Both neurospecific and nonspecific brain proteins were thus shown to participate in the processes accompanying formation of the memory trace. The results are evidence that the structure most concerned in conditioned reflex formation in a Y-shaped maze is the visual cortex. Even the level of the neurospecific protein P_2, which is quite "inert" in the other structures tested, changes in this part of the brain. The reason may perhaps be that conditioned stimulus in this model of learning was light.

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IS THE PRESYNAPTIC ACTION OF CARBACHOL LINKED WITH ACTIVATION OF THE POSTSYNAPTIC MEMBRANE?

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Besides depolarization of the postsynaptic membrane (PSM) of the muscle fiber, carbachol also causes a decrease in the quantum composition of end-plate potentials (mEPP) [2, 3]. This last fact is in harmony with data in the literature showing that other cholinomimetic drugs possess a presynaptic action [2, 6-8, 13]. Solution of the problem of whether the presynaptic action of cholinomimetics is due to their direct effect on motor nerve endings or whether it is brought about indirectly through ionic shifts is an essential preliminary to the understanding of the mechanism of this effect. In particular, the view is sufficiently widely held that the presynaptic action of cholinomimetics is linked with depolarization of motor nerve endings by potassium ions leaving the muscle fiber during activation of its PSM [9]. However, there is no factual evidence in support of the role of K^+ in the mechanisms of the effect of carbachol or of other cholinomimetic drugs on evoked liberation of mediator. Moreover, there are serious grounds for considering that a decrease in mEPP, taking place in the presence of carbachol, is due to the direct action of the mimetic on nerve endings [3].

To shed light on the role of end-plate depolarization in the mechanism of the presynaptic effect of carbachol, the investigation described below was undertaken with the following aim:

KEY WORDS: nerve-muscle preparation; carbachol; presynaptic action; end-plate depolarization.

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to compare minimal concentrations of carbachol required to produce pre- and postsynaptic effects; to study the presynaptic action of carbachol on a "divided" nerve–muscle preparation – an object in which the depolarizing action of the mimetic on the PSM is not exhibited; to compare the minimal concentration of D-tubocurarine (DTC), when the presynaptic action of carbachol is blocked, with the concentration required to prevent activation of PSM.

EXPERIMENTAL METHOD

Experiments were carried out on an isolated nerve–muscle preparation of the sartorius muscle of pond frogs in the winter period. Synaptic potentials were derived by the standard microelectrode technique. During the experiment the nerve–muscle preparation was kept in a transparent plastic bath with a capacity of 5 cm^3, containing running Ringer's solution (rate of flow 8 ml/min) of the following composition (in mM): NaCl 115.0, KCl 2.5, $CaCl_2$ 1.8, $NaHCO_3$ 3.0. Action potentials and contractions of the muscle were blocked either by reducing the Ca^{++} concentration in the Ringer's solution to 0.9 mM and adding Mg^{++} up to 5 mM (a "magnesium" preparation) or by dividing the muscle fibers transversely (the "divided" preparation) [1, 4, 5]. In the "divided" muscles mEPP was calculated by determining the coefficient of variation of amplitude of the EPP, whereas in the "magnesium" preparation this was done by the direct method, i.e., by dividing the mean amplitude of the EPP by the mean amplitude of the miniature EPP (mEPP). If the amplitude of the EPP exceeded 4 mV, a correction was introduced beforehand for nonlinearity of mEPP summation [10]. When the dose–effect curve was plotted for PSM the initial values of the membrane potential (MP) in different muscle fibers were standardized, reducing them to the level of –90 mV [12]. The experimental results were subjected to statistical analysis by Student's t-test and Wilcoxon's test.

EXPERIMENTAL RESULTS

One way of studying the problem of the role of PSM depolarization in the development of the presynaptic action of carbachol is to compare changes in mEPP with the magnitude of PSM depolarization as a result of the action of different concentrations of the mimetic. As a first step the dose–effect curve was plotted for the PSM (Fig. 1). On the basis of the results, the following concentrations were chosen for studying the effect of carbachol on mEPP: $5 \cdot 10^{-7}$ M, at which no depolarization of PSM took place, $1 \cdot 10^{-6}$ M, a concentration depolarizing PSM by not more than 2 mV, and $5 \cdot 10^{-6}$ M, a concentration reducing MP of the muscle fiber in the region of the synapse by an amount of about 10 mV.

The experiments to study the presynaptic action of carbachol in these doses were performed on "magnesium" preparations. The results of these experiments are given in Table 1. In all concentrations used carbachol significantly depressed mEPP. In the lowest concentration ($5 \cdot 10^{-7}$ M), although it had no appreciable postsynaptic action, carbachol depressed mEPP by 12.7%, whereas in a concentration of $1 \cdot 10^{-6}$ M, depolarizing PSM by only 1.5 ± 0.4 mV, it reduced evoked liberation of the mediator by 10.4% of its initial vaule. In a concentration of $5 \cdot 10^{-6}$ M carbachol reduced mEPP by 30.1 ± 5.2%, depolarizing PSM by 9.6 ± 1.4 mV.

To answer the question of the contribution of PSM activation to the presynaptic action of the mimetic in a concentration of $5 \cdot 10^{-6}$ M, it was decided to study the effect of this

TABLE 1. Effect of Carbachol on Quantum Composition of EPP (mEPP)

$5 \cdot 10^{-7}$ M				$1 \cdot 10^{-6}$ M				$5 \cdot 10^{-6}$ M			
mEPP		MP, mV		mEPP		MP, mV		mEPP		MP, mV	
before action of carbachol	after 15 min of action	before action of carbachol	after 15 min of action	before action of carbachol	after 15 min of action	before action of carbachol	after 15 min of action	before action of carbachol	after 15 min of action	before action of carbachol	after 15 min of action
8,2	6,3	76,3	76,3	14,6	12,8	95,0	92,2	50,7	36,8	70,7	61,0
4,7	3,1	84,1	84,0	16,1	15,6	89,1	89,0	30,8	21,3	80,0	70,2
7,7	7,1	63,8	63,8	9,9	5,7	81,5	80,2	21,3	13,0	71,1	53,7
23,3	24,0	58,0	57,7	19,0	16,5	78,2	77,4	21,7	20,1	68,3	60,9
11,7	9,8	66,4	66,5	20,6	18,8	82,8	80,4	42,2	28,9	68,4	58,3
5,3	3,6	88,7	88,6	6,4	7,2	75,3	74,1	16,1	9,0	73,8	67,6
21,8	18,5	90,3	90,4	21,0	19,7	74,3	72,1	13,9	8,1	75,1	68,3
11,8	10,3	75,4	75,3	15,4	13,8	82,3	80,8	28,1	19,6	72,5	62,9
P<0,02		P>0,25		P<0,05		P<0,01		P<0,002		P<0,001	

Legend. Here and in Table 2, mean value (M) shown below the line.

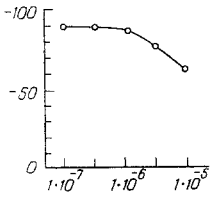

Fig. 1. Depolarization of end plate of muscle fiber depending on carbachol concentration. Abscissa, carbachol concentration (in M); ordinate, value of MP (in mV).

TABLE 2. Effect of Carbachol ($5 \cdot 10^{-6}$ M) on Quantum Composition of EPP (mEPP) and on MP of Muscle Fiber (in percent of control) in the Presence of DTC ($1 \cdot 10^{-7}$ g/ml)

mEPP after action of carbachol for 15 min		MP after action of carbachol for 15 min	
Ringer's solution	Ringer's solution + DTC	Ringer's solution	Ringer's solution + DTC
72,6	102,3	85,9	86,8
69,1	126,7	86,5	83,6
61,0	102,9	76,0	88,6
92,6	111,8	87,3	92,5
68,5	127,6	84,9	90,8
55,9	122,7	91,4	87,5
70,0	115,7	85,3	88,3
	P<0,05		P>0,1

concentration of carbachol on mEPP in the "divided" nerve–muscle preparation, i.e., under conditions when carbachol had no significant postsynaptic action. Transverse division of the muscle brought MP of the muscle fiber closer to the equilibrium potential for response to the cholinomimetic, whereas activation of chemoreceptors of PSM evoked a much smaller degree of depolarization than in the intact muscle.

mEPP was reduced by 41.5 ± 4.1% 15 min after the beginning of action of carbachol ($5 \cdot 10^{-6}$ M), but there was no change under these circumstances in MP of the muscle fiber. Comparison of these data (Wilcoxon's test) with the results of a change in mEPP in the previous series of experiments revealed no significant difference between them ($P > 0.05$). This fact is evidence that the decrease in the mEPP under the influence of carbachol in a concentration causing depolarization of PSM of the muscle fiber by not more than 10 mV, was not connected with the postsynaptic action of the mimetic.

The sensitivity of motor nerve endings to DTC is higher than the sensitivity of the PSM [11, 14, 15]. Hence it follows that, by using small doses of DTC, it is evidently possible to choose the concentration of the drug which would modulate the pre- and postsynaptic action of carbachol to different degrees. To test this hypothesis experiments were carried out on nerve–muscle preparations blocked by reducing the Ca^{++} concentration in the Ringer's solution and adding Mg^{++}. DTC was added to this solution in a concentration of $1 \cdot 10^{-7}$ g/ml. After 60 min mEPP was calculated by the "direct" method and the solution was replaced by a similar solution containing carbachol ($5 \cdot 10^{-6}$ M). The results of these experiments are given in Table 2. They showed that tubocurarine completely abolished the presynaptic action of the mimetic (mEPP did not change significantly under the influence of carbachol), whereas the postsynaptic effect was preserved (PSM was depolarized by 7.7 ± 1.1 mV, i.e., to the same degree as in medium without DTC).

The results of these experiments thus show that the pre- and postsynaptic effects of carbachol within the concentration range of $1 \cdot 10^{-7}$–$5 \cdot 10^{-6}$ M develop separately in the neuromuscular synapse of the frog, i.e., that depolarization of PSM (and, in particular, outflow of K^{+}) are not essential for the realization of the effect of carbachol on nerve endings.

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ROLE OF THE VESTIBULAR SYSTEM IN THE REGULATION OF COLD TREMOR

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The main source of heat production in homoiothermic animals is cold tremor (CT). Central regulation of CT is effected by the hypothalamic center, with the participation of brainstem systems forming postural muscular activity [5-7, 10]. It is natural to suggest that these systems may also have a definite influence on activity of the muscle during CT.

In the investigation described below the role of the vestibular component of the postural control system in the regulation of CT was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 132 cats anesthetized with a chloralose-urethane mixture (50 and 500 mg/kg, respectively). During the experiment the animals were kept in a constant temperature chamber (temperature 18-20°C) in a frame, fixing them in a "standing" posture, or they lay freely on their side. Activity of the limb muscles (flexors: biceps brachii, sartorius; extensors: triceps brachii, triceps surae) and the dorsal cervical muscles (trapezius, rhomboideus) was investigated. Methods of derivation of muscle potentials and of stimulation and destruction of the vestibular system were described by the writers previously [1, 2]. To analyze changes in muscle electrical activity during CT evoked by stimulation of the vestibular system, the function of motor units (MU) of the ipsilateral sartorius muscle was investigated before and 5 and 10 min after destruction of the labyrinth by the method in [4]. Throughout the experiment the rectal and subcutaneous temperature was recorded continuously on the N-3020-3 automatic writer.

EXPERIMENTAL RESULTS

In anesthetized animals in an ambient temperature of about 20°C the subcutaneous and rectal temperature fell, with the subsequent appearance of bioelectrical temperature-regulating activity in the muscles (CT). Muscular activity developed in the limb flexors and neck muscles. It was absent in the limb extensors. Stimulation of the vestibular system was applied during CT against the background of a stable integral electromyogram (EMG).

The investigation showed that during unilateral stimulation of the vestibular system by a pulsed current and also during caloric stimulation a bilateral decrease or cessation of electrical activity took place in the neck muscles and limb flexors. During stimulation of the labyrinth no evoked responses were observed in the limb extensors. In the cervical muscles suppression of activity predominated on the contralateral side relative to the side on

KEY WORDS: cold tremor; vestibular system; motor units.

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