THE ROLE OF MESENCEPHALIC CHOLINERGIC SYSTEMS IN THE MECHANISM OF NICOTINE ACTIVATION OF THE ELECTROENCEPHALOGRAM

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That certain motor manifestations of nicotine's effect are central in origin is clear from many reports [2,7,8, 9, 10, 15, 16], and nicotine hyperkinesia is now being used to find drugs for treatment of Parkinsonism. Nicotine's effect on the bioelectric activity of the brain is less well understood – particularly the physiological mechanisms of such changes. Longo and colleagues [16, 17], using a l'encephale isolé preparation, have shown the electroencephalographic changes following nicotine administration to be central in nature. The recently evolved theory, that the cholinergic mechanisms of the reticular formation of the brain stem participate in the processes activating cortical activity [3,5,6,13,18,20] make this question particularly important.

The purpose of this investigation was to study the central nervous system mechanisms involved in this activating effect of nicotine. Experiments were conducted on animals with the brain stem sectioned at different levels, and the nicotine's reaction with cholino- and adrenolytics was studied in animals with intact brain and with sectioned brain stem.

EXPERIMENTAL METHODS

The bioelectric activity of the brain was studied in 120 animals with intact brain (acute experiments on cats, chronic experiments on rabbits) and in 80 animals (cats and rabbits) with different brain stem sections. A bipolar lead was used; steel electrodes were inserted into the boney regions corresponding to the sensorimotor and visual regions of the cerebral cortex. The biocurrents were recorded with a Kaiser 8-channel, ink-writing electroencephalograph. The background bioelectric activity and the "after" reaction to light flickering at various frequencies were investigated, as well as the "arousal" reaction to stimulation of the sciatic nerve by square-wave electric pulses or to sound stimulation. Operative interventions were done under ether anesthesia, and the cats with intact brain were immobilized with Diplacin and given artificial respiration. The brain stem was sectioned either mechanically (as described in [6]), or by electrolysis at the following levels: 1) l'encephale isolé, i.e., spinal cord sectioned between the I and II cervical vertebrae [10, 11]; 2) pons, in the region of the trigeminal nerve nuclei (trigeminal section [20]); 3) cerveau isolé [11, 12], i.e., between the anterior and posterior tubercula of the lamina quadrigemina; 4) from in front of the anterior tubercula of the lamina quadrigemina (dorsally) to directly behind the mammillary bodies (ventrally) – premesencephalic section. Posthumously, the mechanical sections were checked visually, the electrolytic sections histologically.

The experimental preparations – nicotine base, Amizyl Benactizine,* Metamysil,* atropine sulfate, Benzacin,* Spasmolytin (Difacil, trasentine), Tropacine, hexamethonium (Hexonium), and Aminazine (chlorpromazine) – were administered intravenously.

EXPERIMENTAL RESULTS

Intravenously injected into rabbits in a dose of 0.4-0.5 mg/kg, nicotine caused phasic changes in the EEG. Twenty to 25 sec after the injection began, the electroencephalogram (EEG) of the anterior portions of the brain showed low-amplitude, high-frequency waves, while the EEG of the posterior sections showed a regular, synchronized rhythm of 4-6 waves per sec. These changes resemble the picture observed with extraneous stimulation or with

*Name not verified – Publisher's Note.

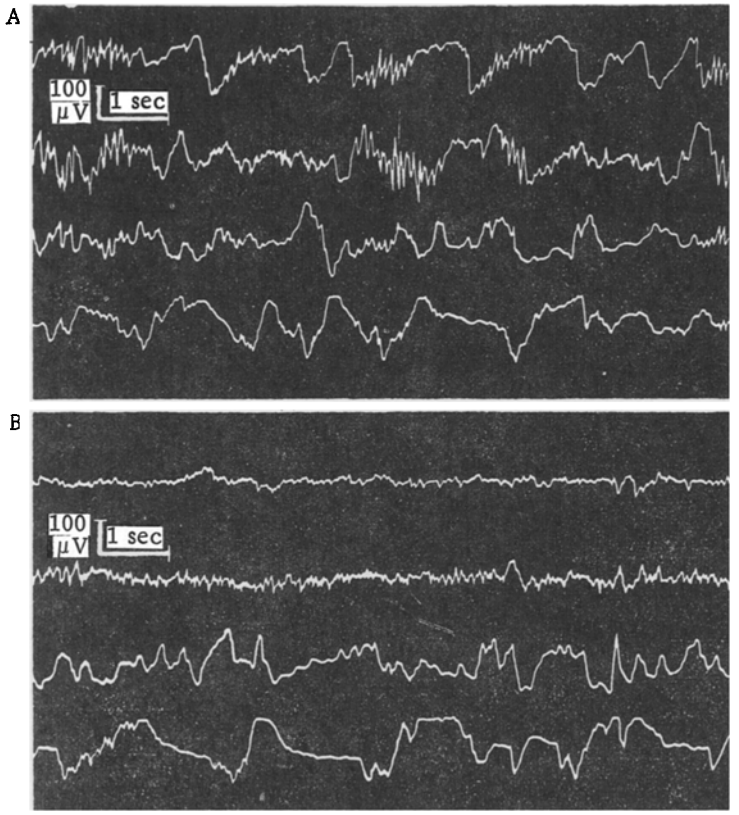

Fig. 1. Effect of nicotine on EEG of a cat with asymmetrical brain stem section. Left side, cerveau isolé, premesencephalic section. Curves (from top to bottom) show: EEG of left sensorimotor, left visual, right sensorimotor, right visual regions of cerebral cortex. a) Before administration; b) after intravenous administration of 0.3 mg/kg nicotine.

stimulation of the reticular formation of the brain stem. After 40-60 sec, the phase of EEG activation was succeeded by a phase of convulsive discharges distinguished by the presence of sharp, high-amplitude waves, either consecutive or alternated with slow waves. Then the number of convulsive discharges gradually decreased, and slow, high waves alternating with "silent zones" (periods of almost no bioelectric potentials) began to predominate in the background activity, signifying the onset of the third, or depressive phase of nicotine's effect. The tonico-clonic convulsions and tremor observed coincided in time with the initial phase of nicotine's effect. The EEG picture observed with nicotine administration was exceptionally variable; although the dose and rate of injection were uniform, the duration and distinction of the phases differed in different animals. Although changes in behavior were only noted with the first administration of nicotine, the changes in the bioelectric activity of the brain also developed with its repeated administration, although the character and duration of the EEG changes could change up to a point of complete disappearance of individual phases.

Nicotine doses of 0.4-0.5 mg/kg poorly suited our purposes (observation of the character of EEG activation and study of the effect of cholinolytics and adrenolytics) because the activation period induced by these doses changed rather quickly into convulsive discharges. In the next experiments, therefore, we used a dose of 0.3 mg/kg, which in rabbits induces in a majority of cases only an activation phase lasting $2\frac{1}{2}$-3 min, with brief convulsions being observed in only a few animals.

In the acute experiment on cats, nicotine caused basically the same changes, but the activation was more lasting in a number of cases, and larger doses (2-5 mg/kg) were required to produce convulsive discharges on the EEG.

In a number of animals, nicotine not only altered the background activity, but caused a marked improvement in the "trace" reaction in response to high-frequency light flicker (30-40 per sec for rabbits, 60-70 per sec for cats).

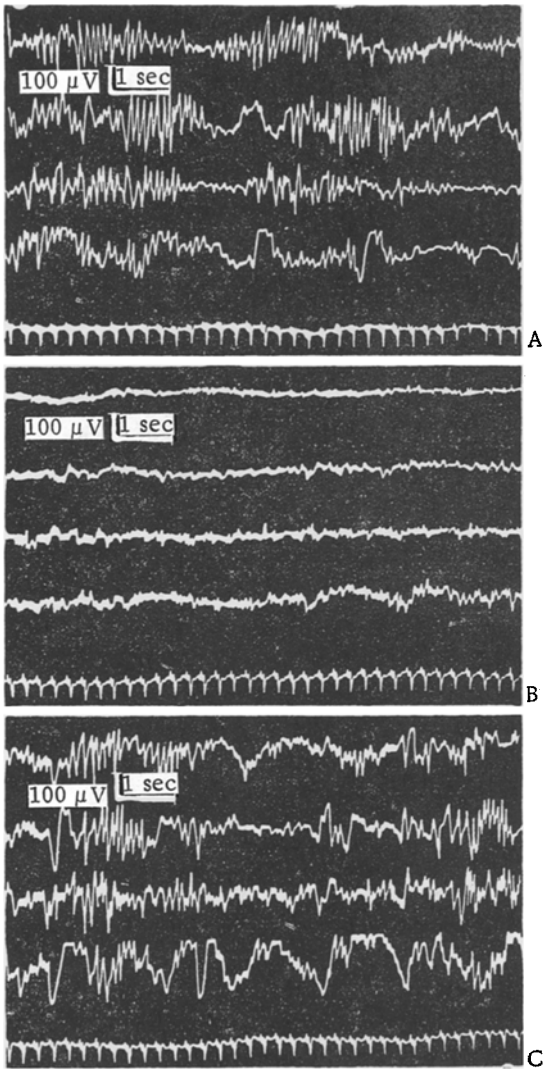

Fig. 2. Effect of Amysil on nicotine EEG activation in a cat with cerveau isolé. Curves (from top to bottom) show: EEG of left sensorimotor, left visual, right sensorimotor, right visual regions of cerebral cortex, ECG. A. Before administration; B) 3 min after intravenous injection of 0.3 mg/kg nicotine; C) 2 min after intravenous injection of 0.3 mg/kg Amysil.

Improvement in the "trace" reaction was only noted during the activation phase; this reaction deteriorated during the depression phase.

In doses of 0.3 mg/kg, nicotine caused bradycardia lasting 2-3 min in the animals, while larger doses caused a 5-6 min bradycardia (number of cardiac contractions decreased to 70-100 from the normal 200-300 per min), distortion of the ECG complex and, in a number of cases, arrhythmia. Preliminary administration of hexamethonium (0.1 mg/kg) prevented the ECG changes but not the EEG activation caused by nicotine.

In the experiments with the sectioned brain stem, we found that with l'encephale isolé and cerveau isolé sections, as well as with sections somewhat more rostral, where part of the reticular formation of the brain stem remained connected with the superjacent sectors, administration of nicotine caused the appearance of the same low-amplitude, high-frequency picture which was observed in the animals with intact brain. Where the section passed in front of the anterior tubercula of the lamina quadrigemina and behind the mammillary bodies at the base of the brain (premesencephalic), nicotine administration did not change the slow, high-amplitude activity characteristic of this section. Nicotine's lack of activating effect in this case was not connected with circulatory disturbance in the superjacent sections of the brain; Corazole caused an EEG picture of convulsive attack and, when methylene blue was introduced into the carotid artery, the brain stained above the section. Preservation of the functional condition of the cortical and subcortical neurons was shown by the presence of the "trace" reaction to light impulses in cases where the connection of the corpora geniculatum laterale with the visual region of the cortex was preserved.

The relation of nicotine's EEG effect to the level of the section was particularly clear in the experiments with unilateral and asymmetrical sections of the brain stem. In cases in which cerveau isolé had been created on one side, leaving the brain stem intact on the other, nicotine administration induced EEG activation bilaterally.

In cases with unilateral premesencephalic section with either intact brain or cerveau isolé on the other side, however, nicotine administration caused unilateral EEG activation (Fig. 1). It should be noted that nicotine consistently caused greater changes in the ECG in the animals with brain sections than in those with intact brains; to prevent this, we injected 0.1 mg/kg hexamethonium (which does not affect the EEG in this dose) before nicotine in a number of cases.

To determine which biochemical structures in the reticular formation of the brain stem were affected to produce nicotine EEG activation, we investigated the reaction of nicotine with substances which selectively block the central m- and n-cholinergic structures and the adrenergic structures. This investigation showed the preliminary administration of Aminazine, which blocks central adrenergic structures, did not affect nicotine EEG activation. The slow, high-amplitude picture with many spindles induced by Aminazine (3-5 mg/kg) was superceded by activation lasting up to 2-3 min after the injection of nicotine (0.3 mg/kg).

However, the EEG changes induces by nicotine were affected by the administration of substances that block the central cholinergic structures. The high-amplitude, slow EEG picture caused by administration of substances that

block the n-cholinergic structures primarily (Spasmolytin and Tropacine in doses of 7-10 mg/kg) could be observed even after the nicotine injection, i.e., these substances prevented nicotine's activating effect. In small doses (5 mg per kg), these substances did not wholly prevent nicotine activation of the EEG, but did shorten its duration. Administered on a background of developed nicotine activation, small doses of Spasmolytin and Tropacine (0.5-1 mg per kg) also showed a blocking effect: the EEG activation was superceded by slow, high-amplitude waves.

Our results, showing that Spasmolytin prevents nicotine's effect in animals with intact brain, are in accord with the literature data [1].

The next experimental series showed that the interaction of the substances selectively blocking the n-cholinergic structures and nicotine did not change under conditions of a sectioned brain stem. Activation induced by 0.3 mg/kg nicotine in an animal with cerveau isolé was changed by the administration of Spasmolytin or Tropacine into slow, high-amplitude activity; the doses required to produce this effect were $1\frac{1}{2}$-2 times smaller than those required in the animals with intact brain. The animals with trigeminal section were the exception, as the doses of the cholinolytics required to prevent nicotine's activating effect were almost the same as those required in the animals with intact brain.

Nicotine's activating effect on the EEG was also prevented and obliterated by substances that block the m-cholinergic systems. After the injection of Benzacine, Metamysil, or Amysil in a dose of 1-2 mg/kg into animals with intact brain, for example, nicotine had almost no effect on the EEG; the slow, high-amplitude activity evoked by the cholinolytics was not changed. Atropine (3-4 mg/kg) shortened, but did not entirely prevent nicotine's activating effect in rabbits and prevented it in cats. Central m-cholinergics also obliterated nicotine-induced EEG activation in doses $\frac{1}{4}$ to $\frac{1}{5}$ as large as those required to prevent it. The effects of these substances were the same in the animals with brain stem sections. As Fig. 2 shows, m-, like n-cholinolytics, eliminated nicotine's EEG effect in animals with cerveau isolé in doses $\frac{1}{3}$ to $\frac{1}{4}$ as large as those required to prevent this effect.

The experiments conducted, especially those with hexamethonium and Diplacin (curarization of the animals) showed the EEG activation to be associated with central action of nicotine rather than with a stimulating action on the autonomic ganglia and the n-cholinergic systems of the striated muscles. The fact that nicotine caused EEG activation under conditions of cerveau isolé, and in cases where the superjacent portions of the brain remained connected, if only partially, with the mesencephalic reticular formation, but did not have an activating effect when the mesencephalon was cut off, suggests that nicotine EEG activation is associated with excitation of the mesencephalic reticular formation.

The fact that preliminary administration of Aminazine could not prevent the development of nicotine activation indicates that adrenergic structures have no part in its mechanism; this is assumed to be true of a number of nicotine's effects in the organism, tremor particularly [12]. The elimination and prevention of nicotine's activating effect by substances that block the central m- and n-cholinergic structures, and the important fact that these relations are preserved in animals with brain stem sections, indicate that nicotine stimulates the cholinergic structures of the mesencephalic reticular formation. When these data are compared with those obtained in previous investigations of a substance stimulating the m-cholinergic structures (arecoline) [4], one can see that the doses of these same n-cholinolytic substances which failed to eliminate arecoline EEG activation successfully eliminated nicotine's effect. One can therefore assume that the structures of the mesencephalic reticular formation that participate in the mechanism of nicotine EEG activation are n-cholinergic. The elimination of nicotine's effect by the m-cholinolytics is probably accomplished at a higher level of the central nervous system.

SUMMARY

Acute and chronic experiments on cats and rabbits demonstrate that nicotine administration causes phasic changes in the bioelectric activity of the brain. In isolated brain and in rostral sections, in which a part of the reticular formation of mesencephalon remains connected to the overlying portions, the administration of nicotine provokes EEG activation as in animals with an intact brain. With the section passing from the dorsal side in front of the superior colliculi of corpora quadrigemina, and from the ventril side – directly behind the corpora mammilaria (premesencephalic section), nicotine administration provokes no change in the slow high-amplitude activity characteristic of the section. The data obtained suggest that the activating effect produced by nicotine on the EEG is connected with the excitation of reticular formation of mesencephalon. The impossibility of preventing nicotine activation by preliminary aminazine administration shows that the excitation of adrenoreactive structures does not participate in the mechanism of this activation. On the basis of studying the interaction of nicotine-provoked activation with substances blocking the central m- and n-cholinoreactive systems (benactisin, bensatsin, metamisyl, atropine, tropacine, trasentin, chlorpromasine) it is suggested that the biochemical structures excited in the reticular formation are n-cholinoreactive.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
