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Effect of Biogenic Amines and Cannabinoids on Bacterial Chemotaxis

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The chemotactic response of *Pseudomonas fluorescens* was significantly enhanced by the stimulants DL-amphetamine and epinephrine. Acetylcholine, a physiological antagonist of epinephrine, and the cannabinoid tetrahydrocannabinol inhibited bacterial chemotaxis. It may be possible to use bacterial chemotaxis as a bioassay in biochemical studies of drug action.

Motile bacteria have primitive chemoreceptors (1). These have been associated with feeding responses (5) and with intermicrobial predation (2). Bacterial chemoreception is stimulated by low concentrations of hydrocarbons and pesticides and inhibited by high concentrations of these chemicals (F. Walsh and R. Mitchell, Bacteriol. Proc., p. 159, 1972). In this report, we describe the effect on chemoreception of a number of common biogenic amines and cannabinoids known for their stimulatory or psychotomimetic action when administered to humans.

The test bacterium was *Pseudomonas fluorescens*. Chemotactic response was determined by using the capillary method of Adler (1). Capillaries with a capacity of 1 μliter (Drummond Scientific Co.) were filled with a solution of 1% nutrient broth (Difco) in 0.1-M potassium phosphate buffer (pH 7.0) and closed with silicone grease at one end. The open tip was placed in a suspension of 10^8 bacterial cells per ml on a glass slide. The bacteria were suspended in 0.1 M phosphate buffer (pH 7.8) with or without drug solution.

Cannabinoids were kindly supplied by R. Mechoulam (The Hebrew University Pharmacy School). The other drugs were obtained from "Assia" Chemical Laboratories Ltd., Tel-Aviv, Israel.

The time period used to test the chemotactic response was 10 min (on the basis of the data shown in Fig. 1). Chemotaxis was recorded by direct microscopy observation of bacterial band in the capillary and by making dilution counts of the bacteria in the capillary on nutrient agar. Drugs were added both to the capillary and to the bacterial suspension at various concentrations (5 to 1,000 μg/ml). The cannabinoids were dissolved in distilled water containing 2% Tween 80, as were the other drugs. The response of *P. fluorescens* to various concentrations of several drugs is presented in Fig. 2. Because the lowest concentration of all drugs which significantly affected chemotaxis was 10 μg/ml, this concentration was selected for comparative studies.

In Fig. 3, the effect of DL-amphetamine concentration on the time of bacterial band appearance in the capillary is shown. When drugs were put in a capillary with phosphate buffer, no effect on chemotaxis was observed, indicating that the drugs do not act as attractants.

The normal response of the *P. fluorescens* to nutrient broth (Difco) was a yield of approximately 120,000 bacteria/capillary. The response to Tween 80, the solvent for the cannabinoids, was 17,000 cells/capillary. No more than 7,000 bacteria entered the capillary when phosphate buffer was substituted for nutrient broth as the attractant. When the drugs (10 μg/ml) dissolved in phosphate buffer were used as attractants, no significant differences were observed.

The structurally related compounds, epinephrine and DL-amphetamine, are known to be stimulants to certain muscles of the central nervous system because of their action at the same neuromuscular receptor sites in humans (3). Table 1 shows their effect on bacterial chemotaxis. The addition of 10 μg of epinephrine per ml caused the chemotactic response of *P. fluorescens* to nutrient broth to increase fivefold. The same concentration of DL-amphetamine resulted in an eightfold increase in the attraction of the bacterium to nutrient

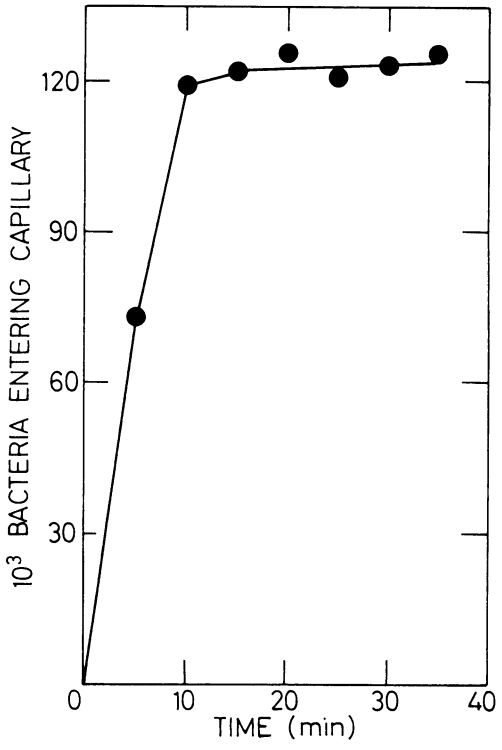

FIG. 1. *Effect of time on chemotaxis of Pseudomonas fluorescens toward a capillary filled with nutrient broth. The initial concentration of bacterial cells in the suspension was 10^8/ml.*

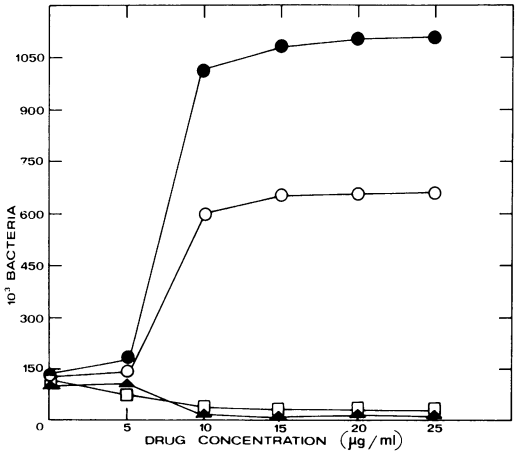

FIG. 2. *Effect of drug concentrations on the response of Pseudomonas fluorescens toward nutrient broth:* (●) DL-*amphetamine;* (○) *epinephrine;* (□) *acetylcholine;* (▲) Δ*1(6)-tetrahydrocannabinol.*

broth. Conversely, acetylcholine, a quaternary ammonium compound, which in man stimulates receptors unaffected by epinephrine or amphetamine (3), inhibited bacterial chemotaxis. Only 40,000 bacteria/capillary were attracted in the presence of 10 μg of acetylcholine per ml, compared with 120,000/capillary in its absence.

The stimulatory effect of DL-amphetamine on bacterial chemotaxis is readily observed by microscopy and Fig. 4a shows the attraction of *P. fluorescens* to a capillary containing nutrient broth. After 10 min, a cloud of bacteria develops at the tip of the capillary. The effect of DL-amphetamine is seen in Fig. 4b. In the same period of time, a much more dense cloud forms at the capillary tip as a result of the stimulation of chemotaxis.

The cannabinoids have a psychotomimetic and sedative effect on humans (3). The active component is Δ1(6)-tetrahydrocannabinol. The three other components of cannabis, cannabinol acetate, cannabigerol, and cannabidiol, are not known to have any effect (4). Table 2 shows the effect of the cannabinoids on chemotaxis of *P.*

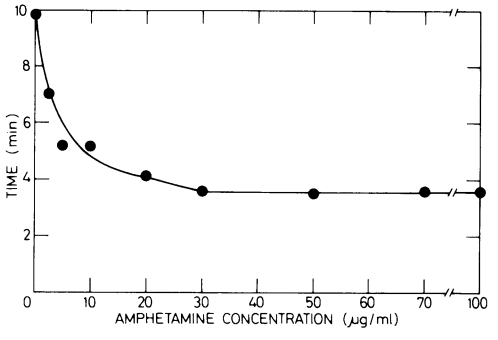

FIG. 3. *Effect of* DL-*amphetamine concentration on the time required for the appearance of a bacterial band at the tip of a capillary dipped in a suspension of Pseudomonas fluorescens (10^8 cells/ml).*

TABLE 1. *Effect of stimulants and sedatives on the chemotactic response of Pseudomonas fluorescens to nutrient broth*

Compound	No. of bacteria attracted/ capillary[a]
Nutrient broth in phosphate buffer (N.B.)	120,000 c
N.B. + epinephrine	600,000 d
N.B. + DL-amphetamine	1,000,000 e
N.B. + acetylcholine	40,000 b
Phosphate buffer	6,500 a

[a] Figures followed by similar letters did not differ significantly at the 5% level by use of Duncan's multiple range test. Each number represents the average of at least seven experiments.

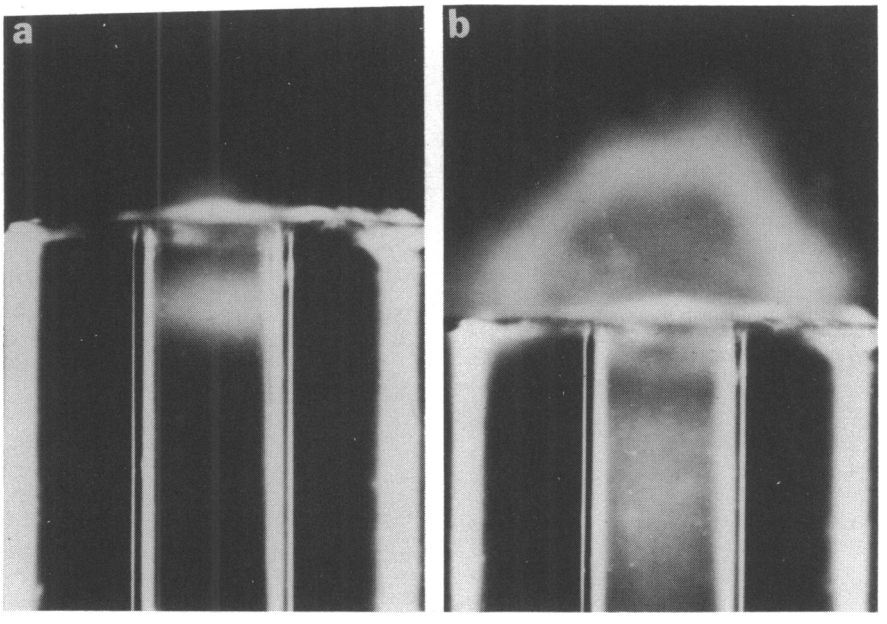

FIG. 4. *(a) Cloud of bacteria formed around tip of capillary containing nutrient broth; (b) stimulation of chemotactic response in the presence of 10 μg/ml of* DL-*amphetamine*

TABLE 2. *Effect of cannabinoids on the chemotactic response of Pseudomonas fluorescens to nutrient broth (dissolved in phosphate buffer)*

Compound	No. of bacteria attracted/ capillary[a]
Nutrient broth (N.B.)	120,000 b
N.B. + cannabinol acetate	120,000 b
N.B. + cannabigerol	140,000 b
N.B. + cannabidiol	160,000 b
N.B. + Δ1(6)-tetrahydrocannabinol	20,000 a
Phosphate buffer	7,000 a
Phosphate buffer with 2% Tween 80	17,000 a

[a] Figures followed by similar letters did not differ significantly at the 5% level by use of Duncan's multiple range test. Each number represents the average of at least seven experiments.

TABLE 3. *Effect of stimulants and sedatives on the chemotaxis of Pseudomonas fluorescens towards* 10^{-3} *M* L-*leucine (in phosphate buffer)*

Compound	No. of bacteria attracted/ capillary[a]
L-Leucine	65,000 b
L-Leucine + epinephrine	280,000 d
L-Leucine + DL-amphetamine	190,000 c
L-Leucine + acetylcholine	12,000 a
L-Leucine + Δ1(6)-tetrahydrocannabinol	6,000 a
Phosphate buffer	8,000 a

[a] Figures followed by similar letters did not differ significantly at the 5% level by use of Duncan's multiple range test. Each number represents the average of at least five experiments.

fluorescens to nutrient broth. Despite the structural similarity of these molecules, only Δ1(6)-tetrahydrocannabinol had any effect on the chemotactic response, causing a dramatic reduction in the number of bacteria attracted from 120,000 per capillary to 20,000 per capillary.

The attraction of the bacteria to specific amino acids such as L-leucine was lower than to that of nutrient broth, but was significant as compared with phosphate buffer (Table 3). Upon chemotaxis, the general effect of the tested drugs towards L-leucine was similar to their effect when nutrient broth was used as attractant (Table 3).

Bacterial motility was measured under a phase-contrast microscope at ×800 magnification by using a Petroff-Hausser bacteria counter and a stopwatch at room temperature. The average motility of 300 bacteria was found to be 15 μm/sec ± 2 μm/sec. No significant difference in motility could be observed in the presence of any of the tested drugs.

Viability of the bacterial cells was tested both

at the beginning and at the end of each experiment by the dilution plate technique by using nutrient agar. None of the drugs used had any effect on cell viability.

Because bacteria are ideal tools for physiological research, it should be possible to utilize bacterial chemotaxis as a bioassay in biochemical studies of chemoreception and in the elucidation of mechanisms of action of biogenic amines, cannabinoids, and other drugs.

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