EFFECT OF ADRENOCHROME ON THE NEURONAL MEMBRANE OF *Helix pomatia*

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The biochemical and physiological effects of adrenochrome, the oxidized form of the neurohormone adrenalin, has now been studied reasonably completely. Inhibition of the activity of some enzymes [3, 6] and also the ability of adrenochrome to induce psychoses in healthy subjects [11] suggest that it participates in the genesis of mental diseases. Nevertheless, information on the effect of adrenochrome on the key membrane mechanisms of neuronal activity that are expressed through electrogenesis is not available. This paper describes an attempt to bridge this gap.

EXPERIMENTAL METHOD

Experiments were carried out on neurons located on the dorsal surface of the subesophageal ganglion complex of *Helix pomatia*. The preparation was placed in a chamber containing physiological saline by Sorokina's method [5]. Two microelectrodes filled with 2.5 M KCl, with a resistance of 5-10 MΩ, were inserted into the neuron. The usual technique of microelectrode recording and membrane voltage clamping was used. The following characteristics of the neuron membrane were determined: resting potential, amplitude and frequency of the action potential, input resistance, inward current, fast and delayed outward currents, steady-state inactivation of the inward and outward currents. The adrenochrome concentration in the solution surrounding the cell was $5 \cdot 10^{-4}$-$20 \cdot 10^{-4}$ M and its action was studied for 60-80 min.

EXPERIMENTAL RESULTS

The effect of adrenochrome on neurons of *Helix pomatia* was studied in 20 experiments. Just as previously [1], transformation of regular spike activity into burst activity and the appearance of oscillations of membrane potential (Fig. 1b) were observed after its application. Volleys of spikes against the background of depolarization were separated from each other by periods of interburst hyperpolarization. Under the influence of adrenochrome silent cells also began to generate burst of spikes. If a neuron initially had bursting activity, after application a change was observed in the rhythm of the bursts (Fig. 2). Membrane depolarization after 60-80 min was 7.06 ± 2.23 mV ($P = 0.05$), and the conductance of the leak channels was increased by 1.33 ± 0.35 times but the amplitude of the action potential fell to 50-60% of its initial value. During the burst the input resistance of the membrane decreased (Fig. 2d).

In 35% of cases periodic short shifts of membrane potential appeared 40-60 min after application of adrenochrome against the background of bursting activity (Fig. 1a). In 25% of cases these shifts took place toward depolarization and were accompanied by a high-frequency discharge (Fig. 2a, b), but in 10% of cases they were accompanied by hyperpolarization.

The voltage clamping method showed a decrease in both inward and outward currents under the influence of adrenochrome. The ratio between the peak values of the inward current after application and the normal value was 0.69 ± 0.10. Steady-state inactivation curves for the inward and outward currents were shifted by adrenochrome toward negative membrane potential values (Fig. 3). Recording of the leak current by a shift of membrane potential toward hyperpolarization by means of a sawtooth pulse revealed a phenomenon of abnormal rectification. Ouabain led to the disappearance of spike activity and of the oscillations of resting potential induced by adrenochrome (Fig. 1b, c).

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Fig. 1

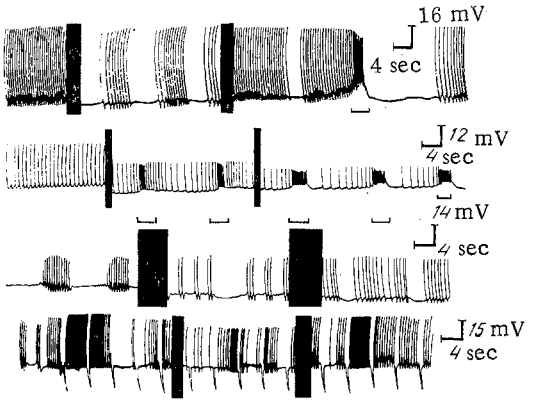

Fig. 2

Fig. 1. Changes in membrane potential after application of adrenochrome (A) and ouabain (B). a) Periodic short shifts of membrane potential against a background of bursting unit activity; b, c) alternate depolarization and hyperpolarization of membrane, inhibition of activity by ouabain.

Fig. 2. Changes in firing pattern under the influence of adrenochrome. a, b) Transformation of regular spike discharge into bursting type, appearance of periodic short shifts of membrane potential (⌣); c) change in rhythm of bursting activity; d) decrease in input resistance of membrane during burst.

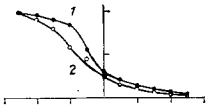

Fig. 3. Steady-state inactivation of fast outward current: 1) before; 2) after application of adrenochrome.

The sensitivity of different cells to adrenochrome varied: Cells of Sakharov's group A and LPa3 [4] reacted to a concentration of $5 \cdot 10^{-4}$ M, whereas adrenochrome had a visible action on cells of the B, C, and RPa5 groups only in a concentration of $20 \cdot 10^{-4}$ M or over.

Some similarity was observed between the action of adrenochrome on the neuron membrane and the effects of convulsants, notably metrazol [7, 9] and strychnine [8, 9]: Bursting activity and abnormal rectification appeared, the amplitude of the action potential and strength of the ionic currents were reduced.

The decrease in amplitude of the action potential under the influence of adrenochrome was probably due to a decrease in the inward current, possibly as a result both of a shift of the steady-state inactivation curve toward negative membrane potential values and of a decrease in maximal conductance of the channels of the inward current.

It has been suggested that bursting activity is due to two interconnected ionic mechanisms: a slow inward current, depolarizing the neuron membrane, and an outward current, causing hyperpolarization [2, 13]. At the peak of hyperpolarization, because of inactivation of the outward current the shift of membrane potential toward the threshold of excitability is accelerated, and this leads to the generation of a burst of spikes. The outward current, activated by depolarization, stops the burst and causes hyperpolarization of the membrane, which is accelerated by the decrease in the inward current, after which the whole cycle is repeated [13]. It has been suggested that the inward current is carried by Ca^{++} ions [10, 12]. An increase in the intracellular concentration of this ion leads to the appearance of abnormal rectification. However, the results do not rule out the possibility that a role in the appearance of bursting activity may also be played by active ion transport due to a decrease in the input resistance of the neuron membrane during the burst. Evidence in support of this view is given by the fact that application of ouabain, which blocks active transport, led to complete disappearance of spike activity and of oscillations of membrane potential.

The mechanism of appearance of periodic short shifts of membrane potential is not clear. It is probably connected with reversible transformations of membrane structures responsible for permeability of sodium potassium ions. Considering the time of appearance of the periodic short shifts of membrane potential, it can be tentatively suggested that these structural changes are not due to the direct action of adrenochrome on the membrane, but are effected through cell metabolism.

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