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European Journal of Pharmacology 430 (2001) 19–23

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Chronic ethanol treatment reduces adenylyl cyclase activity in human erythroleukemia cells

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Received 19 April 2001; received in revised form 28 August 2001; accepted 30 August 2001

Abstract

Characteristic changes of platelet membrane adenylyl cyclase activity have been described in men with alcoholism. We studied the occurrence of these changes in human erythroleukemia (HEL) cells after chronic ethanol treatment. Chronic treatment of the HEL cell with ethanol (50 or 100 mM) for 48 h resulted in significant reduction of prostaglandin E_1-stimulated adenylyl cyclase activity. The acute ethanol (200 mM, 5 min) enhancement of adenylyl cyclase activity was significantly reduced after chronic ethanol treatment. We also observed a reduction in phorbol-12,13-dibutyrate (PDB) enhancement of prostaglandin E_1-stimulation after chronic ethanol treatment. Chronic ethanol treatment (50 or 100 mM) reduced the activity of adenylyl cyclase in response to stimulation by acute ethanol to a greater extent than that of after acute PDB. The increase in cAMP formation by ethanol and PDB was only evident when prostaglandin E_1 was present and under basal conditions (when no stimulatory agent was present) ethanol up to 200 mM, and PDB up to 1 M, had no significant effect on adenylyl cyclase activity. The reduced capacity of ethanol and/or PDB to stimulate adenylyl cyclase activity after chronic ethanol treatment suggests the involvement of a common denominator in the action of ethanol and PDB.

Keywords: Ethanol; Adenylyl cyclase; HEL cell

1. Introduction

Chronic ethanol treatment produces biochemical adaptations in the central nervous system, which underlie the development of tolerance to and dependence on alcohol. The mechanisms underlying these effects of ethanol on the central nervous system are poorly understood but appear to involve alterations in cellular membrane structure and function. Studies of the chronic effects of ethanol have shown it to cause changes in the abundance or functioning of various receptors, neurotransmitters, ion channels and transmembrane signaling system (Little, 1991). Recent work has focused on the interaction between ethanol and the adenylyl cyclase signaling system in cells. Adenylyl cyclase serves as an important signal transduction element in neurons and other cells of mammals, as well as having a critical part in signal transduction in lower organisms. Previous studies with fresh and cultured mammalian cells have shown that the adenylyl cyclase signal transduction pathway is affected by ethanol (Tabakoff and Hoffman, 1998). Acute ethanol exposure in vitro potentiates receptor-stimulated adenylyl cyclase activity in cell membranes and enhances receptor-stimulated accumulation of intracellular cAMP (cyclic 3′, 5′-adenosine monophosphate) in intact cells (Tabakoff and Hoffman, 1998). In contrast, chronic ethanol exposure in vivo or in vitro often diminishes adenylyl cyclase activity as well as cAMP accumulation. The responsiveness of the adenylyl cyclase enzyme to stimulation by neurotransmitters was also shown to be diminished in the brain of animals after long-term ingestion of alcohol (French et al., 1975; Saito et al., 1987).

Attempts have been made to provide a valid model of brain enzyme activity after chronic ethanol treatment in a tissue that is easily accessible in humans. Blood platelets are an accessible tissue that reflects the activity of many enzymes found in the brain. However, the study of platelet adenylyl cyclase activity is limited by the impossibility of maintaining these cells in vitro for long periods of drug treatment. The human erythroleukemia (HEL) cells are a human leukemic cell line that retain a number of features

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01370-X

of megakaryocyte/platelet lineage and have been used extensively as a model cell system for studying signal transduction processes in platelets (Brass, 1992). In the present study, we used the HEL cells in order to extrapolate the chronic effects of ethanol on adenylyl cyclase activity in platelets. In addition to its therapeutic values, knowledge of these alcohol-induced changes could provide objective indexes of effects of chronic alcohol consumption.

2. Materials and methods

2.1. Materials

[2-^{3}H]Adenine (15–25 Ci/mmol) was from Amersham International (UK). 3-Isobutyl-1-methylxanthine (IBMX) was purchased from Calbiochem. Phorbol 12,13-dibutyrate (PDB), was purchased from Alexis (San Diego, CA, USA) and dissolved at high concentration in appropriate solutions, and kept frozen until used. All other products were purchased from Sigma (St. Louis, MO, USA).

2.2. Cell culture

HEL cells (American Type Culture Collection, Rockville, MD, USA) were grown in suspension culture in RPMI 1640 (GIBCO-BRL, Grand Island, NY, USA) medium containing fetal calf serum (10% v/v). Cells were maintained at 37 °C in a humidified atmosphere with 95% air, 5% CO_2 at a density between 0.5 and 1×10^6 cell/ml. HEL cells have a doubling time of about 24 h in log phase growth and reach a saturation density of approximately 1×10^6/ml. For cAMP measurement, cells were pelleted by centrifugation ($200 \times g$ for 5 min) and resuspended in serum-free RPMI 1640 without phenol red pH indicator, supplemented with 20 mM HEPES, pH 7.4, at a cell concentration of 1×10^6/ml. Aliquots (0.4 ml) of the cell suspension were added to each well (24-well plates) and allowed to equilibrate for 30 min at 37 °C before the start of the assay.

2.3. Chronic ethanol treatment

Cells were maintained in the presence of ethanol for 48 h. To minimize ethanol evaporation, the flasks were placed in a glass container holding the same concentration of ethanol as the flask. The medium was removed daily and replaced by fresh medium containing the appropriate concentration of ethanol. Control cells were maintained in ethanol-free medium and received the same schedule of medium changes. After maintenance of cells in absence or presence of ethanol for the indicated time, the medium was removed and the cells were incubated for 30 min in ethanol-free medium. The cells were then placed in 24-well plate and cyclic AMP assay was performed.

2.4. Cyclic AMP accumulation assay

cAMP formation was measured by monitoring the conversion of [^{3}H]ATP into [^{3}H]cAMP after preloading of the intracellular ATP pool with [^{3}H]adenine as previously described (Yoshimura and Tabakoff, 1999). The cells were treated with the phosphodiesterase inhibitor, IBMX (500 μM), for 10 min before the addition of prostaglandin E_1 (10 μM), which was prepared in ethanol-containing solution that resulted in a final concentration of 8.6 mM ethanol in the assay mixture containing prostaglandin E_1. A further 5 min of incubation was allowed after the addition of prostaglandin E_1 and then the reaction was stopped using trichloracetic acid. [^{3}H]ATP and [^{3}H]cAMP were separated by sequential chromatography using Dowex 50 and neutral alumina columns as described by Salomon et al. (1974), and radioactive material was quantified with a Beckman LS 6000TA liquid scintillation counter. cAMP production was calculated as a fraction of the available pool of [^{3}H]ATP converted to [^{3}H]cAMP as follows: cAMP accumulation over time (%) = $A/(A+B) \times 100$, where A is [^{3}H] (cpm) recovered in the cAMP fraction and B is [^{3}H] (in dpm) recovered in the ATP fraction. Results are reported as means ± standard error of mean (S.E.M.). All assays were performed in triplicate and statistical analysis of the data was performed with an analysis of variance (ANOVA) followed by Student's *t*-test using the SigmaStat statistical program (Jandel). The criterion for significance was $P < 0.05$.

3. Results

3.1. Effects of prostaglandin E_1 on cAMP formation in HEL cells

The effects of various concentrations of prostaglandin E_1 on cAMP accumulation were first examined in HEL cells pulse-labeled by incubation with [^{3}H]adenine (Fig. 1).

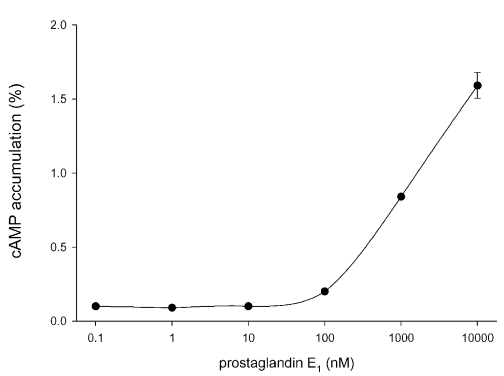

Fig. 1. Concentration dependence of prostaglandin E_1 effects on cAMP accumulation in HEL cells. Cells were incubated for 5 min with various concentrations of prostaglandin E_1 and [^{3}H]cAMP accumulation during this time were measured as described in materials and methods. Results are from a representative experiment, with triplicate determinations.

When prostaglandin E_1 (0.1–10,000 nM) was added to the HEL cells and incubated for 5 min, it resulted in a dose-related increase in cAMP formation. The stimulating effect of prostaglandin E_1 on adenylyl cyclase activity began at 100 nM and continued steadily up to 10 μM.

3.2. Effects of chronic ethanol treatment on prostaglandin E_1 stimulation of adenylyl cyclase activity in HEL cells

When cells were chronically treated with either 50 or 100 mM ethanol for 2 days, the response of the HEL cells to stimulation by 10 μM prostaglandin E_1 was significantly reduced by 34% (from 1.95 to 1.45) and 82% (from 1.95 to 1.07), respectively (Fig. 2).

3.3. Effects of chronic ethanol treatment on acute ethanol enhancement of prostaglandin E_1 stimulation of adenylyl cyclase activity in HEL cells

Acute addition of ethanol to various cell types has been shown to enhance the prostaglandin E_1-stimulated cAMP accumulation. In our previous work, we showed that when ethanol (25–200 mM) was added simultaneously with prostaglandin E_1, there resulted a dose-related increase in prostaglandin E_1-stimulated cAMP formation (Rabbani et al., 1999). The percentage enhancement of prostaglandin E_1-stimulated adenylyl cyclase activity by 200 mM ethanol in cells not chronically treated with ethanol was 46% (Fig. 3). When the HEL cells were chronically treated with 50 or 100 mM ethanol for 48 h, the percentage enhancement of the prostaglandin E_1-stimulation of adenylyl cyclase activity by 200 mM ethanol (acute) was decreased by 33% and 80%, respectively.

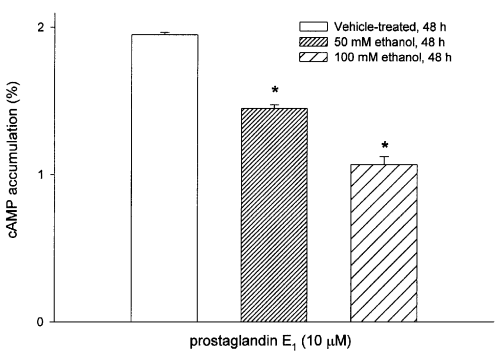

Fig. 2. Effect of chronic ethanol treatment on prostaglandin E_1-stimulated adenylyl cyclase activity in HEL cells. Cells were incubated in the presence or absence of ethanol (50 or 100 mM) for 48 h, and then washed and resuspended in ethanol-free medium before being challenged with prostaglandin E_1 (10 μM). Cells were incubated for 5 min in the presence of prostaglandin E_1 and the [^{3}H]cAMP accumulation during this time was measured as described in materials and methods. Basal adenylyl cyclase activity (% conversion of [^{3}H]ATP to [^{3}H]cAMP) was 0.1 ± 0.01 (means $\pm$ S.E.M., $n = 3$) and did not vary with chronic ethanol treatment. With 10 μM prostaglandin E_1, the activity increased to 1.59 ± 0.09% conversion (means $\pm$ S.E.M.). Results represent means $\pm$ S.E.M. from three experiments. $^*P < 0.05$, compared with vehicle-treated control.

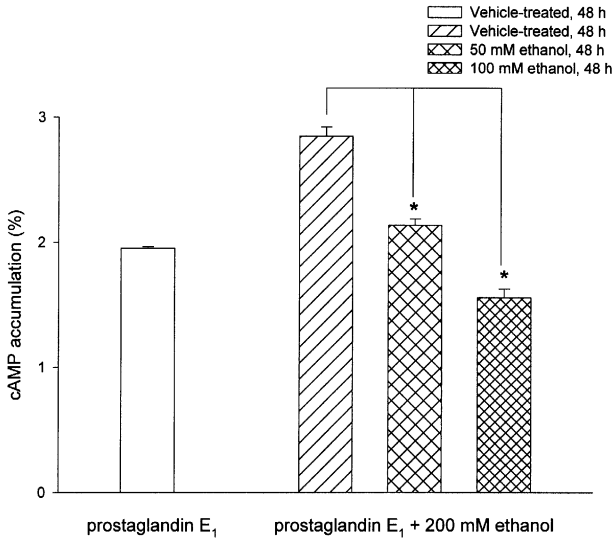

Fig. 3. Effect of chronic treatment on ethanol enhancement of prostaglandin E_1-stimulated adenylyl cyclase activity in HEL cells. Cells were incubated in the presence or absence of ethanol (50 or 100 mM) for 48 h, and then washed and resuspended in ethanol-free medium before being challenged with acute ethanol (200 mM) plus prostaglandin E_1 (10 μM). The [^{3}H]cAMP accumulation during the 5-min incubation period in the presence of prostaglandin E_1 was measured as described in materials and methods. Results represent means $\pm$ S.E.M. from three experiments. $^*P < 0.05$, compared with the corresponding control.

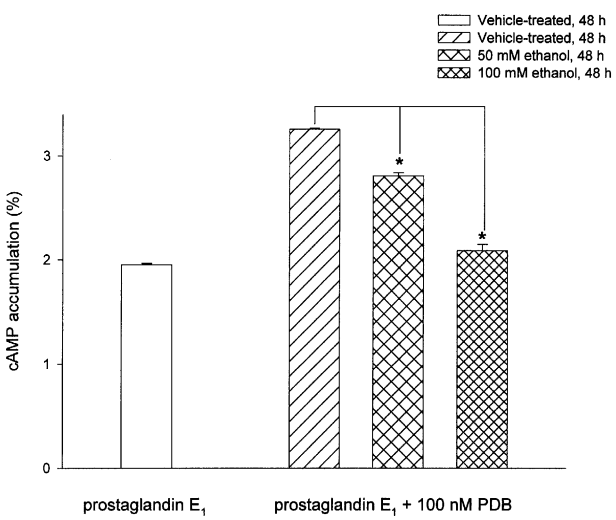

Fig. 4. Effect of chronic treatment on PDB enhancement of prostaglandin E_1-stimulated adenylyl cyclase activity in HEL cells. Cells were incubated in the presence or absence of ethanol (50 or 100 mM) for 48 h, and then washed and resuspended in ethanol-free medium before being challenged with acute ethanol (200 mM) plus prostaglandin E_1 (10 μM). The [^{3}H]cAMP accumulation during the 5-min incubation period in the presence of prostaglandin E_1 was measured as described in materials and methods. Results represent means $\pm$ S.E.M. from three experiments. $^*P < 0.05$, compared with vehicle-treated control.

3.4. Effects of chronic ethanol treatment on PDB enhancement of prostaglandin E_1 stimulation of adenylyl cyclase activity in HEL cells

Treatment of the HEL cells (not previously exposed to ethanol) with PDB resulted in a dose-dependent enhancement of cAMP accumulation (Rabbani et al., 1999). The percentage enhancement of prostaglandin E_1-stimulated adenylyl cyclase activity by 100 nM PDB in cells not chronically treated with ethanol was 65% (Fig. 4). However, when the HEL cells were chronically treated with 50 or 100 mM ethanol for 48 h, the percentage enhancement of the prostaglandin E_1-stimulation of adenylyl cyclase activity by 100 nM PDB was decreased by 16% and 56%, respectively.

3.5. Combined effects of ethanol and PDB on prostaglandin E_1 stimulation of adenylyl cyclase activity in HEL cells chronically treated with ethanol

To gain an insight into the mechanism of adenylyl cyclase activation by ethanol and PDB, we asked whether the combined effects of ethanol and PDB on adenylyl cyclase are also affected by chronic ethanol treatment. In HEL cells not chronically treated with ethanol, combined effects of ethanol (200 mM) and PDB (100 nM) resulted in 102% enhancement of cAMP accumulation (compared with prostaglandin E_1 stimulation alone, Fig. 5). The combined effects of ethanol and PDB in HEL cells chronically treated with 50 or 100 mM ethanol for 48 h, i.e. the percentage enhancement of prostaglandin E_1 stimulation of adenylyl cyclase activity, was reduced by 14% and 56%, respectively (Fig. 5).

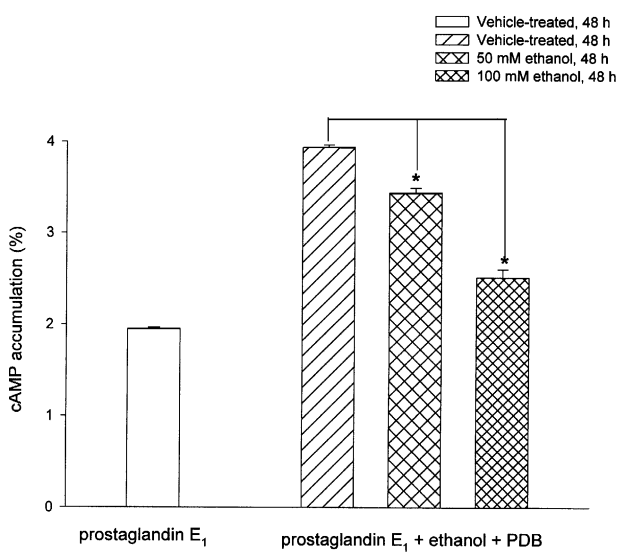

Fig. 5. Effect of chronic treatment on the combined addition of PDB and ethanol on adenylyl cyclase activity in HEL cells. Cells were incubated in the presence or absence of ethanol (50 or 100 mM) for 48 h, and then washed and resuspended in ethanol-free medium before being challenged with acute ethanol (200 mM) plus prostaglandin E_1 (10 μM). The [^{3}H]cAMP accumulation during the 5-min incubation period in the presence of prostaglandin E_1 was measured as described in materials and methods. Results represent means ± S.E.M. from three experiments. * $P < 0.05$, compared with vehicle-treated control.

In all the abovementioned experiments, the increase in cAMP formation caused by ethanol and PDB was only evident when prostaglandin E_1 was present. Under basal conditions (when no stimulatory agent was present) ethanol up to 200 mM and PDB up to 1 M had no significant effect on adenylyl cyclase activity.

4. Discussion

In agreement with previous findings, we showed that ethanol and/or PDB (a protein kinase C activating phorbol ester), when added acutely, both enhance receptor-stimulated adenylyl cyclase activity. In our previous study, we showed that this enhancing effect of ethanol and PDB on adenylyl cyclase activity in HEL cells is dose-dependent (Rabbani et al., 1999). The stimulation of adenylyl cyclase by ethanol and/or PDB required concurrent stimulation by a G_s-coupled receptor agonist (prostaglandin E_1). Ethanol has also been found to enhance G_s-stimutated adenylyl cyclase activity in membranes prepared from various tissues and cultured cells. The effects of ethanol on adenylyl cyclase activity in the membrane, however, may not predict the effect of ethanol on cAMP accumulation in intact cells. For example, Rabe et al. (1990) showed that adenylyl cyclase activity in membranes prepared from two distinct subclones of PC12 cells was enhanced by ethanol, but that cAMP accumulation in intact cells of one of the subclones was inhibited by ethanol.

Our results showed that after chronic exposure of HEL cells to ethanol (50 or 100 mM), the sensitivity of adenylyl cyclase activity to stimulation by prostaglandin E_1 was reduced. The concentration of ethanol at 50 mM is within the range of the blood alcohol level in intoxicated humans. When the cells were exposed to a similar schedule of chronic ethanol treatment, the percentage enhancement of prostaglandin E_1-stimulated adenylyl cyclase activity normally observed with acute ethanol and/or PDB was also reduced. The reduced capacity of ethanol and/or PDB to stimulate adenylyl cyclase activity after chronic ethanol treatment suggests the involvement of adaptive membrane changes. Previous work on neurotransmitter-stimulated adenylyl cyclase activity in the brains of animals fed ethanol has demonstrated a diminished responsiveness of adenylyl cyclase activity to stimulation by guanine nucleotide, isoproterenol, or norepinephrine (French et al., 1975; Saito et al., 1987). The reduced capacity of ethanol to stimulate adenylyl cyclase activity after chronic ethanol treatment was also reported for the platelets of alcoholic subjects (Tabakoff et al., 1988). It has also been shown that the adenosine-stimulated adenylyl cyclase activity in lymphocytes of alcoholics is lower than those in the controls (Diamond et al., 1987). The difference in adenylyl

cyclase activity may be interpreted either as reflecting a response to long-term consumption of ethanol, or as an inherent characteristic of persons with alcoholism. It is not possible from these data to predict how long these changes will last, however, data from other studies indicate the dissipation of most changes after 24 h of withdrawal (Saito et al., 1987). In the present study, we did not observe any difference in basal adenylyl cyclase activity in HEL chronically treated with ethanol as compared with that of the controls.

In addition to ethanol, PDB also was found to lose its capacity to stimulate adenylyl cyclase activity after chronic ethanol treatment. The decrease in adenylyl cyclase activity that we observed after chronic ethanol treatment could have been due to several factors. The fact that both ethanol and PDB lose their acute stimulating effects reflects the involvement of a common denominator in the action of ethanol and PDB. It has been shown that the acute effects of ethanol on adenylyl cyclase activity in HEL cells are dependent on protein kinase C, and compounds such as staurosporine could block the stimulating effects of ethanol on adenylyl cyclase (Rabbani et al., 1999). Chronic ethanol treatment could therefore, result in down-regulation of protein kinase C and consequent loss of ethanol and PDB effects. However, down-regulation of protein kinase C is unlikely to account fully for the reduction in ethanol and PDB stimulating effects on adenylyl cyclase activity. Previous work has shown an increase in the expression of δ and ε protein kinase C after chronic ethanol treatment in a neural cell line (Roivainen et al., 1994). Further studies are required to determine the exact role of protein kinase C following chronic ethanol treatment. Adaptation at receptor or second messenger level could be another explanation for the appearance of a reduction in ethanol and PDB effects. Since both ethanol and PDB require concurrent stimulation by a G_s-coupled receptor agonist (prostaglandin E_1) and are not capable of stimulating adenylyl cyclase activity by themselves, changes at the receptor, G-proteins, level could also be an important consequence of chronic ethanol treatment. Ethanol, acutely, has been shown to have profound effects on various receptors and G-proteins (Hoffman and Tabakoff, 1990).

Acknowledgements

The authors thank Dr. Masami Yoshimura for helpful consultation. This work was supported by the Banbury Foundation.

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