Reports and Letters

Antagonism of 5-Hydroxytryptamine by Chlorpromazine

In the course of studies of the mechanism of the vascular injury induced in rats by a group of agents that damage mast cells and liberate histamine (ovomucoid, 48/80, dextran, and testis extract), it was discovered that 5-hydroxytryptamine as well as histamine is capable of producing hyperemia and edema when injected subcutaneously into rats (*1*). It was also found that 5-hydroxytryptamine or a related substance is associated with mast cells and is liberated along with histamine by the "histamine liberators" (*2*). It has been demonstrated that dibenamine inhibits the *in vitro* action of 5-hydroxytryptamine on the rat colon (*3*). We therefore tested it *in vivo* as an antagonist of the edematous response to 5-hydroxytryptamine and found it a potent inhibitor. It is known that chlorpromazine has antihistaminic activity (*4*) and adrenergic blocking properties similar to those of dibenamine (*5*). Consequently, we examined chlorpromazine for its anti-5-hydroxytryptamine properties and antihistaminic properties *in vivo* in the rat and *in vitro* on the rat colon and found it to be a potent 5-hydroxytryptamine antagonist as well as an antihistaminic (*6*).

Female albino rats (Sprague-Dawley) weighing 160 to 200 g were used in these experiments. Subcutaneous injections of the various agents in solution in freshly made 0.86-percent NaCl were given in the dorsal skin of the paws of rats. Saline was used for control injections. The subcutaneous injections were made by carefully inserting a 27-gage needle between the third and fourth digits of the paws to about the midpoint of the dorsum of the paw; 0.05 ml was injected in the fore paw and 0.10 ml in the hind paw. Each side of the rat, fore and hind paw, was used to measure the action of a single edema-producing agent; two agents were thus tested simultaneously in each rat. Four or more rats were used to test each dose of an agent, and the injections were rotated from rat to rat so that the right and left sides were used an equal number of times for each agent. Evans blue, 0.5 ml of a 0.4-percent solution in 0.86-percent saline, was injected via the tail vein immediately prior to the local injection. Intense bluing of the skin is an evidence of leakage of plasma protein because the dye is bound to the plasma protein. There was little or no bluing of saline-treated paws. The following concentrations of the agents were used: 5-hydroxytryptamine (*7*), 1 μg/ml; and histamine, 200 μg/ml (each as the free base). These concentrations of the edema-producing agents gave approximately the same response as measured either by the increase in tissue water content or by the grossly evident swelling and bluing of the skin (*2*). The local vascular response was graded grossly from 0 to 4+, 2 hours after local injection; at this time the induced edema was still maximal and the swelling from saline injection had largely disappeared. The agreement between the gross observations and the measurements of edema by increase in tissue water, estimated by removal of the skin and drying to constant weight at 100°C for 5 days, was excellent.

Chlorpromazine (*8*) was tested in doses of 0.5 to 3.0 mg/kg of body weight. The drug was administered in 0.86-percent saline solution via the tail vein 15 minutes prior to the local injection of histamine or 5-hydroxytryptamine. With a dose of chlorpromazine of 0.5 mg/kg, little decrease in vascular injury was observed. At doses of 1.0 and 1.5 mg/kg, the action of both histamine and 5-hydroxytryptamine was completely abolished. The 3.0 mg/kg dose produced an observable lethargy, convulsions, and occasional death in the animals.

Chlorpromazine was also tested on the rat colon as antagonist to 5-hydroxytryptamine and acetylcholine. The method used was that of Dalgliesh, Toh, and Work (*9*). Atropine was omitted from the bath fluid. The bath volume was 16 ml. For the test, the strip was standardized to give about one-half maximal contraction to each of the stimulants. For 5-hydroxytryptamine, the quantity was 0.025 μg and for acetylcholine it was 0.05 μg. Exposure of the strip to 10 μg of chlorpromazine for 3 minutes inhibited the reaction to both stimulants by 50 per cent or more. Addition of 20 μg of chlorpromazine to the bath reduced the activity to almost nothing. Complete recovery of activity then required about 30 minutes. Essentially the same result was obtained with strips from 3 rats.

The full range of the specific pharmacological antagonisms of chlorpromazine has not been worked out. The known activities have recently been summarized (*10*). They are mild histamine, acetylcholine, noradrenalin antagonism and powerful adrenalin inhibition. To this we now add the 5-hydroxytryptamine antagonism (*11*). This action is of considerable interest in terms of the rapidly appearing evidence of the importance of this agent as a neurohumor (*12*). Chlorpromazine has been used with good effect in a variety of conditions including mental disease (*13*), nausea and vomiting (*14*), and alleviation of pain (*15*). We suggest that the demonstrated antagonism of chlorpromazine to 5-hydroxytryptamine may contribute to an understanding of the diverse actions of the drug and that the antagonism merits further investigation.

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References and Notes

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6. This work was supported in part by grants from the American Heart Association and grant H-1073 from the National Heart Institute of the U.S. Public Health Service.
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11. Since this report was submitted, it has come to our attention that the antagonism of chlorpromazine to 5-hydroxytryptamine, with the nictitating membrane of the cat as the test object, has been described by J. Lecomte, *Arch. intern. pharmacodynamic* 100, 457 (1955).
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Method for Distinguishing Intact Cells from Free Nuclei

In recent communications, Brown (*1*) and Allfrey and Mirsky (*2*) have stressed the lack of any convenient and reliable method for distinguishing whole cells from free nuclei, particularly in the case of small lymphocytes with very scanty cytoplasm. We wish to draw attention to a simple method that has given promising results with many types of material that we have examined. This is based on