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KNJIGA XVII

ODJELJENJE MEDICINSKIH NAUKA
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MEĐUNARODNI SIMPOZIJUM

O EKSPERIMENTALNOM TREMORU

1 — 3. APRIL 1971. GODINE

Urednik
PAVEL ŠTERN,
redovni član Akademije nauka i umjetnosti
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I. JURNA

DRUG INDUCED RIGIDITY AND TREMOR IN THE RAT

During the last years much evidence has been accumulated in favour of the view first formulated by Barbeau (1962) that the motor symptoms in Parkinson's disease may result from an imbalance between monoaminergic and cholinergic mechanisms of the brain. Hornykievicz and coworkers (Ehringer and Hornykiewicz, 1960; Birkmayer and Hornykiewicz, 1964) found a marked reduction in the content of monoamines, especially dopamine, in the neostriatum of Parkinson patients. Accordingly, application of L-DOPA as the precursor of dopamine alleviates the extrapyramidal motor syndrome (Barbeau et al., 1962; Birkmayer and Hornykiewicz, 1961, 1962). On the other hand, reduced monoaminergic activity may also be compensated by drugs with cholinolytic properties, as for instance the classical antiparkinson drug atropine.

Under experimental conditions a parkinson-like state of motor disturbance is induced by drugs which either depress monoaminergic (dopaminergic or noradrenergic) or increase cholinergic activity. Impairment of monoaminergic transmission may result from a depletion by reserpine or tetrabenazine of monoamines in the brain (Carlsson et al., 1957; Quinn et al., 1959; Bertler, 1961; Pletscher et al., 1962; Carlsson, 1966) or from block of the respective receptor sites by chlorpromazine, haloperidol or phenoxybenzamine. Enhancement of cholinergic transmission will follow from an inhibition by physostigmine of cholinesterase activity. In the rat the application of each of the drugs mentioned produces rigidity, tremor and akinesia (Steg, 1964; Roos and Steg, 1964; Arvidsson et al., 1966; Jurna et al., 1969). Since drugs interferring with monoaminergic transmission are also known to be able to develop a Parkinson syndrome in man, they might be considered as useful tools for the study of changes in motor activity underlying this type of extrapyramidal disorder, and to test drugs on a possible antagonistic action.

The following part deals with the results obtained when investigating two of the main motor symptoms in experimental parkinsonism, that is rigidity and tremor.

Rats were operated under halothane anaesthesia. The spinal cord was exposed, the dorsal roots supplying the calf muscles were prepared

for electrical stimulation, and thin filaments were isolated from the corresponding ventral roots, care being taken that their greater part was left unsevered. The animals were fixed rigidly and the spinal cord was covered with warm paraffin oil. All drugs were injected into one of the tail veins.

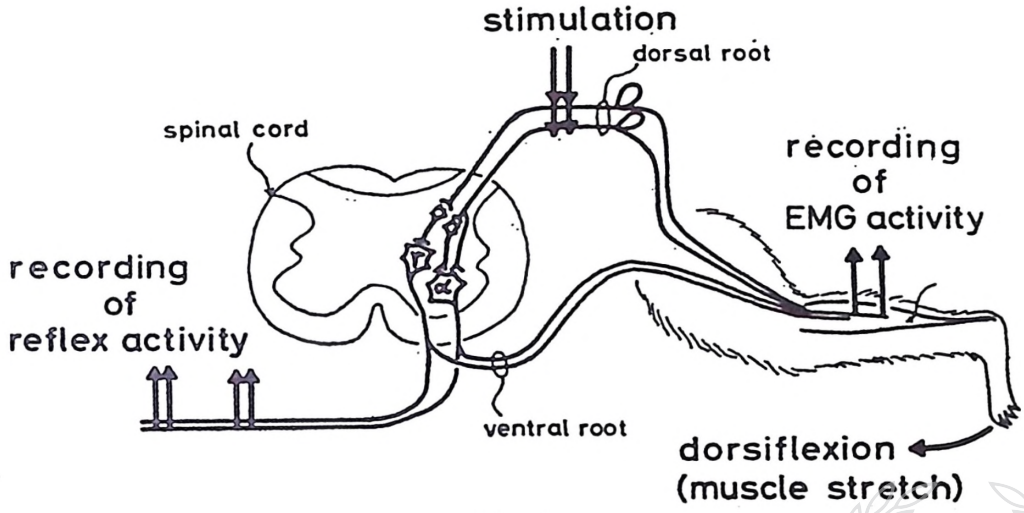

Fig. 1

Schematic representation of the experimental situation

Fig. 1 illustrates the experimental situation. The dorsal root was mounted on a pair of stimulating electrodes. A ventral root filament was placed on two pairs of recording electrodes, one pair being situated closest to the spinal cord, the other more distally. The distance between the two pairs of recording electrodes was about 5 mm. The activity recorded from the ventral root filaments was displayed on a dual beam oscilloscope, where the upper and lower beam presented the potentials led off with the proximal and distal pairs of electrodes respectively. In this way discharges from alpha and gamma motoneurones could be identified by differences not only in shape and amplitude, but also in conduction velocity of the potentials. Reflex discharges were elicited by stimulation of the corresponding dorsal root with single rectangular pulses of supramaximal strength. Electromyographical activity was recorded from the calf muscles at rest and during stretch produced by dorsiflexion of the foot.

In the untreated rat alpha reflex discharge is very low and gamma activity is high *(Fig. 2)*. After the administration of a high dose of reserpine (10 mg/kg) alpha activity increases markedly and gamma activity is depressed (S t e g, 1964; R o o s and S t e g, 1964; A r v i d s s o n et al., 1966). Moreover, reserpine reduces the latency between the stimulus artifact and the first alpha reflex response (J u r n a and L a n z e r, 1969). Simultaneously with the increase in alpha motor activity rigidity develops manifesting itself by tonic activity in the electromyogram during sustained muscle stretch.

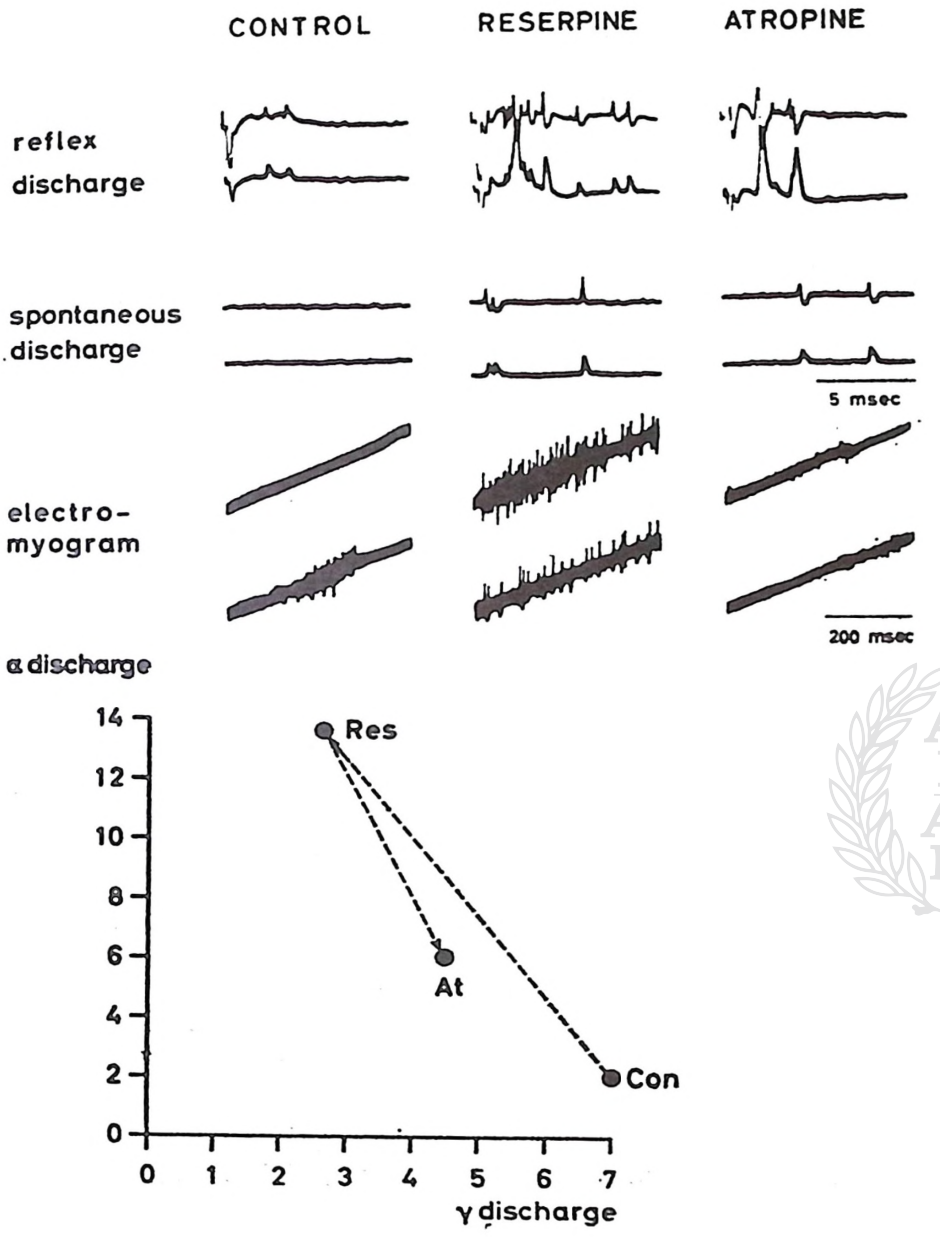

Fig. 2

Effect of reserpine and atropine on motor control. Upper row of recordings: reflex discharge led off from ventral root filament following stimulation of corresponding dorsal root with single impulse. Middle row of recordings: spontaneous discharge from ventral root filament. Upper tracings: recording made with electrode proximal to spinal cord; lower tracings: recording made with distal electrode. Recordings made with running beam on stationary film. — Lower row of recordings: electromyographical activity led off from the calf muscle. Recordings made with running beam on running film. — First column of recordings: control recordings showing little alpha but high gamma

Similar results have been obtained with other drugs impairing monoaminergic transmission, such as chlorpromazine, haloperidol, phenoxybenzamine or tetrabenazine, and also with physostigmine (Roos and Steg, 1964; Arvidsson et al., 1966; Jurna et al., 1969). Therefore, parkinson-like rigidity induced in the rat is of the alpha type, in contrast to rigidity resulting from intercollicular decerebration, which is of the gamma type. Interestingly enough, chlorpromazine has been shown to reduce gamma activity also in the decerebrate preparation (Henatsch and Ingvar, 1956).

Drugs which either stimulate monoaminergic or depress cholinergic activity abolish rigidity and normalize the disturbed control of spinal motor activity in the rat (Roos and Steg, 1964; Arvidsson et al., 1966; Jurna and Lanzer, 1969; Jurna et al., 1969). This is achieved with typical anti-parkinson drugs as atropine *(Fig. 2)*, biperiden, trihexyphenidyl and caramiphen, or with L-DOPA *(Fig. 3)* or metamphetamine.

The similarity between experimental rigidity and the corresponding clinical symptom in Parkinsonism is furthermore stressed by the observation that rigidity is abolished by removal of the striatum, but not by decortication, ablation of the cerebellum, lesion of the vestibular and red nuclei, nor by pyramidectomy (Arvidsson et al., 1967).

Another group of drugs which has found widespread application in the study of substances on possible effectiveness in Parkinson's disease, although as yet almost exclusively with respect to suppression of tremor activity, consists of tremorine and its active metabolite oxotremorine. Both tremorigenic agents have been reported to produce tremor and spasticity in various animal species (Everett, et al., 1956; George et al., 1962). They raise the concentration of acetylcholine in the brain (Holmstedt et al., 1963; Holmstedt and Lundgren 1964; Lundgren and Malmberg 1968) and could therefore be expected to evoke changes in spinal motor control similar to those following the application of physostigmine.

In fact, tremorine and oxotremorine induce a state of marked rigidity in the rat which is signalled by the appearance of tonic activity in the electromyogram *(Fig. 4)* and an increased resistance against dorsiflexion of the foot. At the same time alpha reflex activity is increased, the latency between the stimulus artifact and the first alpha discharge is shortened, and spontaneous bursts of alpha discharges can be observed (Jurna et al., 1970). Moreover, rigidity and alpha hyper-

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reflex discharge, spontaneous gamma activity and phasic activity in the electromyogram during quick stretch of muscle. Second column of recordings: recordings made 30 min after the injection of reserpine 10 mg/kg, showing high alpha and low gamma activity, shortening of the interval between stimulus artifact and first alpha discharge, spontaneous alpha activity, and tonic activity during sustained muscle stretch. Third column of recordings: recordings made 40 min after the injection of atropine 5 mg/kg, showing depressin of alpha and electromyographical activity. — The diagram represents the mean values of the number of alpha and gamma reflex discharges per sweep during the control period Con, after reserpine Res, and after atropine At. (from Jurna and Lanzer, 1969).

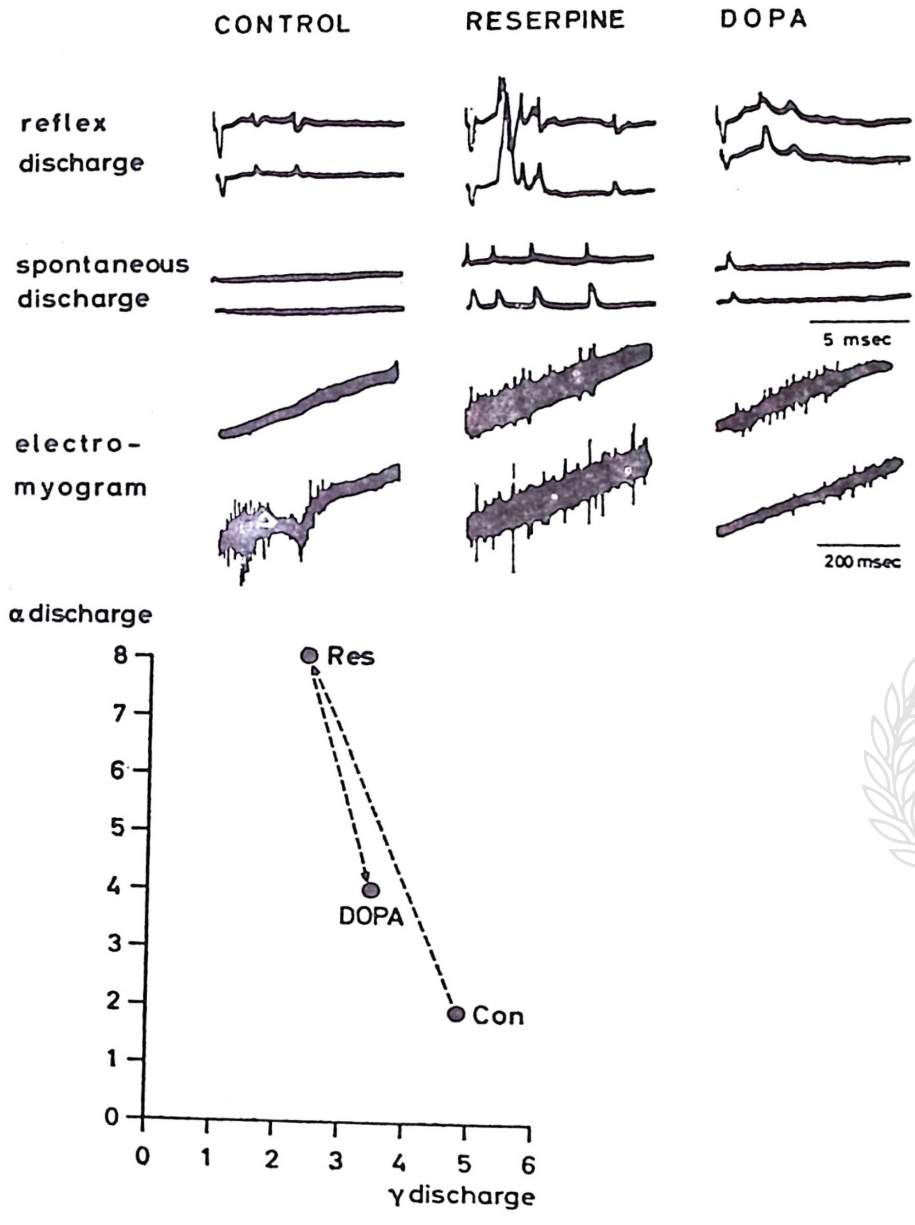

Fig. 3

Effect of reserpine and Dopa on motor control. Recordings as in Fig. 2. The record represented in the third column were taken 40 min after the injection of Dopa 100 mg/kg. The diagram represents the mean values of the number of alpha and gamma reflex discharges per sweep during the control period Con, after reserpine Res, and after Dopa. (from Jurna and Lanzer 1969).

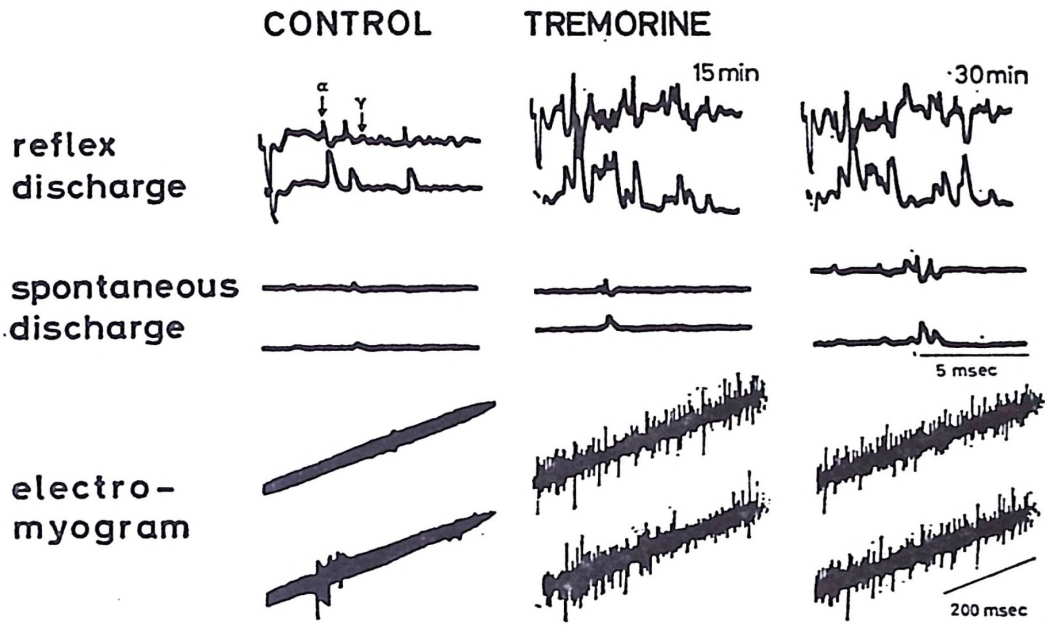

Fig. 4

Effect of tremorine on alpha and gamma motor discharges and electromyographic activity. Recordings as in Fig. 2. First column of recordings: control recordings showing little alpha and high gamma reflex discharge (an alpha and a gamma discharge are indicated by arrows), spontaneous gamma activity and phasic activity during quick muscle stretch. Second and third column of recordings: recordings made 15 and 30 min after the injection of tremorine 10 mg/kg, showing increased alpha reflex activity, shortening of the interval between stimulus artifact and first alpha discharge, spontaneous alpha discharges and increased spontaneous gamma discharges, and tonic activity in the electromyogram. (from Jurna et al., 1970).

activity from tremorine or oxotremorine are depressed by antiparkinson drugs with cholinolytic properties *(Fig. 5)* which have been shown to antagonize also reserpine and physostigmine rigidity.

Thus, as far as experimental rigidity is concerned, the changes in motor control produced by reserpine, physostigmine and tremorine or oxotremorine are identical.

With respect to the generation of tremor activity, however, the situation is different. Reserpine as well as tremorine or oxotremorine produce tremor activity, but obviously in different ways. As has been mentioned before, reserpine stimulates spontaneous alpha activity and depresses gamma activity. Therefore, tremor resulting from the administration of a high dose of reserpine must be operated by the alpha motor system which may be found to discharge in a burst-like fashion *(Fig. 6)* Similar bursts of alpha activity are also observed after an application of metamphetamine.

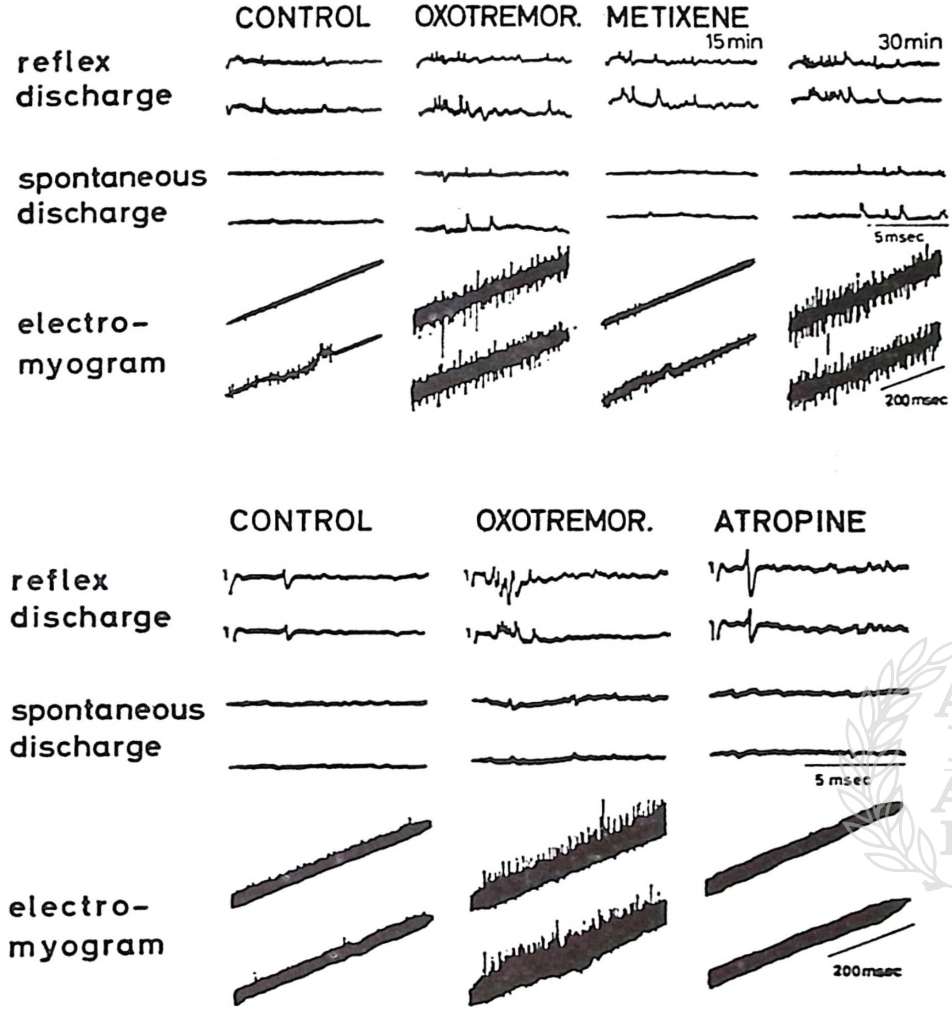

Fig. 5

Effect of oxotremorine on motor control and its depression by metixene and atropine. Both sets of recordings as in Fig. 2. First column: control recordings. Second column: recordings made 15 min after the begin of the infusion of oxotremorine 40μg/kg/min.
Following columns: recordings made 15 min and 30 min after the injection of metixene 1 mg/kg or 15 min after the injection of atropine 5 mg/kg. (from Jurna et al., 1970).

Tremorine and oxotremorine, on the other hand, do not depress but increase the spontaneous activity of the gamma motoneurones which discharge in bursts. Spontaneous alpha activity may also appear after tremorine or oxotremorine, but this is probably due to an activation by way of the so called gamma loop (gamma motoneurone —

muscle spindle — afferent fibre — alpha motoneurone; Granit 1955). The importance of the gamma motor system in tremorine and oxotremorine tremor is confirmed by the observation that drugs depressing the gamma motor hyperactivity induced by both tremorigenic agents,

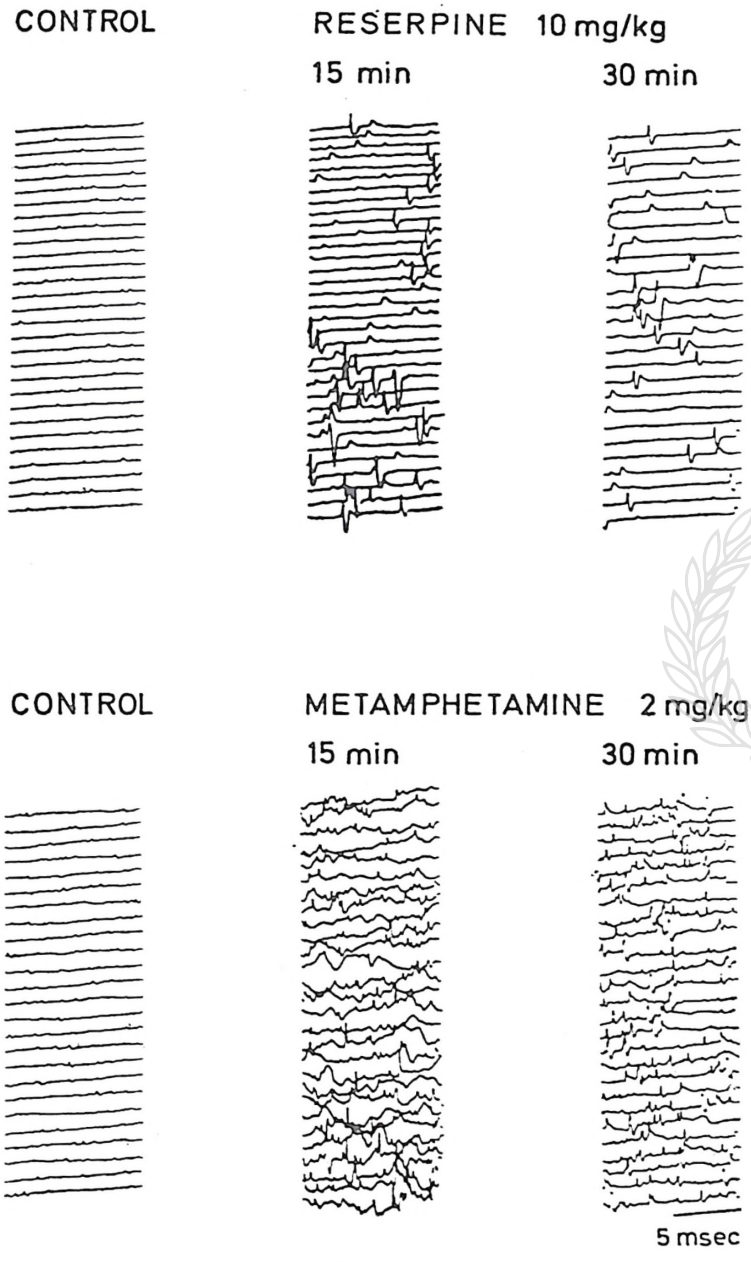

Fig. 6

Burst-like increase of spontaneous alpha discharges induced by reserpine and metamphetamine. Discharges were led off from ventral root filament. The recordings were made with running beam on running film.

such as atropine, biperiden, metixene or the adrenergic beta receptor blocking agent propranolol, abolish tremor activity, whereas drugs which inhibit exclusively alpha reflex hyperactivity, such as l-dopa depress tremor intensity but not tremor generation (Jurna et al., 1970).

The difference in the way of tremor generation by drugs which uniformly produce parkinson-like rigidity is not surprising if it is borne in mind that there are two final pathways mediating changes in spinal motor control, the alpha and the gamma system. Consequently, similar motor symptoms may be brought about by changes in activity of one or the other system, as is well illustrated by rigidity resulting from either intercollicular or anemic decerebration, which is of the gamma or the alpha type respectively. It indicates, however, that different supraspinal structures are responsible for the initiation and maintenance of parkinson-like rigidity and tremor.

With respect to the Parkinson syndrome the results may be summarized as follows *(Fig. 7)*.

	rigidity	tremor	akinesia
reserpine	α	α	present
oxotremorine	α	γ	absent

Fig. 7

Summary of the results

Rigidity of the alpha type is produced by drugs either depressing monoaminergic or increasing cholinergic transmission, and by tremorine or oxotremorine. Tremor resulting from an application of reserpine is due to bursts in alpha motor activity, whereas oxotremorine tremor is operated by the gamma motor system. And finally, parkinson-like akinesia is only produced by drugs interferring with monoaminergic transmission, but not by oxotremorine.

I. JURNA

RIGIDITET I TREMOR MIŠA IZAZVAN LIJEKOVIMA

KRATAK SADRŽAJ

Iz dosadašnjih saznanja izvučen je zaključak da Parkinsonova bolest može rezultirati iz nedostatka ravnoteže između monoaminergičnog i holinergičkog sistema u mozgu, gdje izgleda da je deplecija dopamina u striatumu jedan od najvažnijih faktora. Bilo da smanjuju monoaminergične funkcije (kao npr. reserpin i hlorpromazin), ili povećavaju

holinergične funkcije (fizostigmin), ti lijekovi na isti način proizvode akineziju, rigiditet i tremor miša. Ovaj tip rigiditeta je nastao uslijed hiperaktivnosti alfa motornog sistema i može se prekinuti antiparkinsonskim lijekovima. Alfa-tip rigiditeta je također prouzrokovan tremorgeničnim agensima tremorinom i oksotremorinom, koji povećavaju koncentraciju acetilholina u mozgu.

Tremor izazvan tremorinom i oksotremorinom nastao je uslijed inhibicije aktivnosti gama motornog sistema. Gama motorni sistem ipak ne snosi odgovornost za aktiviranje tremora nakon reserpina, jer je taj alkaloid reducirao gama motorna ispražnjavanja.

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DISCUSSION

Bruggencate: 1) what are the effects of tremorine in a spinal animal? 2) there is now so much evidence accumulating for a physiological role of the so called alpha-gamma linkage (Granit, 1968; Hagbarth and Vallbo, 1968) that the dissociation between alpha-gama-activity you get, looks in a way »abnormal«. Do you think that this dissociation between alpha and gamma-activity could be related somehow to the inability of patients, suffering from rigor, to preform movements?

Jurna: 1) Tremorine had no effects on alpha or gamma motor activity in spinalized rats in the dose used. 2) There are only the results obtained on alpha and gamma reflex discharge under the influence of tremorine and oxotremorine which at first sight indicate a dissociation of alpha-gama linkage, because of the increase in alpha reflex discharge and the unchanged or sometimes even increased gamma reflex discharge. It could well be, however, that gamma reflex discharge actually is not unchanged but reduced the reduc-

tion being masked by the increase in spontaneous gamma activity, which is always observed after application of both tremorigenic drugs. In the recordings of »reflex« alpha and gamma activity real reflex activity could not bedistinguished from intermixed spontaneous activity. Depressed gamma activity, as it seen for instance in reserpine rigidity, might indeed contribute to some extent to inability to perform graded movements.

Vizi: In what concentration did you use propranolol? Propranolol has got a strong local anaesthetic effect. Thus, what do you think the effect of propranolol in abolishing tremor is exerted via beta-receptor, or it is a simple local anaesthetic action?

Jurna: Propranolol was tried in varying doses in experiments, in which only the electromyogram was recorded. The minimal dose to reduce tremor activity was 0.5 mg/kg. Suppression of tremor generation was observed after a dose of 2 mg/kg. I doun't know whether in this dose the local anaesthetic action of propranolol will have to be taken into consideration. It is quite improbable, however, that the antitremor effect of the drug is due to a local anaesthetic action, because procaine does not abolish tremor generation but only depresses tremor intensity by way of reducing alpha hyperactivity.

Schnieden: I was interested in your results with propranolol. Have you tried other beta blocking agents such aspractalol?

Jurna: We have also performed experiments with pronethalol and obtained similar results as with propranolol.

Bernard: It was stated by the author that propranolol depresses tremor activity after oxotremorine. Is this a central or a peripheral effect of propranolol?

Jurna: Probably it is a central effect. It is assumed that tremor activity after the administration of tremorine or oxotremorine results from gamma hyperactivity. Now alpha and gamma activity was recorded from centralroot filaments so that peripheral effects were eliminated.

Dimitrijević: 1) You stated there are two motor outputs alpha and gamma motor system: where you define this output in the level of ventral filaments or muscles? 2) You mentioned that oxotremorine could induce spasticity. What you call by spasticity? Please if you give your operating definition.

Jurna: 1) We identified alpha and gamma motor units in ventral root filaments. 2) The term. spasticity was used by EVERETT et al. 1956 to describe the increased muscular tone observed after application of tremorine. I have called the motor symptom consisting of an increased resistance against passive movements of feet and legs and tonic activity in the electromyogram during sustained stretch of the muscles after tremorine or oxotremorine »rigidity« because it is very similar to the respective symptom after reserpine or physostigmine. I confess, however, that I would not make the choice of one or the other term a point of serious argumentation.

György: May I ask you how does influence reserpine the effect of OT (is there a potentiation or an antagonism)?

Jurna: We have shown a reduced response to oxotremorine after pretreatment of rats with propranolol which would fit in with your findings on the inhibition of the gamma discharges. We have also shown that reserpine will reduce oxotremorine tremor. In no case was there an abolition of the tremor only a reduction in intensity.

Štern: My experiments with preparation CIBA 28882 doun't prove that rigidity is caused by activation alpha-motor system. This substance inhibites muscle spindle and gamma-cells. Fluphenasine given after mentioned preparation produces tremor rigor. After distroying alpha-cells with p-bromphenilacetilurea fluphenazine cause rigor.