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O EKSPERIMENTALNOM TREMORU

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TREMOR, AMINES AND TRACE METALS

Professor Stern has kindly asked me to talk on the pathophysiology of tremor as it relates to amines and trace metals. It is always difficult to understand the delicate biochemistry of any illness, but the subject becomes even more hazardous when one attempts to delineate the chemical changes in the brain underlying any one symptom. Studies on Parkinson's disease during the last 10 years (1—4) have implicated disturbances in putative neurotransmitters, particularly dopamine, serotonin, and acetylcholine. The only clear relationship with symptoms is that between dopamine and akinesia, but this statement does not imply that other transmitters are not also involved as a consequence.

Thus in attempting to delineate the biochemical substrate of tremor we are facing a challenge that probably surpases our presently available knowledge. We will therefore confine our remarks to outlining broad experimental results and possible avenues worth exploring.

ANU ВіН АНУ БиХ

A — TREMOR

The first task we should undertake is to define in physiological terms the problem we want to solve. Doing so for tremor is not easy. Clinically, as is well known, there are numerous forms of tremor, from the slow 3—8/sec. resting alternating movements of the hands and feet, classically associated with Parkinson's disease, to the rapid, fine intention or attitude tremor occasionally involving the head, which has received the name »essential familial tremor«, or to the large-amplitude »flapping« tremor of Wilson's disease and hepatic failure. If one also considers the tremor seen in delirium tremens, in hyperthyroidism, in anxiety or stress, it is difficult to imagine that these various manifestations of the phenomenom rest upon a common physiological or biochemical substratum. And yet such a proposal has been made on occasion. It is not our purpose here to review this subject in detail. Suffice it to recall that, at one time or another, every level of the neuraxis has been thought to be involved in the pathophysiology of tremor, from the gamma system of the spinal cord to the cortex. The search for a so-called »tremorigenic center« has not ended, but it now focusses mainly on

the brain stem reticular formation and a loop involving the basal ganglia, thalamus and cortex. This last proposal, best studied by the group of Cordeau, Jasper and Lamarre (5—7) calls for a reverberating circuit between these brain areas which in itself would constitute the »tremorgenic center« and which would utilize the cortico-spinal pathway as a common output. The evidence for this hypothesis rests with the demonstration by micro-electrodes in the tremor monkey and in human Parkison's disease of cells firing at the appropriate 3/8 sec. rate (8). These cells are found mainly in the posterior part of the ventro-lateral nucleus of the thalamus and in some-areas of the subthalamus. Other neighboring cells with rhytmic properties are also observed in this area, but they usually fire in response to a peripheral stimulus such as touch or movement of a joint.

It should be remembered that the ventro-lateral nucleus of the thalamus is also the area that must be destroyed for the best results in both parkinsonian and intention tremor syndromes. It is thus a reasonable proposal to assume for our discussion that what we will, somewhat clinically, call the »pulsating« cells of this nucleus are a necessary component of the »tremorigenic« system to analyse. It is also probable that, under ordinary circumstances, these cells function at an intensity insufficient to produce clinical symptoms. To create tremor would not require direct damage to this area, since this would eliminate the »pulsating« cells from the circuit, but rather one of three circumstances: triggering by an excitatory input from another center, removal of usually inhibitory dampening system, or both mechanisms working in series.

The available evidence indicates that the ventro-lateral nucleus of the thalamus receives inputs directly from the basal ganglia (mainly globus pallidus), the cerebellum (dentate nucleus) and indirectly from the reticular formation and perhaps the substantia nigra reticulata. At least two of these systems are facilitating, but even their output is carefully controlled and monitored by an inhibitory servo-mechanism. Thus the main output of the basal ganglia is through the globus pallidus to the VL, but this output is controlled by the inhibitory action of the dopaminergic circuit from the substantia nigra. A similar inhibitory mechanism (utilizing GABA as a probable neurotransmitter from the Purkinje cells) also monitors the dentato-rubro-thalamic output in the cerebellum. Finally it is well known from the studies of the Swedish group (9) that there exist parallel serotoninergic and noradrenergic ascending pathways from the brain stem reticular formation to the thalamus. These pathways have been shown by Jouvet and others (10, 11) to be involved in sleep-waking mechanisms.

How these multiple incoming pathways interact at the level of the VL and how they activate the »pulsating« tremorigenic cells in this nucleus is the still unresolved problem. We have seen that most putative neurotransmitters, particularly acetylcholine, dopamine, serotonin, and noradrenaline are involved in some way in this mechanism, but their relationship and interaction is far from clear. At one time or another, most of these substances have been implicated in the mechanism of tremor. It is thus important for us to look closely at the evidence.

B — AMINES

a) Noradrenaline

Many years ago Schwab and his collaborators (12) obtained a marked increase in the tremor of Parkinson's disease patients after infusions of noradrenaline and adrenaline. This finding has been confirmed by others (13). It probably acts by the same unspecific mechanism as that postulated during fear or anxiety, where it is equally observed that all forms of tremor are enhanced. The studies of Andén et al. (9) have indicated that the ascending noradrenergic pathway from the brain stem reticular formation to the centre median and unspecific nuclei of the thalamus is probably involved in the alerting and awakening mechanisms (11). Such mechanisms would only indirectly contribute to stimulation of the VL »pulsating« cells by modifying the response threshold.

b) Dopamine

The best known tremor syndrome, of course, is Parkinson's disease, and in this illness the role of dopamine has been conclusively demonstrated. However a common misunderstanding has arisen which assumes that this amine is directly involved in the pathogenesis of all the symptoms of Parkinson's disease. That this is not completely so should be evident from a number of experiments and clinical observations:

(a) the only established relationship between the urinary concentration of dopamine and a clinical symptom is with akinesia (16);

(b) a specific decrease in dopamine produced by alpha-methyl-paratyrosine results only in hypokinesia in the animals so treated, unless previous damage had been inflicted to the brain stem area (17);

(c) specific blocking of dopaminergic (adrenergic) receptors, such as with haloperidol results only in hypokinesia.

Thus the specific low striatal dopamine syndrome in man and animals is *hypokinesia*. This, of course, is also the symptom best influenced by L-DOPA treatment (4). The above observations, however, should not be taken to indicate that dopamine cannot be involved in the pathogenesis of tremor. It is known, for example, that L-DOPA can occasionally be very useful in alleviating parkinsonian tremor (4). It would appear that a dopamine deficiency is acting more as a facilitating factor to another mechanism. Nor is dopamine hyperactivity a likely culprit. L-DOPA overdosage, in the presence of previous brain stem damage, has brought out chorea, tics or dystonia, but never the denovo production of tremor (4). Whenever tremor had persisted despite treatment, L-DOPA resulted in an increase in the amplitude of this movement with essentially no change in the basic rhythm. However in »essential intention tremor«, L-DOPA worsens the condition.

c) Acetylcholine

Although acetylcholine is the best known neurohormone and the only one for whom most of the criteria of a central transmitter substance have been met, there is yet no agreement as to its precise physiological function. Shute and Lewis (18) have shown that the previously postulated thalamo-cortical projections are at best indirect and through the capsula externa. Moreover Poirier and collaborators (19) have indicated that the most important cholinergic pathway may be the one from the striatum to the substantia nigra, thus completing a closed circuit with the dopaminergic nigrostriatal pathway. Such a possibility would be compatible with the experiments of Ernst (20) who produced gnawing in animals (a symptom usually associated with dopaminergic striatal stimulation) after implantation of eserine in the substantia nigra.

Stern and collaborators (21) and many others have shown that ACh infusion can cause tremor in animals. It is well known, of course, that standard anticholinergic medication will be useful in partially controlling the parkinsonian tremor. On the other hand they usually increase essential attitude tremor. However recent studies by Coyle and Snyder (22) indicate that these substances also affect dopaminergic systems and add complications to the accepted mechanism of action of anticholinergic drugs.

Although there is no doubt that acetylcholine is involved in tremorigenic mechanisms, it is not clear yet at what level it could be acting. The most likely mechanism is by saturating muscarinic receptors. In the short fiber integrating areas of the central nervous system, dopamine and acetylcholine appear to be playing the role assigned to noradrenaline and serotonin in longer fiber groups. Thus dopamine and acetylcholine should be considered the respective central modulators of the ergotrophic and tropotrophic nervous system and are always in precarious balance especially in integrating areas such as the striatum (27). The possible role of dopamine as a presynaptic inhibitor of ACh release strengthengs this interrelationship.

d) Serotonin

This amine appears to be a likely candidate for playing a direct role in tremor production. It has been found in lower than normal concentration in the brain of parkinsonian patients (23) and in reserpine induced states where a tremor is frequently observed. It is highly concentrated in the brain stem, hypothalamus and limbic areas. It has been implicated in the regulation of sleep and of mood. Previous studies from our group (24) had shown that its synthesis is probably not normal in Parkinson's disease and that some parkinsonian tremors could be decreased with the precursor 5-hydroxytryptophan. According to Stern, the specific serotonin receptor blocking agent LSD-25 can also produce tremor (25), while 5-HTP can inhibit oxotremorine induced tremor. Moreover, Poirier and collaborators (26) have indicated that serotonin

may be an important neurotransmitter in the rubro-olivo-cerebello-rubral loop which they postulate is the essential physiological system in tremor.

Thus there is some evidence implicating many of the putative neurotransmitters in the production of tremor. Although the evidence is strongest for acetylcholine, it is impossible to disregard the others. Moreover there is now increasing agreement for important interactions between the various neurohormones, especially in centers where they are all found in high concentration, such as the striatum (27).

From the scattered facts reviewed above, can a useful pattern emerge? It is more physiologically useful to consider what happens at specific receptor sites, rather than to examine brain concentrations of any given amine, which are only imperfect reflections of a static state. With such a point of view, we could envision the *dynamics of tremor production* as involving:

(1) understimulation of the *serotonin* receptors;

(2) decreased stimulation of the *dopamine* receptors;

(3) hyperstimulation of the *acetylcholine* receptors;

(4) possibly also hyperstimulation of *noradrenergic* receptors.

The anatomic sites for each of these receptors are not yet clearly defined, and are probably multiple. However the above observations agree with the known interactions between neurotransmitters. Such interactions reveal a dopamine-acetylcholine antagonism in the striatum (24) as well as a serotonin-noradrenaline antagonism in the brain stem and spinal cord. Thus studies with Orzeck in our laboratory (29) have indeed shown that if low striatal concentrations of acetylcholine are produced in the rabbit by reserpine (with increased release), a return to normal levels (or release rate) can occur after giving L-DOPA or apomorphine to the animals, but not after 5-HTP. Conversely, Van Woert and co-workers have shown that the increased cholinergic sensitivity present in parkinsonian patients can be reversed by treatment with L-DOPA (30). The parallel pathways but opposing actions of serotonin and noradrenaline in the brain stem and spinal cord and their respective functions in the sleep-waking cycles have already been referred to (9, 10).

Thus under normal conditions the »pulsating« cells in the VL could be under a modulating influence of opposing serotonin and noradrenergic pathways from the brain stem, through the unspecific thalamic relays. In the presence of decreased serotonin stimulation (receptor block such as with LSD; damage to serotoninergic pathways from the brain stem) the cells would become more susceptible to stimulation by noradrenaline.

Normally these »pulsating« cells maintain their tone through a modulating input from the basal ganglia. Such an input is usually sufficient to avoid excessive firing because of the filtering function of the striatum. In the presence of high cholinergic activity, such as that induced by a defect in the controlling dopaminergic pathway, the »pulsating« cells are activated and the force of their synchronized output is such that the appropriate cortical motor area is stimulated and clinical tremor results.

It should be emphasized that, to date, it is impossible to state which neurotransmitter actually modulates the »pulsating« cells from the striatum. We only know that part of this system is cholinergic.

Thus the biochemical pathophysiology of tremor cannot involve only a single system or neurotransmitter. The tremorigenic center in the thalamus is under regulating influences from the brain stem. Some of this mechanism probably involves output from the rubro-olivo-cerebellar-rubral loop of Poirier (26), from the substantia nigra reticulata and from the reticular formation. Serotonin may be an important neurohormone in these pathways. GABA, the cerebellar inhibitory transmitter could play a controlling role similar to that of dopamine in the striatum. Damage to the serotoninergic pathways thus results in hyperreactivity of the »pulsating« VL cells.

Under normal conditions the output of the »pulsating« cells is kept in check by the restraining influence of the striatum. If the cholinergic component of this system loses its own controlling mechanism (the dopaminergic nigro-striatal system), the input from the basal ganglia overstimulates the VL cells. In the presence of a previous state of hyperreactivity of these cells, the bursts of firing necessary for clinical tremor are produced.

C — TRACE METALS

In the light of such a delicately balanced system, it is difficult to visualize what the various trace metals found in these regions are doing, and to what extent the production of tremor is mediated through them, as suggested by Prof. Stern (14, 15) from many pharmacological experiments. The trace metals are indeed known to be involved as co-enzymes in many metabolic functions and as necessary constituents of binding, release and transport mechanisms. It is thus unlikely that they normally undergo major abrupt changes. We would then like to postulate, in slight variance with Prof. Stern, that whenever a drug, or substance, causes a change in trace metals, it is not exerting its action directly through this effect, but through modifications in the underlying balance between neurotransmitters. This is true of course for chronic experiments. In acute toxicity metabolic cell damage may also play a role. Let us look at the evidence:

1) There is no doubt that the basal ganglia and mainly the globus pallidus and substantia nigra reticulata contain an abundance of trace metals. This is particularly striking in the case of iron.

2) A tremor similar to that of Parkinson's disease can be produced with tremorine and LSD-25, both substances which are said by Prof. Stern to lower brain iron. However both substances also act on amines (31).

3) A form of intention tremor, more like that seen in Wilson's disease, is produced by diethylcysteamine, penicillamine, thioacetamide or alpha-methyl-dopa (14, 15). These drugs are said by Prof. Stern to mobilize copper in the brain. Alpha-methyl-dopa, of course, also inhibits aminoacid decarboxylase which would result in a decreased formation

of serotonin. Using atomic absorption, we unfortunately have been unable to confirm changes in the brain copper concentration of mice after thioacetamide (Table 1), but have clearly demonstrated an increase

Table 1*

EFFECT OF THIOACETAMIDE ON BRAIN COPPER

DRUG	Cu (wet wt.)	Cu (dry wt.)
SALINE	3.61	17.51
THIOACETAMIDE	3.80	18.61

* Data from Donaldson, J,; Minnich, J., and Barbeau, A. (1971).

in serotonin concentration (Table 2), in the same animals. With alpha--methyl-dopa, as with thioacetamide, it can be surmised that serotonin receptors were understimulated.

Table 2*

EFFECT OF THIOACETAMIDE ON BRAIN SEROTONIN BRAIN CONTENT

	(μg./gm.)	
(A) CONTROL ANIMALS (NaCl)	0.62	P
(B) THIOACETAMIDE (170 mg./kg. x 3 days)	0.72	$> < 0.001$
(C) NaCl + 5-H. T. P. (150 mg./kg.)	1.20	
(D) THIOACETAMIDE + 5-H. T. P.	1.93	$> < 0.001$

* Data from Donaldson, J.; Minnich, J., and Barbeau, A. (1971).

4) Costa and collaborators (32) have been able to produce in monkeys (with chronic manganese intoxication) a brain decrease in dopamine and serotonin and the clinical picture of akinesia and tremor.

5) Chronic injections of iron in rats cause a decrease in dopamine and serotonin and a rigid-tremulous symptomatology (33).

6) Chronic copper toxicity, such as in Wilson's disease, is apparently accompanied by increased striatal concentration of dopamine.

7) Finally, we have recently been able to obtain pure akinesia and a specific striatal dopamine deficiency in dogs treated with a magnesium dificient diet (17 ppm/days) for 35 days (34) (Table 3).

Table 3

CAUDATE AMINES AFTER A LOW MAGNESIUM DIET
(Dogs — μg/gm. tissue; 6 animals/group)
(mean ± S. E. M.)

	DOPAMINE	NORADRENALINE	SEROTONIN
CONTROL ANIMALS	4.93±0.47	0.072±0.01	1.12±0.11
LOW Mg ANIMALS	2.68±0.39*	0.057±0.04	1.10±0.11

* $p < 0.01$ from control.

Thus trace metals may be important in the production of the symptomatology of extrapyramidal diseases, but only as contributing or trigger factors in induced changes in amine metabolism or through the production of subtle amine imbalances. In acute toxic experiments, such metals-could probably also cause damage through anoxic or metabolic cell changes.

Thus like Prof. Stern, we feel that it is important to concentrate on a rigourous study of trace metals and ions in the pathogenesis of various diseases of the extrapyramidal system, but such studies should be of *chronically* induced minimal changes. Acute loading experiments probably will not contribute much to our understanding of these mechanisms. In this light we are presently studying the effect of chronic magnesium deficiency on the production of electrolyte and trace metal changes in the brain, on the concentration of enzymes and amines in different regions of the brain, and finally on the possibility of cell damage in specific areas. This model may serve for the study of Parkinson's disease. Similar models with copper, iron, manganese, potassium or calcium chronic loading or deficiencies, are also contemplated. The theoretical basis for the expected amine changes in such models cannot be reviewed in detail here, but is summarized in Table 4.

Table 4

AMINES AND EXTRAPYRAMIDAL DISEASES
(STATE OF RECEPTOR STIMULATION REQUESTED FOR POSSIBLE MODELS OF SPECIFIC DISEASES)

DISEASE	DOPAMINE	SEROTONIN	ACETYLCHOLINE
PARKINSON	low	low	high
DYSTONIA	high	low	normal
ESENTIAL TREMOR	normal	low	low
CHOREA	high	slightly high	low

АНУ ВиН

SUMMARY

An hypothesis of the pathophysiology of tremor is presented: parkinsonian tremor is probably due to an increased synchronized output from normally »pulsating« cells in the ventro-lateral nucleus of the thalamus and parts of the subthalamus. The activation of these cells may originate from damage to incoming (probably serotoninergic) pathways from the brain stem; the increased synchronized firing could result from removal of the dopaminergic filter mechanism in the striatum, with secondary hyperactivity of the cholinergic mechanism and hyperstimulation of cholinergic muscarinic receptors.

Such imbalance in multi-neurotransmitter systems may be the consequence of faulty enzymatic machinery. Defects in trace metals, or

electrolytes could be responsible for these mechanisms, but it is likely that such changes are produced slowly. Chronic loading, or deficiency, experiments have more chances of solving this equation than the almost toxic variations obtained during acute pharmacological manipulations. Theoretical models of the amine imbalance in various extrapyramidal disorders are given. Induced variations in trace metals or electrolytes should endeavour to produce such models clinically and biochemically. Preliminary results from a magnesium deficiency study are given.

A. BARBEAU, J. DONALDSON I J. L. MINNICH

ULOGA METALA U TRAGOVIMA KATEHOLAMINA U PRODUKCIJI TREMORA

KRATAK SADRŽAJ

Osim tremora u Parkinsonovoj bolesti, važnu tačku za kompletnu kliničku prezentaciju predstavljaju atitudinalni i intencionalni tremor u ostalim ekstrapiramidalnim poremećajima. Poznato je da će L-dopa doprinijeti redukciji Parkinsonovog tremora, dok će ga alfa-metil-dopa povećati; prava uloga serotonina još uvijek nije ispitana, jer se tremor može izazvati redukcijom ovog amina (Reserpin) u moždanom sadržaju ili visokim dozama prekursora 5-HTP. Tremorin i oksotremorin, koji prouzrokuju tremor, modificiraju moždani sadržaj dopamina, serotonina i acetilholina.

Isti paradoks postoji u eksperimentalno proizvedenom tremoru nakon egzogenih ili nutritivnih modifikacija tragova metala u moždanom sadržaju (željezo, mangan, bakar i magnezijum) ili nakon lijekova kao što su tremorin, karbon tetraklorid, tioacetamid, alfa-metil-dopa, L-dopa.

Prezentirana su ispitivanja na korelaciji između tragova metala i kateholamina u mozgu i pokušano je da se pokaže da simptom »tremor« rezultira iz kompleksne interakcije različitih važnih neurotransmitera i modulatora. U tim interakcijama relativna koncentracija različitih tragova metala (kao koenzimi i regulatori u sinaptičnoj membrani i transportu) može da bude »trigger« mehanizam.

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DISCUSSION

Bruggencate. I found your remark very interesting, that one needs a lesion within the rubro-dentato-rubro loop as an additional factor to produce tremor. As you certainly know, the organisation of midbrain-cerebellar systems is rather complex. Thus, N. ruber not only affects the inferior olive but also other cerebellopetal systems, for instance the VSCT. Lesion within the midbrain certainly can influence also this system. Doun't you think that

it would be important to differentiate further between the various possibilities particuary since olive-cerebellar fibres are ending as climbing fibres, and the VSCT as mossy fibres? (And since nobody knows what these different fibres mean to the cerebellum).

Barbeau: I agree with you that the finite geography and histochemistry of this loop still has to be mapped out. I refer you to the excellent anatomical and physiological studies of Poirier and his collaborators who have been investigating the production of tremor after lesion of this loop in monkeys and cats.

Štern: I am very glad to see that prof. Barbeau found that Serotonin is important in the therapy of Parkinson's disease. Ten years ago in one of my works. I found together with collegue Gašparević that 5-Hydroxitryptofan and not L-DOPA abolishes the tremor of mice caused by tremorin.

Barbeau: Thank you prof. Stern. Another was to interfere with serotonin is to use LSD-25 which, as you know, also produces tremor.

Schnieden: 1) Could you clarify one point in your talk. You postulated that a decrease of 5-HT and dopamine and a rise in ACh is important in Parkinsonian tremor. Does this postulate apply only to slow resting tremor, orto both forms? 2) Could you comment one the pathophysiology of essential tremor?

Barbeau: Thank you for your questions. To the first question: We have mainly been considering Parkinsonian resting tremor when talking about the effects of a decreased dopamine stimulation upon the release of ACh and the saturation of the muscarinenic receptors. The effect of the brain-stem serotonin pathway lesion is common to any sort of tremor: resting or intention (action). We would suspect that another servo-mechanism, similar to that using dopamine for the striatum, is operative in the cerebellum and we postulate that for intention tremor GABA plays the same controlling role as dopamine for rest tremor.

To the second question: I have no personal studies concerning the pathophysiology of essential tremor. I can only say that this form of tremor is made worse clinically by anticholinergic drugs as well as by L-DOPA. I would suspect that cholinergic receptor stimulation is decreased in the presence of possibly increased dopamine receptor stimulation.

Vizi: Have you got any idea of what is the role of magnesium level in Parkinsons disease? We have found that high Mg^{2+} (9.3 mM) is capable of inhibiting the ACh release induced by nicotine (5×10^{-6} g/ml) from the nerve terminals. Nicotine was used to mimic the pathological firing of neurone. Furthermore, it is generally accepted, that the actual concentration of Mg^{2+} can influence the ACh release. Could you tell us, whether during low Mg^{2+} level the probably increased ACh release is the factor which plays some role in producing of disturbances of extrapyramidal system?

Barbeau: Thank you for your remarks. We entirely agree that the most likely explanation for the action of Mg is upon the release mechanism of the neurotransmitter. However, the mode of action may be much more complex than we thought. During our Mg deficiency studies we also produces a decrease in serum Potassium concentrations. Since the diet was not deficient in Potassium, a shift towards intracellular pools is possible and may also have to be considered. It is too early to say how Mg deficiency may contribute to extrapyramidal system.

György: You have mentioned the role of 5-HT in the parkinsonian tremor. Have you any data about the activity of methysergid or p-chlorophenilalanine: how they influence the function of extrapyramidal system or the drug-induced experimental tremor or rigidity?

Barbeau: We have no definite data on this point yet, but we should be reminded that p-chloro-phenilalanine is not specific to the brain-stem serotonin pathways. The limbic system, also rich in 5-HT, would also be affected. It is thus difficult to use such pharmacologic tools for the understanding of any given symptom, unless topical localized studies are carried out.