

THE EFFECT OF DDT ON MUSCLE DYSTROPHY AND ITS ROLE IN PRODUCING REST TREMOR*

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Although the toxic properties of DDT were extensively studied, its pharmacological effects remained rather obscure. In this report the author presents his own results of the studies of the effects of DDT on muscles and discusses the results of other authors. The aim of the experiments was mainly to find out the mechanism of tremor provoked by DDT, to study it and to introduce such model of tremor for other studies. The author was able to show that DDT significantly improves muscular function in an experimental model of muscular dystrophy. In the author's opinion further research into pharmacological effects of DDT might lead to its wider use as a good therapeutic agent.

In this report we want to show our results in pharmacological investigation of the effect of DDT (1, 2). While the toxicity and the pesticidal power of this substance were investigated in detail (3), its other pharmacological properties have been rather neglected. The starting point in this work is the known fact that DDT can provoke the rest tremor (RT) (4). We examined this property of DDT by trying to explain the mechanism of the tremor and find its antagonists. Knowing that DDT causes a faster conduction of the motor neuron's (5), we investigated the influence of DDT on muscular dystrophy. This was necessary because we showed earlier that oxotremorine (2) and Recosen (6, 7) (which are activators of acetylcholintransferase) (8, 9) as well as the poison of black widow (that releases ACh from its vesicles) (10) help in cases of muscular dystrophy (11).

DDT TREMOR

It has been known for a long time that DDT provokes RT (4). It is also known that the intensity of the tremor depends on the concentration of DDT in the nervous tissue (12). Tremor appears when the brain and cerebellum are destroyed even below the cut line of the spinal cord (4).

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DDT does not change the activity of cholinesterase neither does it reduce the quantity of ACh in the nervous tissue (13), or affects the activity of cholinacetylase (14). DDT prolongs the period of postsynaptic potentials and acts as the antagonist of tetrodotoxin (5).

We showed earlier that there are various methods for producing tremor in animals (15, 16). It seems that DDT tremor does not depend on extrapyramidal system, but merely on the spinal cord (3). These experiments were done in order to find out the mechanism of tremor provoked by DDT and the antagonists of this tremor. In this way we may discover another model of tremor. It will also be useful to know more about pharmacological effects of DDT and, perhaps, its clinical application. It should be pointed out that in distinction to all other models of experimental tremor this tremor lasts more than 24 hours.

All the experiments were carried out on Wistar rats and DDT 100 mg/kg solved in olive oil was administered per os. The investigated substances were given five minutes after DDT or when tremor was well developed. Only two substances were given 24 hours before DDT. We measured also ACh level in corpus striatum. This was necessary because it is known that ACh in corpus striatum is very important for the genesis of certain kinds of tremor (17).

We examined the effect of the following substances: atropine sulphate, methylatropine nitrate, caramiphen, ethyl benztropine bromide, oxotremorine, guanethidine, α -methyl-dopa, penicillamine, 4-(1-naphthylvinyl)-pyridine, decaborane, glycine, aminooxyacetic acid, mephenesine, diazepam, γ -hydroxybutyrate, α -methyl-m-tyrosine, p-chloroamphetamine, substance P.

Only mephenesine and diazepam, which are inhibitors of the polysynaptic reflexes, were able to prevent the DDT tremor completely. However, the next day, when the substances stopped acting the tremor appeared again. Anticholinergic substances had no effect and those that stop the tremor of the Willson type (18), increased DDT tremor. Glycine, the substance that abolishes the tremor provoked by cyclopium toxine (19), which also acts on the level of the spinal cord, was without effect too. The increase of dopamine caused by γ -hydroxybutyrate inhibited the DDT tremor but again not completely.

DDT does not change the level of ACh in the whole brain or in corpus striatum.

From these results we can draw a conclusion that DDT acts on the level of the spinal cord. In other words, this substance affects the spinal polysynaptic reflexes. The destruction of supraspinal structures does not affect the DDT tremor. *Tripod* even suggests that DDT acts like strychnine (21). It is known that mephenesine and diazepam are the antagonists of strychnine (22). It is interesting that γ -hydroxybutyrate which increases the quantity of dopamine in the brain (20) by reducing the cholinergic functions, can slightly diminish the DDT tremor. A local application of atropine in the striatum of the rat has a weak effect on the same kind of tremor. So, the supraspinal mechanisms are of secondary importance

in the case of DDT tremor. *Hrdina et al.* (23), even found that DDT reduces the level of ACh in corpus striatum but they administered higher doses of DDT than we did. To conclude we can say that DDT tremor is a special model of experimental tremor originating at the level of the spinal cord, without any influence on extrapyramidal system.

As we pointed out at the beginning, oxotremorine (6) and recosen (7) as well as the poison of black widow increase the cholinergic transmission from the nerve to the muscle and cause better functioning of dystrophic muscles (11). That is why it was interesting to investigate the effect of DDT in that sense. We have already said that DDT is the antagonist of tetrodotoxin (5) and that it enhances the conduction through the axone of motor neurones.

It is interesting to note that DDT has already been used for therapeutic purposes e. g. in barbiturate intoxication (24).

The dystrophy of hind legs of the rat was provoked according to the method of *Selye* (25). The animals were given 25 mg/kg of DDT per os, or 10 mg/kg dissolved in 0,2 ccm oil. DDT was administered from the first day of ligation and the animals were sacrificed after 15 days. In these 15 days a very high degree of dystrophy was developed. The same group of muscles of the hind leg was isolated in the controls, and in the experimental group and their weights were balanced. After that the muscles were taken for the histological control.

Our experiments have shown that DDT increases significantly the weight of dystrophic muscles (2).

The histological examinations showed that the dystrophic changes in the muscles of the experimental group were less pronounced than in the muscles of the control group. The number of necrosis was decreased in the muscles of treated animals (1). The metabolites of DDT (DDD and DDE) had no effect. They were examined under the same conditions as DDT and the same doses were applied (10 and 25 mg per os) (26).

It is evident that the weight of dystrophic muscles increases although DDT does not change the quantity of ACh in the brain (13). *Hrdina et al.* (23) have found that, in higher doses, DDT even decreases ACh level in striatum. We have stated that DDT increases the conduction in axone, so probably the release of ACh from the vesicles is faster. It is well known that DDT is the antagonist of tetrodotoxin (5). It seems that the number of spontaneous impulses from motor neurones is increased. So, the trophic effect on muscle is probably due to this mechanism.

Richer et al. (27) have shown that glycine is the only aminoacid of the plasma that is significantly lowered in blood after DDT application. We mention this fact because we were able to show in our former experiments that a decrease of glycine in medulla spinalis produces a rigor (28, 29), due to the absence of the inhibitory influence of glycine upon α -motor cells (30). Glycine was, perhaps, reduced in the spinal cord as well. It is possible that a decrease in glycine enhances the central effects of DDT.

It is for sure that this kind of dystrophy is not the same as the one that appears in Erb's disease, but it is a fact that the poison of black widow acted when applied to this model (11). It also stimulates the release of ACh from the vesicles at the end of motor neurones (10). The inhibition of this phenomenon results in the expressive atrophy of the striated muscles (31). It is known, for example, that the application of botulinus toxin inhibits vesicle draining at the endings of motor nerves (32). These findings indicate the neurogenic origin of Erb's disease. The subject has been discussed recently by *Law* and *Atwood* (33).

It should also be stressed that DDT significantly prolongs the life of mice intoxicated with botulinus toxin (26). Theoretically, this was to be expected because DDT must obviously increase the drain of ACh from motor end-plates. However, it does not act in the same way as lathroductus toxin. Some data found in the literature indicate that DDT may directly influence the ACh drain from its vesicles (34).

It is interesting that *Mac Comas* supposes that DMP might be based on the shortage of motor end-plates (35).

Finally, we wish to mention our recent experiments that we consider, perhaps, most important for our whole research in the field (26). Not long ago *Mendel et al.* (36, 37) were able to show that the binding of rat's aorta (at the site recommended by *Selye*) (25) and subsequent application of serotonin lead to the kind of dystrophy very much similar to the Duchenne type of DMP. (The similarity is both histological and biochemical). (Creatine phosphokinase, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, lactic dehydrogenase were increased).

We were able to show that DDT in this model significantly improves muscle function. While the control muscles were of yellowish color, the muscles treated with DDT were of normal color. In vivo a great difference was seen between experimental and control muscle groups. We were able to measure this difference objectively by suspending the rats on the net. Hence we can conclude that *Selye's* method represents, to a certain extent, a good model for DMP, but less good than the new method recommended by *Mendel et al.* (36, 37).

We think that a further investigation of DDT might also be interesting for the therapy. *Deichman* (38) has recently proved that DDT may be given to humans. The administration of 25 mg/kg of DDT per os over a period of 21 months in one group of 24 volunteers did not bring about any clinical or pharmacological changes.

Finally, we want to forward three questions:

1. Is DMP a kind of chronic state of botulinus intoxication?
2. Is it possible to use DDT in the therapy of muscular dystrophy?
3. Is there a correlation between the saturation of organism with DDT and the manifestations of muscular dystrophy?

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Sažetak

UČINAK DDT-ja NA MIŠIČNU DISTROFIJU I NJEGOVA ULOGA
U IZAZIVANJU TREMORA

Iako su toksična svojstva DDT-ja mnogo proučavana, njegovo je farmakološko djelovanje još nedovoljno poznato. U ovom je radu autor prikazao rezultate svojih istraživanja o djelovanju DDT-ja na mišiće. Ujedno raspravlja i o rezultatima drugih autora koji su proučavali slične učinke DDT-ja.

Glavna svrha eksperimenata s DDT-jem bilo je proučavanje mehanizma tremora izazvanog u pokusnih životinja kako bi se dobivena iskustva mogla primijeniti u sličnim studijama te eventualno iskoristiti tremor izazvan DDT-jem kao model u farmakološkim istraživanjima.

Autor je pokazao da DDT znatno poboljšava funkciju mišića u eksperimentalno izazvanoj mišićnoj distrofiji. Daljnja istraživanja farmakološkog djelovanja DDT-ja mogu, po autorovu mišljenju, pospješiti upotrebu DDT-ja kao lijeka u terapiji nekih bolesti.

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