RHYTHMIC TREMOR OF SKELETAL MUSCLE, CAUSED BY THIOL POISONS

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Since the end of the last and the beginning of this century there has been knowledge of a group of substances capable of producing prolonged rhythmic tremor of skeletal muscles of the frog(guanidine [8], aminopyridine [7], tetraethylammonium [10], diphenol [5, 6], and others). These contractions can be prevented or diminished by curarization and do not occur in denervated muscle when enough time has elapsed for the degeneration of the stimulating nerve endings [9]. These substances are capable of markedly enhancing muscle response to concentrations of potassium ions which ordinarily cause only threshold contractions; the sensitization is reciprocal, as potassium ions in their turn markedly accentuate contractions caused by the above-named agents; it disappears with curarization, and within two weeks following denervation of the muscle [1, 2].

The above considerations provide a basis for believing that the substances named above sensitize the peripheral nervous apparatus to ions of potassium and facilitate the liberation of acetylcholine from its labile combination with the stimulated albumin of the nerve-muscle synapse. The precise chemical reaction between guanidine and other substances, causing nervous tissue to cause rhythmic muscular contractions, and their relation to exact biochemical structure is as yet not clear. Therefore, it is of importance to consider the fact that the total effect is produced by substances known as agents affecting the thiol radicals of the albumins.

In the isolated diaphragm of rats contractile activity appears under the influence of Salyrgan, as well as mercuzal, first reported in 1953-1954 by Kushinsky and his co-workers[11, 12].

Independently of these authors, in 1954 we discovered that organic mercurials – mercuzal and parachloromercuribenzoate of sodium – are capable of evoking rhythmic activity of isolated skeletal muscles in frogs (straight abdominal muscle and the sartorius).

Of the substances capable of eliciting rhythmic contractile activity of these muscles, only for mercuzal and the parachloromercuribenzoate of sodium do we have the knowledge of the chemical reactions in which they participate: both poisons block the sulfhydryl groups in the albumins, forming mercapto groupings.

Both the thiol poisons named, when used experimentally in 1:5000 concentrations, cause contractions of the tonic muscles. This concentration is twice that causing rhythmic contracting activity. The contractions of the sartorius muscle do not start even with a concentration of 1:1000.

Experiments with Mercuzal

The contractile ability of the sartorius muscle arises when mercuzal is used in 1:10,000-1:5,000 and stronger concentrations. It is characterized by two types of tremor. The first type, as a rule, arises in 5-10 minutes. They are occasional, repetitious after 2-5-8 minutes, with their amplitude as shown (Fig. 1, A).

Contractions of the second type occur 10-20 minutes later, against the background of the disappearance of the first type, being much lower in amplitude but occurring more frequently, and often reenforcing each other (Fig. 1, B).

The second type is observed as the first type diminishes; after disappearance of the first type, the second type continues 30-70 minutes.

Contractions of the first type, besides those coinciding with tremor, are caused by the above-named substances acting upon the movement-controlling nerve endings, especially those occurring under the influence of tetraethylammonium. Contractions of the second type are more comparable with those arising in sartorious muscle immersed in 0.6% solution of sodium chloride.

We set ourselves the task of determining to what extent the hyperkinesis arising under the influence of mercuzal, depends as a function upon the moving nerve and components.

In the first series of experiments we studied the effects of curare. Under the influence of curare (firm of Suchardt) in concentrations of 1:200,000 to 1: 50,000, tremor of the first type in the isolated sartorius muscle is diminished or totally disappears immediately on application, but 20-40 minutes later there appear contractions of the second type; nevertheless these are much weaker than in the control, i. e., the action of curare somewhat suppresses the second type.

In the second series of experiments (April, 1954) we denervated the muscles of one limb of a frog.

Under ether anesthesia the abdomen skin was cut on the right side, separating the muscles. The inner organs were moved by blunt dissection to the left, exposing the right lumbar plexus; all nerves of this plexus were elevated on a hook and severed with scissors. The skin and muscles were then sewn with sterile silk. In the ensuing days, in order to prevent wound sepsis an aqueous solution of sulfathiazole was introduced into the frog's stomach [4]. Fifteen to twenty days after the operation – a length of time sufficient for the degeneration of the nerve endings at the muscles [2, 9] – the experiments were performed. In each frog the sartorius was isolated on both sides, one serving as the control. Altogether there were 10 experimental efforts with 10 controls.

In the control muscles, under the influence of mercuzal there arose tremor of the first and second type (Fig. 1, A, B). In the denervated muscles, in 7 experiments out of 10 there were observed contractions of the second type; tremor of the first type did not occur (Fig. 1, B).

In the third series of experiments we observed the influence of mercuzal upon muscle immersed in a solution of physiological sodium chloride.

Earlier we had observed that guanidine is incapable of causing its characteristic influence on muscle contractions when the muscle contractions were immersed in an 0.6% solution of sodium chloride. When Ringer's solution was substituted for the physiological saline, guanidine was capable of causing the characteristic muscle contraction. This is connected with the fact that the contracting activity of muscle under the influence of guanidine is associated with the presence of potassium in Ringer's solution. Increasing the concentration of calcium chloride (1:2000-1:1000) depresses mecuzal-caused tremor of the first type, just as it has been known for a long time to do with guanidine [9]. Upon the action of mercuzal on muscle immersed in warmed physiological saline, tremor of the first type did not occur. Against the background of "salt"-caused tremors, it is difficult to explain contractions of the second type; as explained above, mercuzal causes these parallel actions. Insofar as in a series of experiments the additional mercuzal reinforced the contractions, it can be thought that this reinforcement is on the basis of mercuzal caused tremor of the second type. Therefore, the mercuzal-induced contractions of the first type, as in the ones due to guanidine, do not occur in physiological saline. Guanidine, on the background of mercuzal-induced contractions of the second type, is incapable of producing any changes whatever in the latter or of producing the contractions characteristic for it. Upon the action of mercuzal on guanidine hyperkinesis, in the beginning there is some accentuation of the latter, and then a

transition of tremors (first type) in another, i. e. cessation of the guanidine-caused and appearance of the mercuzal-caused contractions of the second type.

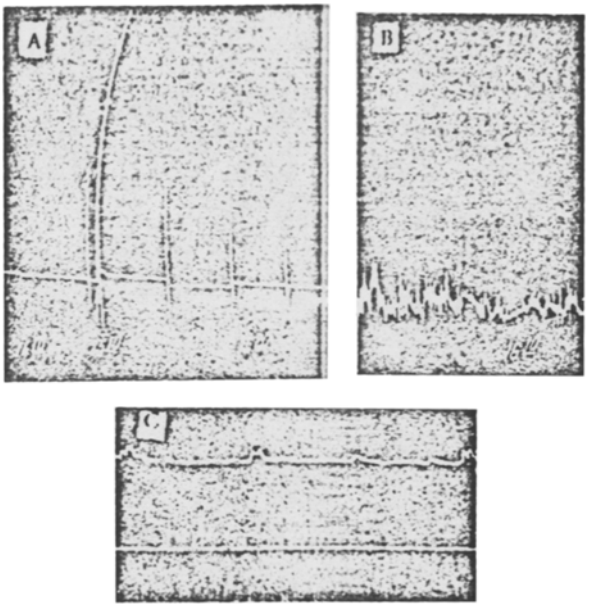

Fig. 1. Application of mercuzail, 1:10,000 concentration to isolated sartorius muscle at point marked by arrow. In 5 minutes a contraction of the first type occurs lasting 45 minutes (A); on the background of persisting first type contractions, contractions of the second type appear, lasting 65 minutes (35 minutes after cessation of the first type contractions)(B). The effect of mercuzal (1:10,000) upon sartorius of frog, denervated 15 days before the experiment, second type contractions appearing after 22 minutes (C). Below is shown the base line.

Experiments with Parachloromercuribenzoate

Another poison blocking thiol groups – parachloromercuribenzoate – like mercuzal, is capable of evoking contractions in isolated skeletal frog muscles (in sartorius, as well as in the straight abdominal muscles) in concentrations of 1:50,000-1:25,000 and stronger. The contractions of the muscles appear more rapidly the stronger is the concentration of the poison, e.g., with the use of 1:50,000 concentration the contractions appear in 45-60 minutes, while with a concentration of 1:10,000 they appear within 2-5 minutes.

The contractions of the sartorius muscles which appear under the influence of parachloromercuribenzoate have an irregular amplitude and a varying rhythm. They either build upon each other, or are very rare, or sometimes very perceptible. The rhythmic activity caused by the poison lasts 30 to 60 minutes (Fig. 2). This is prevented or markedly diminished by mercaptosuccinic acid (its sodium salt is used) – a substance containing free thiol groups and being an antidote to thiol poisons [3]. However, it must be noted that to counteract the effect of thiol poisons much lower concentrations are needed to prevent than to suppress an already existing effect.

The use of the mercaptosuccinic acid on already developed contractions first reenforces and then suppresses them.

The simultaneous use of both poisons upon the action of the muscles exerts reenforcing action of one upon the other. Simultaneous concentrations used were: mercuzal 1:200,000, parachloromercuribenzoate 1:100,000;

in these strengths separately no effect was produced upon the muscle; together they synergized a well-developed rhythmic activity in the sartorius muscle of the frog.

Contractions caused by parachloromercuribenzoate are comparable with activity upon immersion in 0.6% saline solution.

The curare preparation (Suchardt) in concentrations of 1:100,000-1:10,000 does not prevent or diminish contractions caused by the parachloromercuribenzoate; they remain without change (muscle contractions stimulated by guanidine, α-aminopyridine, tetraethylammonium, diphenols, all stimulants of nerve endings in muscle, are suppressed by 1/10 as much curare).

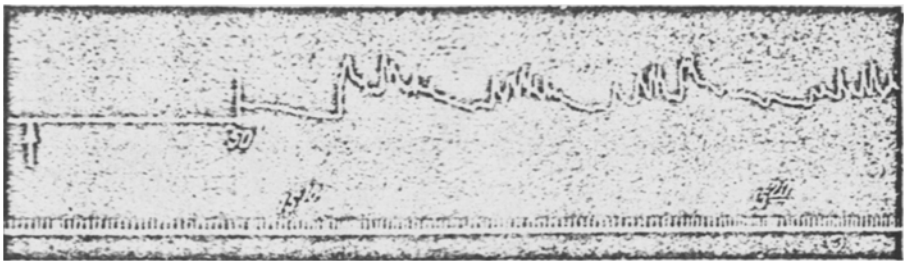

Fig. 2. Effect upon sartorius of frog of 1:50,000 parachloromercuribenzoate (applied at point marked by arrow). After 50-minutes there appeared low amplitude waves, some rare, some superimposing on each other. Below is the second scale (5 seconds).

When parachloromercuribenzoate acts on muscles denervated 15-20 days prior to the day of the experiment (Fall, 1954), long enough to permit nerve degeneration in the denervated muscle the contractions are observed just as they are on the control side.

Kushinsky and his co-workers [11, 12] explained the contractile ability of the rat diaphragm, produced under the influence of Salyrgan, as an anticholinesterase activity of the poison. Salyrgan-induced contractions were equated with eserine or Prostigmine-like mechanisms. Such an explanation does not hold in case of the isolated sartorius muscle of the frog, as in this case neither eserine nor Prostigmine can produce rhythmic muscle contractions. It must be assumed that the mechanism of action of the poisons being studied is different.

We observed that mercuzal produces in isolated skeletal frog muscle rhythmic contractions different from those caused by parachloromercuribenzoate and characterized by two types of contractions. First type contractions depend on the intactness of the nerve endings, while the second type does not so depend. Mercuzal-induced contractions of the first type, just as with guanidine, are quite sensitive to various pharmacologic agents; they do not occur in either curarized muscle, or in denervated muscle; neither are they observed in muscle preparations immersed in 0.6% saline.

V. M. Karasik theorizes that guanidine, without disturbing acetylcholine synthesis, weakens its bonds with "stimulated" albumins of the neuromuscular synapse, which leads to the liberation of acetylcholine and the appearance of a muscular contraction. This theory can be developed with the mercuzal action of muscle contractions of the first type. Most complex albumins contain sulfhydryl groups; they are not only present in the albumin but also form complexes with acetylcholine, participating in its binding. It is possible that mercuzal is a powerful thiol poison, competing with acetylcholine for the sulfhydryl group and replacing it in the albumin complex, the freeing of the acetylcholine leading to the muscle contractions.

Following prolonged activity (30-60 minutes) by mercuzal, apparently the neuromuscular synapse is blocked, which eliminates muscular activity of the first type. At this time guanidine is unable to produce its characteristic contractions.

Much less clear is the blockage by the thiol groups and the role of mercuzal in the contractions of the second type, also, contractions due to parachloromercuribenzoate influence. The participation in such a blockage by these organic mercurials cannot be disputed.

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