ELECTROMYOGRAPHIC RECORDING OF HYPERKINESIAS CAUSED BY GUANIDINE, TETRAETHYLAMMONIUM, α-AMINOPYRIDINE, AND DIHYDROXYBENZENES IN WARM-BLOODED ANIMALS

(UDC 616.8-009.12-02 : 615.7-07 : 616.74-073.97)

A. I. Podlesnaya

Division of Pharmacology (Head, Active Member AMN SSSR Professor S. V. Anichkov), Institute of Experimental Medicine, USSR Academy of Medical Sciences. Leningrad. Scientific Director of Research Active Member AMN SSSR Professor V. M. Karasik
(Presented by Active Member AMN SSSR S. V. Anichkov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 3, pp. 73-77, March, 1966
Original article submitted November 5, 1964

Hyperkinesia of an isolated skeletal muscle of cold-blooded animals evoked by guanidine [11, 12], tetraethylammonium [16], dihydroxybenzenes [6, 7], α-aminopyridine [8], and certain thiol poisons [2-5] arises as a result of the influence of these substances on the motor nerve endings on the skeletal muscles. V. M. Karasik [1] suggested that the onset of guanidine hyperkinesia may be explained by rupture of the bond between acetylcholine and the protein reserving it by guanidine, thus facilitating the liberation of the acetylcholine, which causes the contractile activity of the isolated muscle.

The use of the method of microelectrode recording of the end-plate biopotentials has shown [15] that guanidine considerably increases the amplitude of the end-plate potential of the curarized muscle. The authors cited concluded that guanidine intensifies the liberation of acetylcholine under the influence of the nervous impulse. This conclusion has been confirmed by other studies [14, 16]. In warm-blooded animals hyperkinesia has been observed following administration of tetraethylammonium and dihydroxybenzenes [6, 7].

In the present investigation an electromyographic recording and pharmacological analysis were made of the hyperkinesia developing in warm-blooded animals following administration of guanidine, tetraethylammonium iodide (TEA), α-aminopyridine, and dihydroxybenzenes (resorcinol, pyrocatechol, and hydroquinone).

EXPERIMENTAL METHOD

Experiments were conducted on 110 male albino mice weighing 18-25 g, anesthetized with ether or sodium amytal. The electrical activity of the muscles were recorded on photographic paper by a "Disa" electromyograph. The action potentials of the gastrocnemius muscles were detected by means of types 13k51 and 13k50 concentric needle electrodes (diameter 0.45 mm, length 20-30 mm). Guanidine, α-aminopyridine, TEA, and dihydroxybenzenes were injected intraperitoneally in aqueous solutions, and the solutions of the dihydroxybenzenes were made up when required.

EXPERIMENTAL RESULTS AND DISCUSSION

All the compounds were found to produce hyperkinesias and to evoke electrical activity in the muscles (Fig. 1), and this activity was most marked and came on soonest (within 1-3 min after injection) in the animals receiving dihydroxybenzenes and α-aminopyridine. TEA and guanidine caused hyperkinesia when injected in near-lethal or lethal doses.

The mean lethal doses of the tested compounds and the doses causing bioelectrical activity in the gastrocnemius muscles are given in the table.

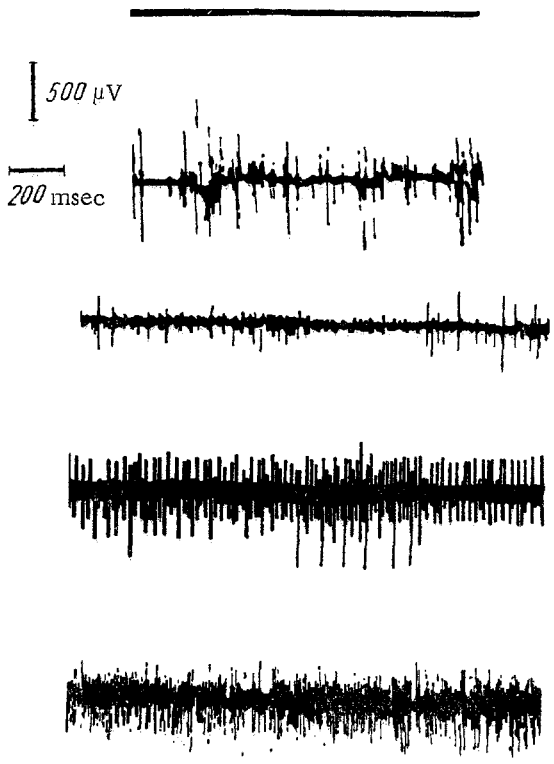

Fig. 1. Bioelectrical activity of gastrocnemius muscle of albino mice after intraperitoneal injection of "hyperkinetic" poisons. From top to bottom: control; resorcinol (150 μg/g); guanidine (350 μg/g); TEA (30 μg/g); α-aminopyridine (20 μg/g). Speed of movement of photographic paper 5 cm/sec; amplification 50 μV/mm.

Comparison of Doses of "Hyperkinetic" Poisons Causing Hyperkinesias and Bioelectrical Activity of the Skeletal Muscles in Albino Mice

Preparation	Dose (in mg/kg)		
	LD_{50}	hyperkinesia	bioelectrical activity of muscles
Guanidine	350	300-400	200
TEA	35	30-40	30
α-Aminopyridine	28	20	20
Dihydroxybenzenes			
resorcinol	215	150	150
pyrocatechol	175	50-100	50-100
hydroquinone	175	100	100

The appearance of bioelectrical activity under the influence of the dihydroxybenzenes and α-aminopyridine coincided with the onset of hyperkinesia. Bioelectrical activity was observed in the gastrocnemius muscles after administration of doses of guanidine and TEA not producing hyperkinesia, although in only 60-66% of cases. Against the background of chloral hydrate and sodium amytal sleep, doses of guanidine and TEA not leading to hyperkinesia evoked bioelectrical activity in the muscles in 100% of cases. Under ether anesthesia neither variant of hyperkinesia developed.

In the experiments conducted on albino mice under sodium amytal anesthesia (100 μg/g) in which the bioelectrical activity of denervated muscles was recorded, it was found that 10-15 days or more after removal of 0.3-0.5 cm of the upper third of the sciatic nerve spontaneous bioelectrical activity developed in the denervated muscle, which differed from that evoked by the tested compounds. Biopotentials of low amplitude and an irregular high-frequency rhythm were recorded in the denervated muscle, whereas the biopotentials evoked by guanidine, TEA, α-aminopyridine, and the dihydroxybenzenes varied in amplitude and were of a lower frequency. The bioelectrical activity of the denervated muscles remained unchanged under the influence of the above-mentioned "hyperkinetic" poisons. Neither bioelectrical activity (Fig. 2) nor hyperkinesia was found in the muscles immediately after resection of the sciatic nerve.

Evidence of the synaptic localization of the action of the "hyperkinetic" poisons was given by the fact that all variants of peripheral hyperkinesia were abolished by curarization of the muscle in frogs. In contrast to this, muscle relaxants (D-tubocurarine, paramyon, succinylcholine, decamethonium), when injected intraperitoneally into mice in doses immobilizing the animals, did not prevent or suppress the bioelectrical activity appearing under the influence of guanidine, α-aminopyridine, TEA, and the dihydroxybenzenes. Likewise no changes were observed in the hyperkinesia and the bioelectrical activity of the muscles after administration of atropine or chlorpromazine. In fact, under the influence of atropine, the pyrocatechol hyperkinesia and bioelectrical activity actually lasted longer.

Hence, the hyperkinesia produced in mice by guanidine, α-aminopyridine, TEA, and dihydroxybenzenes, unlike the hyperkinesia observed in the isolated muscle of cold-blooded animals, reacts with far greater difficulty to pharmacological agents.

The hyperkinesia found in mice after intraperitoneal injection of guanidine, TEA, α-aminopyridine, and the dihydroxybenzenes differed from that seen in cold-blooded animals. Whereas in the frog, after destruction of the

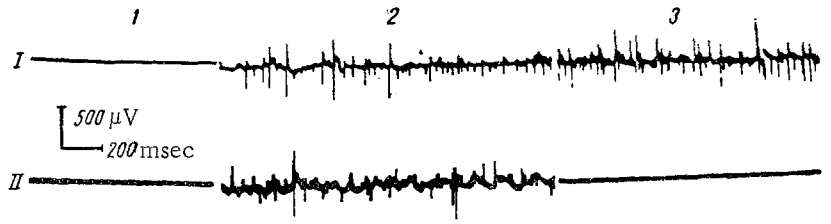

Fig. 2. Bioelectrical activity of the right (I) and left (II) gastrocnemius muscle of a mouse. 1) Initial background; 2) pyrocatechol (100 μg/g intraperitoneally); 3) resection of sciatic nerve (in left limb). Speed of movement of photographic paper 5 cm/sec; amplification 50 μV/mm.

central nervous system, stronger spasms develop than when the nervous system is intact, and they are observed in the isolated muscle, in warm-blooded animals hyperkinesia arises only when the central nervous system is intact.

In the warm-blooded animals neither muscle relaxants, nor barbiturates, nor cholinolytics (atropine, chlorpromazine) depress the bioelectrical activity of muscles produced by "hyperkinetic" poisons. The lack of any such depressant effect of the muscle relaxants is evidently associated with the fact that their doses inhibiting voluntary movements in animals are too small to suppress or prevent the supermaximal impulse activity evoked by the "hyperkinetic" poisons. This hypothesis is supported by investigations carried out in 1963 [10]. These showed that the motor end plate transforms the presynaptic impulse in a manner which depends on the number of excited nerve fibers. The neuro-muscular spread of excitation during submaximal stimulation corresponding to a physiological impulse activity is particularly labile for synaptic poisons, and even very small doses of D-tubocurarine (0.1 mg/kg) totally inhibit these impulses. During supermaximal stimulation the same doses of D-tubocurarine have no such effect.

So far as the influence of the centrally acting drugs on the pharmacologically evoked bioelectrical activity of the skeletal muscles is concerned, this may evidently be attributed to synergism of the barbiturates and cholinolytics with the "hyperkinetic" poisons, the mechanism of which is not understood.

The fact that hyperkinesia and bioelectrical activity of the muscles in mice, like the hyperkinesia of the isolated muscle in cold-blooded animals, do not arise in chronically denervated muscles, suggests that the "hyperkinetic" poisons may act on the neural part of the synaptic structures. According to the suggestion made by V. M. Karasik [1] and to experimental findings [13, 15, 16], guanidine, TEA, the dihydroxybenzenes and also, evidently, α-aminopyridine facilitate the liberation of acetylcholine under the influence of the nervous impulse from its complex with the protein holding it in reserve.

The onset of hyperkinesia and of bioelectrical activity of the muscles under the influence of the "hyperkinetic" poisons in the intact organism may be attributed either to the more intensive liberation of acetylcholine in the neuro-muscular synapses in the presence of a constant flow of impulses from the central nervous system, or to an increase in the flow of impulses into the synapses from the central nervous system, causing an increase in the liberation of acetylcholine in the neuro-muscular synapses.

Hydroquinone is known [9] to influence the evoked potentials of the cortex, the thalamus, and the mesencephalic reticular formation.

LITERATURE CITED

1. N. V. Ermakov and N. B. Medvedeva, Fiziol. zh. SSSR, No. 2, 191 (1954).
2. V. M. Karasik, In book: Pharmacology of New Therapeutic Drugs [in Russian], Leningrad (1953), p. 151.
3. Kh. S. Koshtoyants, Proteins, Metabolism, and Nervous Regulation [in Russian], Moscow (1951).
4. A. I. Podlesnaya, Byull. éksper. biol., No.7, 44 (1956).
5. A. I. Podlesnaya, In book: Annual Report of the Institute of Experimental Medicine of the AMN SSSR for 1955 [in Russian], Leningrad (1956), p. 185.
6. I. E. Sterin, Fiziol. zh. SSSR, 20, 2, 330 (1936).
7. I. E. Sterin, Fiziol. zh. SSSR, 20, 4, 678 (1936).
8. G. I. Tsobkallo, Farmakol. i toksikol, 5, No. 6, 32 (1942).

9. G. Bartharion and H. Laborit, Agressologie, 4 (1963), p. 157.
10. W. Erdmann, H. Ridal, and G. Schmidt, Arch. exp. Path. Pharmak., Bd. 244, S. 351 (1963).
11. H. Fühner, Arch. exp. Path. Pharmak., Bd. 58, S. 1 (1907-1908).
12. F. Gergens and E. Baumann, Pflug. Arch. ges. Physiol., Bd. 12, S. 205 (1876).
13. K. Koketsu, Am. J. Physiol., 193 (1958), p. 213.
14. J. Loeb and W. F. Ewald, J. biol. Chem., 25 (1916), p. 377.
15. M. Otsuka and M. Endo, J. Pharmacol. exp. Ther. 128 (1960), p. 273.
16. M. Otsuka and Y. Nonomura, Ibid., 140 (1963), p. 41.

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.