TREMORINE HYPERKINESIA AS A MODEL FOR ASSESSMENT OF THE CHOLINOLYTIC ACTIVITY OF DRUGS

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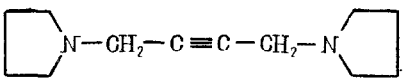
Several authors [6, 7] have reported that tremorine (1,4-dipyrrolidine-2-butine), in doses of 5-20 mg/kg, causes a marked tremor of central origin, salivation, and other toxic manifestations indicating excitation of the muscarine-like (M) and nicotine-like (N) cholinergic systems in various species of experimental animals. The central and peripheral cholinomimetic action of tremorine is suppressed fairly effectively by various cholinolytics [6, 8]. However, the data in the literature on this subject do not give a clear idea of the efficacy of preparations with differences in the selectivity of their action on the M and N cholinergic receptors.



In the present study the antagonism between tremorine and various cholinolytic substances differing in their M and N cholinolytic activity was studied in order to determine whether the effect of these substances on the N cholinergic system can be assessed. In all the experiments the antitremorine activity of the cholinolytics was evaluated by comparison with their ability to prevent the effects of arecoline poisoning.

EXPERIMENTAL METHOD

The investigation was conducted on albino mice of both sexes weighing from 17 to 23 g. Aqueous solutions of the cholinolytics were injected subcutaneously in a volume of 0.1 ml/10 g body weight. The magnitude of the dose was varied by suitably adjusting the concentration. Each dose was tested on five animals. The aqueous solution of tremorine was injected intraperitoneally, also in a volume of 0.1 ml/10 g body weight, 5 min after injection of the cholinolytic. The degree of antagonism was assessed by the alternative analysis of two of the indices of tremorine poisoning—tremor and salivation. As in the experiments with arecoline, these two indices were analyzed for 30 min from the moment of injection of the tremorine. Arecoline was injected subcutaneously. The numerical results were analyzed by the method of Miller and Tainter.

EXPERIMENTAL RESULTS

Preliminary experiments established that the optimal dose of tremorine for mice is 10 mg/kg. After the intraperitoneal injection of this dose of the drug, when 1-3 min had elapsed the mice salivated profusely and showed a marked tremor, sometimes resembling convulsions. These symptoms were observed in the animals for several hours, and they then gradually diminished and disappeared. When tremorine was injected into mice in smaller doses, the tremor appeared less clearly. After injection of doses exceeding 20 mg/kg, the animals died. The intraperitoneal method of injection was chosen because of the need for rapid development of the poisoning. When tremorine is injected subcutaneously the tremor and salivation appear considerably later.

Under the influence of arecoline a similar picture of poisoning developed after the subcutaneous injection of the drug in a dose of 17 mg/kg. In this case the tremor was characterized by movements of higher frequency and of

Mean Effective Doses of Cholinolytics (ED_{50} in mg/kg) Preventing Effects of Poisoning by Tremorine and Arecoline in Albino Mice

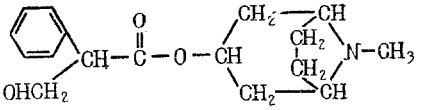
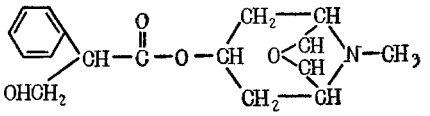
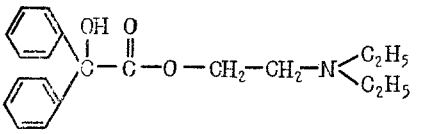
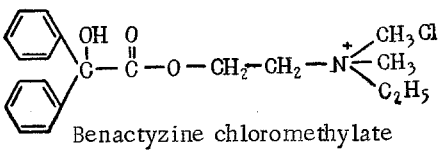
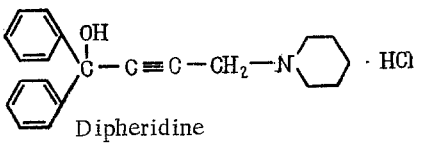
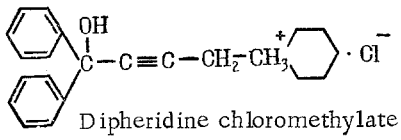
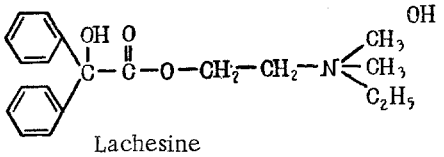
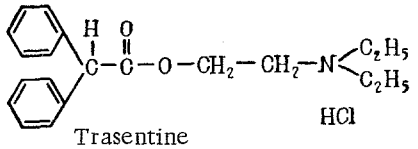
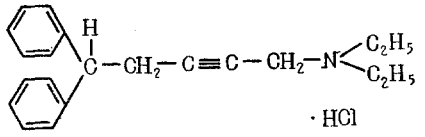
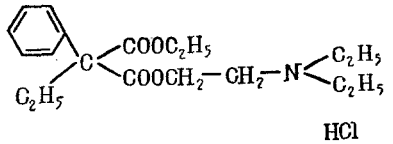
Serial No.	Cholinolytic	Tremorine		Arecoline	
		tremor	salivation	tremor	salivation
1	 Atropine · $H_2SO_4H_2O$	3,0±0,5	0,27±0,05	1,2±0,2	0,06±0,01
2	 Scopolamine · HBr	0,17±0,02	0,016±0,004	0,18±0,02	0,02±0,002
3	 Benactyzine · HCl	2,0±0,5	5,8±0,7	0,5±0,04	1,9±0,3
4	 Benactyzine chloromethylate	—	0,46±0,07	—	0,13±
5	 Dipheridine	9,7±0,5	14,5±2,1	1,8±0,27	1,25±0,24

Table (Continued)

Serial No.	Cholinolytic	Tremorine		Arecoline	
		tremor	salivation	tremor	salivation
6	 Dipheridine chloromethylate	–	1,9±0,18	–	0,24 ± 0,03
7	 Lachesine	–	0,15 ± 0,02	–	0,21 ± 0,04
8	 Trasentine	30,0 ± 2,0	–	28,2 ± 3,9	51,0 ± 4,0
9	 Amiphine	–	–	–	–
10	 Antitremorine	17,0 ± 1,0	–	18,5 ± 1,1	–

smaller amplitude. For the investigation of their ability to suppress tremor hyperkinesia and salivation, ten cholinolytic preparations of different chemical structure were selected (see the table).

As the table shows, the selected cholinolytics differed greatly in their ability to block the central and peripheral M and N cholinergic systems. For example, atropine, scopolamine, and benacytzine are typical M cholinolytics; trasentine and dipheridine possess, besides their action on the M cholinergic system, a marked effect also on the N cholinergic receptors; preparation IT-369 (amiphine) has no ability to act on M cholinergic receptors, but possesses a selective action on the central N cholinergic system [2]; three preparations (Nos. 4, 6, and 7) were quaternary salts; finally, antitremorine was chosen for comparison, as a substance possessing high activity, according to reports in the literature [9] in relation to abolishing the tremorine hyperkinesia. Assessment of the central nicotinolytic activity of antitremorine showed that this substance blocks the central N cholinergic receptors in low doses; nicotine tremor in rabbits can be prevented by administration of antitremorine in a dose of 4-5 mg/kg.

The results of experiments to study the antagonism between the above-mentioned cholinolytics and tremorine and arecoline are given in the table.

The investigated cholinolytics were equally capable of preventing tremorine and arecoline hyperkinesia. Cholinolytics with a marked central M cholinolytic activity were most effective in preventing the tremorine hyperkinesia. Scopolamine occupied first place among them, followed by benactyzine, atropine, and dipheridine. These substances occupied the same order in respect of their central antiarecoline activity. The weaker central M cholinolytics such as antitremorine and trasentine, had to be given in large doses to prevent the tremorine hyperkinesia. The fact that these substances possess central nicotinolytic activity shows clearly that this activity is not important for preventing the tremorine hyperkinesia. This was demonstrated more strikingly by the action of amiphine, possessing no M cholinolytic activity, for despite the presence of a marked selective activity towards the central N cholinergic receptors, it could not prevent tremorine hyperkinesia even in a dose of 100 mg/kg.

The most active antagonists of tremorine, both at the periphery and in the experiments with arecoline, were the quaternary compounds.

The results of the experiments with tremorine and arecoline showed that rather higher doses of the cholinolytics were needed to prevent the toxic effects of tremorine. This model is evidently more "rigid" than the arecoline model, especially in relation to the abolition of salivation. It is not by accident that the cholinolytics exhibiting weak antiarecoline activity at the periphery (trasentine, for example) did not prevent tremorine salivation.

The results obtained demonstrate that the hyperkinesia and salivation arising in mice under the influence of tremorine owe their origin to excitation of the central and peripheral M cholinergic system.

The ability of the cholinolytics to prevent these effects of tremorine is clearly correlated with their M cholinolytic activity as established in experiments with the "arecoline model." Consequently, tremorine poisoning in mice can be used as a test for evaluation of the central and peripheral M cholinolytic activity of drugs as an alternative to the "arecoline model." However, like the latter, this method cannot give any indication of the nicotinolytic properties of the tested compounds. For this reason doubts may be expressed regarding the adequacy of the use of tremorine poisoning as a test for screening drugs for treatment of parkinsonism, for according to the findings of Bovet and Longo [5], a clear correlation exists between the activity of drugs against parkinsonism and their central nicotinolytic activity.

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