The results show that the cAMP level in platelets of blood incubated with nicotinic acid is increased more on average (almost threefold) than their PGE_2 content (by 36-15%) relative to the initial concentration of the same parameter in control blood samples.

The results of these experiments suggest that the mechanism of the inhibitory action of nicotinic acid on platelet aggregation is due to its ability to act directly on these blood cells and to disturb their homeostatic balance of regulators of platelet aggregation, chiefly in the direction of an increase in the synthesis of its inhibitor, cAMP. This conclusion is also supported by the ability of nicotinic acid to inhibit the formation of thromboxane A_2, a highly active inhibitor of platelet aggregation, in the platelets.

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SEASONAL DIFFERENCES IN THE ACTION OF MORPHINE AND NALOXONE ON THE RESPONSE OF *Helix* NEURONS TO DOPAMINE

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KEY WORDS: morphine; naloxone; responses to dopamine; mollusk neurons.

Investigations of the membrane mechanisms of effects of agonists (morphine and enkephalins) and antagonists (naloxone) of opiate receptors have shown that opiates can not only act on the membrane potential of *Helix* neurons [3, 4], but can also weaken the response of these neurons in a naloxone-dependent manner to serotonin [1] and dopamine [2], by a noncompetitive mechanism.

Considering the abundant data in the literature on seasonal differences in intracellular metabolism and the state of neuronal reception in mollusks [6, 10, 11] and also data [13] on seasonal differences in the effect of opiates on the content of cyclic nucleotides in the brain of these animals, it appeared interesting to study seasonal differences in the action of morphine and naloxone on functional activity of molluscan neurons and, in particular, on their response to dopamine.

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EXPERIMENTAL METHOD

Snails (*Helix pomatia*) were obtained from the Biological Products Factory in Pushkin, Moscow Region, to which they are usually sent from the Crimea in summer (June-September). The animals were kept at 4°C. In late summer the animals began to crawl actively when placed in a warm room (20°C). In winter and spring the mollusks became apathetic, their peristome was covered by the epiphragm, and they did not always start to move even when placed in very warm water (40°C). Full details of the technique were described previously [1, 2].

EXPERIMENTAL RESULTS

Responses of neurons to dopamine were studied at a membrane potential level near to the threshold for action potential generation. Under these circumstances rapid depolarization or hyperpolarization of the membrane to dopamine, reversible on rinsing and reproducible on repeated presentation, could be recorded.

In the winter and spring (January-April) sensitivity of the neurons to dopamine was reduced, and to obtain high-amplitude responses to dopamine comparable with those in summer and the fall (5-15 mV) the neurotransmitter had to be presented in a concentration one order of magnitude higher ($1 \cdot 10^{-5}$ M, Fig. 1).

Morphine, in a concentration of $1 \cdot 10^{-5}$ M, reduced dopamine-induced depolarization of the neurons and this effect was seen most strongly in the summer-fall (June-November, Fig. 2), when in the presence of morphine the depolarizing responses to dopamine averaged 34 ± 6% (n = 1) of their initial value, and the hyperpolarizing responses 69 ± 4% (n = 21). The effects of morphine were considerably ($P < 0.02$) weakened in winter and spring, and reached a minimum in February-March, when the values for depolarizing and hyperpolarizing responses to dopamine in the presence of morphine were 76 ± 13% (n = 7) and 96 ± 3% (n = 8) respectively. The depolarizing responses to dopamine in the presence of morphine in December-January and April-May reached 57 ± 8% (n = 9) and 42 ± 5% (n = 10) respectively, whereas the hypopolarizing responses were 83 ± 6% (n = 5) and 88 ± 5% (n = 6) of their initial value.

In the summer and fall naloxone ($1 \cdot 10^{-5}$ M), an antagonist of opiate receptors, while it did not affect the response of the neurons to dopamine when added to the perfusion fluid, prevented the action of morphine, so that the response to dopamine under these conditions virtually reached its initial value (Fig. 1). In the winter and spring, together with weakening of the effects of morphine, an inhibitory effect of naloxone on the dopamine response of the neurons was found (Fig. 1). In the presence of $1 \cdot 10^{-5}$ M naloxone, the depolarizing response to dopamine averaged 47 ± 12% (n = 11) and the hyperpolarizing response 63 ± 12% (n = 6) of the original value. In some cases, lower concentrations of naloxone ($1 \cdot 10^{-6}$ M) gave similar effects. On repeated presentations of naloxone (5-10 times) gradual weakening of its effect was observed.

The modification of the inhibitory effect of morphine and naloxone on the response of *Helix pomatia* neurons to dopamine at different seasons of the year, discovered in this investigation, is evidence of the adaptability of the opiate-neurotransmitter system and its connection with the functional state of the animal (hibernation, waking).

Weakening of the effect of morphine in the winter and spring coincides with lowering of the sensitivity of the neurons to dopamine; this can evidently be logically explained by inhibition of activity of the enzyme systems of the neuron (adenylate cyclase, protein kinase) during anabiosis. Since one of the known mechanisms of action of opiates is lowering of adenylate cyclase activity [13, 15], a change in the initial activity of this enzyme in the winter and spring can evidently explain the seasonal weakening of the effect of morphine on the dopamine response of *Helix* neurons, which is known to be associated with the cyclic nucleotide system [9].

Another explanation of this phenomenon may be that it is connected with seasonal plasticity of the opiate receptors. The basis for this suggestion is the observations of Stefano and Hiripi [13] who, in experiments on the nervous system of freshwater mollusks, found seasonal variations in the effect of morphine on the pGMP level although its initial level was unchanged. Correlation between responses of the opiate receptors of the rat brain and the biological rhythms of the animal has been observed by Naber et al. [8].

The present experiments showed that seasonal variability is a feature of the effects not only of morphine, but also of naloxone, which in the summer and fall exhibits the properties

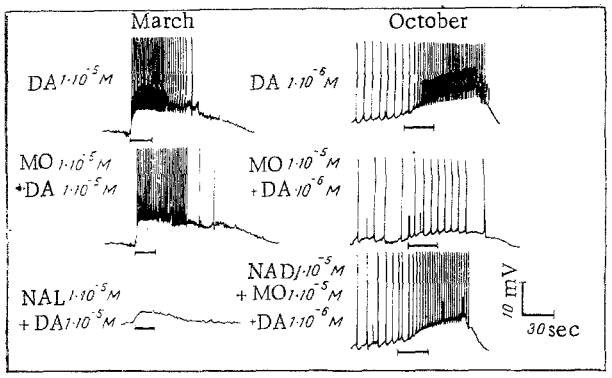

Fig. 1. Effect of morphine and naloxone on response of neurons of F group in visceral ganglion of *Helix pomatia* to dopamine in winter-spring and summer-fall seasons. DA) Dopamine, MO) morphine, NAL) naloxone. On right: results of an experiment in October. Response to $1 \cdot 10^{-6}$ M DA was 7.5 mV (top trace). In the presence of $1 \cdot 10^{5}$ M MO, DA-response was lowered to 2.5 mV (middle trace). NAL in a dose of $1 \cdot 10^{-5}$ M prevented effect of MO so that DA-response under these conditions was 6.5 mV (bottom trace. Lines below trace denote duration of presence of DA in perfusion fluid. On left: results of an experiment in March. Response to $1 \cdot 10^{-5}$ M MO, DA-response was 6.5 mV (middle trace). NAL in dose of $1 \cdot 10^{-5}$ M lowered DA-response to 2.5 mV (bottom trace).

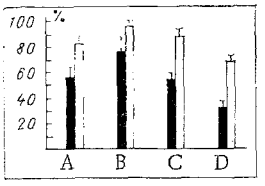

Fig. 2. Mean responses of *Helix pomatia* neurons to dopamine in the presence of $1 \cdot 10^{-5}$ M morphine, observed at different times of the year. Black columns) depolarizing, white columns) hyperpolarizing responses to dopamine. A) December-January, B) February-March, C) April-May, D) June-November.

of a specific antagonist of opiate receptors, whereas in the winter and spring it can have a direct influence on responses of neurons to dopamine.

It is interesting to compare these findings with those obtained by Hungarian workers, who showed that naloxone abolishes inhibition of spike responses to dopamine of neurons RPal [12] and RPa2 [14] of active specimens of *H. pomatia*, whereas in experiments to culture spinal neurons of mice and brain neurons of rats selective inhibition of GABA-responses by naloxone was found [5, 7]. The authors cited suggest that naloxone may perhaps act on dopamine and GABA receptors.

These properties of naloxone, and also data on the plasticity of the opiate system and its possible link with the functional status of the animal must evidently be taken into serious consideration during research into the opiates and their antagonists and their clinical application.

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