Experiments on mice thus showed that retinoic acid, in the form of MR and 13-cis-isomers of methylretinoate, have a modulating action on certain parameters of humoral immunity. MR stimulates antibody production in response to the action of bacterial (*E. coli*) antigens and lowers the parameters of nonspecific antiviral defense — the serum inhibitor and interferon levels. Both isomers reduced the titer of anti-influenzal antibodies.

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CENTRAL CHOLINOLYTIC EFFECT OF TROPANE DERIVATIVES: CORRELATION BETWEEN STRUCTURE AND ACTION

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Besides an aliphatic chain, in the structure of the known cholinolytics which are tropane derivatives (atropine, scopolamine, and so on), there are also an aromatic ring and a hydroxyl group. It is considered that both aryl and hydroxyl radicals are necessary for tropane derivatives to exhibit their cholinolytic properties [8, 9]. This conclusion was drawn from a study of peripheral cholinolytic properties of atropine and its analogs, the effect of which was estimated according to the degree of mydriasis. We know that the acetylcholine receptors of the neocortex are of the muscarinic type. The muscarinic cholinolytic atropine reduces the responses of cortical neurons to acetylcholine (ACh) [5, 7].

The aim of the present investigation was to study whether the presence of the aryl radical and hydroxyl groups is essential for tropane derivatives to exhibit their central cholinolytic activity. For this purpose two substances synthesized in the Department of Organic Synthesis, Institute of Pharmacology, Academy of Medical Sciences of the USSR, under the working name of LK-11 [dihydrochloride of the β-(N-morpholyl)priopionic tropine ester] and LK-14 [trihydrochloride of the β-(N-methylpiperazinyl)propionic ester of tropine], were studied. Neither compound has aryl and hydroxyl radicals, although the tropane structure and the ester grouping are preserved.

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TABLE 1. Correlation Between Different Types of Responses of Sensomotor Cortical Neurons to Microiontophoretically Applied ACh, LK-11 and LK-14

ACh	LK-11			LK-14		
	+	−	No effect	+	−	No effect
+	0	36	10	0	18	1
−	4	2	2	5	1	0
No effect	0	1	9	0	0	5

Legend. Numbers indicate number of neurons, +) increase in discharge frequency, −) decrease in discharge frequency.

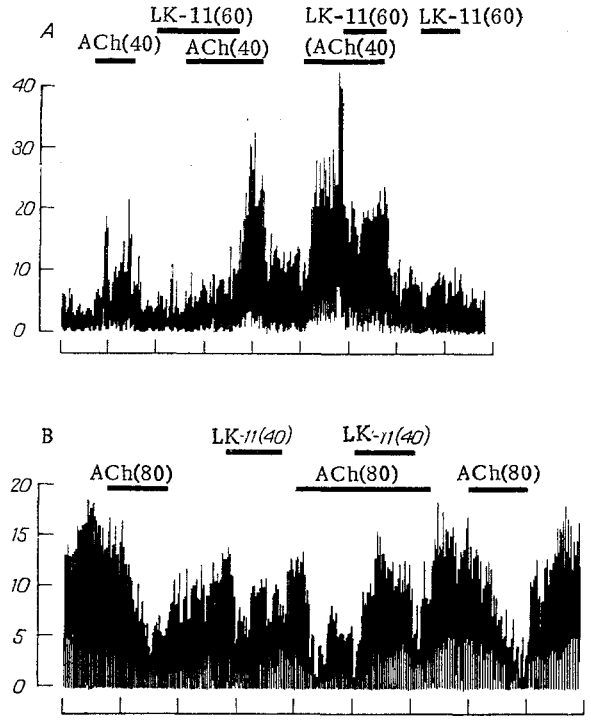

Fig. 1. Decrease in excitatory (A) and inhibitory (B) response of sensomotor cortical neuron to ACh under the influence of microiontophoretically applied LK-11. Abscissa, time (in min); ordinate, spike discharge frequency (spikes/sec). Horizontal lines indicate duration of microiontophoretic application of substances, numbers in parentheses show strength of microiontophoretic current (in nA). A, B) Two different neurons.

EXPERIMENTAL METHOD

Experiments were carried out on 21 adult rabbits weighing 3-3.5 kg. The animals were immobilized with diplacin (5 mg/kg) and artificially ventilated. The test substances were applied to the membrane of single neurons of the sensomotor cortex by microiontophoresis. For this purpose seven-barreled glass microelectrodes were used, one barrel of which served for extracellular recording of action potentials (AP) of the neurons. The other barrels were filled with aqueous solutions of 0.3 M ACh chloride, pH 4.0, 0.3 M atropine sulphate, pH 4.0, 0.3 M LK-11, pH 4.0, 0.3 M LK-14, pH 4.0, and 0.3 M noradrenalin hydrochloride, pH 4.5. One barrel of the microelectrode was filled with 2M NaCl and was used to exclude current artifacts.

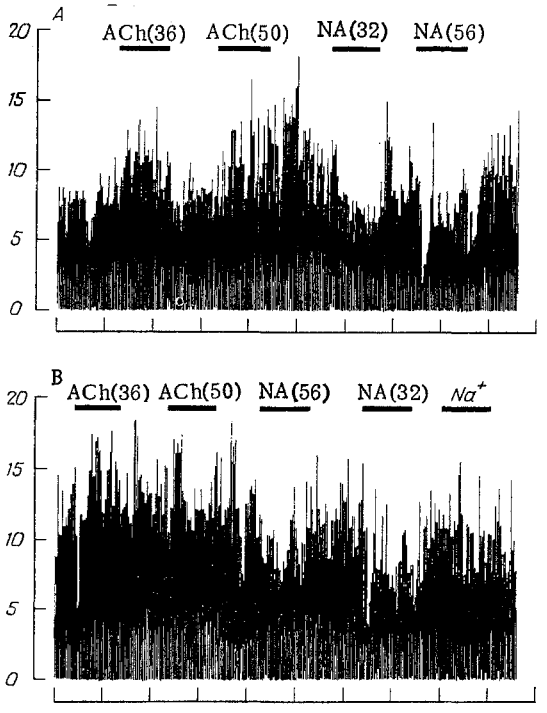

Fig. 2. Decrease in excitatory response of neuron to ACh and preservation of magnitude of inhibitory response to noradrenalin (NA) under the influence of intravenous injection of LK-11. A) Before injection of LK-11, B) 6 min after intravenous injection of LK-11 in a dose of 2 mg/kg. Na^+) Passage of outward current of 100nA through barrel of microelectrode filled with 3M NaCl. Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

The main action of the two tropane derivatives (LK-11 and LK-14) when applied by microiontophoresis to single neurons of the sensomotor cortex was inhibition of spike activity (Table 1). This was expressed as a decrease in the spontaneous discharge frequency. Some neurons gave an excitatory response or no response whatever to the test substances. The direction of the change in unit activity brought about by LK-11 and LK-14 was opposite, in the overwhelming majority of cases, to that observed on microiontophoresis application of ACh. Most neurons excited by ACh were inhibited by LK-11 and LK-14. Neither substance reduced the amplitude of AP even when the spike discharge frequency was considerably reduced.

It will be clear from Fig. 1A that LK-11 and LK-14 reduced responses of the neurons to ACh. When ACh was applied by microiontophoresis to the neuron with a current of 40 nA it had a marked excitatory action: The frequency of AP increased from 2-3 to 10 spikes/sec. ACh, applied to the cell by a current of the same strength 1 min after preliminary application of LK-11, no longer exerted its original activating effect. After the end of application of LK-11, the excitatory action of ACh was quickly exhibited, and was actually stronger than its original effect. It is also shown in Fig. 1 that when LK-11 was applied to the cell against the background of previously developing acetylcholine excitation, in this case definite antagonism was observed between LK-11 and ACh, and was expressed as a marked decrease in the excitatory response to ACh. When LK-11 alone was applied, unit activity was not significantly altered. A similar decrease in the excitatory effect of ACh under the influence of LK-11 and LK-14 was found in 16 of 19 neurons tested. Only in three of 19 neurons was the cholinolytic effect of the tropane derivatives absent.

After intravenous injection of LK-11 and LK-14 in a dose of 2-10 mg/kg, the excitatory effect of ACh also was reduced. The inhibitory response of the neuron to noradrenalin (NA) remained unchanged. Inhibition of the response to ACh continued for not less than 40 min after systematic injection of LK-11. Similar results were obtained for three other neurons.

Not only the excitatory, but also the inhibitory response of the neuron to ACh was found to be almost completely blocked by microiontophoretic application of LK-11, even when

LK-11 itself had an inhibitory action on unit activity (Fig. 1B). This was found in seven of seven neurons investigated. However, it must be noted that in most cases LK-11 and LK-14 had an excitatory effect on neurons that were inhibited by ACh.

It follows from these results that tropane derivatives which have no aryl radical or hydroxyl groups, and which contain other radicals (morpholine, piperazine) in their structure, connected through an ester grouping with the C_3 of tropane, have a central cholinoltyic action similar to that of the typical cholinolytic atropine.

These results are in agreement with the idea expressed by Pfeiffer [10], according to which the decisive conditions for interaction of cholinomimetics and cholinolytics with the specific ACh-sensitive receptor is the distance between the nitrogen atom and the oxygen atoms of the carboxyl group. However, the present writers showed previously that even tropane itself, with no ester groups, can interact with central acetylcholine receptors by an agonist-antagonist mechanism [1]. Compounds with ester groups attached to the C_3 atom of tropane (atropine, LK, and so on) evidently possess exclusively cholinolytic properties and are unable to activate the acetylcholine receptor.

Preservation of the amplitude of AP after microiontophoretic application of LK-11 and LK-14 is evidence that the compounds have no local-anesthetic (membrane-stabilizing) properties. Evidently the presence of an aryl radical in the anesthesiophore grouping is necessary for these properties to be exhibited, as is the case in molecules of cocaine and atropine [4]. Like other workers [7], we found by microiontophoretic application of atropine to neurons that it has a local-anesthetic effect. It was exhibited as a decrease in amplitude of AP, or even by the total blocking of spike generation when comparatively weak microiontophoretic currents were used (under 30 nA). This creates considerable difficulties when an attempt is made to assess the cholinolytic effect of atropine quantitatively and to compare it with the effect of the tropane derivatives studied.

The opposite effect of tropane derivatives and ACh on activity of the same neurons may be due to depression of cholinergic activity by LK preparations in the cortex. With a decrease in spontaneous excitatory cholinergic influences on cortical neurons exerted, for example, by the ascending reticulocortical activating system (the alerting system), an inhibitory effect may be expected, whereas with a decrease in the background inhibitory cholinergic influences, an excitatory action of LK-11 and LK-14 can be expected. Atropine, on microiontophoretic application, also can reduce and increase unit activity in the brain [6].

Considering the results of previous investigations obtained by the summation of nervous impulses method, the conditioned reflex method, and so on [1-3], and also the results of the present investigation, it can be expected that the tropane derivatives studied possess considerable neurotropic activity.

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