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Research Article

Sensitivity of Diatom *Ulnaria ulna* to Catecholamines and their Possible Reception

Victoria V Roshchina*

Laboratory of Intracellular Signaling, Institute of Cell Biophysics, Federal Research Centre, Pushchino Scientific Centre for Biological Research, Russian Academy of Sciences, Pushchino, Moscow Region, Russia

***Corresponding Author:** Victoria V Roshchina, Laboratory of Intracellular Signaling, Institute of Cell Biophysics, Federal Research Centre, Pushchino Scientific Centre for Biological Research, Russian Academy of Sciences, Pushchino, Moscow Region, Russia.

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Abstract

Catecholamines-dopamine, noradrenaline and adrenaline found in unicellular plant organisms such as diatom algae *Ulnaria ulna* can regulate the cell amount in the population. The compounds were oxidized on the air oxygen to toxic red aminochromes and then via chain reaction to black pigments melanins that decreased the cell population. The most primitive single-cellular organisms like diatoms exhibit a marked sensitivity to the neurotransmitters and the blockers of animal adrenoreceptors, indicating a possible reception mechanism similar to that observed in mammals.

Keywords: *Adrenaline; Agonists; Antagonists; Biogenic Amines; Diatoms; Dopamine; Luminescence Microscopy; Microspectrofluorimetry; Noradrenaline*

Introduction

Chemical interactions in water biocenosis may occur with the participation of the compounds known as neurotransmitters in mammalians. Neurotransmitters secreted by plants, animals, or microorganisms are exogenous signals [1]. They may play the important signaling role in the normalization and manifestation of stress. The appearance of biogenic amines in cell secretions is a reaction to environmental factors, especially to stress. The presence of dopamine, containing in green algae *Ulvaria obscura,* prevents the organisms from the eating by herbivorous snails, crustaceans and urchins [2,3]. There are experimental data dealt with the determination of biogenic amines dopamine and histamine in algae *Chara vulgaris* [4,5]. The data with *Chara* demonstrated the influence of the compounds on animal organisms - planarians and molluscs [4,5]. A new approach enabling biotechnologists to increase the yield of *Chlorella vulgaris* biomass by means of biogenic amines (serotonin, dopamine and histamine) has been represented that are known to stimulate growth of various unicellular organisms [6,7]. In biotechnological terms, the data obtained hold some promise with regard to developing a relatively economical technique of boosting *Chlorella* biomass production [7].

Among water inhabitants the simple diatom *Ulnaria ulna*, which is met in fresh water, also produced biogenic amines-dopamine, histamine and serotonin [8]. This model cellular system seems to be useful for analysis of possible reactions to exogenous neurotransmitters and antineurotransmitter compounds [9]. 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 water due to algae and animals [8]. 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 [10]). However, excess of serotonin (10^{-4} M) decreased the population of the cells. In some experiments with *Ulnaria ulna* exogenous acetylcholine, histamine and serotonin stimulated the population growth, while their antagonists blocked the process. Among the acetylcholine antagonists were d-tubocurarine and muscarine, while antagonists for histamine and serotonin - tavegyl and inmecarb, relatively [9]. 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. 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 [2,3]. It was the fact that attracts our attention to all catecholamines - dopamine-noradrenaline and adrenaline.

Purpose of the Study

The aim of the article to test effects of these exogenous catecholamines on the *Ulnaria ulna* reactions as possible sensor for the compounds during three weeks after the beginning of the treatment.

Materials and Methods

The object of study color-less diatoms *Ulnaria ulna* (Nitzsch) Compere from seven. Bacillariophyta (lines 2-419 and 2-903) cultured in 100 ml vessels on a nutrient medium that included KH_2PO_4 6.63, $CaCl_2$ 6.51, NaCl 3.47, $MgCl_2$ 5 µg/L and silica gel (Fluka, Austria) 2 µg/L as a silicon source [8]. The free water living cell is well-seen under luminescence microscope as a red-fluorescing organism due to the presence of chlorophyll (Figure 1a).

Growth of culture was controlled according to autofluorescence at 680 nm related to the chlorophyll which contained in the photosynthetic organisms (Figure 1a). The main parameter for observation is the amount of the red fluorescing cells of each probe on slaids in 10 fields of the luminescence microspectrophotometer/microfluorimeter MSF-15 (LOMO, Russia) and luminescence microscope Leica DM 6000 B (USA-Austria). Number of red-light fluorescent cells in 10 fields of view of a luminescent microscope were counted for every variant (three repetitions). The amount of cells were expressed as mean + statistic error. The main control probes from the growth vessels were given after 7, 14 and 21 days of the beginning of the experiments (Figure 1b).

Reagents: In the experiments, dopamine, labetalol and prazosin (Sigma-Aldrich, USA), mesatonum or phenylephrine (Dal'chimpharm, Russia), noradrenaline and adrenaline (MedPharm, Russia) were used.

Results and Discussion

Catecholamines dopamine, noradrenaline and adrenaline, known neurotransmitters in mammalians, never were analysed as active exogenous water-contained components of biocenosis. Toxic effects of dopamine for diatom *Ulnaria ulna* population shown earlier [8] attracted our attention, and we studied the cell amount in the solutions of dopamine, noradrenaline and adrenaline during three weeks (Figure 2). Three concentrations 10^{-6}, 10^{-5} and 10^{-4} M were used. Dopamine decreased the amount of diatom cells during first 7 day after the beginning of the experiment, especially strong at highest concentration 10^{-4} M. The external medium looked as dark black, perhaps, due to the formation of black pigment melanin [11]. The inhibition in the amount of cells also demonstrated in the variants with noradrenaline or adrenaline too. However, the colour of the adrenaline medium was changed (rose component appeared) in highest concentration. The same picture was seen for noradrenaline just after 14 days of experiments. After 14 and 21 days of the experiments we saw rose colour in medium at 10^{-4} M. In most cases, there was the complete absence of red-fluorescing living cells at this concentration. Moreover, in variant with adrenaline the medium became black, due to melanin formation. It should mark that either noradrenaline or adrenaline completely

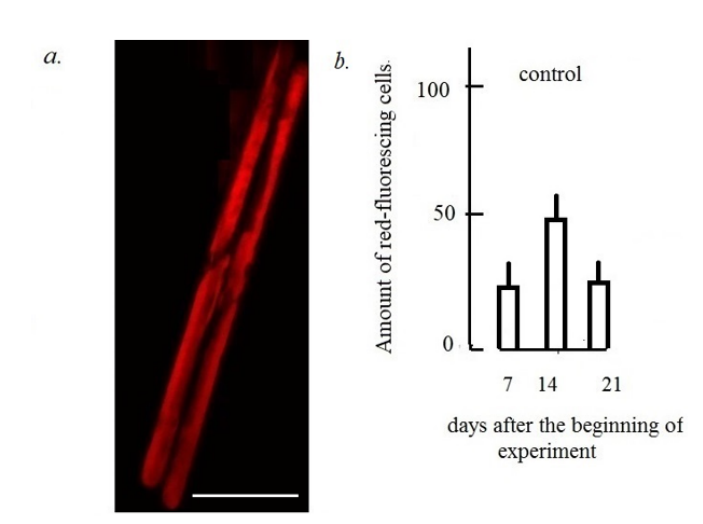

Figure 1: *Image of fluorescing diatom Ulnaria ulna in transmitted an excitation by light 430 nm (a) and the amount of the red-fluorescing cells in control without treatment (b) was counted in control during 7, 14 and 21 days after the beginning of experiment. Bar = 15 µm.*

restored population of cells in small concentration 10^{-6} M in the medium. However, in variants with highest concentrations of dopamine and noradrenaline it did not happen. The difference in the color changes of medium appear to be due the presence of third OH- group in noradrenaline and adrenaline, unlike molecule dopamine that has only two OH- groups (lesser oxidated neurotransmitter may be fast oxidized).

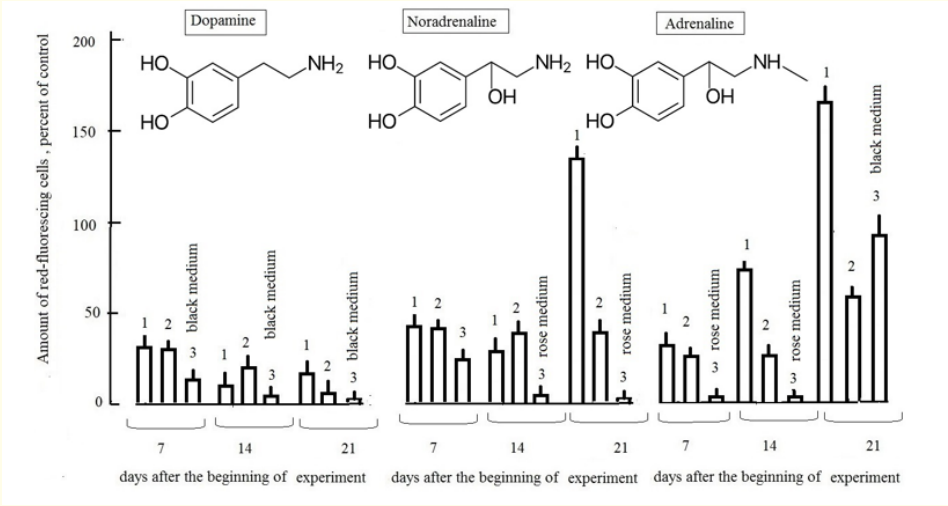

Figure 2: *Effect of exogenous neurotransmitters in concentrations 10^{-6}, 10^{-5} and 10^{-4} M (1, 2, 3, accordingly) on the development of diatoms Ulnaria ulna L.*

The visible oxidation process in the medium containing catecholamines may be connected with a chain reaction of any catecholamine autooxidation in base medium with formation of superoxide-anion radicals [11]. The ending products of the process are red-colored aminochromes (dopaminechrome, noradrenochrome, adrenochrome) and after following oxidation - black pigments melanins. Similar pictures we saw in medium, where diatoms develop that inhibited their population. This event is linked with quinone-like oxidation. The received results with diatoms may be related to free-radical reactions occurred in the medium and also - with mechanisms of adrenoreception, like in mammalian organisms that will be considered below.

If to use reagents acted on the mammalians' adrenoreceptors one can discussed the possible reception of catecholamines by diatom *Ulnaria ulna* plasmatic membrane. Addition of compounds acted on adrenoreceptors of mammalians showed possible effects on the diatom reception (Figure 3). Mesatonum (phenylephrine), adrenomimetic, linked with α-adrenoreceptor, inhibited the diatoms' development to 7 days after beginning of the experiment, but only after 14 days of experiments, and at 21 days stimulated the growth of the population. On the contrary, blocker of α-adrenoreceptor prazosin inhibited the diatom population at all exposures, except weak restoration process after 21 days. This is fact that the reagent blocked α -adrenoreceptor-like component in the algae. Labetalol, which blocked β-adrenoreceptors in mammalians, decreased the amount of the diatom cells only in first 7 days of experiments and later stimulated the population growth at all used concentrations in various degree.

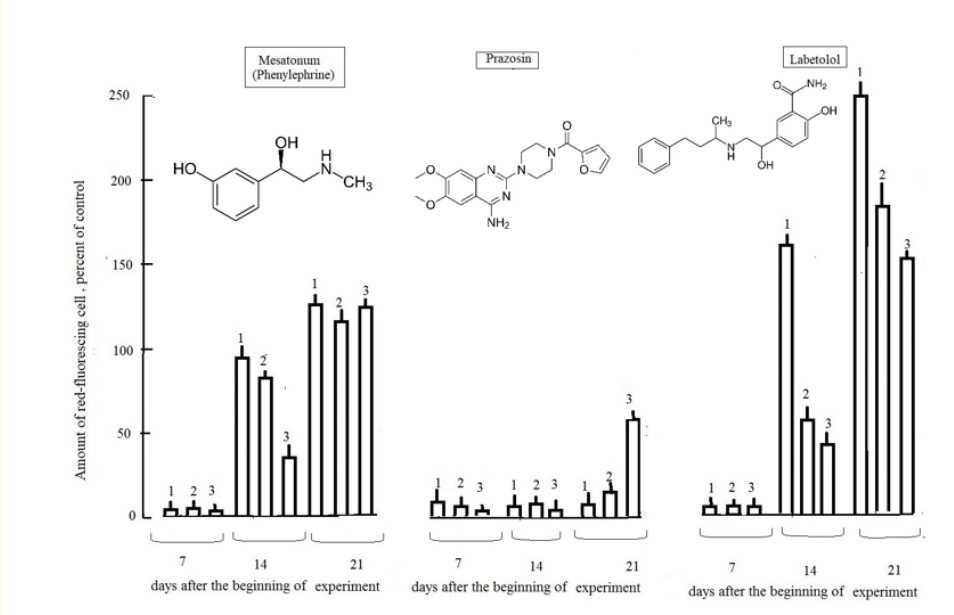

Figure 3: *Effect of exogenous reagents linked with various mammalian adrenoreceptors on growth and development of diatoms Ulnaria ulna L.*

Mesatonum and labetalol inhibited the cell population only to 7 days of the experiment, then the restoration occurs - in weak degree for first compound and more effective for the second one, especially in concentrations 10^{-6} - 10^{-5} M. The most effective block in the α-reception as we saw peculiar, when prazosin was used. Weak increased in cell amount was only after 21 day after the beginning of the experiments. Thus, the most primitive single cell organisms exhibit a marked sensitivity to neurotransmitters and compounds blocked their adrenoreceptors, indicating a possible mechanism of reception similar to that observed in animals.

In the discussion of the neurotransmitters behavior in diatoms, it should mark that 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. If to compare with effects of acetylcholine, serotonin and histamine [9], they can stimulated the population even in small concentrations, unlike catecholamines, which can be oxidized in base medium up to red pigments aminochromes and later, in chain reaction - to black pigments melanins [11]. If the medium has pH < 7, oxidation is weak, if any, but highest pH leads to the formation of toxic aminochromes. Similar toxic reaction for many animal organisms was observed for ocean multicellular algae *Ulvaria obscura* [2].

According to Oleskin with co-workers [6,7], unicellular algae *Chlorella vulgaris* strain ALP was cultivated in the medium included 1, 10, or 100 μM of dopamine, histamine, or serotonin and counted using a light microscope. 1 and 10 μM (but not 100 μM) dopamine increased the cell number in the *C. vulgaris* culture at early cultivation stages. Serotonin caused a slight increase in biomass yield at a concentration of 10 μM, but not at the other tested concentrations. Histamine is the most efficient growth stimulator at concentrations of 1 and 10 μM, but not at a concentration of 100 μM, which even proved inhibitory to the algae 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* [4,5] cells released dopamine and histamine that may act on the behavior of planarians and molluscs. Since animals often produce biogenic amines in response to stress or injury, the data give grounds for the suggestion that planktonic algae growth can be regulated by the neurotransmitters.

Receptor-linked mechanisms needed using of the receptors blockers. In *Ulnaria ulna* all used antagonists d-tubocurarine, muscarine (acetylcholine receptor blockers), yohimbine (dopamine receptor blocker), tavegyl (histamine receptor blocker), and inmecarb (serotonin receptor blocker) reduced the number of cells to varying degrees [9]. Yohimbine [9] and above-used reagents linked with adrenoreceptors of mammalians (Figure 3) can inhibit the growth of the diatom population that confirm possible existence of the similar receptors in *Ulnaria ulna*.

Conclusion

Exogenous neurotransmitters catecholamines-dopamine, noradrenaline and adrenaline have been found to regulate the growth population of unicellular plant organisms such as diatom algae *Ulnaria ulna*. The compounds can be oxidized on the air oxygen to toxic red aminochromes that decreased the cell population. The most primitive single-cellular organisms like diatoms exhibit a marked sensitivity to the neurotransmitters and the blockers of animal adrenoreceptors, indicating a possible reception mechanism similar to that observed in mammals.

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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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