Austin Environmental Sciences

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Austin Publishing Group

Special Article: Biodiversity and Conservation

Reactions of Diatom Ulnaria Ulna on Neurotransmitters and Their Antagonists

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Received: April 13, 2023
Accepted: May 17, 2023
Published: May 24, 2023

Abstract

Exogenous compounds known as neurotransmitters acetylcholine, dopamine, histamine and serotonin and their antagonists d-tubocurarine, muscarine, yohimbine, tavegyl and inmecarb were tested on unicellular diatom algae *Ulnaria ulna*, where they demonstrated signaling and regulatory functions. The most primitive single-cellular organisms like diatoms exhibit a marked sensitivity to neurotransmitters and their antagonists, indicating a possible reception mechanism, similar to those observed in mammals.

Keywords: Acetylcholine; Antagonists; Biogenic amines; Diatoms; Dopamine; Histamine; Luminescence microscopy; Reception; Serotonin

Introduction

In biocenosis, neurotransmitters secreted by plants, animals, or microorganisms are exogenous signals. Chemical interactions, involving biogenic amines and acetylcholine, may play the important signaling role in the normalization and manifestation of stress [1,2]. Exogenous neurotransmitters may regulate the growth and development of not only highly organized multicellular plant organisms, but also unicellular ones, and thereby play a significant role in plant life. However, there is small information how it takes place in water medium. It is known experimental data dealt with the determination and effects of biogenic amines in unicellular algae *Chlorella* [3] and in multicellular algae *Ulvaria obscura* and *Chara vulgaris* [4,5]. The data demonstrated the influence of the compounds on animal organisms - planarians and mollusks [4,5]. The dopamine in green algae *Ulnaria obscura* prevented the organism from the eating by herbivorous snails, crustaceans and urchins [6].

Among water inhabitants the simple unicellular diatom algae *Ulnaria ulna* demonstrated the cholinesterase activity [7] that relates to hydrolysis of exogenous acetylcholine secreted with other organisms.

Moreover, this object produced biogenic amines- dopamine, histamine and serotonin itself [8]. It seems useful in our present study to observe this model cellular system for analysis of its reactions on exogenous neurotransmitters and their antagonists in order to know possible sensitivity and receptor mechanisms.

Materials and Methods

The object of study of diatoms *Ulnaria ulna* (Nitzsch) Compere from seven. Bacillariophyta (lines 2.0-419 and 2.0-903) cultured in 100ml vessels on a nutrient medium that included KH_2PO_4 6.63, $CaCl_2$ 6.51, NaCl 3.47, $MgCl_2$ 5µg/L and silica gel *(Fluka*, Austria) 2µg/L as a silicon source [8].

Growth of culture was controlled according to autofluorescence at 680 nm related to the chlorophyll which contained in the photosynthetic organisms (Figure 1). The main parameter for observation is the amount of the red fluorescing cells of each probe on slaids in 10 fields of the microspectrophotometer/microfluorimeter *MSF-15* (LOMO, Russia) and luminescence microscope Leica DM 6000B (США-Австрия). Number of red-light fluorescent cells in 10 fields of view of a luminescent

Citation: Roshchina VV. Reactions of Diatom Ulnaria Ulna on Neurotransmitters and Their Antagonists. Austin Environ Sci. 2023; 8(1): 1092.

microscope were counted on an object slide. Results were expressed statistically with a standard error of mean + SEM of 4 replications (n=4 object slides) for each variant and control.

In the work, we used following reagents: neurotransmitters acetylcholine chloride, dopamine (*Sigma-Aldrich*, USA), histamine (*Fluka*, Switzerland) and serotonin (*Sigma-Aldrich*, USA) and their antagonists d-tubocurarine, muscarine, yohimbine, (*Sigma-Aldrich*, USA) tavegyl (clemastin) and inmecarb (*ICN Pharmaceuticals*, USA).

The main probes from the growth vessels were given after 7 days of the beginning of the experiments.

Results and Discussion

The unicellular diatom *Ulnaria ulna,* despite its simple structure, has a noticeable sensitivity to exogenous acetylcholine and biogenic amines, which are always present in the pond due to algae and animals (Figure 2). After 7 days of the experiment, histamine and serotonin in a concentration of 10^{-6} M stimulated the growth of the population in a comparison with the control without any additions, while acetylcholine - in a much higher concentration 10^{-4} M (perhaps, released cholinesterase hydrolyzed the small amounts of the substrate). However, excess of serotonin (10^{-4} M) decreased the population of the cells. Unlike acetylcholine, histamine and serotonin, dopamine reduces the number of cells at all tested concentrations, while serotonin - only at 10^{-4} M. Moreover, exogenous dopamine of the probe during 1 day of the experiments was transforming to dopaminechrome (rose color of the medium at concentrations 10^{-5}-10^{-4} M), and later to black melanin. We supposed that the cause of the blocking effects connected with toxic action of dopaminechrome. The formation of dopaminechrome was observed in the sea multicellular algae *Ulvaria obscura* that may be a part of survival strategy from animals' defense [6].

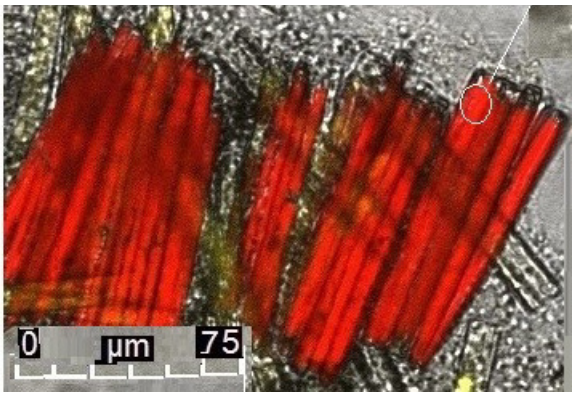

Figure 1: Images of fluorescing diatom *Ulnaria ulna* after excitation by light 430 nm. Amount of the red-fluorescing cells was counted.

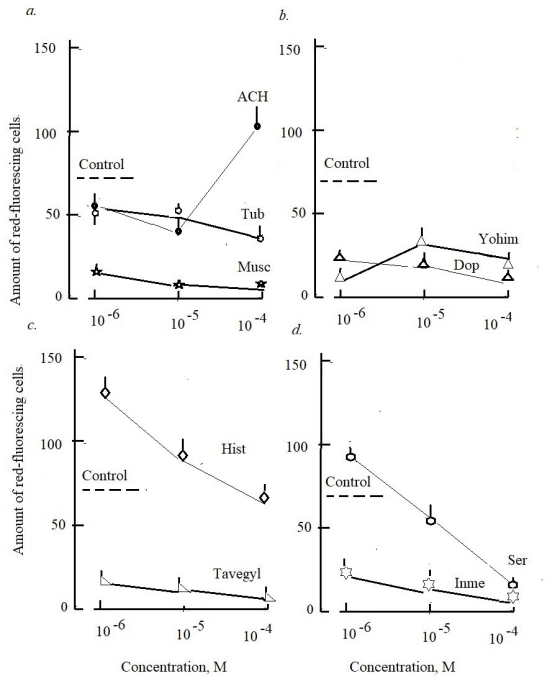

Figure 2: Effect of exogenous neurotransmitters and their antagonists on growth and development of diatoms *Ulnaria ulna* L. Control - without additions 63±6 cells. ACH –acetylcholine, Tub and Musc – tubocurarine and muscarine, Dop and Youhim – dopamine and yohimbine, Hist – histamine, Ser and Inme – serotonin and inmecarb.

Antagonists of our studied neurotransmitters acting on the appropriate receptors of mammalians also were used in the work (Figure 2): d-tubocurarine and muscarine (acetylcholine receptor blockers), yohimbine (dopamine receptor blocker), tavegyl (histamine receptor blocker) and inmecarb (serotonin receptor blocker). D-Tubocurarine and muscarine reduced the number of cells to varying degrees (Figure 2a) by preventing the connection of acetylcholine with cholinoreceptor. Most significant drop in the growth was observed for muscarine that relates to muscarine type of animal cholinoreceptor, while the inclusion of nicotinic receptors linked the the d-tubocurarine blocking seems to be not so big. In experiments where before acetylcholine 10^{-4} M addition, the antagonist d-tubocurarine was added, we saw rarely yellow cells lack of chlorophyll. It means that nicotinic cholinoreceptor are also present in the diatoms. This tendency we observed and after 14 days of experiments.

Another picture was for dopamine antagonist yohimbine acting on adrenoreceptors (Figure 2b). Toxic effects of dopamine combined with inhibitor yohimbine. Preliminary treatment with the antagonist in all used concentrations and then addition of dopamine has given only negative effects with yellow cells without chlorophyll and completely inhibition of population.

If we used tavegyl against histamine receptors (Figure 2c), all blockade of the population growth was seen similar with variant of yohimbine-dopamine link. Preliminary addition of tavegyl before histamine did not revised the antagonist negative effect. Only yellow cells were rarely seen.

Analogous picture was in the case of the serotonin antagonist – inmecarb (Figure 2d) that completely blocked the growth of population either without serotonin or after post-inhibitory addition of serotonin. The addition of neurotransmitters did not lead to an increase in the number of cells in the samples. Thus, the most primitive single-celled organisms exhibit a marked sensitivity to neurotransmitters and their antagonists, indicating a possible mechanism of reception similar to that observed in mammals.

Exogenous biogenic amines affect the growth and development of not only highly organized multicellular plant organisms, but also unicellular ones, and thereby play a significant role in plant life. According to [3], unicellular algae *Chlorella vulgaris* strain ALP was cultivated in the medium included 1, 10, or 100µM of dopamine, histamine, or serotonin and counted using a light microscope. Serotonin caused a slight increase in biomass yield at a concentration of 10µM, but not at the other tested concentrations. 1 and 10µM (but not 100µM) dopamine increased the cell number in the *C. vulgaris* culture at early cultivation stages. Histamine is the most efficient growth stimulator at concentrations of 1 and 10µM, but not at a concentration of 100µM, which even proved inhibitory to the algal culture.

The data obtained demonstrate that the neurochemicals exert a stimulatory influence on the growth of the *Chlorella* culture at relatively low (micromolar) concentrations In multicellular systems such as algae *Chara vulgaris* [5,6] cells released dopamine and histamine that may act on the behavior of planarians and mollusks. Since animals often produce biogenic amines in response to stress or injury, the data give grounds for the suggestion that planktonic algae can benefit, in terms of growth rate, from the substances released by stressed or wounded representatives of aquatic fauna [3]. In biotechnological terms, the data obtained hold some promise with regard to developing a relatively economical technique of boosting *Chlorella* biomass production [3].

Communication between the organisms of various kingdoms in water biocenosis with a participation of neurotransmitters takes place in any case [9,10]. Neurotransmitters may be considered as communicative agents in aquatic ecosystems [10]. It should mark that sometimes in water systems there are big amount of serotonin [11] and histamine [12] as the source of toxicity and stress. The contribution of algae excretions with the neurotransmitters may also influence the health of the biocenosis and organisms served for human nutrition.

Conclusion

Exogenous compounds known as neurotransmitters acetylcholine, dopamine, histamine and serotonin demonstrated signaling and regulatory functions in unicellular plant diatom *Ulnaria ulna*. The most primitive single-cellular organisms like diatoms exhibited a marked sensitivity to neurotransmitters and their antagonists, indicating a possible reception mechanism, similar to those observed in mammals.

Author Statements

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Victoria V. Roshchina, the author of main conception of the work, receiver of all experimental data, and she has written the paper.

Data Availability

The datasets generated during and/or analyzed during the current study are available in the [NAME] repository, [PERSISTENT LINK TO DATASETS].

Ethics Approval

There is no research involving animals, their data or biological material.

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