CENTRAL ANTAGONISM BETWEEN GANGLIOLYTIC AGENTS AND NEOSTIGMINE METHYLSULFATE

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The study of the interrelations between cholinergic substances and anticholinesterase preparations has been advanced by the demonstration of several new factors in the physiology and pharmacology of the central nervous system. Data have been obtained which confirm the cholinergic nature of the functions of the mesodiencephalic reticular formation [6, 9, 10], the presence of choline-reactive structures in the cerebral cortex has been established [4], and the location and character of the central action of acetylcholine and its antagonists – atropine, scopolamine, etc. – have been defined more precisely.

At the same time it should be remembered that the overwhelming majority of works of this kind are associated with the use of "tertiary" cholinolytics. It appears that the explanation of this is to be sought in the fact that the central action is considerably weakened in passing from the "tertiary" nitrogen derivatives to the "quaternary" ones, since the penetration of the latter into the central nervous system is impeded. Nevertheless realization of this fact should not limit research on the central mechanisms in the case of "quaternary" gangliolytics, which certainly have an effect on the central nervous system [1, 2, 5, 7].

The purpose of the present investigation was to study the interaction between the "quaternary" gangliolytics and neostigmine methylsulfate, with the help of methods that reflect the functional state of the central nervous system.

METHODS

For determination of functional changes in the central nervous system, recording of biopotentials of the cortex and subcortical regions was employed. Action potentials from the frontal lobe of the cortex were picked up with unipolar leads, carried to a balanced amplifier of a cathode-ray oscilloscope, and recorded photographically with a motion picture camera. Unipolar recording of action potentials of the subcortical region was carried out on decerebrate cats. The active electrode was inserted in the direction of the floor of the fourth ventricle, as determined by brain section. In the second series of experiments, the latent period of a flexor reflex was determined by the method of V. V. Zakusov [3]. Cutaneous stimulation in the femoral region was brought about with the help of needle-shaped electrodes. The current from an electronic rectifier plugged into the line was used as a stimulus. The stimulus intensity was determined from the voltage. Time was indicated automatically by a type EMS-4 millisecond clock. Various derivatives of the di-iodide of hexamethylene-bis-(trimethylammonium), substituted with cations and anions, were used as gangliolytics. The experiments were performed on 26 rabbits and 14 cats.

RESULTS

Changes in the bioelectrical activity of the brain occurring under the influence of these gangliolytics were used as indicators of functional shifts in the central nervous system. In rabbits, these changes were usually of a phasic character. Upon intravenous administration of gangliolytics in doses of 0.5-5 mg/kg obliteration of the slow waves and increased amplitude and frequency of the fast waves (to 55-75 μV and 20-25 per second) were initially noted. In the second phase, which came on 5-45 minutes after injection of the substance, a considerable reduction of the bioelectrical activity of the frontal lobe of the cortex was seen, and the electroencephalogram assumed a flattened character. Changes in the bioelectrical activity of the cerebral cortex were recorded for 1-4 hours from the time of injection of the gangliolytic.

The bioelectric activity of the hypothalamic region of the cat brain also underwent substantial changes under the influence of these preparations. Both intravenous injection and injection directly into the cavity

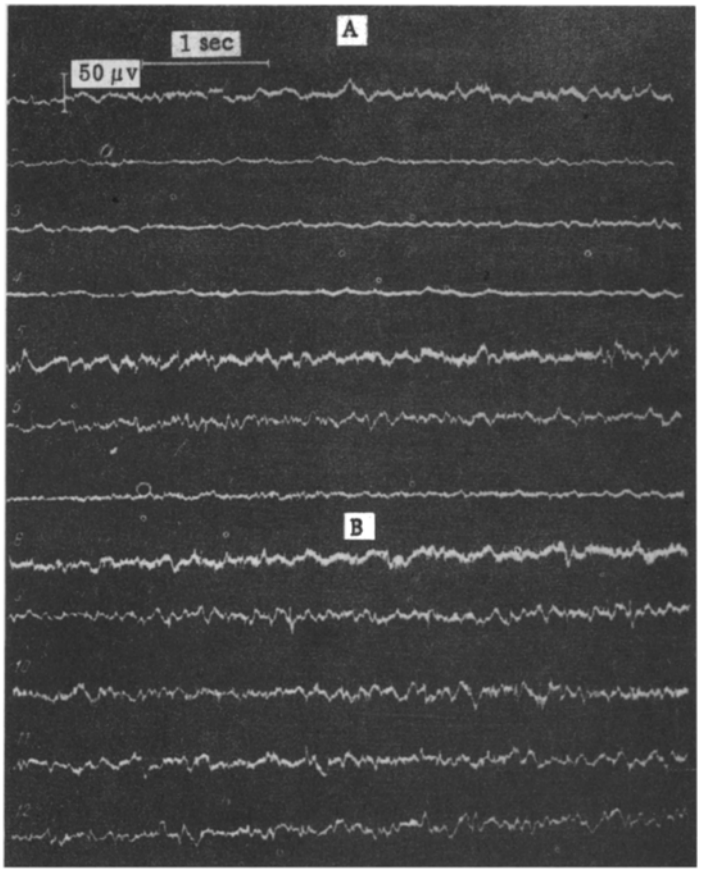

Fig. 1. Effect of gangliolytics and neostigmine methylsulfate on bioelectrical activity of the cerebral cortex of the rabbit. Record of action potentials of frontal lobe. A) Effect of di-p-amino-benzoyl salt of hexamethylene-bis-ethyldimethylammonium in a dose of 5 mg/kg. 1) Normal; 2,3,4,5,6, 7) 5,10,20,30,60 and 90 minutes after injection of the preparation. B) Effect of the same preparation against a background of neostigmine methyl-sulfate (0.2 mg/kg intravenously). 8) normal; 9,10,11, 12) 5, 10,30, and 60 minutes after injection of gangliolytic.

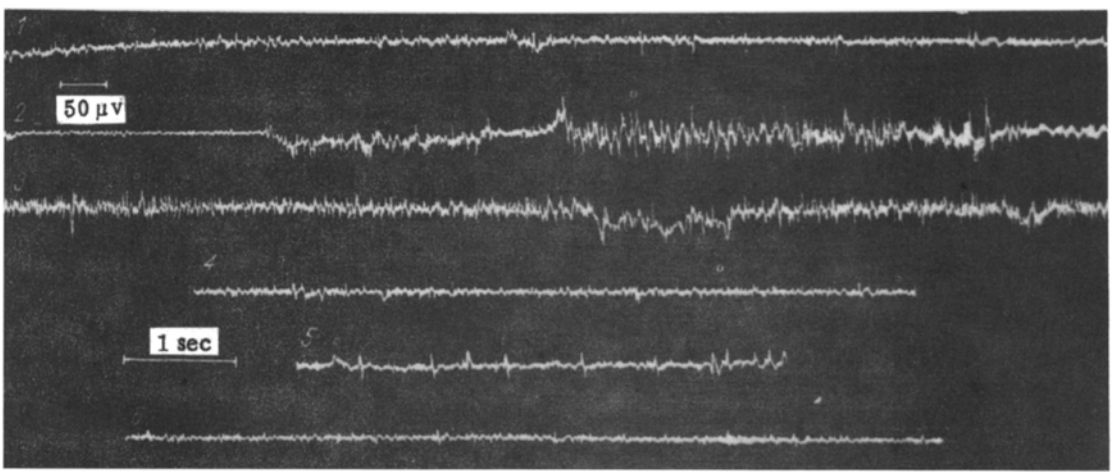

Fig. 2. Effect of gangliolytics and neostigmine methysulfate on bioelectric activity of hypothalamic region of brain cat. 1) Normal; 2, 3, 4-3, 10 and 30 minutes after injection of 1 mg/kg of di-pyridine-β-carboxyl salt of hexamethylene-bis-triethylammonium into cavity of 4th ventricle; 5, 6) 3 and 10 minutes after injection of the same preparation against a background of neostigmine methyl-sulfate (40 γ/kg into the cavity of 4th ventricle).

of the 4th ventricle were accompanied by depression of bioelectrical activity, and reduction of the amplitude of all components of the electroencephalogram. In a number of experiments in which relatively small doses of gangliolytic were injected into the cavity of the 4th ventricle (0.1-1 mg/kg) synchronous, peak-shaped waves with a frequency of 3-40 per second and an amplitude of 30-60 μV were seen to appear.

It was established that the character of the anion had a substantial effect on the duration and depth of the functional shifts in the central nervous system, as determined by the electroencephalogram.

The salts of "quaternary" nitrogen derivatives substituted with anions of nitrogen-containing cyclic radicals – β-pyridine-carboxyl, p-nitrobenzoyl, p-aminobenzoyl – were more active in this respect than their halogen homologs.

The data obtained served as a basis for conducting experiments in which the effect of neostigmine methylsulfate on changes on the electroencephalogram produced by gangliolytics was studied. It was first established that neostigmine methylsulfate administered intravenously in doses of 0.05-0.1 mg/kg produced no substantial changes in the bioelectrical activity of the cortex and subcortical regions. We also found corresponding data in the literature [6, 8].

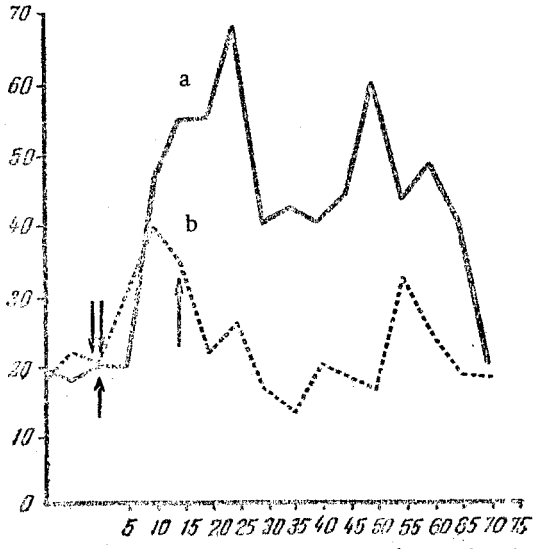

Fig. 3. Effect of gangliolytics and neostigmine methylsulfate on the latent period of flexor reflex of the rabbit. a) Following injection of dipyridine-β-carboyxl salt of hexamethylene-bis-triethylammonium (1 mg/kg intravenously); b) following injection of the same preparation against a background of neostigmine methylsulfate (0.05 mg/kg intravenously). Arrows indicate the moment of injection of gangliolytic (↑) and neostigmine methylsulfate (⇓). Along abscissa – reflex time in milliseconds; along ordinate – duration of experiment in minutes.

In various versions of the experiment – with preliminary, simultaneous, and subsequent administration of neostigmine methylsulfate – it was established that this compound significantly reverses the bioelectrical reaction to a gangliolytic. The slow variations, with a frequency around 3-4 per second and amplitude around 50-60 μv, remained even after administration of gangliolytic; the rhythm of the oscillations with moderate frequency and amplitude (of the order of 10-30 μv and 10-20 per second) was somewhat accelerated, but did not reach the control levels in a single experiment. The second phase of action of the gangliolytic – depression of the bioelectrical activity of the cortex – was completely absent (Fig. 1).

The antagonistic effect of neostigmine methylsulfate on the central actions of the gangliolytic also appeared in experiments on cats in which injections were made into the 4th ventricle. It should be noted that neostigmine methylsulfate was active not only against the inhibitory effects of these substances on bioelectrical activity of the hypothalamic region of the brain, but also towards the excitatory action which was observed in several instances when comparatively small doses of gangliolytics were injected into the cavity of the 4th ventricle. The large amplitude and rapid peak-shaped rhythm characterizing the excitation of bioelectrical activity of the subcortical region of the brain was absent following a single injection of neostigmine methyl sulfate and gangliolytic (Fig. 2). Repeated injections of small doses of gangliolytic into the cavity of the 4th ventricle of the same animal, made for purposes of control, showed that when the interval between injections is not less than an hour, the excitatory action of the preparations of this series remains, as determined by the electroencephalogram.

The central antagonism between these substances also was manifested in suppression of the motor reaction which occurred in several experiments when the ganglionic blocking agent was injected into the cavity of the 4th ventricle.

In the second series of experiments we studied the effect of neostigmine methylsulfate on the changes in the latent period of the flexor reflex observed in rabbits following the administration of gangliolytics. In control experiments, it was established that substances of this group injected intravenously in doses of 0.5-5 mg/kg produce a distinct slowing of the conduction of impulses through the synaptic apparatus of the spinal cord. The latent period of the reflex was 18-34 milliseconds in various rabbits. When the ganglioplegic was injected, an increase in the latent period ensued as early as 5-10 minutes after injection and remained for from 30 minutes to 4 hours. The latent period of the flexor reflex increased in various experiments to 50-190 milliseconds, which was 2-4 times longer than the original values.

Preliminary (10-15 minutes previously) intravenous injection of neostigmine methylsulfate in a dose of 0.05 mg/kg, which somewhat prolonged the latent period of

the reflex in the majority of experiments, reversed the similar effect of the gangliolytics.

In those experiments in which the effect of gangliolytics was still seen, it was considerably weaker and briefer against a background of neostigmine methylsulfate. The mean values shown in Fig. 3, calculated on the basis of 5 experiments with uniform characteristics, show the antagonistic action of gangliolytics and neostigmine methylsulfate on the latent period of the flexor reflex in the rabbit (Fig. 3).

The antagonistic effect of these substances was also displayed in the fact that the fasciculations of the muscles of the trunk and extremities and the quickened breathing seen in rabbits after injection of neostigmine methylsulfate were suppressed by gangliolytics.

The data presented confirm the presence of a central antagonism between "quaternary" gangliolytics and neostigmine methylsulfate. Such interrelations appear at various functional levels of the central nervous system – in the cortex, the subcortical region, and the spinal cord. These facts give evidence of the cholinolytic nature of the central action of ganglionic blocking agents – the derivatives of "quaternary" ammonium bases.

SUMMARY

The interrelations between gangliolytics – derivatives of the quaternary ammonium bases – and neostigmine methylsulfate were investigated by methods reflecting the functional state of the central nervous system. As shown by experiments in which cerebral biopotentials were recorded in rabbits and cats, neostigmine methylsulfate considerably reverses the bioelectrical reaction to gangliolytics. The antagonism between these substances is also demonstrated by the fact that neostigmine methylsulfate reverses the retardation of synaptic impulse transmission along the spinal cord caused by gangliolytics. The present data point to the fact that the central action of the series of compounds investigated is cholinergic in nature.

LITERATURE CITED

[1] B. B. Voznesenskii, Farmakol. i toksikol. 21, 4, 7 (1958).*

[2] P. P. Denisenko, Gangliolytics and Substances that Block Neuromuscular Junctions (Leningrad, 1958) p. 21 [in Russian].

[3] V. V. Zakusov, Fiziol. zhurn. SSSR 23, 2, 276 (1937).

[4] M. Ya. Mikhel'son, E. K. Rozhkova, and N. V. Savateev, Byull. eksperim. biol. i med. 37, 2, 7 (1954).

[5] M. L. Tarakhovskii, Farmakol. i toksikol. 20, 1, 33 (1957).*

[6] P. B. Bradley and J. Elkes, J. Physiol., 1953, v. 120, Proceeding p. 14.

[7] A. S. Doutas and M. J. Nickerson, J. Pharmacol. a. Exper. Therap., 1957, v. 120, p. 147.

[8] W. H. Funderburk and T. J. Case, Electroencephal. a. Clin. Neurophysiol., 1951, v. 3, p. 213.

[9] F. Rinaldi and H. E. Himwich, Arch. Neurol. a. Psychiat., 1955, v. 73, p. 387, p. 396.

[10] W. C. Wescoe, R. E. Green, B. P. McNamara, and others, J. Pharmacol. a. Exper. Therap., 1948, v. 92, p. 63.

*Original Russian pagination. See C. B. Translation.