ROLE OF cAMP IN THE GENERATION OF EXCITATORY RESPONSES OF *Helix* NEURONS TO SEROTONIN

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UDC 612.822.3-08:[612.822.3.015.2:577.123.3].014.46:[615.357:577.175.823

KEY WORDS: molluscan neurons; cAMP; serotonin.

The study of the role of cAMP as an intracellular transmitter during generation of electrophysiological responses of neurons to neurotransmitters is a topical problem in neurobiology. Large molluscan neurons, for which cAMP has been shown to be a mediator of serotonin- (5-HT-) induced changes in K conductance [6-8, 10], are a convenient model with which to study this problem. Meanwhile the role of cAMP in responses to 5-HT connected with changes in Na conductance has received less study. Data in the literature on this problem are few in number and contradictory in nature [3-5].

Accordingly, the aim of the present investigation was to compare effects of intracellular injection of cAMP and extracellular application of 5-HT to *Helix* neurons and to study the effect of theophylline, an inhibitor of cyclic nucleotide phosphodiesterase, on these responses.

EXPERIMENTAL METHOD

Resection of the preparation and intracellular injection of cAMP by microiontophoresis through a multibarreled microelectrode were described previously [1]. The neurons were identified in accordance with Sakharov's classification [2].

5-HT and acetylcholine (ACh) (10^{-7}-10^{-5} M, from Serva, West Germany), and also theophylline (10^{-4}-10^{-3} M) were added to the solution surrounding the preparation. The intervals between injections of cAMP, and also between applications of the transmitters, were not less than 5 min.

EXPERIMENTAL RESULTS

In 17 experiments conducted on cells RPa2 and V4 cells in the F region, 5-HT induced rapid and reversible monophasic or biphasic membrane depolarization, the amplitude of which rose as a linear function with an increase in membrane potential (MP) (Fig. 1a). The reversal potential (RP) of the responses to 5-HT ($E_{5\text{-HT}}$) and to cAMP was determined from the point of intersection of the volt-ampere characteristic (VAC) of the membrane in the control solution and in the presence of 5-HT. $E_{5\text{-HT}}$ varied in different neurons from +10 to −30 mV (Fig. 1c).

Intracellular injection of cAMP into these same neurons caused rapid and reversible membrane depolarization, which increased in amplitude with an increase in MP parallel to the increase of the responses to 5-HT (Fig. 1b). RP of the cAMP responses (E_{cAMP}) varied from +10 to −30 mV. The values of $E_{5\text{-HT}}$ and E_{cAMP} coincided in seven of 17 cases (Fig. 1c). In the remaining 10 experiments they differed from each other by 5-15 mV.

Theophylline, in a concentration of $1 \cdot 10^{-4}$-$5 \cdot 10^{-4}$ M had no marked effect on MP of the neurons, but in 12 of 14 experiments it increased the amplitude and duration of both 5-HT- and cAMP-dependent depolarization by 1.5-2 times (Fig. 2). The effect of potentiation of the responses to 5-HT by theophylline can be regarded as specific, for theophylline had no such effect on ACh-induced depolarization. If the theophylline concentration was increased to 10^{-3} M, it was then able to induce membrane depolarization comparable in amplitude with that induced by 5-HT. When theophylline was rinsed out of the preparation the responses of 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. 103, No. 6, pp. 657-659, June 1987. Original article submitted December 10, 1986.

0007-4888/87/0006-0741$12.50 © 1987 Plenum Publishing Corporation

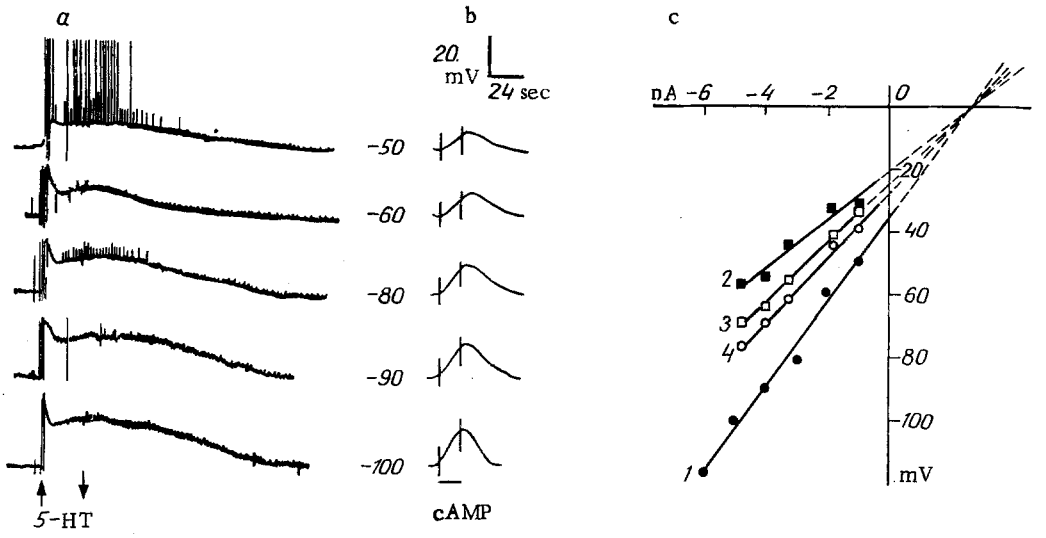

Fig. 1. Dependence of amplitude of 5-HT response (a) and cAMP-response (b) on MP level (neuron in F region). a, b) Values of MP indicated in millivolts near each trace. Arrow pointing upward indicates time of application of 5-HT (10^{-5} M to chamber, arrow pointing downward indicates time of starting flow and beginning of rinsing out of 5-HT (here and in Fig. 3). Time of microiontophoretic injection of cAMP (injection current 30 nA) indicated by a continuous line beneath the trace, and can also be seen from the artifacts due to on and off of the microiontophoretic current on the traces; c) graphs showing amplitude of 5-HT and cAMP responses as a function of MP, corresponding to traces in a and b. 1) Control VAC of neuron; 2) VAC of fast phase of response to 5-HT 3) VAC of slow phase of response to 5-HT; 4) VAC of cAMP response. RP of both phases of response to 5-HT and cAMP was determined by projecting the point of intersection of the straight lines, 2, 3, and 4 with the straight line 1 on the voltage axis. In this particular cell $E_{5\text{-HT}}$ for both phases of the response and E_{cAMP} coincided and their value was 0 mV.

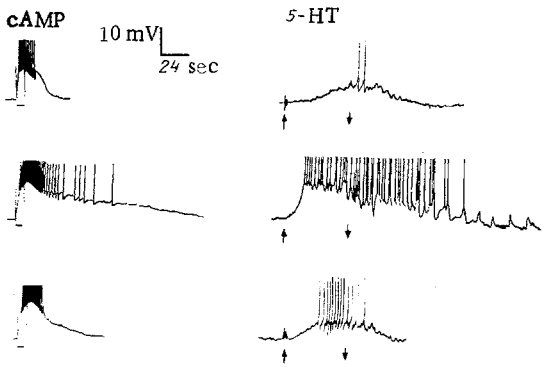

Fig. 2. Potentiating effect of theophylline on cAMP response (a) and 5-HT response (b) (neuron RPa2). MP = −60 mV. Top traces — cAMP and 5-HT responses in control; middle traces — in the presence of theophylline ($5 \cdot 10^{-4}$ M); bottom traces — 20 min after beginning of rinsing out of theophylline. Increase in amplitude and duration of cAMP and 5-HT responses can be seen in the presence of theophylline.

neurons to 5-HT and to cAMP were reduced to their initial values after 20-40 min in seven of 12 experiments.

In three experiments conducted on neurons in the G region, during application of 5-HT membrane hyperpolarization was recorded with $E_{5\text{-HT}}$ between −70 and −80 mV. Intracellular injection of cAMP into these neurons, on the other hand, induced membrane depolarization with E_{cAMP} between 0 and −30 mV (Fig. 3).

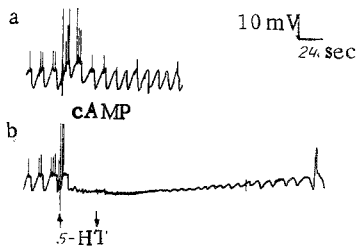

Fig. 3. Depolarization in response to cAMP (a) and hyperpolarization in response to 5-HT (b) of a neuron in the G region. MP = −60 mV. Oscillation of MP can be seen, disappearing after application of 5-HT and membrane hyperpolarization.

Comparison of the effect of intracellular injection of cAMP and extracellular application of 5-HT on *Helix* neurons showed that the effects in most experiments were similar in principle, namely, rapid and reversible membrane depolarization with RP of between +10 and −30 mV. The values of RP indicate the multi-ionic nature of the responses and participation of changes in membrane conductance for two (Na^+, K^+) or three (Na^+, K^+, Cl^-) ions in their genesis. This must evidently be the explanation of the incomplete coincidence of $E_{5\text{-HT}}$ and E_{cAMP} in some of the experiments. The results satisfy one of the principal criteria for mediation of the response to the neurotransmitter by a secondary messenger [9] and they suggest involvement of the cAMP system in the generation of excitatory responses of *Helix* neurons to 5-HT.

The potentiating effect of theophylline on serotonin-induced depolarization, and also its ability, in high concentrations, to simulate serotonin depolarization, in our view, confirm the hypothesis that cAMP is the intracellular mediator of serotonin-induced depolarizing responses.

However, this problem cannot be solved by electrophysiological experiments alone. Its final solution requires biochemical experiments to be undertaken on isolated identified neurons, in order to study stimulation of adenylate cyclase by 5-HT and prevention of this stimulation by blockers of serotonin receptors.

In neurons in the G region, responding to 5-HT by hyperpolarization, the opposite effect was produced by cAMP, namely membrane depolarization; at first glance this rules out the possibility of a connection between 5-HT- and cAMP-responses in these neurons. However, since the cAMP response, to judge from RP, may also include a K^+ component, this connection, in our view, cannot be ruled out. The possible role of cAMP in the realization of inhibitory 5-HT responses, due to an increase in K-conductance, evidently requires special study.

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