THE EFFECT OF CERTAIN CHOLINOLYTIC DRUGS ON THE EEG OF THE RABBIT WHEN INJECTED INTO THE CEREBRAL VENTRICLES

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Acetylcholine, when injected into the carotid artery, and anticholinesterase preparations, when injected intravenously, lead to the appearance of an activation reaction in the electroencephalogram (EEG) [1, 9, 12, 14]. The presence of cholinergic synapses has been reported in the structures responsible for the development of this reaction. In consequence of the presence of the blood-brain barrier, it may be postulated that substances injected into the cerebral ventricles may come into contact with substances not reached when they are injected into the blood stream. In this way the situation and the pathways of their action in the central nervous system may differ. We have found [5] that acetylcholine, carbachol, and galanthamine, when injected into the cerebral ventricles, produce a clear and persistent activation reaction in the EEG of the rabbit, lasting up to 2-3 h. This reaction is suppressed or prevented by intravenuous injection of the central cholinolytics benactyzine chloride hydro and atropine. The peripheral cholinolytic, oxyphenonium bromide, when injected intravenously, has no effect on the changes in the EEG produced by intraventricular injection of the cholinomimetic drugs.

These findings demonstrate the central action of the tested cholinomimetic preparations when injected into the cerebral ventricles and confirm the hypothesis that cholinergic synapses are present in the mechanisms responsible for activation. However, to prove the mediator action of acetylcholine when injected into the cerebral ventricles, it is necessary to investigate the interaction between the cholinomimetic substances to be tested and cholinolytics administered by the same route (intraventricularly), so that the likelihood that they act on the same structures is increased. We have carried out chronic experiments to investigate the action of the cholinolytic drugs benactyzine hydrochloride, atropine, and oxyphenonium bromide, injected into the lateral ventricle of the brain, on the background electrical activity of the brain and certain autonomic indices, and also to study their action on the changes in the EEG caused by injection of cholinomimetic drugs into the cerebral ventricles.

EXPERIMENTAL METHOD

Investigations were carried out on rabbits with bipolar electrodes implanted permanently in the brain. Action potentials were recorded from the sensorimotor, parietal, and occipital regions of the cortex, the hippocampus, the reticular formation, the anterior and posterior hypothalamus, and the septal region. The drugs were injected through a cannula inserted into the lateral ventricle, in 0.1-0.2 ml of sterile physiological saline. The potentials were recorded on a 15-channel Alvar electroencephalograph. Besides the EEG, simultaneous recordings were made of the respiration and ECG, and observations were made on the animal's general condition and behavior. Altogether 75 experiments were performed on 20 animals. The method is described in detail in our previous communication [5].

EXPERIMENTAL RESULTS

The background EEG of the rabbit in our experiments corresponded to that described by other authors [4, 11]. Two types of activity can be distinguished in the EEG of the rabbit: an activity characteristic of a state of sleep or rest (slow, irregular, high-amplitude waves and spikes), and an activation or arousal reaction – an activity characteristic of an active waking state (a regular, synchronized rhythm with a frequency of 4-7/sec, in the hippocampus,

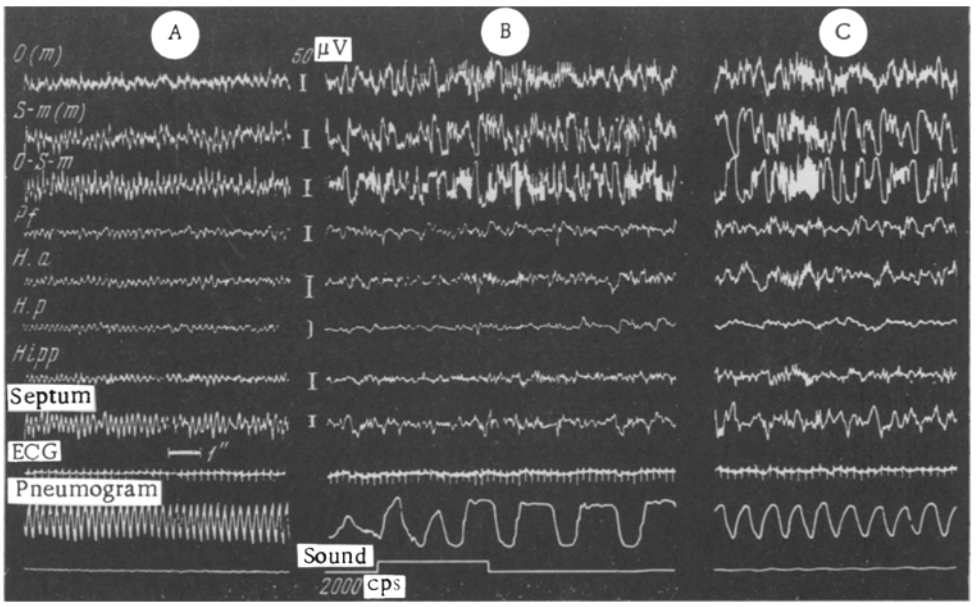

Fig. 1. EEG of a rabbit after injection of cholinomimetic substances into the cerebral ventricle. A) Initial background; B) 5 min after injection of 2 mg benactyzine hydrochloride into lateral ventricle; C) 5 min after intraventricular injection of 10 μg carbachol; O) occipital region; P) parietal region; S-m) sensorimotor region; R_f) reticular formation of mesencephalon; Ha) anterior hypothalamic region; Hp) posterior hypothalamic region; Hipp) hippocampus; Sept) septum.

reticular formation of the mesencephalon, and posterior divisions of the cortex, desynchronization in the cortical leads, especially marked in the sensorimotor region). When the animal was accustomed to the experimental conditions, its EEG was dominated by the characteristic picture of sleep or rest, and this gave way to activation during the application of sensory stimuli.

Benactyzine hydrochloride (a central cholinolytic acting mainly on muscarine-like cholinergic systems) was injected into the lateral ventricle of the rabbit's brain in a dose of 1.5-4 mg before and after intraventricular injection of carbachol. Chlorpromazine caused a clearly defined picture of sleep or rest in the EEG of the intact rabbit 1-3 min after intraventricular injection, with suppression or a considerable increase in the thresholds of the activation reaction in response to sensory stimulation. The animals' behavior in these circumstances preserved all the signs of an active waking state, and they sometimes showed signs of excitation. Against this background, injection of cholinomimetic drugs into the cerebral ventricle did not evoke the usual activation reaction (Fig. 1). When benactyzine hydrochloride was injected intraventricularly against the background of activation caused by the preliminary intraventricular injection of acetylcholine, carbachol, or galanthamine, after 2-3 min the EEG assumed the picture of sleep or rest, with a considerable increase in the thresholds or a blocking of the activation reaction to external stimuli (sound, light, touch). Hence benactyzine hydrochloride, when injected into the cerebran ventricle, just as when injected intravenously, suppressed or prevented the action of cholinomimetic substances injected into the cerebral ventricles on the EEG.

Atropine (a central muscarine-like cholinolytic) was injected into the cerebral ventricle in doses of between 200 μg and 4 mg. Altogether 17 experiments were carried out, in which atropine was injected into the ventricle of an intact animal. In 9 experiments atropine caused a clear activation reaction 5-10 min after injection, lasting throughout the experiment (30-60 min, Fig. 2). In 2 of the 9 experiments the onset of activation was preceded in the first 5-10 min by a tendency towards sleep or rest, with an increase in the thresholds of the activation reaction.

In 5 experiments, after injection of atropine a clear picture of activation persisted. In 3 experiments, after injection of atropine (in a dose less than 1 mg) intraventricularly, alternation of rest and activation continued to take place in the EEG as in the original tracing, i.e., atropine had no visible effect on the EEG. Besides this tendency towards activation, in 6 experiments (in a dose greater than 1 mg) atropine caused the appearance of convulsive discharges in all leads; in 3 of these experiments convulsive fits were observed. After the periods of paroxysmal activity the EEG showed a picture of activity of low amplitude, with ill-defined, flattened waves (Fig. 2).

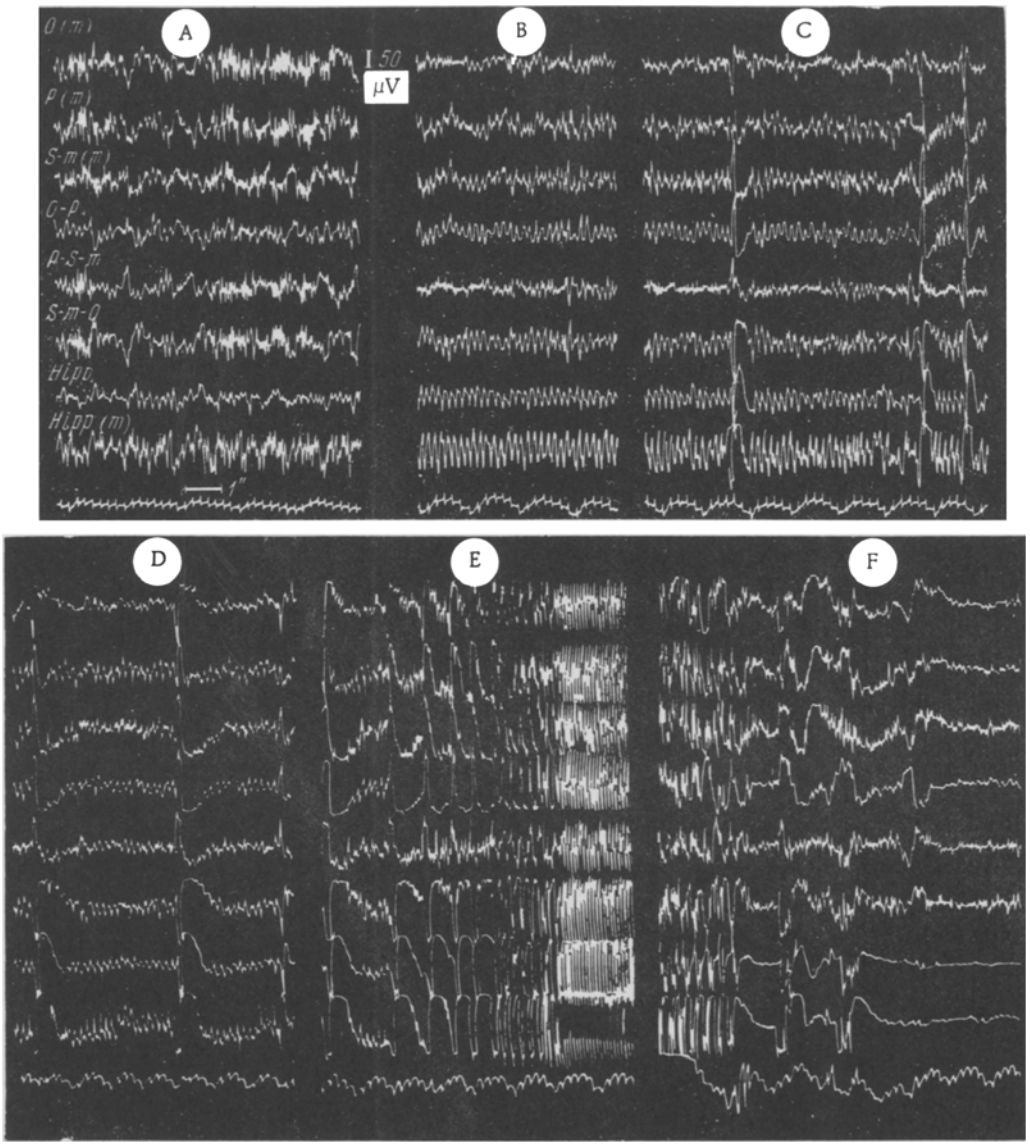

Fig. 2. Activation reaction in the EEG after injection of atropine into the lateral cerebral ventricle. A) Initial background; B, C, D, E, F) after injection of 2 mg atropine.

To determine the degree to which cholinergic and adrenergic links of the activating reticular formation [7, 8, 12, 13] are concerned in the production of the activation reaction evoked by intraventricular injection of atropine, we investigated the effect of intravenous injection of cholinolytics (benactyzine, hydrochloride, atropine) and an adrenolytic (chlorpromazine) on this activation.

After intravenous injection of 3-6 mg chlorpromazine against the background of the pattern of sleep or rest produced in the EEG, with an increase in the thresholds of the activation reaction, the intraventricular injection of atropine (1-2 mg) led in 3 experiments to a distinct tendency towards activation in all leads, and in one experiment to activation in the hippocampus only. In this experiment a clearly defined synchronized rhythm with a frequency of 4-6/sec was observed, characteristic of activation in the hippocampus, and at the same time slow, high-amplitude, irregular waves and spikes were seen in the other leads, especially in the cortex. When chlorpromazine was injected after the intraventricular injection of atropine (1-2 mg), activation persisted sometimes in all leads, sometimes only in the hippocampus. Chlorpromazine had no effect on the convulsive discharges and motor spasms evoked by atropine.

Benactyzine, hydrochloride, and atropine, injected intravenously (1-3 mg/kg), suppressed the activation of the EEG after intraventricular injection of atropine, causing the appearance of a picture of sleep or rest in the EEG (in some cases with a blocking of the activation reaction), but had no effect on the convulsive discharges.

Oxyphenonium bromide (a peripheral cholinolytic) was injected intraventricularly (dose 1-4 mg) in 10 experiments. When injected intravenosuly (up to 5 mg/kg), oxyphenonium bromide had no effect on the ECG [1, 3]. When injected intraventricularly, in 6 of 10 cases oxyphenonium bromide caused, after 5-7 min, considerable changes in the EEG towards the picture of sleep or rest, with complete blocking of the activation reaction to external stimuli in 2 of the 6 cases. The animal remained active at this time, and in some experiments the picture of sleep or rest in the EEG after intraventricular injection of oxyphenonium bromide was accompanied by motor excitation.

In two experiments only a very slight tendency for the pattern of the EEG to change to predominance of rest or sleep was recorded; in two experiments oxyphenonium bromide (in a dose less than 2 mg) had no effect on the EEG. Carbachol, when injected intraventricularly against the background of the development in the EEG of a picture of sleep with blocking of the activation reaction due to intraventricular injection of oxyphenonium bromide, did not produce an activation reaction in the EEG. Besides the development of a picture of rest or sleep in the EEG, in 9 experiments oxyphenonium bromide caused convulsive discharges. They appeared almost regularly every 3-4 sec, and then increased in frequency, changing into bursts of continuous convulsive activity with a frequency of up to 40/sec. After the frequency of the convulsive discharges had increased in this way, for a short period a sparse activity with a greatly reduced amplitude was recorded. In 2 cases convulsive fits developed, and in 6 cases marked motor excitation was present.

Intraventricular injection of the test substances led, in some cases, to a marked slowing of the pulse and arrhythmia, usually arising 5-10 min after the injection, often simultaneously with the development of convulsive discharges. Bradycardia and arrhythmia developed more often after the injection of atropine and oxyphenonium bromide. Dilatation of the pupil was observed most frequently after injection of oxyphenonium bromide; benactyzine hydrochloride and atropine in most experiments did not produce dilatation of the pupil.

These investigations showed that benactyzine hydrochloride, when injected intraventricularly, as also when injected intravenously [6], suppresses the activation reaction in the EEG evoked by sensory stimulation or by cholinomimetic drugs, and produces a picture of sleep or rest. Oxyphenonium bromide has a similar action when injected intraventricularly, although when given intravenously it has no action on the EEG [3]. Both drugs, when injected intraventricularly, prevent the action of cholinomimetic drugs injected by the same route on the EEG. These facts confirm the mediator action of acetylcholine when injected intraventricularly, and prove the existence of cholinergic synapses in the system responsible for the appearance of the activation reaction.

The effect of oxyphenonium bromide, when injected intraventricularly, on the EEG demonstrates the impermeability of the blood-brain barrier to this compound and confirms that cholinolytic drugs, when injected intraventricularly, exert their action directly (and not through the general circulation).

The fact that benactyzine hydrochloride and oxyphenonium bromide, when injected intraventricularly, caused a picture of sleep or rest in the EEG with blocking of the activation reaction shows that the activating action of atropine is due, not to its cholinolytic properties, but apparently to some special effect of atropine on the central nervous system. The activating action of atropine on the central nervous system, in contrast to the sedative action of the other muscarine-like cholinolytic, scopolamine, was mentioned in the older textbooks of pharmacology. Signs of excitation are observed in persons with atropine poisoning. Evidently the intraventricular injection of atropine creates a high concentration of the drug in the corresponding divisions of the central nervous system, which leads to the manifestation of its distinctive activating action. When injected intravenously, atropine suppresses the activation reaction evoked by intraventricular injection of atropine. When injected intraventricularly, atropine evidently does not reach in adequate amount the cholinergic structures which it blocks when injected intravenously.

The convulsions caused by atropine and oxyphenonium bromide are evidently brought about, not by the cholinolytic properties of these substances, but by their special action. This is shown by the absence of convulsions following injection of benactyzine hydrochloride and also by the fact that atropine, when applied locally to the cortex, suppresses the convulsive discharges caused by acetylcholine, but intensifies the discharges caused by the local application of curare and penicillin [10].

The bradycardia and arrhythmia observed in some experiments after intraventricular injection of the cholinolytic substances are evidently central in origin, for the peripheral action of these drugs is to produce tachycardia. The bradycardia is probably associated with blocking of the cholinergic elements of the central nervous system responsible for the production of sympathetic reactions [2].

SUMMARY

A study was made of the effect produced by the central cholinolytics – amyzyl and atropine, and the peripheral cholynolytic – oxyphenonium bromide on the rabbit's EEG following their administration into the cerebral ventricles. By intraventricular administration, amyzyl and oxyphenonium bromide reproduced a picture of sleep or rest in the EEG, preventing and depressing the activating effect of cholinomimetric agents introduced into the cerebral ventricles. Atropine administered intraventricularly reproduced the activation picture in the EEG. Atropine and oxyphenonium bromide administration into the cerebral ventricles was followed by convulsive discharges in the EEG and by motor convulsions in a number of experiments. The activating effect of atropine is due to its peculiar action on certain brain structures, but not to its cholinolytic effect. The action of amyzyl and oxyphenonium bromide administered intraventricularly pointed to the existence of cholinolytic synapses in the system of the activation reaction and to the mediator effect of acetylcholine in intraventricular administration.

LITERATURE CITED

1. R. Yu. Il'yuchenok and M. D. Mashkovskii, Fiziol. zh. SSSR, 11, 1352 (1961).
2. G. N. Kassil', Byull. éksper. biol., 27, 2, 108 (1949).
3. M. D. Mashkovskii and S. S. Liberman, Farmakol. i toksikol., 3, 216 (1959).
4. L. A. Novikova and D. A. Farber, Abstracts of Proceedings of a Conference on Problems in the Electrophysiology of the Central Nervous System [in Russian], Moscow (1958), p. 91.
5. F. M. Berger, C. D. Hendley, and T. E. Lynes, Proc. Soc. exp. Biol. (New York), Vol. 92 (1956), p. 563.
6. M. Bonvallet, P. Dell, and G. Hiebel, Compt. rend. Soc. Biol., Vol. 147 (1953), p. 1162.
7. M. Bonvallet, P. Dell, and A. Hugelin, J. Physiol. (Paris), Vol. 46 (1954), p. 262.
8. P. B. Bradley and J. Elkes, Brain, Vol. 80 (1957), p. 77.
9. W. H. Funderburk and T. J. Case, Electroenceph. clin. Neurophysiol., Vol. 3 (1951), p. 213.
10. V. G. Longo, G. P. Berger, and D. Bovet, J. Pharmacol. exp. Ter., Vol. 111 (1954), p. 349.
11. F. Rinaldi and H. Himwich, Arch. Neurol. Psychiat., Vol. 73 (1955), p. 396.
12. A. B. Rothballer, Electroenceph. clin. Neurophysiol., Vol. 8 (1956), p. 603.
13. R. P. White and E. A. Daigneault, J. Pharmacol. exp. Ther., Vol. 125 (1959), p. 339.
