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NEUROPHYSIOLOGICAL CORRELATES OF THE DOPAMINERGIC CILIO-INHIBITORY MECHANISM OF *MYTILUS EDULIS*

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SUMMARY

The neurophysiological regulation of gill ciliary activity by the CNS of the bivalve mollusc *Mytilus edulis* was studied by recording electrophysiological activity of the branchial nerve while simultaneously observing ciliary activity of the lateral ciliated cells of the gill by stroboscopic microscopy. The addition of dopamine to the visceral ganglion slowed and stopped ciliary activity by increasing the firing rate of the cilio-inhibitory dopaminergic neurones of the visceral ganglion which innervate the gill. This could be antagonized at the ganglion by pre-applications of ergonovine or methysergide, or by prior treatments of intact animals with the neurotoxin 6-hydroxydopamine. The study confirms earlier work showing the inhibitory functioning of dopaminergic neurones of the CNS and demonstrates the manner in which they may exert their effects.

INTRODUCTION

Ciliary activity of various bivalve molluscs exhibits spontaneous starting, stopping and changing of beating frequencies, which has suggested that neuronal pathways innervate the ciliated cells of the gill (Nelson, 1960; Aiello & Guideri, 1964; Takahashi & Murakami, 1968). The most frequently studied bivalve with respect to ciliary activity has been *Mytilus edulis*. Peripherally, dopamine and serotonin are considered to be neurotransmitters responsible for decreasing or increasing ciliary activity, respectively (Aiello, 1960, 1970; Gosselin, 1961, 1966; Gosselin, Moore & Milton, 1962; Aiello & Guideri, 1964, 1965, 1966; Paparo & Aiello, 1970; Malanga, 1971,

1974, 1975; Takahashi, 1971; Stefano, Catapane & Stefano, 1977). Stimulating the CNS electrically or by superfusion of dopamine or serotonin also alters ciliary activity (Catapane, Stefano & Aiello, 1978). The CNS and gill of *M. edulis* contain dopamine and serotonin, and the enzymes responsible for their synthesis and degradation (Blaschko & Milton, 1960; Welsh & Moorehead, 1960; Aiello & Guideri, 1966; Stefano & Aiello, 1975; Stefano, Catapane & Aiello, 1976; Stefano & Catapane 1977*a*, *b*; Stefano, Hiripi & Catapane, 1978). The visceral ganglion contains approximately 15 μg/g of dopamine and 20 μg/g of serotonin (Haley, Stefano & Catapane, 1978) while the gill has about 0·50 μg/g of dopamine (Malanga, Wenger & Aiello, 1972) and 1 μg/g of serotonin (Aiello & Guideri, 1966; Stefano & Catapane, 1977*a*). The accumulated pharmacological evidence demonstrates that the gill lateral cilia of *M. edulis* are innervated by cilio-inhibitory dopaminergic neurones and cilio-excitatory serotonergic neurones originating from the visceral ganglion and innervating the gill via the branchial nerve (Catapane, 1976; Catapane *et al.* 1978). The elegant electrophysiological study of Murakami & Takahashi (1975) has demonstrated that a ciliary arrest response is the result of a transient depolarization of the lateral cells as a result of nervous activity of the branchial nerve which can summate resulting in a prolonged arrest response. The response appears to be calcium dependent (Motokawa, Murakami & Takahashi, 1975).

The purpose of this study was to obtain electrophysiological correlates of ciliary regulation, and determine the extent to which the central nervous system regulates ciliary activity.

MATERIALS AND METHODS

Most specimens of *M. edulis* used for these experiments were collected from the shores of Long Island Sound at New Rochelle, N.Y. at low tide and maintained in the laboratory for up to 2 months in an Aquarium Systems temperature-regulated aquarium. Artificial sea water (ASW) was prepared from synthetic sea salts (Instant Ocean Aquarium Systems) dissolved in deionized distilled water and kept at a specific gravity of 1·024 ± 0·001, a pH of 7·2 ± 0·2 at 15–16 °C. Other specimens for experiments performed at the Marine Biological Laboratory, Woods Hole, Ma., were collected from the supply department there and maintained in natural flowing sea water.

VG-preparations used in these experiments were prepared as described by Catapane *et al.* (1978) with a modification to enable electrophysiological recordings to be made. The posteriorly directed fibres of the branchial nerve were carefully freed from the connective tissue sheath and the gill axis and drawn into the lumen of a suction electrode.

The determination of lateral ciliary beating rates were determined stroboscopically as has been described (Catapane *et al.* 1978). Simultaneous electrophysiological recording from the branchial nerve of VG-preparations were performed by conventional techniques by means of platinum suction electrodes, Grass P-511 and P-7A preamplifiers and displayed on a Tektronics 502A Oscilloscope and a Grass Poly 7 Oscillograph. Minimum bandwidth filtering was used to eliminate unwanted signal from the recordings. Permanent records of the electrical activity were made on the

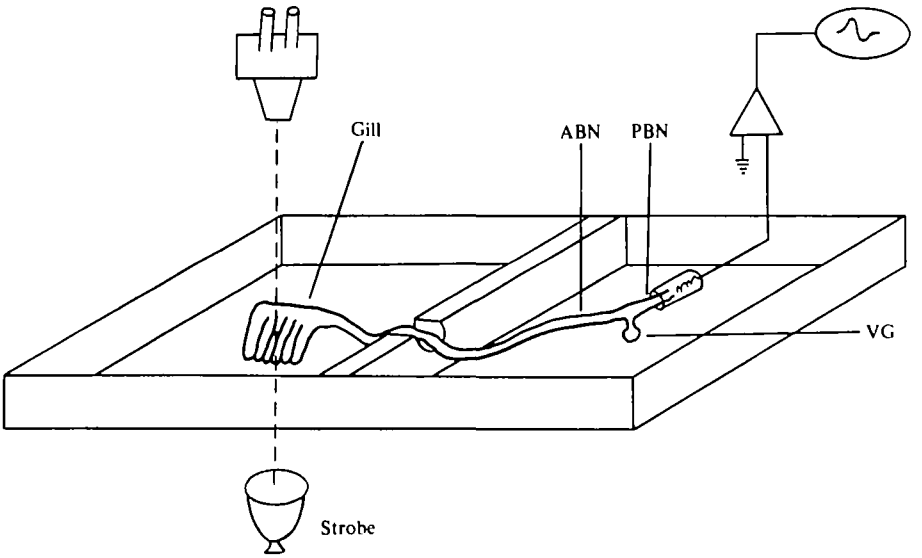

Fig. 1. Chamber for recording nerve activity while observing ciliary activity. Lateral cilia are observed with stroboscopic light while the anterior portion of the gill is bathed in its own part of the chamber. The activity of the posterior branch of the branchial nerve (PBN) is recorded by a suction electrode, a.c. preamplifier and an oscilloscope and oscillograph. The visceral ganglion (VG) is also bathed in its own part of the chamber and the anterior branch of the branchial nerve (ABN) passes over a watertight barrier to innervate the gill. All other parts of the gill have been removed.

oscillograph (from which the spiking rate in impulses/min was determined) while simultaneously observing ciliary activity of the same preparations (Fig. 1).

As described earlier (Catapane *et al.* 1978) observation chambers were used in which a Plexiglass barrier coated with petroleum jelly separated the visceral ganglion from the gill so that chemical stimulations could be specifically applied to either ganglion or gill without leakage to the other chamber.

Dopamine and 6-hydroxydopamine (6-OHDA) were obtained from Sigma, ergonovine (ERG, ergometrine, lysergic acid propanolamide) from Lilly Labs and methysergide (MS, 1-methyl-D-lysergic acid butanolamide) from Sandos Pharmaceuticals. Each was freshly prepared in filtered degassed ASW containing 10 mg% ascorbic acid buffered to pH 7·0 with sodium bicarbonate (ASWA) according to the method of Malanga (1975). The posterior adductor muscles of animals pretreated with 6-OHDA were injected through a notch cut into the dorsal posterior margin of the valves in proximity to the posterior adductor. Each animal was injected with 10 μl of a 10 μg/μl solution of 6-OHDA every other day for 6 days. These animals were maintained in individual 600 ml beakers of aerated ASW at 15–16 °C with daily changes of water for the period. Control animals were injected with vehicle minus the drug and identically treated.

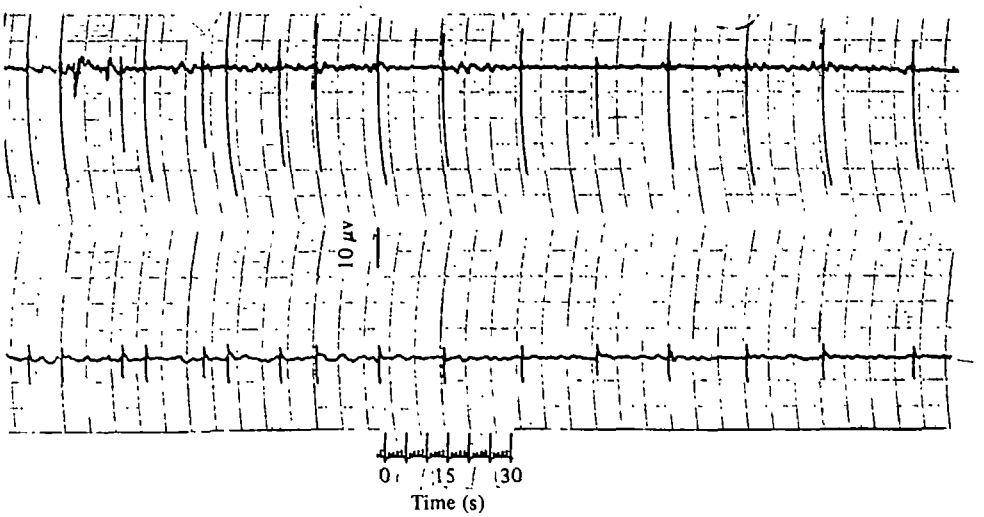

Fig. 2. Electrical activity recorded from the branchial nerve of VG preparations. The top tracing was recorded from the posterior section of the nerve, while the bottom was recorded from the anterior section.

OBSERVATIONS AND RESULTS

Action potentials in the branchial nerve

The amplitude of action potentials recorded from the branchial nerve by means of suction electrodes varied from about 5 to 100 μV between preparations, but remained fairly constant for an individual preparation. The rise time of the spikes, as determined from high speed oscilloscope recordings ranged from 20 to 30 ms. When the branchial nerve was bisected, spikes could be recorded only from that portion of the nerve still attached to the visceral ganglion and not from the portion attached to the gill, indicating that impulses were from the visceral ganglion, travelling toward the gill.

As the branchial nerve emerges from the visceral ganglion it divides into two branches, one travelling posteriorly and another branch travelling anteriorly, each innervating a different portion of the gill. To determine if the nervous activity recorded from the posteriorly directed branch was representative of the activity along the entire nerve, preparations were set up with a suction electrode at each branch (Fig. 2). Examination of the recordings of 12 preparations showed that the rise times and temporal distribution of spiking were the same in both branches of the nerve, although a difference in amplitude of the action potentials was seen. Those from the anteriorly directed branch were consistently smaller.

Effects of drugs on the visceral ganglion

Preliminary experiments indicated that the superfusion of dopamine onto the visceral ganglion caused a decrease in lateral ciliary activity. Therefore 18 preparations with initially high basal rates of ciliary beating were tested for the effects of superfusing dopamine (10^{-6} to 10^{-4} M) onto the visceral ganglion after observing basal ciliary activity for 10 min. Fig. 3 shows the effect of 1×10^{-4} M dopamine. The mean ciliary

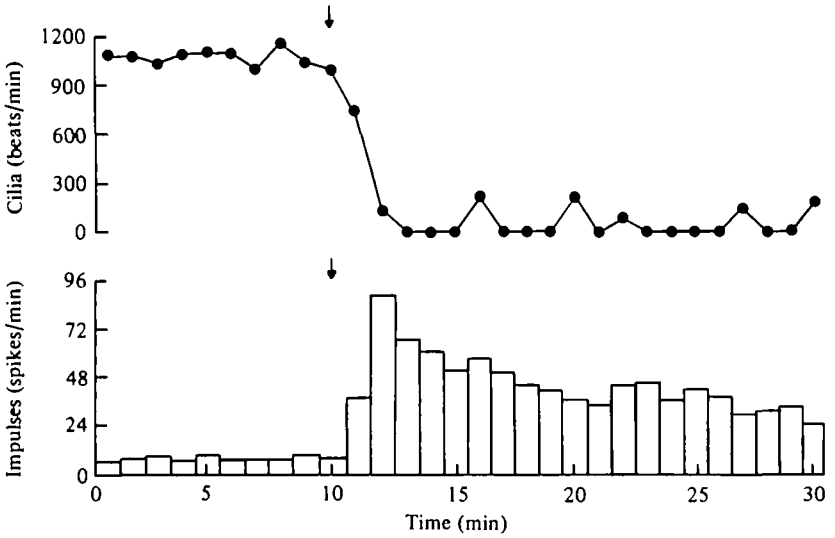

Fig. 3. The change in beating rate of the gill lateral cilia (upper) and of impulse activity of the branchial nerve (lower) in response to the addition of 1×10^{-4} M dopamine to the visceral ganglion. The arrows indicate the time of drug addition. $N = 5$. A signed-ranks test indicates $P < 0{\cdot}01$ for comparison of activity before and after drug addition for both upper and lower figures. The sem's of ciliary beating in beats/min and nerve firing rate in spikes/s are comparable to those in Fig. 4.

rate before the addition of dopamine was 859 beat/min. The mean rate for the 20 min following dopamine was 73 beat/min. Within 3 min of adding dopamine the cilia completely stopped and remained stopped except for brief bursts of beating, mainly during the last few minutes of observations. The basal spiking rate before dopamine was 8 spikes/min. Within 3 min of applying dopamine it increased to 68 spikes/min. Throughout the 20 min following dopamine, impulse firings averaged 48 spikes/min. Fig. 4 displays how cilio-inhibition and spiking rate increase as a function of the dopamine concentration applied to the visceral ganglion.

The effects of preadministering dopamine antagonists to the visceral ganglion also were examined. ERG (10^{-7} to 10^{-5}M) and MS (10^{-6} to 10^{-5}M) were superfused onto the visceral ganglion for a 10 min period followed by superfusion of dopamine (10^{-5} to 10^{-4} M). ERG and MS produced a graded antagonism to both the cilio-inhibition and the increased nerve impulse firings caused by dopamine applications (Fig. 4).

The effects of the chemical neurectomy agent 6-OHDA, which selectively destroys dopaminergic neurones, were studied as follows. 6-OHDA (100 μg in 10 μl) was injected into the posterior adductor muscle every other day for 6 days. Fig. 4 shows that the ability of dopamine to depress ciliary rates and increase impulse activity was markedly decreased. The highest concentration of dopamine tested (1×10^{-4} M) insignificantly altered basal activity as can be seen from Fig. 5 which shows the an responses of eight preparations from animals pretreated with 6-OHDA.

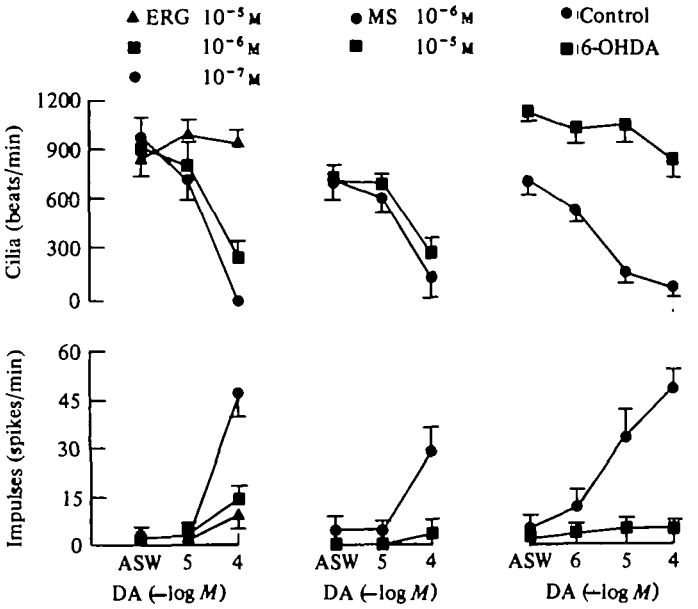

Fig. 4. Graph of the beating rate of lateral cilia (upper) and the firing rate of branchial nerve fibre (lower) in response to different concentrations of dopamine (DA) in the presence of different concentrations of ergonovine (ERG) or methysergide (MS) or after injection of the intact animal with 6-hydroxydopamine (6-OHDA) as described in the text. For each point $N = 5$ and the S.E.M's are indicated by a bar in one direction.

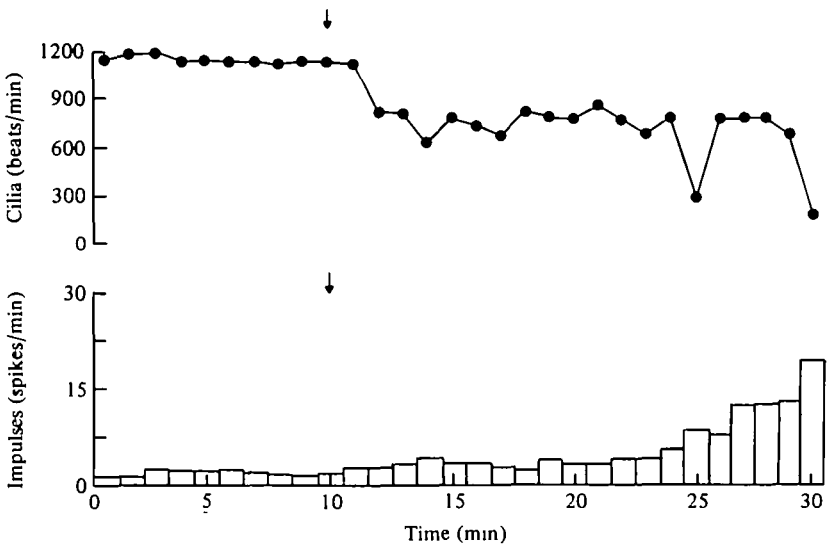

Fig. 5. Figure showing the change in beating rate of the gill lateral cilia (upper) and impulse activity of the branchial nerve (lower) in response to the addition of 1×10^{-4} M dopamine to the visceral ganglion of animals which were pretreated with 6-OHDA. N is 8. The arrows indicate the time of the drug addition.

DISCUSSION

Pharmacological studies of the gill of *M. edulis* have demonstrated that dopamine slows and stops lateral ciliary activity when superfused onto the gill (Paparo & Aiello, 1970; Malanga, 1974; Catapane *et al.* 1978). Cilio-inhibition also is observed: as a result of high frequency electrical stimulation of the branchial nerve which innervates the gill (Paparo & Aiello, 1970; Stefano *et al.* 1977; Catapane *et al.* 1978); by superfusion of the visceral ganglion with dopamine; and by electrically stimulating the preganglionic cerebrovisceral connective with high frequencies (Catapane *et al.* 1978). It also has been shown by us (1978) that the cilio-inhibitory effect of dopamine and of electrical stimulation to the branchial nerve can be antagonized by ERG. Dopamine's actions at the visceral ganglion and the effects of cerebrovisceral connective stimulation is blocked by both ERG and MS. These findings and the data of earlier works cited above, which have been described in some detail by Catapane *et al.* (1978), strongly suggest that dopaminergic neurones originating from the visceral ganglion are responsible for cilio-inhibition. The synaptic arrangements of the visceral ganglion are as yet unknown.

About 2–3 mm after the branchial nerve emerges from the visceral ganglion it forks and sends one branch anteriorly along the gill axis and another posteriorly. The nerve is composed of numerous fine fibres (Aiello & Guederi, 1965) assumedly arising from separate neurones, a portion of which displays specific monoaminergic fluorescence (Stefano & Aiello, 1975).

It is clear from the observations and results that the application of dopamine to the visceral ganglion increases impulse activity while decreasing ciliary activity. Based upon this and earlier studies (Catapane *et al.* 1978) it appears that at the visceral ganglion dopamine is acting as an excitor of the cilio-inhibitory circuits. It could be acting directly on dopamine neurones or on unidentified interneurones. Because VG-preparations show some impulse and ciliary activity, a degree of regulation appears to reside in the visceral ganglion. The work of Hamburg & Paparo (1975), Paparo, Hamburg & Cole (1975), Paparo & Morris (1976) and Hamburg, Paparo & Morris (1976) on the recordings of impulses in the visceral ganglion itself is not applicable to the present findings for reasons previously discussed in detail (Catapane *et al.* 1978). They did not show the site of their recordings or demonstrate that the recordings were related to lateral ciliary activity in any manner.

That cilio-inhibition results as a consequence of an increase in nervous activity of the branchial nerve agrees with the finding of Murakami & Takahashi (1975) that the ciliary arrest response was the result of transient depolarizations which could summate at a frequency of 20–40 Hz when the branchial nerve was electrically stimulated. In our study increased firing of the branchial nerve was produced by bath applications of dopamine onto the visceral ganglion where its actions could be blocked by either ERG or MS. It would appear that the recorded activity represents the activity of those fibres which carry inhibitory impulses to the lateral cilia because there is a graded dose/response relationship between the concentration of dopamine applied to the visceral ganglion and the rate of firing of the fibres in the nerve and correspondingly to the degree of cilio-inhibition that occurs, and because the time course of ciliary inhibition corresponds to the onset and duration of increased spiking. The

simplest circuitry needed to account for the observations would consist of dopaminergic neurones which themselves are excited by dopamine from other dopaminergic neurones. At no time during this study were high basal spiking activity of VG-preparations seen. This also would indicate that the cilio-inhibitory dopaminergic neurones must be driven by other neurones which are not present in the visceral ganglion.

6-OHDA has been extensively studied in mammalian systems and shown to be a potent destructor of catecholamine-containing neurones (Johnson, Fuxe & Daley, 1972). Its effects in invertebrates has not been as well documented. Treatment of the CNS of *M. edulis* with 6-OHDA results in a decrease in dopamine-containing structures and an increase in endogenous serotonin (Stefano *et al.* 1976). Unpublished data from our laboratory shows that reduction in DA levels can approach -65% as compared to controls without being lethal. In animals whose dopamine neurones were destroyed with 6-OHDA or whose dopamine content was depleted with α methyl-paratyrosine, an inhibitor of dopamine synthesis, basal ciliary activity tended to be higher than that of control and animals were less responsive to stimuli which produce cilio-inhibition (Stefano *et al.* 1977). In the present study 6-OHDA treatment depressed the dopamine induced firing of fibres in the branchial nerve and the resulting inhibition of lateral cilia.

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