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Interaction of Neurons at the Level of Cell Bodies in the Snail CNS. Heterogeneity of the Neuroactive Environment

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Experiments on the CNS of snail *Lymnaea stagnalis* in which a cell isolated from the serotonin cluster PeA was used as a mobile sensor neuron demonstrated the presence of neuroactive factors at the surface of the cellular "cortex" of the pedal ganglion. Apart from the previously known factor serotonin, effective concentrations of a factor suppressing the electrical activity of PeA were found at this site, along with a depolarizing factor which, unlike serotonin, narrowed PeA action potentials. The ability of these factors to control the electrical activity of the sensor neuron demonstrates the possible involvement of chemical agents in the intercellular space of the "cortex" in neuronal signaling.

KEY WORDS: interneuronal signaling, isolated neuron, *Lymnaea stagnalis*.

Cell bodies (the soma) in the CNS of many neurons in invertebrates are located at the peripheries of ganglia (sometimes termed the cellular cortex), their processes entering the depths where they form a fibrous plexus, the neuropil. The neuropil is regarded as the substrate for interneuronal chemical interactions, which, as we have suggested, are also important at the cell body level for the functioning of neuronal ensembles in the typical invertebrate ganglion.

Our preceding studies on the mollusk brain (the pond snail) addressed the PeA group of serotoninergic neurons, using an isolated neuron of this type as a sensor to detect the presence of neuroactive factors in the cell's environment. We then demonstrated that serotonin (5-HT) secreted by the neuron body during serotoninergic arousal acts on the excitatory somatic autoreceptors on these cell bodies and on neighboring neurons, thus facilitating the integration of the PeA neuron pool as a whole [2].

Some observations have already demonstrated that the effects the neuroactive environment on the cell bodies of PeA neurons do not lead to the excitatory actions of 5-HT: in three of 42 cases, the sensor neuron responded to application to PeA cell with inhibition rather than excitation [2]. The presence of a non-serotonin excitatory factor (or factors) is evidenced by the results obtained from our own unpublished experiments in which the serotonin receptor blocker mianserin, even at very high doses, did not always completely eliminate excitation of the isolated neuron when it was applied to PeA cells.

The aim of the present work was to seek significant evidence of the heterogeneity of the neuroactive environment acting on the cell surface in the PeA cluster of pedal ganglia in the pond snail. The primary task was to develop an experimental system allowing each of these factors to identified reliably.

It is particularly difficult to discriminate the depolarizing effects of unknown factors from those of 5-HT. During the experiments developing the model system, we used a series of pharmacological agents, particularly antagonists of 5-HT receptors and transporters, as well as substances acting on ion channels; this allowed us to select a satisfactory discriminating feature – changes in the action potential duration. The action potentials of PeA cells are known to be widened by 5-HT [3]. In our experiments, conversely, this potential was narrowed by the depolarizing factor studied here (which is henceforth termed narrowing factor). Discrimination of the effects of 5-HT and narrowing factor in terms of the width of this potential was particularly clear in conditions of preliminary widening of the potential in the isolated neuron with quinine at a concentration of 10 μM. These development experiments also showed that the inhibitory factor was best detected on the background of the excitatory action of exogenous 5-HT.

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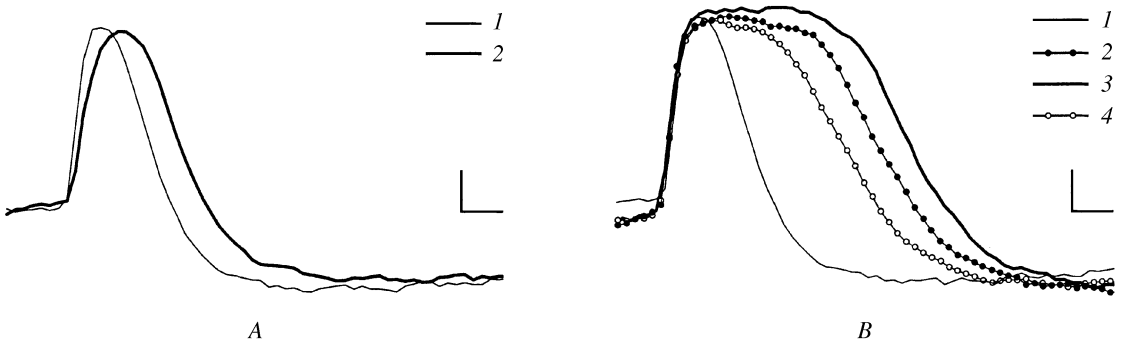

Fig. 1. Sequential changes in the durations of the action potentials of isolated PeA neurons during a single experiment. In *A*: *1*) in the absence of treatment; *2*) in the presence of 1 μM 5-HT; in *B*: *1*) in the absence of treatment; *2*) in the presence of 10 μM quinine; *3*) on approximation to a capillary filled with 5-HT in the presence of 10 μM quinine; *4*) on approximation to the PeA area of the CNS in the presence of 10 μM quinine. Calibration: 10 mV, 5 msec.

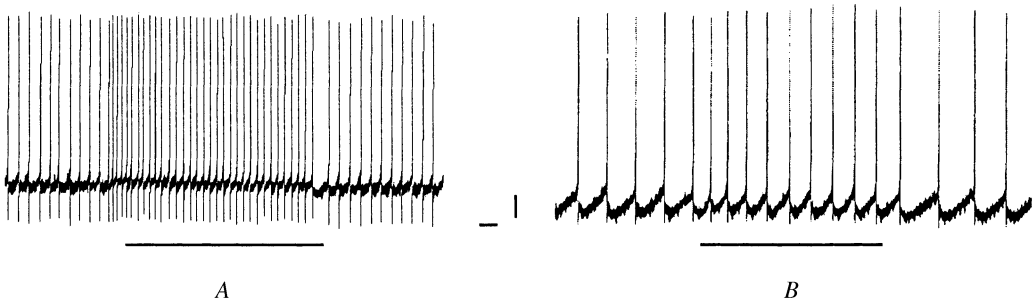

Fig. 2. Changes in the electrical activity of an isolated PeA neuron on approximation (thick continuous line) to a capillary filled with 5-HT. *A*) In the absence of quinine; *B*) in the presence of 10 μM quinine. Calibration: 10 mV, 10 sec.

METHODS

Studies were performed using adults of the greater pond snail *Lymnaea stagnalis* (*Pulmonata, Basommatophora*) reared in aquarium cultures. The CNS (lacking the buccal ganglia) was isolated and the left cerebropedal and cerebropleural connectives were cut to provide a convenient approach to the paired groupings of PeA neurons located in the medial parts of the left and right pedal ganglia [6]. After proteolytic digestion (Pronase E, Sigma; 4 mg/ml, 7–35 min) followed by washing with Ringer's solution for snails (50 mM NaCl, 1.6 mM KCl, 4 mM $CaCl_2$, 8 mM $MgCl_2$, and 10 mM Tris-HCl, pH 7.4), CNS preparations were placed in a 1.5-ml flow chamber.

A single PeA cell was isolated as described by D'yakonova [1]. A recording microelectrode was inserted into the cell body and this was slowly moved to the side until complete rupture of the neurite branch from the neuropil was obtained. The isolated neuron was established in the initial position at a distance of 0.5–1 mm from the pedal ganglia and was then used as a sensor for monitoring the pericellular environment, as in [2]. Under visual control, the neuron was brought to selected parts of the CNS surface.

A flow of Ringer's solution at a rate of 0.7–1 ml/min was maintained throughout the experiment. Quinine (Calbiochem) and 5-HT (5-hydroxytryptamine, Sigma) were delivered with the flow. In some experiments, the isolated neuron was also placed close to the tip of a capillary filled with 1 mM 5-HT. Approximation of the isolated neuron to the ganglion and capillary filled with 5-HT was performed alternately, several times in each experiment.

The microelectrode was filled with KCl (0.1 or 3 M) and had a resistance of 20–60 MΩ. The neuron membrane potential was recorded using a Mikromed amplifier and was passed through an analog-to-digital converter for recording on an IBM PC 486DX4-S.

RESULTS

The baseline activity of the isolated PeA neuron has been described previously [2]. In the absence of external

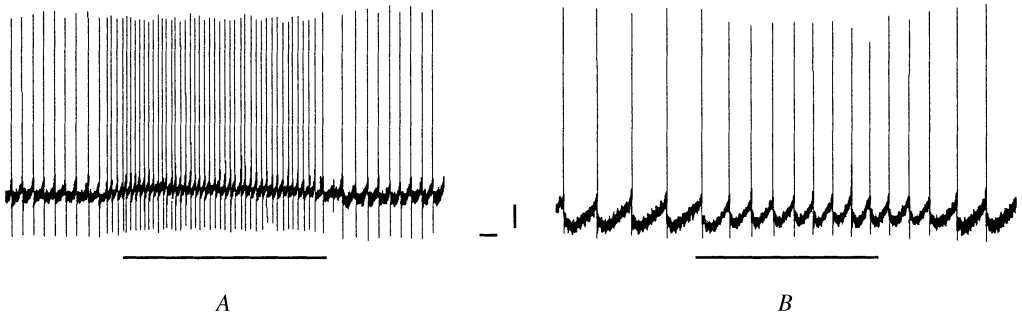

Fig. 3. Changes in the electrical activity of an isolated PeA neuron on approximation (thick continuous line) to the PeA area of the CNS. *A*) In the absence of quinine; *B*) in the presence of 10 μM quinine. Calibration: 10 mV, 10 sec.

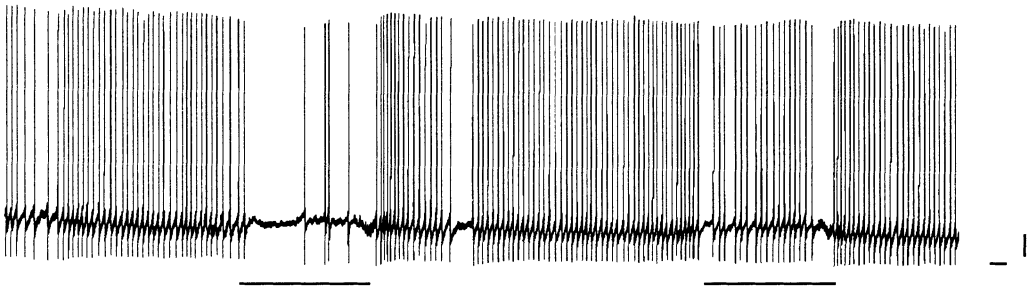

Fig. 4. Changes in the electrical activity of an isolated PeA neuron on approximation (thick continuous line) to the PeA area of the CNS in the presence of 0.5 μM 5-HT. The trace was started at the moment of onset of the action of 5-HT. Calibration: 10 mV, 10 sec.

influences, the isolated cell had a stable mean action potential frequency throughout the experiment.

Effects of quinine. The presence of 10 μM quinine in the flow led to gradual changes in the activity of the isolated PeA neuron (n = 10). There was a decrease in the action potential frequency of the isolated PeA neuron, with an increase in their duration (Fig. 1, *B*) and an increase in the postspike hyperpolarization. This increase in postspike hyperpolarization correlated with a decrease in the hyperpolarization forming part of the repolarization potential (cf. baseline activity before quinine and in the presence of quinine, Figs. 2 and 3). These changes, progressively increasing during exposure to quinine (20–30 min), were reversible, such that the initial form of the electrical activity was restored after 5–10 min of washing.

Effects of 5-HT in the absence and presence of quinine. The isolated 5-HT neuron responded to exogenous serotonin (0.01–100 μM) with an increase in the duration (Fig. 1, *A*) and frequency of action potentials. This type of response was also seen when the test neuron was brought close to the capillary filled with 5-HT. In the presence of quinine, the widening action of 5-HT remained marked (Fig. 1, *B*), and the frequency-increasing effect was somewhat weaker (Fig. 2) (n = 6).

Effects of approximating the isolated neuron to the PeA area in the absence and presence of quinine. Approximation of the isolated PeA neuron to the PeA area of the CNS generally evoked increases in the frequency and duration of action potentials in this neuron, this being reminiscent of the effect of exogenous 5-HT (cf. Fig. 2, *A* and Fig. 3, *A*). Approximation in the presence of quinine evoked similar depolarization, accompanied by increases in the action potential frequency. However, in this case the duration of action potentials decreased (Fig. 3 and Fig. 1, *B*) (n = 9, alternate approximation of the isolated PeA neuron to the PeA area of the CNS and the capillary filled with 5-HT, seen in six of nine experiments).

Effects of approximation of the isolated neuron to the PeA area in the presence of 5-HT. For detection of the inhibitory factor, the preparation was exposed to 0.5 μM 5-HT, which induces excitation of PeA neurons, including the isolated neuron [2, 3]. If the isolated neuron was brought to the PeA area of the CNS within the first minute of exposure to 5-HT, the action potential frequency in the isolated neuron decreased (n = 6, Fig. 4). The inhibitory effects of the PeA area on the sensor neuron were maximal in the first seconds after approximation, and then subsided. Inhibition became more marked with repetition of approximation.

DISCUSSION

Successful selection of experimental conditions in the present study allowed us to demonstrate that the surfaces of the cell bodies of PeA neurons in the pond snail have two neuroactive factors – constrictive and inhibitory. We have previously found extracellular 5-HT in this part of the CNS surface [2]. It is significant that all three substances are present in this location at concentrations sufficient to have significant influences on the electrical activity of the sensor neuron. Thus, neurons occupying this part of the brain are subjected to regulatory influences from the environment adjacent to their cell bodies. These points are in good agreement with the notion that the pericellular environment of the neuron is a "cocktail" of endogenous neuroactive substances.

In preliminary (as yet unpublished) experiments, we approximated a PeA or another isolated neuron to one of the buccal ganglia of the pond snail, and these experiments also showed strong changes in the electrical activity of the sensor neuron. Thus, the region containing PeA neurons in the pond snail studied here does not appear to represent any exception. It would seem that control of neurons by the heterogeneous neuroactive environment acting on cell bodies is a quite general situation.

Continuation of the studies leads to new questions. We do not know the cellular sources, the chemical natures, nor the regulatory functions of the constrictive and inhibitory factors. An explanation is also required of which changes they induce in the operation of channels involved in electrogenesis in the PeA cell membrane. Experiments on identified neurons in *Planorbis* (a mollusk) ganglion and the leech (a ringworm) showed that somatic secretion of neurotransmitter occurs in the "cortex" of the ganglion [4, 5]; it was found in the case of one type of secretory vesicle in the leech neuron that transmitter release correlated not so much with individual action potentials as with their frequency [4]. These results are in good agreement with our data indicating that chemical signaling between cell bodies may be important in the invertebrate ganglion cell body zone and its intersomatic space. Thus, the neuropil does not appear to be the only location for network-based interneuronal interactions to take place.

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