ATYPICAL RESPONSES OF *Helix pomatia* NEURONS TO DOPAMINE

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Much information has accumulated in the last two decades to show that in certain cases the action of a mediator on the postsynaptic membrane may be based, not on increased ionic permeability of the membrane (a decrease in its input resistance — R_{in}), as has hitherto been considered but, conversely, by a decrease in ionic permeability (increased R_{in}). Effects such as these may arise in cases when the initial ionic permeability of the membrane is relatively high and interaction between neuromediator and receptor leads to closing of ionic channels.

To distinguish them from the classical variety these responses were described as atypical. Atypical responses have been found both in higher animals in experiments with acetylcholine [15], dopamine (DA) [4, 7], GABA [10], and other mediator amino acids [9], and in mollusks in experiments with serotonin [5], GABA [12], glutamate, and histamine [1].

This paper describes atypical responses of *Helix pomatia* neurons to DA.

EXPERIMENTAL METHOD

Experiments were carried out on neurons on the dorsal surface of isolated subesophageal ganglia of *H. pomatia*. The ganglia were placed in perfusion solution containing (in mM): NaCl 120, KCl 5, $CaCl_2$ 6, $MgCl_2$ 3.5, pH 7.5-7.8. The cells were identified according to Sakharov's classification [2]. An apparatus for microelectrode research from Nihon Kohden (Japan) was used. The microelectrodes were filled with 2M potassium citrate solution. The membrane was polarized by passing a current of the required polarity through the recording microelectrode, using the bridge circuit of an MÉZ-8101 amplifier. The relative value of R_{in} was determined from the amplitude of the hyperpolarizing stimulus. DA was added to the perfusion fluid to a final concentration of 1-10 μM. The interval between applications of DA was not less than 5 min.

EXPERIMENTAL RESULTS

DA, added to the perfusion fluid in a concentration of 1-10 μM, induced a rapid (latent period 0.5-5 sec), reversible, dose-dependent change of membrane potential (MP) and R_{in} in several neurons.

Investigation of dependence of the amplitude of the DA-responses on the MP level and direct recording of their reversal potentials (E_r) was rendered difficult in many experiments because of the rectifying properties of the membrane. In most cases these were exhibited in MP levels exceeding —30 Mv (delayed rectification), so that E_r with more positive values than —30 mV could not be measured directly, and were determined by extrapolation of the straight line representing amplitude of the DA response as a function of MP to the abscissa. With MP between —35 and —85 mV, the value of R_{in} was unchanged in many experiments, so that it was possible to measure directly those values of E_r which were below the resting potential.

Depolarization to DA in response to a fall of R_{in} on average by 35% (18 experiments), was recorded on neurons RPa_2, RPa_4, B_4, B_5, etc. The amplitude of responses to DA increased during membrane hyperpolarization and decreased during depolarization; E_r varied from —15 to —20 mV (four measurements, Fig. 1a).

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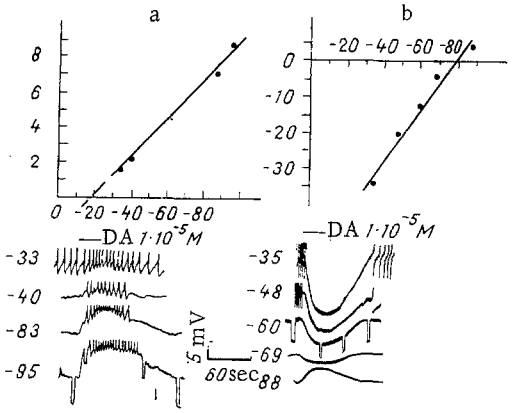

Fig. 1. DA responses developing with a decrease in R_{in}. a) Amplitude of DA depolarization as a function of MP, E_r = —18 mV, cell B_4. Abscissa, MP (in mV); ordinate, amplitude of DA responses (in mV). Traces of DA response given below. Each trace corresponds to a point on the graph. Values of MP shown on left of traces. Downward deflection of beam on bottom trace indicates measurement of R_{in}. Horizontal line above top trace shows duration of DA application. b) Amplitude of DA hyperpolarization as a function of MP. E_r = —80 mV, cell in A region. Legend as in Fig. 1a.

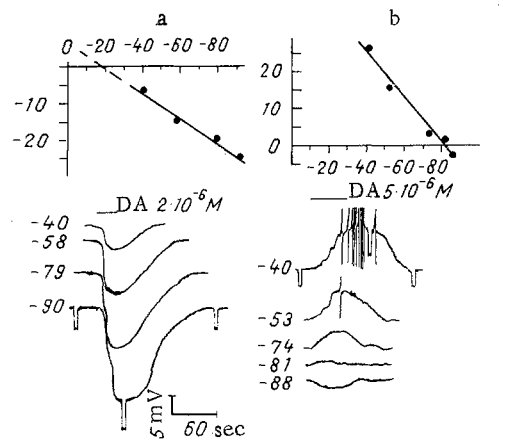

Fig. 2. DA responses developing with an increase in R_{in}. a) Amplitude of DA hyperpolarization as a function of MP, E_r = —18 mV, cell in D region; b) amplitude of DA depolarization as a function of MP, E_r = —83 mV, cell in F region. Legend as in Fig. 1a.

Hyperpolarization to DA, accompanied by a fall in R_{in} on average by 30%, was recorded on neurons RPa_3, RPa_4, LPa_3, B_4, B_6, etc. The amplitude of the responses decreased as a linear function of membrane hyperpolarization. E_r was —81 ± 2 mV (15 measurements, Fig. 1b).

In three experiments on neurons in the G and D regions dopamine induced membrane hyperpolarization which developed with an increase of R_{in} by 20-40%. The amplitude of the response was a linear function of the MP level, but opposite to that observed for DA responses developing with a decrease in R_{in}, namely: The response increased during artificial membrane hyperpolarization; E_r of the responses was between —15 and —20 mV (Fig. 2a).

Depolarization to DA accompanied by an increase in R_{in} by 20-50% was recorded on neurons LPa_2, F, and D in six experiments. The response decreased with membrane hyperpolarization and was reversed when MP was —83 ± 7 mV (Fig. 2b).

Complex responses to DA, the first phase of which consisted of depolarization with a decrease in R_{in}, and a second phase consisted of a sharp fall in depolarization with an increase

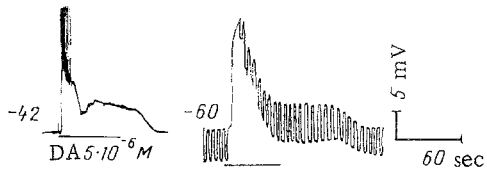

Fig. 3. Biphasic response of B_2 cells to DA at different MP levels. First phase — rapid depolarization is accompanied by a decrease in R_{in}, second phase — a decrease in depolarization is accompanied by an increase in R_{in}, as the right trace shows. Legend as in Fig. 1a.

in R_{in} relative to its initial value, were found on cells B_2 and G (two cases). The amplitude of both phases of the response increased with hyperpolarization of the membrane (Fig. 3).

DA is one of the mediators in the molluscan nervous system [2]. Various workers have shown that exogenous DA causes excitation (depolarizing) or inhibitory (hyperpolarizing) responses of mulluscan neurons. Specific agonists and antagonists have been found for excitatory and inhibitory DA responses, suggesting that these responses are mediated by different types of DA receptors [3, 14].

The present investigation showed that DA responses in most experiments developed with a marked increase of ionic permeability of the membrane; E_r for DA depolarization was between −15 and −20 mV, suggesting that these responses are Na^+, K^+ in nature, whereas reversal of DA hyperpolarization occurred at MP −83 ± 7 mV, and was evidently connected with an increase in permeability for K^+. In cases when the initial R_{in} was unchanged between MP values −35 and −85 mV the amplitude of the DA response was a linear function of MP, and this confirms the potential-dependence of DA-sensitive ionic channels.

Besides DA responses developing with an increase of ionic permeability, DA depolarization and DA hyperpolarization due to a reduction of ionic permeability of the membrane also were recorded. These results confirm once again the idea which has developed in the literature, that one mechanism of neuromediator action may be a decrease in ionic membrane permeability.

The idea that such a mechanism of neuromediator responses is possible was first expressed by Kuffler in 1960 [8], and it was confirmed in 1961 by Grundfest in experiments with serotonin on lobster skeletal muscle [6]. Later, atypical responses to various neuromediators, including to DA, were found on neurons of higher animals [4, 7, 9-11, 15].

Atypical responses on molluscan neurons were first found in experiments with serotonin [5]. Both depolarizing atypical responses with E_r between −20 and −30 mV (β-responses) and hyperpolarizing responses with E_r of −75 mV (α-responses) were found. Later, atypical responses were described on molluscan neurons for GABA [12], glutamate, and histamine [1] also.

The decrease in membrane permeability under the influence of a neuromediator suggests the existence of a special population of chemosensitive channels, initially open, and closed under the influence of the neuromediator (atypical channels).

Recording atypical responses to neuromediators on completely isolated molluscan neurons, not exposed to synaptic influences [1], confirms this view and rules out the previous explanation according to which the initially increased membrane permeability was due to synaptic influence [5].

In two experiments mixed responses to DA were found, the initial phase of which developed with an increase in permeability and the subsequent phase with a decrease. These results are evidence that the usual ionic channels in the membrane of a single neuron, which open under the influence of DA, may coexist with atypical DA channels. Similar results were obtained on cultures of mouse spinal neurons in experiments with glutamate and homocysteic acid [9]. Other evidence in support of this view is also given by results showing that in the same neuron acetylcholine induces responses with increase membrane permeability, whereas glutamate, histamine, and serotonin induce atypical responses [1].

The formation of atypical responses to neuromediators is evidently determined not only by the presence of atypical channels in the neuron membrane, but also by the presence of special receptors in the membrane. This conclusion was drawn previously [5] from the fact that antagonists of ordinary responses to a neuromediator are not antagonists for atypical responses.

Like the state of other neuromediator and neuromodulator receptors, the state of atypical receptors (their activation or inhibition) depends on the functional state of the cell and of the organism as a whole. For example, Sakharov [13] has shown that atypical responses to serotonin are recorded on neurons of active snails and are replaced by responses with an increase in membrane permeability in snails in a state of anabiosis.

LITERATURE CITED

1. S. A. Gapon, "The action of mediator substances on identified neurons of gastropod mollusk *Planorbis corneus*," Author's Abstract of Candidate's Dissertation, Leningrad (1981).
2. D. A. Sakharov, The Genealogy of Neurons [in Russian], Moscow (1974).
3. P. Ascher, J. Physiol. (London), 225, 173 (1972).
4. J. P. Gallagher et al., Nature, 283, 770 (1980).
5. H. M. Gershenfeld and D. Paupardin-Tritsch, J. Physiol. (London), 243, 427 (1974).
6. H. Grundfest, Biophysics of Physiological and Pharmacological Actions, Washington (1961).
7. P. L. Herrling, Brain Res., 212, 331 (1981).
8. S. W. Kuffler, Harvey Lect., 54, 176 (1960).
9. J. F. MacDonald and J. M. Wojtowicz, Can. J. Physiol. Pharmacol., 58, 1393 (1980).
10. M. L. Mayer et al., Brain Res., 222, 204 (1981).
11. S. Minota et al., Brain Res., 216, 224 (1981).
12. A. Nistri and A. Constanti, Prog. Neurobiol., 13, 117 (1979).
13. D. A. Sakharov and G. N. Korobtsov, Experientia, 32, 588 (1976).
14. J. M. Van Rossum, Fed. Proc., 37, 2415 (1978).
15. F. F. Weight and A. Padjen, Brain Res., 55, 225 (1973).

EFFECT OF LONG-TERM ESTRADIOL ADMINISTRATION IN DIFFERENT DOSES ON ITS RECEPTORS IN THE RAT UTERUS

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The effect of a single dose of estradiol on the concentration of its receptors in target organs (homospecific regulation) has been studied by many workers [1, 3, 8]. However, it must be pointed out that the stimulating action of the steroid under these circumstances is not the only possible mechanism of regulation by hormones of the level of their receptors in the tissues. A qualitatively different picture of the regulatory effect of estrogens may be obtained by their repeated administration. Under natural conditions target tissues are always subjected to the continuous influence of steroids secreted by the gonads and, in addition, experiments under such conditions provide suitable models of the development of certain pathophysiological states arising during long-term hormone therapy.

The aim of this investigation was to study the effect of long-term administration of different doses of estradiol on the concentration of estrogen receptors in the rat uterus, and to compare it with levels of this hormone in the blood serum and uterine tissue cytosol.

EXPERIMENTAL METHOD

Experiments were carried out on three groups of rats: 1) normal noninbred female rats (age 3-6 months) in the stage of estrus; 2) androgen-sterile females with closed vagina, which were injected on the 2nd-3rd day after birth with testosterone propionate in a dose of 50-100 μg per animal; 3) ovariectomized rats 3-4 weeks after operation. Estradiol benzoate was injected intramuscularly into the rats in the form of an oily solution during the first half of the day for 7-8 days daily in doses of 1 and 10 μg. Control animals were given injections of the pure oily solution. The animals were killed 1 day after the last injection and receptor binding of estradiol in the uterine tissue was determined by the method described in [2,

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