katada T, Ueda Y, Homcy CJ, Ishikawa Y (1994b): Differential activation of adenylylcyclase by protein kinase C isoenzymes. J Biol Chem 269:16554–16558.

Krauss SW, Ghirnikar RB, Diamond I, Gordon AS (1993): Inhibition of adenosine uptake by ethanol is specific for one class of nucleoside transporters. Mol Pharmacol 44: 1021–1026.

Krauss SW, Diamond I, Gordon AS (1994): Selective inhibition by ethanol of the type 1 facilitative glucose transporter (GLUT1). Mol Pharmacol 45:1281–1286.

Li W, Mischak H, Yu J-C, Wang L-M, Mushinski JF, Heidaran MA, Pierce JH (1994): Tyrosine phosphorylation of protein kinase C-δ in response to its activation. J Biol Chem 269:2349–2352.

Pieroni JP, Harry A, Chen J, Jacobowitz O, Magnusson RP, Iyengar R (1995): Distinct characteristics of the basal activities of adenylyl cyclases 2 and 6. J Biol Chem 270:21368–21373.

Slater SJ, Cox KJA, Lombardi JV, Ho C, Kelly MB, Rubin E, Stubbs CD (1993): Inhibition of protein kinase C by alcohols and anesthetics. Nature 364:82–84.

Taussig R, Iniguez-Lluhi JA, Gilman AG (1993a): Inhibition of adenylyl cyclase by Gi alpha. Science 261:218–221.

Taussig R, Quarmby LM, Gilman AG (1993b): Regulation of purified type I and type II adenylylcyclases by G protein beta gamma subunits. J Biol Chem 268:9–12.

Taussig R, Tang WJ, Hepler JR, Gilman AG (1994): Distinct patterns of bidirectional regulation of mammalian adenylyl cyclases. J Biol Chem 269:6093–6100.

Yoshimura M, Tabakoff B (1995): Selective effects of ethanol on the generation of cAMP by particular members of the adenylyl cyclase family. Alcohol Clin Exp Res 19:1435–1440.

Zimmermann G, Taussig R (1996): Protein kinase C alters the responsiveness of adenylyl cyclase to G protein α and βγ subunits. J Biol Chem 271:27161–27166.