also is reduced in the opposite direction compared with changes in the presence of GABA. In that case, in the presence of THIP only a change in K_d ought to be observed. However, saturation analysis in the presence of THIP revealed a decrease not only of affinity, but also of density of the receptors, which cannot be explained by antagonistic relations between GABA and THIP. It is likewise not clear why the inhibitory action of THIP is exhibited only in a buffer of low molarity.

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MUSCARINIC AGONISTS HAVE NO EFFECT ON SPONTANEOUS QUANTUM TRANSMITTER RELEASE FROM FROG MOTOR NERVE ENDINGS

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The study of the mechanisms regulating transmitter release from nerve endings through the intervention of presynaptic autoreceptors is of great interest [2, 5, 14]. Both spontaneous and evoked quantal acetylcholine secretion in the neuromuscular junction of warm-blooded animals and in synapses of amphibians is modified by the action of cholinomimetics through activation of receptors of the nicotine type [7-11, 13]. There is evidence in the literature that both nicotinic and muscarinic receptors may participate in the mechanism of this effect. However, reduction of the frequency of miniature end-plate potentials (MEPP) under the influence both of carbachol (CCh), a mimetic which can activate both nicotinic and muscarinic receptors, and also purely muscarinic agonists, namely muscarine and metacholine, has been described, so that the authors cited could postulate the existence of receptors of muscarinic type on frog motor nerve endings [12]. There is also evidence that inhibition of spontaneous transmitter secretion in frogs takes place in the presence of the nicotine mimetic suberyldicholine [4], whereas the muscarine antagonist atropine does not abolish the decrease in frequency of MEPP induced by CCh [1]. In the present investigation, to remove the contradictions in the question of the existence of muscarinic receptors on frog motor nerve endings, the effect of several substances, differing essentially in their chemical structure, but possessing high muscarinomimetic activity, was tested on spontaneous quantal acetylcholine release.

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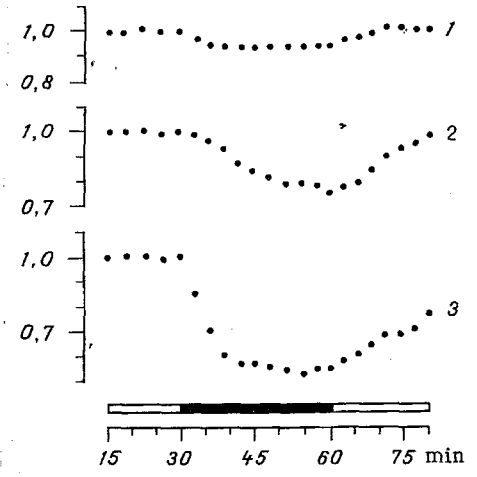

Fig. 1

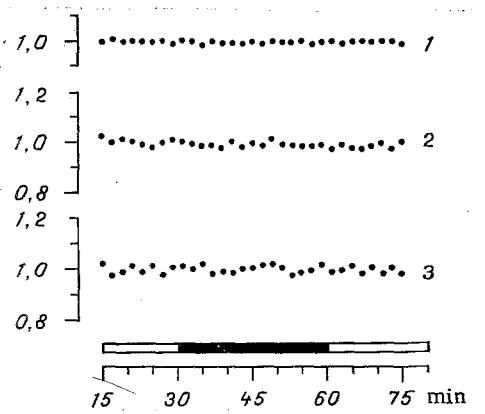

Fig. 2

Fig. 1. Changes in membrane potential (1), and amplitude (2) and frequency (3) of MEPP under the influence of CCh (3.5 μM). Here and in Fig. 2: abscissa, time (in min); ordinate, ratio of above parameters to corresponding control value. Black rectangle indicates time of CCh application.

Fig. 2. Membrane potential (1), and amplitude (2) and frequency (3) of MEPP in the presence of methylfurmethide (5 μM). Black rectangle indicates time of application of methylfurmethide.

EXPERIMENTAL METHOD

Experiments were carried out on a nerve-muscle preparation of the frog *Rana temporaria* in the fall and winter. The frogs were kept at 10°C. The isolated muscle with the nerve leading to it were placed in a bath with a capacity of 1.5 cm^3, through which flowed Ringer's solution (20.5°C; pH 7.3) at the rate of 5 ml/min; the composition of the Ringer's solution was (in mM): NaCl 113.0, KCl 2.5, $CaCl_2$ 1.8, $NaHCO_3$ 3.0. A solution with 10 mM KCl was used in some experiments. In this case the iso-osmolarity of the solution was maintained by a corresponding decrease in the NaCl concentration. Synaptic potentials were recorded with the aid of standard microelectrodes, beginning not earlier than 40 min after the preparation was placed in the bath. The substances for testing were added after the amplitude and frequency of MEPP and the membrane potential of the muscle fiber had stabilized. Control experiments showed that when these conditions were observed the mean values of the parameters recorded remained constant for 2 h. MEPP were analyzed by means of a measuring and calculating system based on the Élektronika DZ-28 computer. The mean amplitude and frequency of the MEPP were determined and histograms of the distribution of amplitudes plotted for every 100 signals. Slow changes of membrane potential were recorded on an automatic writer.

The following products were used: carbachol (from Koch-Light, England), methylfurmethide (USSR origin), oxotremorine (From Serva, West Germany), methacholine (from Sigma, USA), and F-2268 (USSR origin).

EXPERIMENTAL RESULTS

In the first series of experiments the effects of CCh on spontaneous release of quanta of transmitter was studied. The original values of mean frequency and amplitude of MEPP were 1.3 ± 0.4 Hz and 0.46 ± 0.1 mV, respectively, and the membrane potential of the muscle fiber in the synaptic zone was 79.5 ± 4.1 mV. The frequency of MEPP fell to 0.67 ± 0.3 Hz and their amplitude to 0.36 ± 0.02 mV after exposure for 20 min to CCh in a concentration of 3.5 μM, and the post-synaptic membrane was depolarized to −74.5 ± 1.6 mM (Fig. 1). Despite the decrease in amplitude of MEPP and the increase in noise of the cholinoreceptive membrane due to the post-synaptic action of CCh, the signal to noise ratio in these experiments could be recorded without loss of MEPP even at its lowest value, as was shown by the absence of differences in the character of distribution of MEPP amplitudes before and after the action of the cholinomimetic. Thus, the action of an agonist capable of activating both muscarinic and nicotinic acetylcholine receptors on the frog nerve-muscle preparation led to a decrease in spontaneous quantal transmitter release by the same degree as was observed in the previous investigations [1, 12].

In the next series the presynaptic action of methylfurmethide, oxotremorine, and methacholine, agonists with high muscarinic specificity [6], was studied. Methylfurmethide in concentrations of $5 \cdot 10^{-7}$-$3.5 \cdot 10^{-5}$ M in Ringer's solution of normal ionic composition, acting for 30 min, had no effect on the frequency and amplitude of MEPP and did not change the membrane potential of the muscle fiber in the zone of the synapse (Fig. 2). Oxotremorine ($5 \cdot 10^{-7}$-$1 \cdot 10^{-5}$ M) and methacholine ($8 \cdot 10^{-7}$-$2 \cdot 10^{-6}$ M) likewise caused neither postsynaptic nor presynaptic effects, even if applied for 1 h.

Since muscarinic receptors possess stereochemical specificity, i.e., they may be activated to different degrees by dextro- and levorotatory isomers [3, 6], the presynaptic effect of L- and D-forms of the muscarinic agonist F-2268 was studied. In concentrations of $3.3 \cdot 10^{-8}$-$5 \cdot 10^{-6}$ M the isomers caused no significant change in the frequency of MEPP.

These investigations indicate that muscarinic agonists of varied chemical structure, in their action on the frog neuromuscular synapse, do not affect the process of spontaneous quantal transmitter secretion in medium with normal ionic composition. These findings do not agree with results obtained by Duncan and Publicover [12].

A similar situation of contradictory information regarding the effect of cholinomimetics on spontaneous transmitter release also was observed in experiments on warm-blooded animals, when some workers describe the facilitatory action of nicotine agonists on quantal acetylcholine secretion whereas others did not observe this effect [14]. The disagreements were removed when it was shown that the presynaptic effect of cholinomimetics depends on the membrane potential of the nerve ending [15]. Preliminary depolarization of the nerve endings by increasing the K^+ concentration in the solution containing the preparation led to the development of a marked facilitatory effect of the cholinomimetics on spontaneous transmitter release [7, 8, 15]. Assuming that the possible cause of the disagreement between our own results and data in the literature [12] could also be the different degree of initial polarization of the nerve endings, the presynaptic action of muscarine mimetics was studied in medium with an increased K^+ concentration. However, raising the K^+ concentration to 10 mM did not lead to the appearance of an effect of metacholine and methylfurmethide on MEPP frequency.

The absence of effect of muscarine agonists on spontaneous quantal acetylcholine secretion, both in medium with normal ionic composition and after increasing the K^+ concentration, is evidence that there are no muscarinic receptors controlling spontaneous transmitter release on frog motor nerve endings, and that the presynaptic action of CCh is not associated with its muscarinomimetic activity.

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