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ANTAGONISM OF SEROTONIN-ACTIVATED ADENYLATE CYCLASE IN THE LIVER FLUKE FASCIOLA HEPATICA BY LEVORPHANOL AND DEXTRORPHAN

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A highly active serotonin (5-HT)* -stimulated adenylate cyclase is present in particles from the liver fluke Fasciola hepatica [1]. This enzyme is activated through a single class of receptors by indoleamines and LSD derivatives. Several recent reports showed that opiates can interact with adenylate cyclase from various tissues [2-4]. In this report we examine the effects of morphine-like drugs upon adenylate cyclase activity in cell free particles from F. hepatica. We report a non-stereospecific inhibition of both basal and serotonin-stimulated cyclase activity and discuss the possible mechanism of this inhibition.

MATERIALS AND METHODS

Particles from liver flukes for adenylate cyclase activity were prepared as described previously [1]. Adenylate cyclase was assayed by the method of Salomon et al. [5] except that 20 μM EGTA was added. Each value is the average of at least duplicate incubations.

The effects of chemical agents on motility were examined subjectively as reported before [6]. Each concentration of a drug was tested on four separate flukes. The motility was evaluated by another investigator and ranked on a 1 to 4 scale. A score of 4 indicated the highest motility.

Biochemicals and radioactive substrates were from the same sources reported before [1]. Levorphanol, dextrorphan and naloxone were gifts from Dr. Brian Cox, Addiction Research Foundation, Palo Alto, CA.

RESULTS AND DISCUSSION

Levorphanol and dextrorphan antagonized the activation of the adenylate cyclase by 5-HT (Fig. 1). Therefore, the antagonistic effect of levorphanol is not stereospecific. By replotting the data presented in Fig. 1 as the Woolf inverse plots (not shown) and fitting the plots by linear regression ($r^2 > 0.99$) it was possible to compute a half-maximal inhibitory concentration of 43 μM for levorphanol and 62 μM for dextrorphan. The effects of levorphanol and dextrorphan on the 5-HT stimulated cyclase were not antagonized but increased in the presence of naloxone. Both morphine and naloxone were shown to inhibit the 5-HT-stimulated adenylate cyclase activity at concentrations even higher than those for levorphanol and dextrorphan (data not shown).

Experiments were carried out to determine whether levorphanol or dextrorphan affects the enzyme activity in the absence of serotonin. No significant inhibition of fluoride-activated cyclase by concentrations of levorphanol or dextrorphan as high as 2.5 mM was found. Basal activity of the adenylate cyclase (enzyme activity with no activators) was decreased and even suppressed by levor-

*Abbreviations: 5-HT, 5-hydroxytryptamine, serotonin; LSD, D-lysergic acid diethylamide; cAMP, cyclic AMP; and EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.

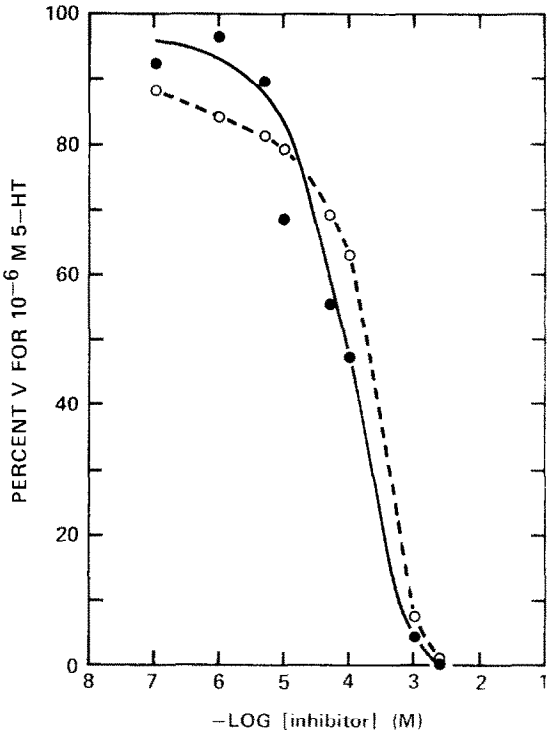

Fig. 1. Inhibition of 5-HT-activated adenylate cyclase by levorphanol and dextrorphan. Levorphanol (●) or dextrorphan (○) was added at the indicated concentrations in reaction mixtures containing 1 μM 5-HT, 100 μM GTP, 100 μM ATP, and 2 mM $MgCl_2$. Adenylate cyclase activity was measured as described in Materials and Methods. Basal velocity was subtracted from all values and the results are expressed as a fraction of the activity due to 1 μM 5-HT alone. One hundred percent adenylate cyclase in both cases was 155 pmoles/min/mg.

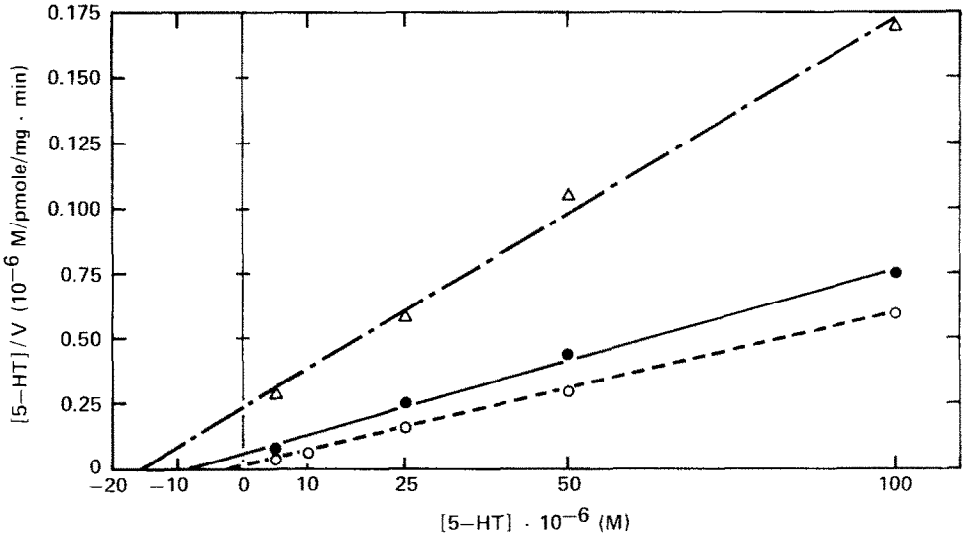

Fig. 2. Woolf plots of the saturation of adenylate cyclase activation by serotonin in the absence or presence of levorphanol. 5-HT at concentrations that varied from 5 to 100 μM was added to adenylate cyclase reaction mixtures containing 100 μM ATP, 100 μM GTP, 2 mM $MgCl_2$ and no (○), 100 μM (●), or 1 mM (△) levorphanol. Adenylate cyclase was assayed at 30° as described in Materials and Methods. Basal velocity was subtracted from all values and the plots were fitted by linear regression ($r^2 > 0.99$).

phanol and dextrorphan. A half-maximal decrease in velocity of basal activity of 250 μM for levorphanol and 218 μM for dextrorphan was found.

The effect of levorphanol on the saturation of activation by serotonin was studied (Fig. 2). The results show an increase of the apparent half-maximal activation constant for serotonin from 2.7 μM with no inhibitor to 8.6 μM in the presence of 100 μM levorphanol or 15.2 μM in the presence of 1 mM levorphanol. Furthermore, maximal activation was decreased from 173 pmoles/min/mg to 142 pmoles/min/mg with 100 μM levorphanol and to 66 pmoles/min/mg with 1 mM levorphanol. The results indicate that the antagonism by levorphanol cannot be explained by pure competition for the binding of 5-HT.

GTP is essential for maximal activation of adenylate cyclase by 5-HT [1]. The effect of levorphanol was tested on the saturation of activation by GTP in the presence of a saturating concentration of 5-HT. The results, presented in Fig. 3, show that the K_A for GTP was unchanged by levorphanol, while the maximal activation was decreased from 414 pmoles/min/mg without levorphanol to 189 pmoles/min/mg with 100 μM levorphanol.

Serotonin, LSD and many other activators of adenylate cyclase stimulate motility in the liver fluke [7]. The effect of levorphanol and dextrorphan was tested on the fluke motility subjectively as described under Materials and Methods. Maximal activation (value of 4) by levorphanol was obtained at 1 mM concentration, while the threshold concentration (minimum effective concentration that caused motility increase) was 10 μM. Dextrorphan also stimulated motility of the fluke with approximately the same potency as levorphanol.

The results presented here show that, while levorphanol acts as an antagonist of serotonin activation of fluke adenylate cyclase, it does not exert its effect through an action on an opiate-like receptor. This is indicated by the fact that the inhibition is not stereospecific and is not reversed by the opiate antagonist naloxone. Levorphanol inhibition appears to occur at a site other than the catalytic component of adenylate cyclase. This is supported by the fact that levorphanol did not inhibit the NaF-activated adenylate cyclase. Furthermore, it inhibited basal activity of the enzyme only at a concentration much higher than those which can inhibit the serotonin-activated enzyme. Kinetic data indicate an action by levorphanol at the serotonin component of the cyclase system, although the inhibition is not strictly competitive with regard to the serotonin binding. Such an effect may also explain why levorphanol causes a decrease in the maximal activation of the cyclase when GTP is titrated in the presence of a saturating concentration of 5-HT. The effect of levorphanol on the serotonin receptor protein presumably influences its ability to associate with the GTP-binding protein. This appears to contrast with the effect of opioids on dopamine-stimulated adenylate cyclase from the brain [4], where morphine does not inhibit the increase in adenylate cyclase activity induced by Gpp(NH)p (a non-hydrolyzable GTP analog). The difference may be due to our contention that the effect of the morphine analogs on the fluke cyclase may be at the serotonin receptors, while it is generally believed that the narcotic receptor in the mammalian system is distinct from the hormone receptor.

Both opiates, while acting as antagonists of 5-HT-activated adenylate cyclase, also stimulated motility of the flukes. These results are in line with what we reported about several other antagonists, including LSD [8]. Unpublished data showed that, like LSD [8], both levorphanol and dextrorphan block the serotonin-induced increase of cAMP in intact organisms.* The relationship between the serotonin receptors that control motility and those that regulate adenylate cyclase deserves more detailed study since it is not necessarily a simple one.

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*S.L. Abrahams and T.E. Mansour, unpublished data.

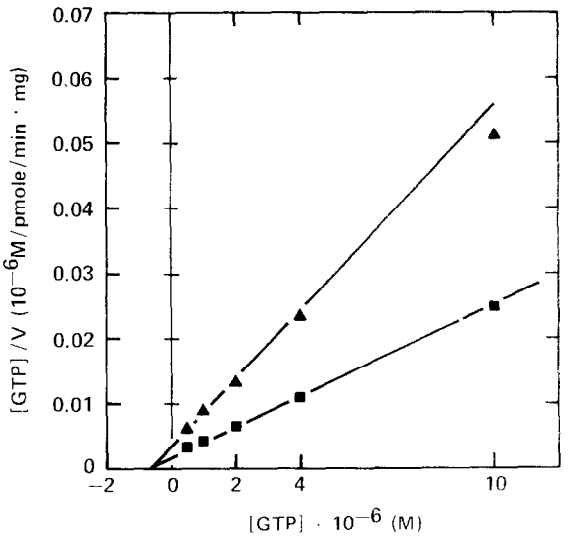

Fig. 3. Woolf plots of the saturation of adenylate cyclase activation by GTP in the presence of a saturating concentration of serotonin, with or without levorphanol. GTP at concentrations between 0.5 and 100 μM was added to adenylate cyclase reaction mixtures containing 100 μM ATP, 100 μM 5-HT, 2 mM $MgCl_2$ and no (■) or 100 μM levorphanol (▲). Adenylate cyclase was assayed at 30° as described in Materials and Methods. The velocity obtained in the presence of 100 μM 5-HT and no GTP was subtracted from all values. The plots were fitted by linear regression ($r^2 > 0.99$). For clarity, values over 10 μM GTP are not shown on the graph but they fit well on the lines.

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