THE EFFECT OF CERTAIN PSYCHOPHARMACOLOGICAL AGENTS ON THE AMEBOID MIGRATION OF HUMAN LEUCOCYTES *IN VITRO*

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THE ameboid migration of human leucocytes in capillary tubes has been shown to be inhibited by relatively low concentrations of hydrocortisone[1]. Further, this inhibitory effect was found to be reversed by the addition of prochlorperazine[2]. The sensitivity of leucocyte migration to these agents suggested that the capillary tube technique might be useful for the study of pharmacological agents at the cellular level. The present report presents some preliminary findings which indicate that certain psychopharmacological agents do have a measurable effect on the ameboid migration of leucocytes.

MATERIALS AND METHODS

In the present experiments freshly drawn, heparinized, human venous blood was separated into cells and plasma by centrifugation. Aliquots of the cells (0·2 ml.) and 0·3 ml. aliquots of the plasma were combined to form the cell suspensions used in these experiments. Serotonin, lysergic acid diethylamide (LSD), and chlorpromazine were dissolved in Hanks's solution[3], and serial dilutions made so that the addition of 0·1 ml. to the cell suspensions would make the desired final concentration. Hanks's solution without the added pharmacological agent was added to the controls. The same cell suspension, plasma, and other reagents were used throughout each experiment.

The preparation of the capillary tubes is described in detail in a previous report[4]. Small bore capillary tubes were filled for about two-thirds of their length by immersing the ends of the tubes in the cell suspension. The unfilled ends of the capillary tubes were sealed, and the tubes were centrifuged towards the sealed ends. This procedure formed a microhematocrit, in which there was a lower layer of packed red cells, an intermediate "buffy coat" of leucocytes, and an uppermost layer of plasma. The tubes were incubated in a vertical position at

37°C overnight, and the leucocytes migrated, by active ameboid motion, through the interstices of the clotted plasma (Fig. 1). The distance which the forward boundary of moving leucocytes migrated from the original buffy coat was measured by means of an ocular micrometer in a microscope. This method measures the fastest cells in the population, which are found to be polymorphonuclear cells.

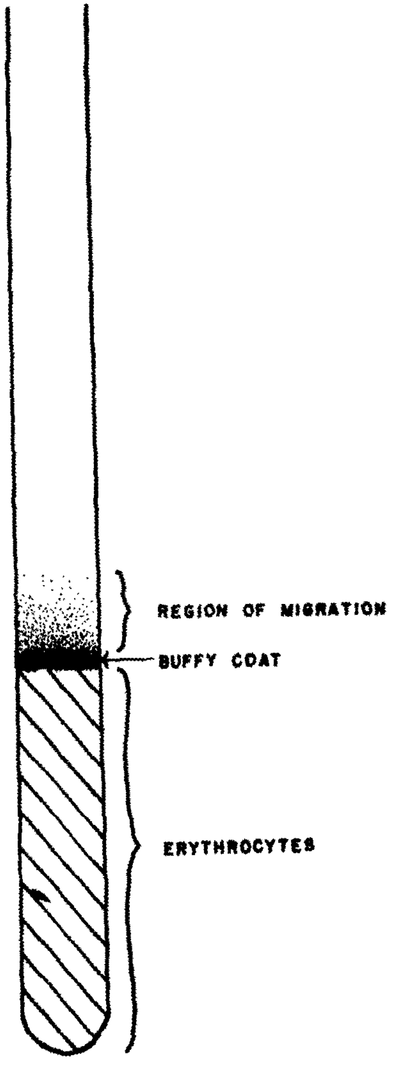

FIG. 1. Diagrammatic representation of the capillary tube method of measuring leucocyte migration.

Ten capillary tubes were prepared for each concentration of the agent tested, and an average value taken. Two identical sets of controls were included in each experiment in order to provide a statistical measure of the internal variation within experiments.

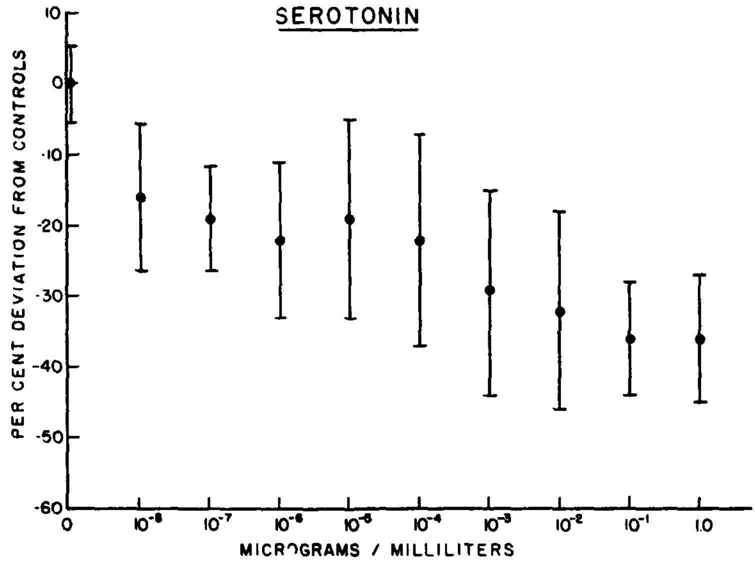

FIG. 2. Effect of serotonin on ameboid migration of leucocytes. Vertical lines on each point represent ± 1 standard deviation.

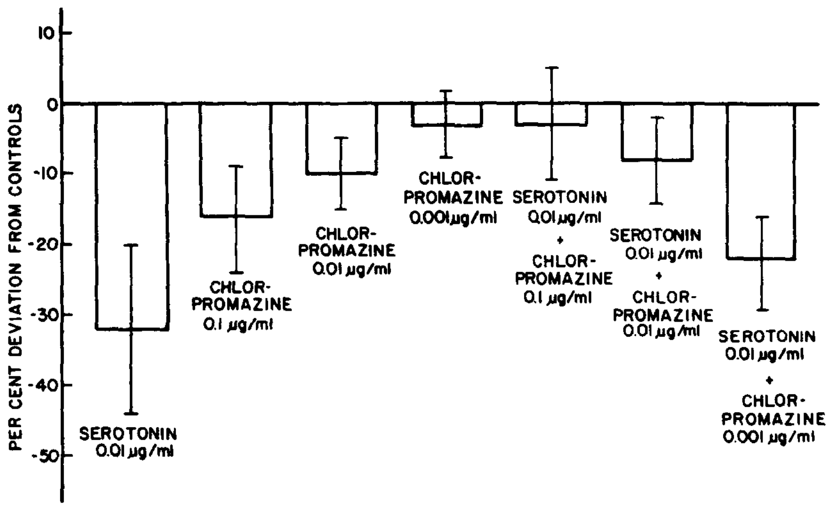

FIG. 3. Effect of serotonin and chlorpromazine alone and in combination on leucocyte migration. Both serotonin and chlorpromazine are able to inhibit leucocyte migration, but when the two substances are added together the inhibitory effect of each is reduced.

RESULTS AND DISCUSSION

The effects of certain specific concentrations of serotonin on leucocyte migration are shown in Fig. 2. In this and in the following figures, the lines above and below the points represent ± 1 standard deviation. Slight inhibition was observed with concentrations of serotonin as low 10^{-8} μg/ml. As the concentration of serotonin was increased, leucocyte migration was further inhibited. With 10^{-1} μg/ml. of serotonin, an average inhibition of 36% was observed. Even at a concentration of 1·0 μg/ml, 100% inhibition was not obtained.

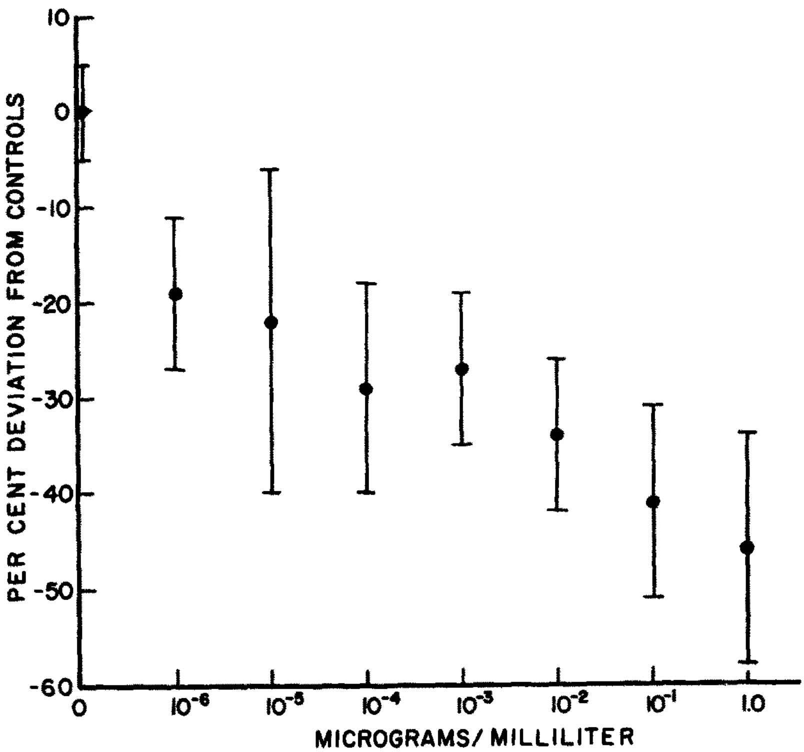

Fig. 4. Effect of a series of concentrations of LSD on leucocyte migration.

In another series of experiments chlorpromazine was added with the serotonin to the cell suspensions. As seen in Fig. 3, 10^{-2} μg/ml. of serotonin alone inhibited leucocyte migration, on the average, by 32%. An average inhibition of 16% was obtained when 10^{-1} μg/ml. of chlorpromazine was added to the leucocyte suspension. However, when 10^{-2} μg/ml. of serotonin and 10^{-1} μg/ml of chlorpromazine were both added to the leucocyte suspension, the inhibition was reduced to an average of 3%. Thus serotonin and chlorpromazine are both able to inhibit leucocyte migration, but when the two substances are added together the inhibitory effect of each is abolished.

The effects of certain specific concentrations of LSD are shown in Fig. 4. Slight inhibition was observed at concentrations as low as 10^{-6}

μg/ml. At a concentration of 10^{-1} μg/ml., an average inhibition of 41% was observed. Even at a concentration of 1·0 μg/ml., however, 100% inhibition did not occur.

The effects of LSD and chlorpromazine added together are shown in Fig. 5. At a concentration of 10^{-2} μg/ml of LSD, 34% inhibition

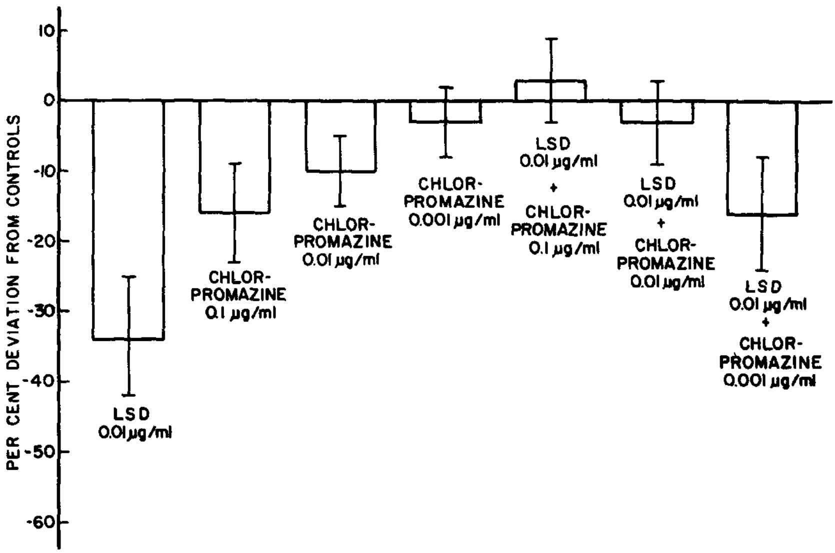

FIG. 5. Effect of LSD and chlorpromazine alone and in combination on leucocyte migration. Both LSD and chlorpromazine are able to inhibit leucocyte migration, but when the two substances are added together the inhibitory effect of each is reduced.

occurred. 10^{-1} μg/ml. of chlorpromazine alone caused an average inhibition of 16%. When 10^{-2} μg/ml. of LSD and 10^{-1} μg/ml of chlorpromazine were added together, no inhibition was observed. Thus, while both LSD and chlorpromazine individually cause an inhibition in leucocyte migration, the two compounds added together do not inhibit leucocyte migration.

Figure 6 shows the results of a series of experiments in which serotonin and LSD were added together. In these experiments 10^{-2} μg/ml. of serotonin alone caused an average inhibiton of 32%, and 10^{-1} μg/ml. of LSD alone caused an average inhibition of 41%. When these same concentrations of serotonin and LSD were added together, the average inhibition was 34%. Thus it appears that the inhibition caused by serotonin and LSD are not additive. When 10^{-1} μg/ml. of chlorpromazine was added with serotonin and LSD, the inhibition was reduced to an average of 3%.

In this study it appears that the ameboid migration of human leucocytes is a sensitive index for the measurement of the action of certain psychopharmacological agents. Serotonin and LSD in relatively low concentrations cause an inhibition of leucocyte migration, and this inhibition may be reversed by chlorpromazine. Further work will be necessary to determine if the study of the ameboid migration of leuco-

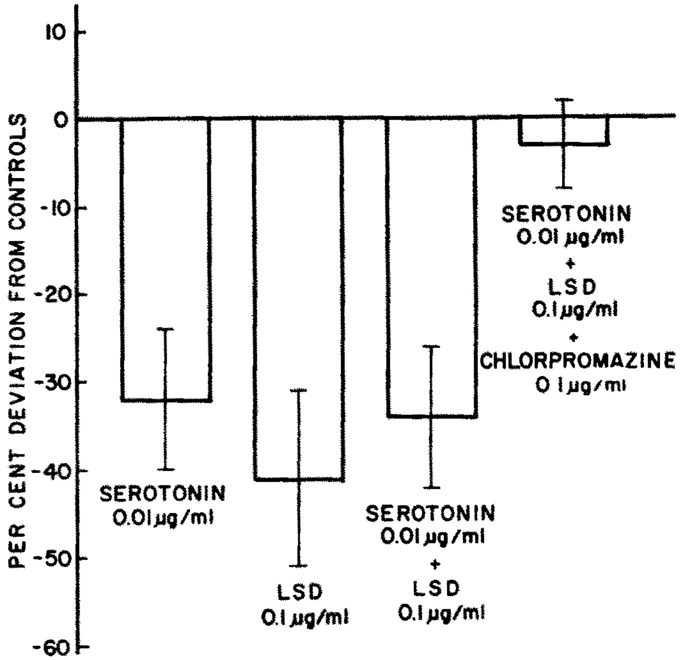

FIG. 6. The inhibition caused by serotonin and LSD added together is not greater than that caused by each agent alone. Chlorpromazine is able to decrease the inhibition caused by serotonin and LSD added together.

cytes will be useful in the elucidation of the mode of action of these agents at the cellular level.

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