clinical and experimental evidence for the importance of histamine in the development of duodenal ulcer disease has been presented since the introduction of H_2-receptor antagonists [12]. Therefore, FMH may well be useful for the treatment of certain kinds of duodenal ulcer disease associated with high HDC activity in humans.

In summary, FMH administered by either i.p. injection or continuous s.c. infusion leads to marked depletion of histamine levels in urine of *Mastomys* bearing transplantable histamine-producing argyrophilic gastric carcinoid. The continuous s.c. infusion of FMH appears to be superior to i.p. injection for suppressing HDC activity in carcinoid cells. The most conspicuous effect of FMH used *in vivo* on tumor-bearing hosts is to suppress the duodenal ulcer(s) that invariably develops in untreated animals.

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Effect of ethanol on inhibition of striatal adenylate cyclase activity

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The activity of adenylate cyclase is under dual regulation coupled to both stimulatory and inhibitory drug receptors [see Refs. 1 and 2]. Addition of a stimulatory drug, such as glucagon, isoproterenol or dopamine, in combination with a short-chain alcohol results in a synergistic activation of adenylate cyclase [3–5]. Although short-chain alcohols also increase basal adenylate cyclase activity [3–5], this increase is only partly responsible for the alcohol-induced increase in dopamine-stimulated activity in the striatum [4]. Furthermore, the presence of dopamine increases the sensitivity of adenylate cyclase to ethanol [4].

Striatal adenylate cyclase is also coupled to opiate and muscarinic receptors, but activation of these receptors attenuates enzymatic activity [6–10]. Short-chain alcohols have been reported to decrease the affinity of muscarinic and opiate receptors for agonists [11–15], but the effects of alcohol on drug-induced inhibition of adenylate cyclase have not been investigated. The present study was, therefore, undertaken to determine the *in vitro* effects of ethanol on the inhibition of striatal adenylate cyclase by morphine, Leu-enkephalin and acetylcholine.

Methods

Female Holtzmann rats were killed by decapitation and the striata rapidly removed. Tissues were homogenized (Brinkmann Polytron) in 2 mM Tris–HCl (pH 7.5) containing 2 mM ethyleneglycolbis(amino-ethylether)tetraacetate (EGTA), and the homogenates were centrifuged at 20,000 *g* for 15 min at 4°. The resulting pellets after resuspension in the Tris–EGTA buffer were again centrifuged, and this procedure was repeated. The final pellet, which was used to measure adenylate cyclase activity, was resuspended in Tris–EGTA (2.2 to 3.9 mg protein/ml)

Adenylate cyclase activity was determined by measuring the conversion of [α-^{32}P]ATP to [^{32}P]cyclic AMP. Reactions were carried out in a final volume of 150 μl containing 50 mM Tris–HCl (pH 7.5), 5 mM cyclic AMP, 2 mM $MgCl_2$, 50 μM GTP, 1 mM theophylline, 100 mM NaCl, 0.1 mM ATP (containing 1–2 million cpm of [α-^{32}P]ATP), 10 mM creatine phosphate, 0.1 mg/ml creatine kinase and various drugs. Eserine (10 μM) was included in all samples containing acetylcholine. Reactions were carried out for 10 min at 30° and were terminated by the addition of 100 μl of a solution containing 50 mM Tris–HCl (pH 7.5), 5 mM ATP and 10% sodium dodecyl sulfate. After incubating the tubes for 15 min in boiling water, sample volumes were increased to 1 ml with water, and a modification [5] of the method of Salomon *et al.* [16] was used to isolate the [^{32}P] cyclic AMP. Protein content was measured by the method of Lowry *et al.* [17] using bovine serum albumin (Fraction V) as a standard. Data were analyzed for statistical significance using Dunnett's test for multiple comparisons [18].

Results and discussion

Addition of ethanol elicited a linear, dose-related increase in adenylate cyclase activity in both the presence and absence of inhibitory drug (Fig. 1). Although stimulatory drugs have been shown previously to increase the sensitivity of adenylate cyclase to activation by ethanol

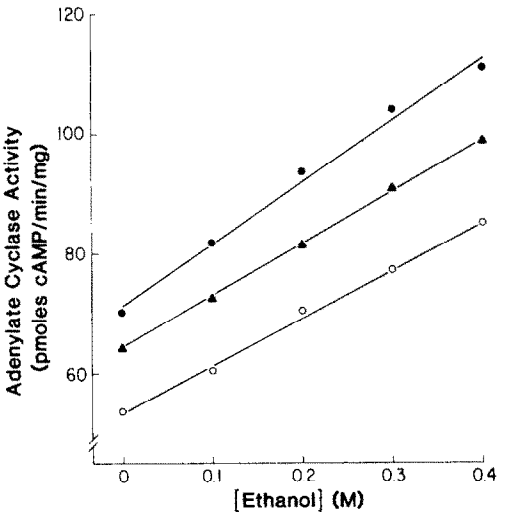

Fig. 1. Effects of acetylcholine and morphine on activation of adenylate cyclase by ethanol. Activation of striatal adenylate cyclase by various concentrations of ethanol was determined in the absence (●) and presence of 100 μM morphine sulfate (▲) or 333 μM acetylcholine chloride (○). Data are plotted as means for seven to eight animals; S.E.M. were less than 7% of the mean.

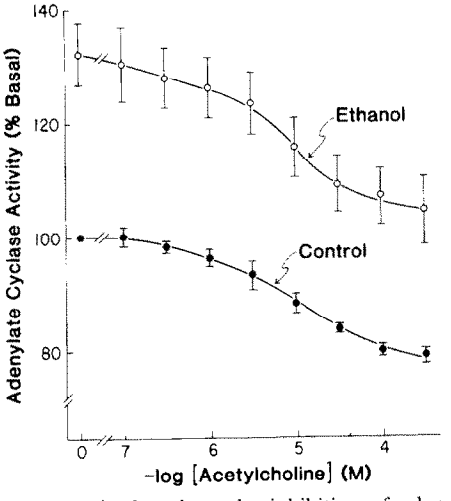

Fig. 2. Effect of ethanol on the inhibition of adenylate cyclase by acetylcholine chloride. Striatal adenylate cyclase activity was measured in the absence (●) and presence of 300 mM ethanol (○). Data are expressed as a percentage of basal which represents enzyme activity in the absence of ethanol and acetycholine (136.5 ± 23.21 pmoles/min/mg). Data are plotted as mean ± S.E.M. for seven to eight animals.

[3–5], inhibitory drugs did not alter significantly the sensitivity of adenylate cyclase to ethanol. The percent stimulation per molar concentration of ethanol was 54 ± 6.3 in control tissue, 37 ± 5.9 in the presence of morphine, and 45 ± 9.3 in the presence of acetylcholine.

In the absence of alcohol, acetylcholine (Fig. 2) and morphine (Fig. 3) caused a dose-dependent decrease in adenylate cyclase activity. Ethanol increased basal activity, but it did not change the maximal percent inhibition elicited by either acetylcholine (21.1 ± 1.65% for control and

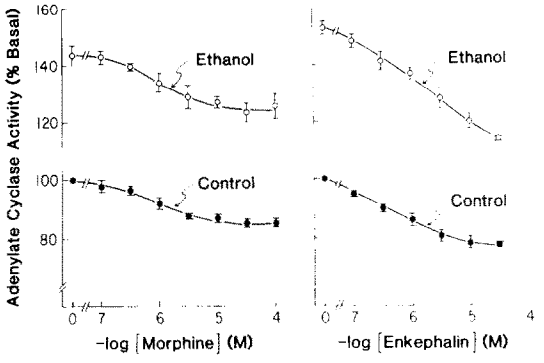

Fig. 3. Effect of ethanol on the inhibition of adenylate cyclase by morphine sulfate (left) and Leu-enkephalin (right). Adenylate cyclase activity was determined in the absence (●) and presence of 300 mM ethanol (○). Data are expressed as a percentage of basal which represents enzyme activity in the absence of ethanol and inhibitory drug. Data are plotted as mean ± S.E.M. for either four (morphine) or six (Leu-enkephalin) animals.

20.6 ± 1.43% in the presence of ethanol) or morphine (14.7 ± 0.77% for control and 12.4 ± 1.65% in the presence of ethanol). Similarly, ethanol had no effect on the IC_{50}* for acetycholine (7.4 ± 2.62 and 7.2 ± 1.99 μM in the absence and presence of ethanol respectively) or morphine (1.0 ± 0.28 and 0.78 ± 0.25 μM in the absence and presence of ethanol respectively). Although morphine-induced reduction in enzyme activity is through the delta rather than the mu receptor [6, 8, 19], the binding of morphine to the delta binding site is complex, with both competitive and non-competitive interactions reported [20, 21]. The *in vitro* effects of ethanol, therefore, were also investigated using Leu-enkephalin (Fig. 3). Ethanol did not change the maximal percent inhibition by Leu-enkephalin (22.1 ± 1.86% for control and 20.9 ± 1.28% in the presence of ethanol) or the IC_{50} value (0.41 ± 0.079 and 0.62 ± 0.170 μM in the absence and presence of ethanol respectively).

In marked contrast to the previously reported synergistic interaction of short-chain alcohols and stimulatory drugs on adenylate cyclase activity [3–5], ethanol did not alter the efficacy of morphine, Leu-enkephalin or acetylcholine to inhibit adenylate cyclase activity. The actions of alcohols on the delta opiate and muscarinic receptors appear to be "competitive" in nature since the affinity for agonists rather than receptor density is decreased [13–15]. Ethanol, however, neither altered the IC_{50} value nor changed the percent inhibition of enzyme activity at any concentration of inhibitory drug.

The lack of an effect of ethanol on the inhibition of adenylate cyclase activity is somewhat surprising since the concentration range of ethanol used in the present study had been shown to decrease the affinity of opiate and muscarinic receptors for their respective agonists [11–14]. The reason for the discrepancy between the reported ethanol-induced decrease in agonist binding and the lack of an effect of ethanol on inhibition of adenylate cyclase is unclear. Different conditions are used to measure adenylate cyclase activity and receptor binding: in particular GTP, sodium chloride and a 30° incubation were used in the adenylate cyclase assay. Although decreasing the assay temperature from 37° to 25° or 24° slightly attenuates alcohol-induced inhibition of [^{3}H]D-Ala,D-Leu-enkephalin binding, both GTP and sodium chloride greatly enhance alcohol-induced inhibition of enkephalin binding [14, 15]. It therefore seems unlikely that the difference in assay conditions is the cause of the discrepant effects of ethanol. Rather, the discrepancy between the effects of ethanol on adenylate cyclase and agonist-binding may be due to an

* Abbreviations: IC_{50}, drug concentrations resulting in 50% inhibition; N_S, stimulatory regulatory subunit; and N_I, inhibitory regulatory subunit.

action on different receptor sites or states. Although inhibition of adenylate cyclase by opiates is through the delta subtype of opiate receptors [6, 8, 19], multiple enkephalin binding sites exist [21]. Thus, alcohol may inhibit binding of enkephalins to a delta binding site that is not coupled to adenylate cyclase. Similarly, acetylcholine inhibits striatal adenylate cyclase through a muscarinic receptor [9], but the muscarinic receptor is coupled to both adenylate cyclase and phosphoinositide metabolism [22]. Alcohol may, therefore, inhibit agonist binding to the form of the muscarinic receptor that is linked to phosphoinositide metabolism rather than to adenylate cyclase.

Both the increase in adenylate cyclase activity by stimulatory drugs and the reduction in enzyme activity by inhibitory drugs require the activation of regulatory subunits [23, 24]. Although N_S and N_I mediate opposite actions on adenylate cyclase activity, the regulatory subunits share a number of similar properties. Both N_S and N_I require GTP and magnesium for activation, and activation involves a dissociation of the subunit into constituent components [23, 25]. Furthermore, N_S and N_I both contain an identical 35,000-dalton (i.e. β) component [26]. However, whereas dissociation of N_S results in activation of the catalytic subunit of adenylate cyclase [23], dissociation of N_I attenuates adenylate cyclase activity by forcing the dissociative activation reaction of N_S toward formation of the inactive holoenzyme [25]. Although ethanol appears to enhance the activation of N_S [5, 27], the present study indicates that ethanol does not alter either the activation of N_I or the reassociation (i.e. inactivation) of N_S. Ethanol thus appears to specifically enhance the dissociative activation of N_S.

In summary, ethanol did not alter the inhibition of striatal adenylate cyclase activity by morphine, Leu-enkephalin and acetylcholine. Thus, while ethanol has been shown to alter the activation of N_S, ethanol does not affect either the activation or activity of N_I. The discrepancy between the previously reported alcohol-induced inhibition of agonist binding and the lack of an effect of ethanol on the inhibition of adenylate cyclase activity may be due to an action of ethanol on different forms of the delta opiate and muscarinic receptors.

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Study of propranolol binding to human α_1-acid glycoprotein by high-performance liquid chromatography

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Propranolol binding to human plasma proteins is known to be high (>90% [1]) with α_1-acid glycoprotein (α_1-AGP) playing a major role in this binding. To determine binding parameters for propranolol bound to human α_1-AGP mostly the equilibrium dialysis (ED) has been used [2–4].

Several high-performance liquid chromatographic (HPLC) methods have been introduced for the study of biomacromolecule–ligand interactions [5–8]. The present paper reports on the application of HPLC as a comparable approach for studying the binding of propranolol to human α_1-acid glycoprotein.