6. S. A. Shelkovnikov, G. A. Savitskii, and V. V. Abramchenko, Fiziol. Cheloveka, 12, No. 6, 1016 (1986).
7. J. Diamond, Adv. Cyclic Nucleot. Res., 9, 327 (1978).
8. A. R. Hughes, M. N. Martin, and T. K. Harden, Proc. Natl. Acad. Sci. USA, 81, No. 18, 5680 (1984).
9. S. Komori, K. Matsuo, Y. Kanamaru, et al., Jpn. J. Pharmacol., 40, No. 1, 1 (1986).
10. S. Komori, Y. Mizutani, and Y. Amano, Jpn. J. Pharmacol., 40, No. 1, 103 (1986).
11. J. F. Krall and A. Morin, J. Cell. Physiol., 129, No. 2, 250 (1986).
12. D. Leiber, M. F. Vesin, and S. Harbon, FEBS Lett., 86, No. 2, 183 (1978).
13. T. J. Torphy, M. Burman, L. B. Huang, et al., Fed. Proc., 45, No. 3, 191 (1986).

LONG-TERM POTENTIATION IN SNAIL NEURONS

O. V. Borisova

UDC 612.815.1/.2.063.019:594.3

KEY WORDS: snail neurons; long-term potentiation; serotonin.

In the investigation to be described below snail *(Helix pomatia)* neurons were used to study long-term plastic changes in the efficiency of synaptic connections. One model of these processes is long-term potentiation — a prolonged increase in the evoked response after application of short high-frequency stimulation. Long-term potentiation was first discovered in the hippocampus [8] and it was later found in other structures of the mammalian brain [10]. The possibility of obtaining such an effect in invertebrates was demonstrated in principle in investigations conducted on the crustacean neuromuscular synapse [7] and on neurons of the marine mollusk *Aplysia* [11, 12]. In investigations also conducted on mollusks it has been shown that an increase in the efficiency of synaptic connections can be brought about by serotonin (5-HT) [1, 5, 9]. The aims of the present investigation were: to study the possibility of formation of long-term (lasting more than 15 min) potentiation of EPSPs recorded in identified neurons of the snail CNS in response to stimulation of a nerve, and to study the effect of 5-HT and of intracellularly injected cAMP on these synaptic responses.

EXPERIMENTAL METHOD

Experiments were carried out on neurons on the dorsal surface of the subesophageal complex of ganglia of *H. pomatia*. The neurons were identified in accordance with Sakharov's classification [4]. A multibarreled microelectrode, one barrel of which served to record membrane potential, a second to pass a polarizing current through the membrane and measure the membrane resistance (both barrels were filled with 2M potassium citrate solution), whereas the remaining barrels were used for microiontophoretic injection of cAMP into the neuron [2], was inserted into the neuron. The resistance of the recording barrel was 10-15 MΩ. The membrane potential was shifted (by passage of a polarizing current) through 10-30 mV from the resting potential toward hyperpolarization, so that responses of the neurons to nerve stimulation and to mediators did not reach the level of action potential generation. The anal nerve was stimulated by tungsten electrodes. 5-HT (Serva) was added to the solution bathing the preparation in sufficient quantity to cause its final concentration to be 1×10^{-5} M. Washing out of the serotonin began 1 min after its application.

EXPERIMENTAL RESULTS

Experiments were carried out on neurons LPa2, LPa3, V1, V2, V4, and V6 and neurons of the F region of the visceral ganglion. In response to stimulation of the anal nerve an EPSP appeared in these neurons, its amplitude being constant during stimulation with a frequency of once every 5 min. After stimulation of the nerve with a frequency of 5 Hz for 30 sec the

Laboratory of Functional Synaptology, Brain Institute, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 651-653, December, 1988. Original article submitted May 26, 1987.

0007-4888/88/0012-1673$12.50

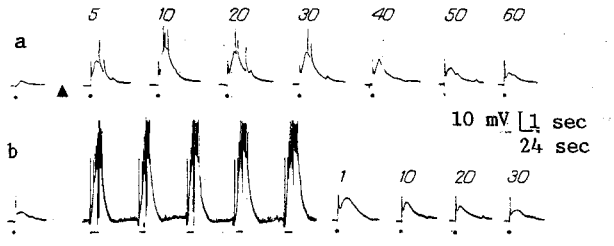

Fig. 1

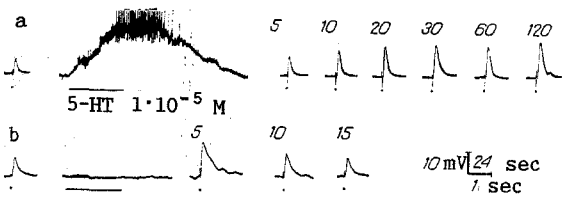

Fig. 2

Fig. 1. Long-term potentiation of EPSP recorded in response to single stimulation of the anal nerve in neuron V5 after tetanization (a) of the same nerve and after intracellular injections of cAMP (b). Numbers above traces show time (in min) after tetanization (a) and after last injection of cAMP (b). Dots below traces indicate times of nerve stimulation. Parameters of tetanization: frequency 5 Hz, duration 30 sec. Neuron membrane potential –70 mV. The line under the recordings denotes the duration of the cAMP injection; injection current 35 nA, cut-off current 3 nA.

Fig. 2. Age amplitudes of the SPSP after application of serotonin ($1 \cdot 10^{-5}$ M). a) Neuron of region; membrane potential 70 mV; serotonin induces reversible membrane depolarization and a prolonged increase in SPSP amplitude. b) Neuron of LPa3; membrane potential 75 mV; the SPSP amplitude returns to the original value 15 min after application of the mediator. The line under the recordings denotes the duration of the incubation of the preparation with the serotonin solution. Numbers above the recordings represent the time (in min) after application of serotonin.

amplitude of the EPSP increased in most neurons (17 of 28 neurons). Testing of the EPSP after tetanization, at a frequency of once every 5 min, showed that the amplitude of the EPSP reverted to its initial value after 30-120 min. Sometimes the amplitude of the EPSP reached its maximal value, not immediately after tetanization, but continued to rise during subsequent testing. An example of the time course of potentiation is given in Fig. 1a. If the amplitude of the EPSP before tetanization is taken as 100%, the amplitude of the maximally potentiated response was 157-800% (259 ± 37%, mean ± error of the mean; n = 17). In the course of tetanization, membrane depolarization could be observed, in some cases reaching the threshold of action potential generation. After the end of tetanization the membrane potential returned to its initial level. In this respect the time course of changes in membrane potential in snail neurons is analogous to that in neurons of the mammalian hippocampus [3]. In control experiments, depolarization of the neuron membrane for 30 sec to the action potential generation level did not induce any after-changes in the amplitude of EPSP.

After the end of long-term potentiation and the return of the amplitude of the EPSP to its original value, 5-HT was applied. Application of 5-HT evoked membrane depolarization in 15 of 32 neurons. After 5 min the 5-HT was washed away and the EPSP amplitude produced by stimulation of the anal nerve increased in 20 of 32 neurons. In 10 neurons (cells V1, V4, large cells in the F region) the amplitude of the EPSP continued to rise during further testing and it remained increased for 2 h or more; in some cases no tendency was found for it to decrease (Fig. 2a). In another group of cells (cells LPa2 and LPa3, unidentified cells of the visceral ganglion — 10 cells altogether) the amplitude of the EPSP returned to its original value 15-30 min after application of 5-HT (Fig. 2b). The amplitude of the maximally potentiated response was 150-666% of the initial value (mean 300 ± 35%, n = 20). The increase in amplitude of the EPSP observed after 5-HT application was not accompanied by an increase in resistance of the neuron membrane. In four neurons (cells V2 and V6) the amplitude of the EPSP fell after 5-HT application by 40-50% and returned to its original level after 15-30 min. No relationship could be found between the effect of 5-HT on the neuron membrane potential and on the amplitude of the EPSP.

Interconnection of the effects of tetanization and of 5-HT, and their intensity, depended on the type of cells. In neurons LPa2 and LPa3 both tetanization and 5-HT caused an increase in EPSP in all experiments. However, if the effects of post-tetanic potentiation lasted on average 50 min, the action of 5-HT as a rule was shorter (15-30 min). Meanwhile, in cells of the visceral ganglion, the effects of tetanization and of 5-HT often did not coincide. For instance, in five cells (two V4 cells, cell V1, two cells in the F region), in which tetani-

zation did not cause potentiation of EPSP to develop, subsequent application of 5-HT led to a prolonged increase in its amplitude. It can be postulated that the presence of a "modulating factor," in this case 5-HT, secreted during high-frequency stimulation of the nerve, is essential for potentiation to develop. In cases when tetanization does not lead to the secretion of this factor in sufficient quantity, potentiation does not develop. The ability of 5-HT to cause an increase in synaptic efficiency [1, 5, 9] and to enhance responses to acetylcholine [6] was demonstrated in investigations conducted both on *Helix pomatia* [1, 6] and on other mollusks [5, 9]. These effects of 5-HT lasted minutes or tens of minutes. Potentiation of synaptic responses after a single application of 5-HT, lasting 2 h or more, has not previously been described, so far as we are aware. However, 5-HT did not increase the synaptic responses of all neurons: In experiments on large unidentified neurons in the F region tetanization led to the development of potentiation of EPSP, but subsequent application of 5-HT caused no changes in its amplitude.

In the present experiments, the whole preparation of the isolated snail CNS was exposed to the action of 5-HT, and we therefore do not know whether the action of 5-HT is linked with presynaptic or with postsynaptic neurons. The experiments of Kandel, conducted on *Aplysia*, showed that facilitation of synaptic transmission caused by 5-HT is mediated through elevation of the cAMP level in the presynaptic neuron [9]. Meanwhile there is evidence [5] of sensitization of postsynaptic receptors to acetylcholine in the course of development of 5-HT mediated heterosynaptic facilitation. Without denying the possibility of a presynaptic action of 5-HT, we wished to discover if cAMP of the postsynaptic neuron participates in the mechanism of onset of long-term potentiation of synaptic responses. We know from the literature that 5-HT is an activator of adenylate cyclase in molluscan neurons and that it raises their cAMP level [9]. In our experiments intracellular microiontophoretic injection of cAMP induced reversible membrane depolarization in the majority of neurons (Fig. 1b). This effect was described by the present writer in more detail previously [2]. Repeated (3-10) injections of cAMP evoked an increase in amplitude of the EPSP evoked by stimulation of the anal nerve (Fig. 1b) in several neurons (cells V4, V5, and LPa3, two cells in the F region) by 52-200%. The amplitude of the EPSP returned to its original value 15-30 min after the last injection of cAMP. Potentiation of the EPSP appeared in all these neurons also after tetanization, and in neurons V4 and LPa3 after application of 5-HT. However, in the majority of neurons (13 or 18 cells) single and repeated injections of cAMP caused no changes in the amplitude of the EPSP.

Thus both short-term high-frequency stimulation of a nerve and short-term perfusion of a preparation of the isolated snail CNS with a solution of 5-HT (1×10^{-5} M) can induce a long-term increase in the amplitude of EPSPs recorded in response to single stimulation of the nerve in many identified and unidentified neurons. The very prolonged increase, lasting several hours, in the amplitude of EPSPs observed in several neurons after a single application of 5-HT is of great interest. Discovery of potentiation of the EPSP after injections of cAMP into the postsynaptic neuron is noteworthy. It can be tentatively suggested that elevation of the cAMP level in the postsynaptic neuron participates in the mechanism of potentiation induced by 5-HT.

LITERATURE CITED

1. P. M. Balaban, I. S. Zakharov, O. A. Maksimova, and M. V. Chistyakova, Neirofiziologiya, 18, No. 3, 291 (1986).
2. O. V. Borisova, E. I. Solntseva, and V. G. Skrebitskii, Byull. Éksp. Biol. Med., No. 3, 291 (1985).
3. V. S. Vorob'ev and V. G. Skrebitskii, Zh. Vyssh. Nerv. Deyat., No. 2, 395 (1981).
4. D. A. Sakharov, The Genealogy of Neurons [in Russian], Moscow (1974).
5. V. M. Storozhuk and I. N. Antonov, Neirofiziologiya, 18, No. 2, 250 (1986).
6. T. M. Turpaev, O. P. Yurchenko, and K. Sh. Rozha, Dokl. Akad. Nauk SSSR, 270, No. 6, 1505 (1983).
7. D. A. Baxter, G. D. Bittner, and T. H. Brown, Proc. Natl. Acad. Sci. USA, 82, 5978 (1985).
8. T. V. P. Bliss and T. Lomo, J. Physiol. (London), 232, 331 (1973).
9. E. R. Kandel and J. H. Schwartz, Science, 218, 433 (1982).
10. K. S. Lee, Brain Res., 239, 617 (1982).
11. S. A. Newlin, T. Werner, and S. H. Schlapfer, Brain Res., 181, 89 (1980).
12. E. T. Walters and J. H. Byrne, J. Neurosci., 5, 662 (1985).