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Chemosensory Responses of a Protozoan Are ied by Modified by Antitubulins

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Modification of a behavioral response of a marine dinoflagellate to chemical cues is described. Negative response to choline was modified by the antitubulins vincristine, vinblastine, griseofulvin, and trifluralin, but not by colchicine. Positive responses to 3,4-dihydroxyphenylalanine were unaffected by these drugs.

The importance of chemosensory responses in the ecology of aquatic microorganisms is seen in a number of recent studies (3, 7, 11). We have developed a method of observing behavioral responses to various chemicals by the heterotrophic marine dinoflagellate *Crypthecodinium* (*Gyrodinium*) *cohnii*, Puerto Rican strain, a free-living saprophyte often seen in samples of decomposing seaweeds (8) and the subject of nutritional and other studies (9). This species grows well on agar slants and tends to imbed in agar gel in biphasic media (6).

Our assay method is based on the increase or decrease in this tendency to imbed in agar gels containing various chemicals. Equal amounts of Ionagar no. 2 gel made with a saline solution (control gel) and with saline plus the tested chemical (experimental gel) were put in different parts of a partitioned petri dish, and a suspension of organisms in saline solution was poured over the gels to a depth of several milliliters and allowed to stand for 3 h. The saline solution consisted of major nonnutritive salts from our defined maintenance medium (5, 6) and had the following composition (g/100 ml): NaCl, 3.0; $MgSO_4$ (anhydrous), 0.35; KCl, 0.08; $CaCO_3$, 0.05; KH_2PO_4, 0.01; pH adjusted to 6.0 to 6.2. Suspended cells were taken from active log-phase cultures and used at densities of approximately 10^5 cells/ml. After standing, the suspension was poured off, and the gels were gently rinsed with distilled water several times and stained with Gram iodine. Densities of remaining imbedded cells in control and experimental gels were compared by counting cells in microscope fields at 40 random locations in each partition and using the nonparametric Wilcoxon ranked-sign test (10).

We have used this method to test for responses to a number of compounds of possible ecological interest. Positive responses (greater tendency to imbed, with $P = 0.001$ that the results were due to chance) were seen with hemin (5×10^{-8} to 5×10^{-5} M), L-fucose (10^{-6} to 10^{-4} M), betaine (10^{-6} to 10^{-5} M), and dimethyl-β-propiothetin (10^{-7} to 10^{-3} M), all of which occur in the vicinity of rotting seaweeds and could serve as chemical cues for suitable natural substrates. Specificity of the responses was seen in that the analogues D-fucose and dimethylacetothetin were inert (no response was detected). Negative response (less tendency to imbed) occurred to choline (10^{-7} to 10^{-4} M) as choline-hydrochloride, choline bitartrate, and choline citrate. A strong positive response was observed with the amino acid 3,4-dihydroxyphenylalanine (5×10^{-7} to 5×10^{-4} M). Further results with a number of sugars, amino acids, amines, and neurochemicals are reported elsewhere (4).

The spatial distribution of imbedded cells is nonrandom. If cells were imbedded at random one would expect sample counts from a particular gel to have a Poisson distribution, with expected value of the mean equal to that of the variance. In all our observations, in both experimental and control gels, the variance of counts was significantly higher than the mean, usually by an order of magnitude. This as well as direct observation indicates that cells were not distributed randomly but rather in clumped distribution (10). This precludes standard (parametric) statistical methods such as the t test and led to the use of the Wilcoxon ranked-sign test; it also suggests that cells may be attracted not only to the agar but also to each other, perhaps through pheromones.

In an effort to find the mechanism(s) of these chemosensory responses we have sought chemicals that would alter or inhibit them. Since microtubules are prominent in the ultrastructure of nerve cells, and many metazoan sensory cells including chemoreceptors have a modified axonemal ultrastructure, we were led to try the

effects of antitubulin compounds. Some of these are known to inhibit specific behavioral responses to sex pheromones in insects (2) and chemotaxis of leucocytes through filters (1). Preliminary studies indicated some effects on negative, but not on positive, responses, and so we have tried the effects of antitubulins on the negative response to choline and, for comparison, the positive response to 3,4-dihydroxyphenylalanine.

Antitubulins were added to the cell suspension 3 h before pouring onto the agar plates. The results are shown in Table 1. The *Vinca* alkaloids vincristine and vinblastine had an effect on the response to choline (10^{-4} M), whereas colchicine did not. This occurred at antitubulin concentrations of 10^{-7} to 10^{-4} M, where differences in the swimming of the protozoan or its ability to imbed in the control agar gel were not detected. When vincristine SO_4 was added the negative response to choline was abolished and experimental and control gels were indistinguishable by our test. When vinblastine SO_4 was added the negative response to choline was replaced by a positive one.

We also tested griseofulvin and trifluralin, both known to inhibit microtubule formation and regeneration in protozoa (12, 13). Griseofulvin (10^{-8} to 10^{-6} M) abolished the negative response to choline. Results to date with the herbicide trifluraline are somewhat variable, but it is clear that there is an effect. In several experiments we observed a strong reversal (change from negative to positive) of the choline response when trifluralin was added at 3×10^{-7} M. We detected no abnormalities of motion or ability to imbed in control gels with griseofulvin or trifluralin at these concentrations.

In comparison experiments we detected no alteration of the positive response to 3,4-dihydroxyphenylalanine in the presence of any of these compounds, which suggest that the receptor(s) and perhaps also the signal-transmitter mechanisms involved may be quite different.

The difference in the effects of *Vinca* alkaloids and colchicine is of interest since the latter is known to act on a different part of the tubulin molecule than the former (13). The fact that griseofulvin and trifluralin, antitubulins with very different chemical structures, are also active is further evidence for microtubular involvement in chemoreception in *C. cohnii*. These results and those with insects (3) and human macrophages (2) suggest that microtubular involvement in chemoreception may be phylogenetically widespread and thus may represent a primitive tubulin function in eukaryotes.

TABLE 1. *Effect of addition of antitubulins to a cell suspension of C. cohnii*[a]

Antitubulin	Choline (10^{-4} M)	DOPA[b] (10^{-4} M)
Control	−	+
Vincristine SO_4 (10^{-7} to 10^{-4} M)	0	+
Vinblastine SO_4 (10^{-7} to 10^{-4} M)	+	+
Trifluralin (3×10^{-7} M)	+[c]	+
Griseofulvin (10^{-8} to 10^{-6} M)	0	+
Colchicine (10^{-7} to 10^{-4} M)	−	+

[a] −, Negative response; +, positive response; 0, random response (no response detected).
[b] DOPA, 3,4-Dihydroxyphenylalanine.
[c] Variable.

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LITERATURE CITED

1. Bandmann, U., L. Rydgren, and B. Norberg. 1974. The difference between random movement and chemotaxis. Exp. Cell Res. 88:63-73.
2. Block, E. F., and W. J. Bell. 1974. Ethometric analysis of pheromone receptor function in cockroaches. J. Insect Physiol. 20:99-103.
3. Chet, I., W. Fogel, and R. Mitchell. 1971. Chemical detection of microbial prey by bacterial predators. J. Bacteriol. 106:863-877.
4. Hauser, D. C. R., M. Levandowsky, and J. Glassgold. 1975. Ultrasensitive responses of a protozoan to epinephrine and other neurochemicals. Science 190:285-286.
5. Hauser, D. C. R., M. Levandowsky, S. H. Hutner, L. Chunosoff, and J. S. Hollwitz. 1975. Chemosensory responses by the heterotrophic marine dinoflagellate *Crypthecodinium cohnii*. Microb. Ecol. 1:246-254.
6. Keller, S. E., S. H. Hutner, and D. E. Keller. 1968. Rearing the colorless marine dinoflagellate *Crypthecodinium* cohnii as a biochemical tool. J. Protozool. 15:792-795.
7. Nohmi, M., and K. Tawada. 1974. The negatively charged protein extract from *Tetrahymena pyriformis* as an attractant in *Amoeba proteus* chemotaxis. J. Cell Physiol. 84:135-140.
8. Pringsheim, E. G. 1963. Farblose Algen. G. Fischer Verlag, Berlin.
9. Provasoli, L., and K. Gold. 1962. Nutrition of the American strain of *Gyrodinium cohnii*. Arch. Mikrobiol. 42:196-203.
10. Siegel, S. 1956. Non-parametric statistics for the behavioral sciences. McGraw-Hill, New York.
11. Straley, S. C., and S. F. Conti. 1974. Chemotaxis in *Bdellovibrio bacteriovous*. J. Bacteriol. 120:549-551.
12. Tartar, V., and D. R. Pitelka. 1969. Reversible effects of antimitotic agents on cortical morphogenesis in the marine ciliate *Conhylostoma magnus*. J. Exp. Zool. 172:201-218.
13. Wilson, L., J. R. Bamburg, S. B. Mizel, L. M. Grisham, and K. M. Creswell. 1974. Interactions of drugs with microtubule proteins. Fed. Proc. 33:158-159.