Međunarodni simpozijum o eksperimentalnom tremoru: 1-3. april 1971. godine

Stern, Pavao

1972

Akademija nauka i umjetnosti Bosne i Hercegovine

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ACADEMY OF SCIENCES AND ARTS OF BOSNIA AND HERZEGOVINA

SPECIAL PUBLICATIONS

VOL. XVII

DEPARTEMENT OF MEDICAL SCIENCES

ISSUE 4.

INTERNATIONAL SYMPOSIUM

ON EXPERIMENTAL TREMOR

1 — 3. april 1971.

Editor
PAVEL ŠTERN,
member of Academy of sciences and arts
of Bosnia and Herzegovina

SARAJEVO
1972

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NEUROMUSCULAR EFFECT OF TREMOGENIC AGENTS IN VITRO*

INTRODUCTION

The site of action of oxotremorine as a cause of many features of Parkinson's disease is in central nervous system (5), in particular in the rat in the midbrain reticular formation (8, 16). Tremorine tremorgenesis is the result of exciation of a pacemaker in the tegmental reticular formation at the mesencephalic-pontine junction (17). At the same time evidence of peripheral involvement has been demonstrated (3, 4, 7). Other substances used as pharmacological tools for the evaluation of antiparkinson drugs (6) as nicotine (1), harmine (20) and physostigmine (15) act in the central and peripheral nervous system also.

Since little quantitative informations are available concerning the intensity of the effect of oxotremorine, harmine, nicotine and cyclopium (19), on neuromuscular transmission in comparission to its effect in central nervous system, it was investigated the influence of tremorgenic agents on the effect of nerve stimulation of isolated vas deferens, urinary bladder and oesophagus of rats and mice. The first preparation is used as a model-system for adrenergic nerve smooth muscle transmission (11), isolated urinary bladder is used as a cholinergic newe smooth muscle preparation (12) and the third model-system is used for striated muscle and cholinergic nerve transmission (14).

METHOD

The animals used were rats and mice. The rats weighted 200 g and mice 25 g. The animals were stunned by a blow on the head and then decapitated. The abdomen and the thorax were opened along the middle line and ductus deferens and urinary bladder were exposed. The hypogastric adrenergic nerve was tied with small peace of peritoneum half cm from ductus deferens. The pelvic nerve was tied around ureter for the pelvic nerve run close to the ureter. .The heart and lungs were removed to

* This work was supported by Federal Grant of Yugoslavia, Beograd.

expose the oesophagus. The longitudinal pair of r a m i i o e s o p h a g e i n e r v i v a g i were tied as near as possible to the oral part the thorax. The organs were put in Tyrode solution first and then were set up in the isolated organ bath. One day oesophagus was set up first, second day urinary bladder was first and third day ductus deferens was first organ that was set up as fresh as possible. The organ bath contained 40 ml of Tyrode solution that was warmed to 32°C±0,5 and bubbled with 95% O_2 and 5% CO_2. The proximal part of ductus deferens was connected with frontal lever. The fundus of urinary bladder was connected with frontal lever too. Frontal lever was connected with proximal part of ductus deferens cardial part of oesophagus and fundus of urinary bladder. The magnification being 4 and the load for the rats organs one gram and for the mice organs half gram. The tied nerve were put through bipolar electrodes as was described (2). Submaximal strength of electrical stimuli, 1 msec. duration at a frequency of 40 Hz were applied every min. or half min. for 2 sec.

Ten control contractions were recorded and the slow infusion of 0,02 ml/min in 40 ml organ bath started. Substances dissolwed in Tyrode solution were infused: Oxotremorine 10^{-2} M, Harmine 10^{-3} M, Nicotine 10^{-3}M and Cyclopium 10^{-3} M/ml. One organ one substance. Three concentrations were found and expressed as a negative logarithm of molar concentration: pSm, pB75 and pB50, they are so called pharmacological constants (18). The first point is pSm, the place on base line where a molar concentration induced the highest contraction. The second point calculated is pB75, concentration that induced 75 percent of control contraction. The third point is pB50 molar concentration of drugs that reduced hight of contraction to the half of control one. As greater pharmacologic constant as greate chemosensitivity.

RESULTS

A. The effect of electrical stimulation

The effect of electrical stimulation of nerves is contraction. The responses can be repeated if the frequency of stimulation was not often and less then 2 in min.

a. *Oesophagus of rats and mice.* The effect of electric nerve stimulation is rapid contraction. The aequal reactions can be repeated for more than two hours induced by constant stimuli.

b. *Urinary bladder of rats and mice.* The effect of stimulation of control organs is slow and uniform contraction if the constant stimulus were applied.

c. *Ductus (Vas) deferens of rat.* The effect of constant stimulation of adrenergic hypogastric nerv is constant contraction, that can be repeated more than an hour if the frequency of the stimulation by the trains of stimuli is not more then 2/min.

B. The effect of tremogenic agents

All substances were infused by infusion pump 0,5 µl/ml/min. The bath was washed out thoroughly after termination of infusion and the record of the control contraction was again taken. The organs taken from mice are more sensitive than from rats.

a. *Oxotremorine.* It induced first increase of the nerve stimulation followed by its decrease and block of the nerve conductance. The organs taken from mice are more sensitive toward oxotremorine then organs taken from rats. The most sensitive is oesophagus of mouse and the least sensitive is the effect of adrenergic nerve stimulation on ductus deferens of rat. The differens between maximal excitatory effect

Tab. 1.

THE INFLUENCE OF THE SLOW INFUSION OF OXOTREMORINE 5×10^{-6} M/min./ml ON THE EFFECT OF NERVE STIMULATION OF ISOLATED ORGANS

Organs		pS_m*	pB_{75}	pB_{50}
Oesophagus	rat	3,65 ± 0,25	3,20 ± 0,16	2,92 ± 0,57
	mouse	4,00 ± 0,01	3,67 ± 0,12	3,66 ± 0,11
Urinary bladder	rat	3,60 ± 0,13	3,11 ± 0,18	— (0/6)**
	mouse	3,66 ± 0,27	3,47 ± 0,19	— (0/6)
Ductus defferens	rat	3,23 ± 0,03	2,82 ± 0,17	— (0/6)
	mouse	3,72 ± 0,15	3,15 ± 0,18	— (0/6)

* pS and pB are negative logarithm of the molar concentration of oxotremorine that induce increase (S) or inhibition (B) of the effect of nerve stimulation.

** Numbers in brackets are frequency of recorded effect in relate to number of experiments.

and considerable inhibitory effect (pB75—pS), is less than one range of value. The greater degree of inhibition was not achived in smooth muscle organs (Tab. 1).

b. *Harmine* induces biphasic effect of all organs used. Exceptions are rat's oesophagus and mouse's urinary bladder. The difference at concentration between maximal effect and considerable decrease is not great as not so difference is not great between other constants (pSm — pB_{75}, pB_{75} — pB_{50}) (Tab. 2 and Fig. 2).

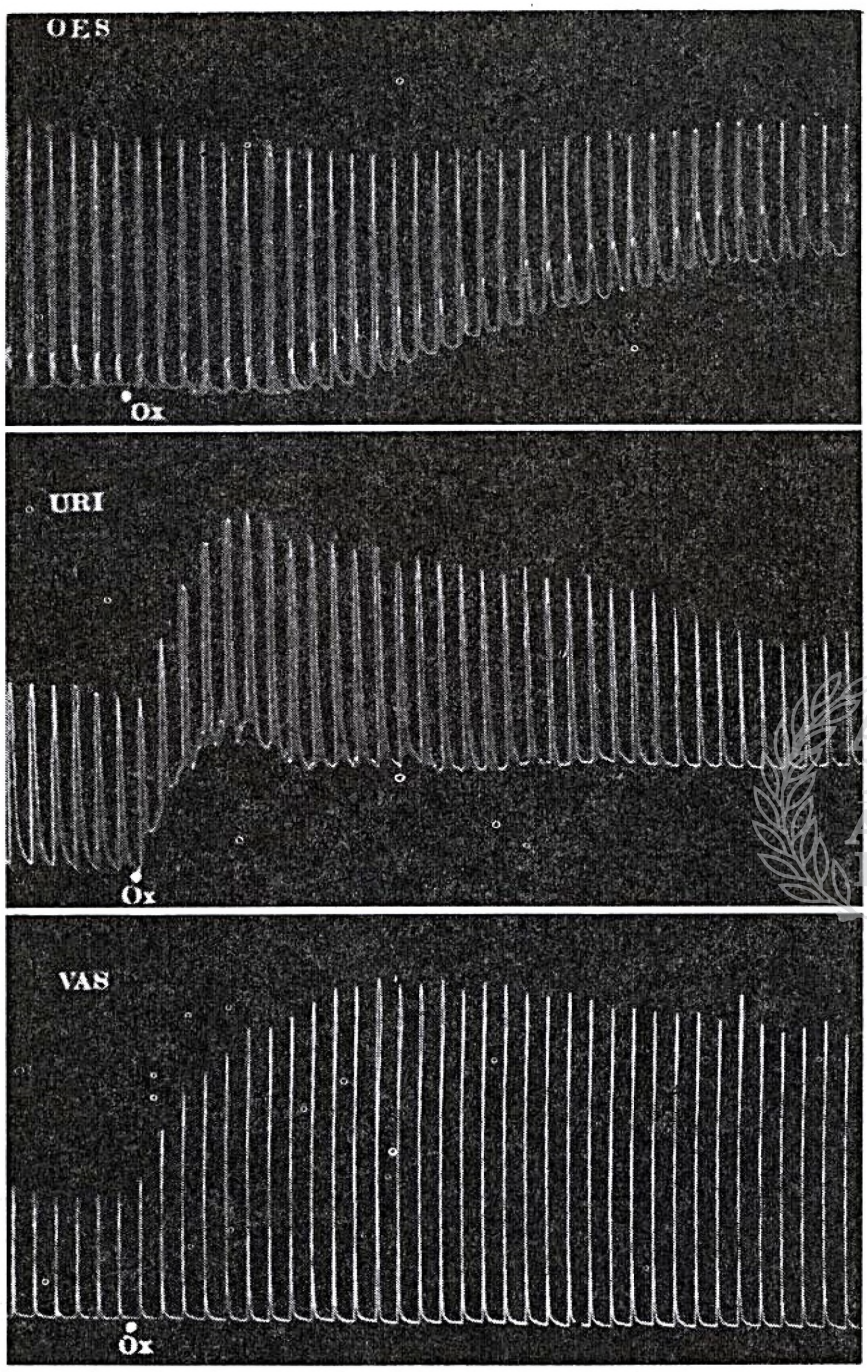

Fig. 1

The contractions of rat's isolated organs induced by the electrical nerve stimulation (5 V ,1 mSec, 40 Hz, every min. for 2 Sec.). At the point Ox infusion of oxotremorine 5 x 10^{-6}M/ml/min stated. Oes is oesophagus, Uri is urinary bladder, vas is ductus deferens.

Tab. 2.

THE INFLUENCE OF THE SLOW INFUSION OF HARMINE 5×10^{-7} M/min./ml ON THE EFFECT OF NERVE STIMULATION OF ISOLATED ORGANS

Organs		pS_m*	pB_{75}	pB_{50}	pB_{25}
Oesophagus	rat	— (0/6)**	5,03 ± 0,24	4,91 ± 0,34	4,59 ± 0,16
	mouse	5,53 ± 0,18	5,26 ± 0,18	5,12 ± 0,15	4,96 ± 0,15
Urinary bladder	rat	5,43 ± 0,13	4,73 ± 0,71	4,61 ± 0,89	4,49 ± 0,09
	mouse	— (0/6)	5,68 ± 0,55	5,03 ± 0,21	4,83 ± 0,99
Ductus deferens of rat		6,20 ± 0,98	5,60 ± 0,13	5,80 ± 0,01	4,84 ± 0,33

* pS and pB are negative logarithm of the molar concentration of harmine that induce increase (S) or inhibition (B) of the effect of nerve stimulation.
** Numbers in brackets are frequency of recorded effect in relate to number of experiments.

c. *Nicotine.* Continual slow infusion of nicotine $10^{-6,3}$ M/ml/min. does not change significantly the effect of nerve stimulation. The effect of nicotine is biphasic on the smooth muscle organs with adrenergic nerve stimulation. The first effect is the increase of stimulation on the vas deferens, pS_m is 5,77 then follows decrease pB_{75} is 5,25 and pB_{50} is 4,88. The difference is 0,5 range of value. The striated muscle of oesophagus responded up on the simultaneous application of drug and electrical stimulation with the smaller contraction then upon the electric stimulation of the nerve only (Tab. 3).

Tab. 3.

THE INFLUENCE OF THE SLOW INFUSION OF NICOTINE 5×10^{-7} M/min./ml ON THE EFFECT OF NERVE STIMULATION OF ISOLATED ORGANS

Organs		pS_m*	pB_{75}	pB_{50}
Oesophagus	rat	5,78 (1/6)**	5,64 (1/6)	5,56 (1/6)
	mouse	5,77 (1/6)	5,25 ± 0,87	5,14 ± 0,63
Urinary bladder	rat	6,10 (1/6)	5,34 ± 0,17	5,16 ± 0,14
	mouse	— (1/6)	4,89 ± 0,54	— (0/6)
Ductus deferens of rat		5,77 ± 0,17	5,25 ± 0,13	4,88 ± 0,55

* pS and pB are negative logaritham of the molar concentration of nicotine that induce increase (S) or inhibition (B) of the effect of nerve stimulation.
** Numbres in brackets are frequency of recorded effect in relate to number of experiments.

d. *Cyclopium.* Slow infusion of cyclopium $10^{-6,3}$ M/ml/min. induced gradual increase of the effect of stimulation. The maximal effect was recorded in concentration $10^{-5,5}$ M. The increased effect was seen in all six preparations. (Fig. 3).

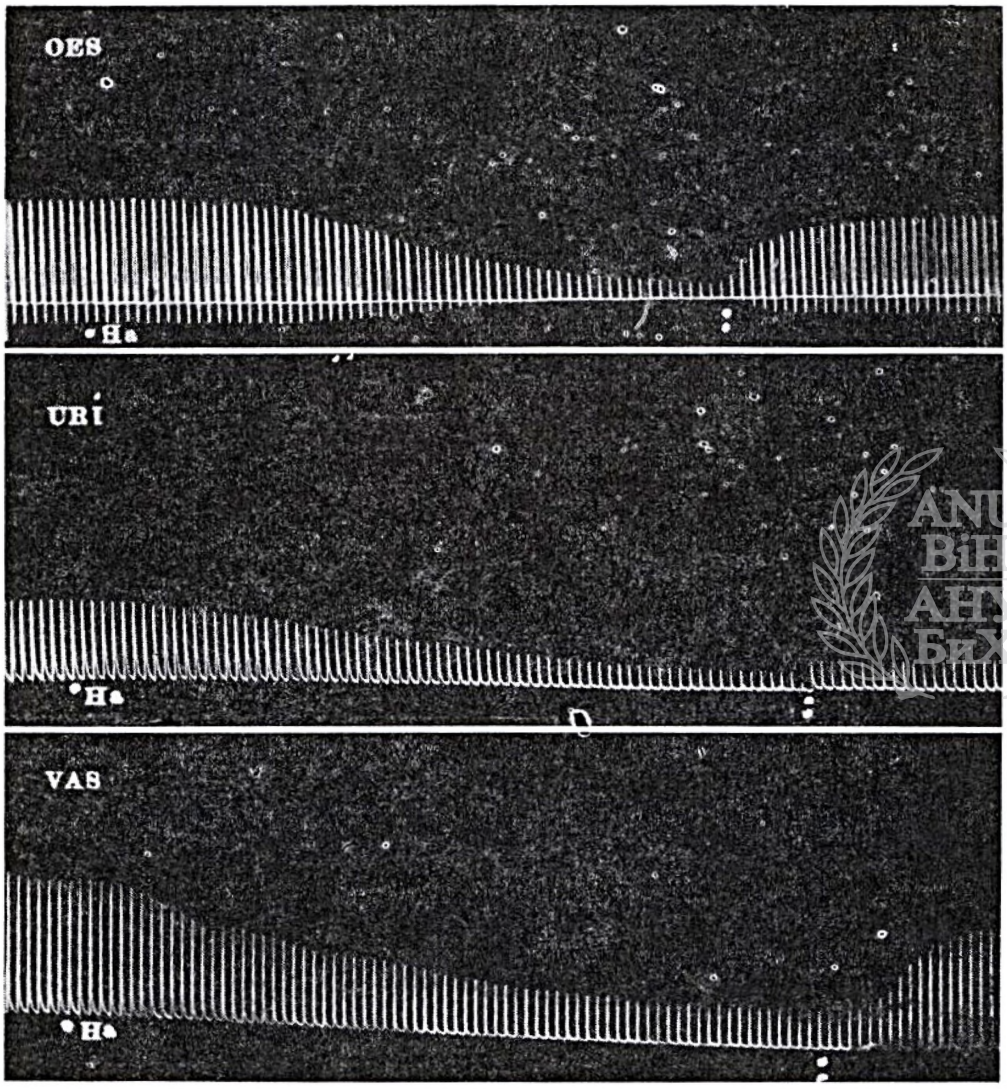

Fig. 2

The contractions of mouse isolated organs induced by the electrical stimulation (5 V, 1 mSec., 40 Hz, every 30 Sec for 2 Sec). At the point Ha infusion of harmine 5×10^{-7} M/ml/min. Oes is oesophagus, Uri is urinary bladder, Vas is ductus deferens.

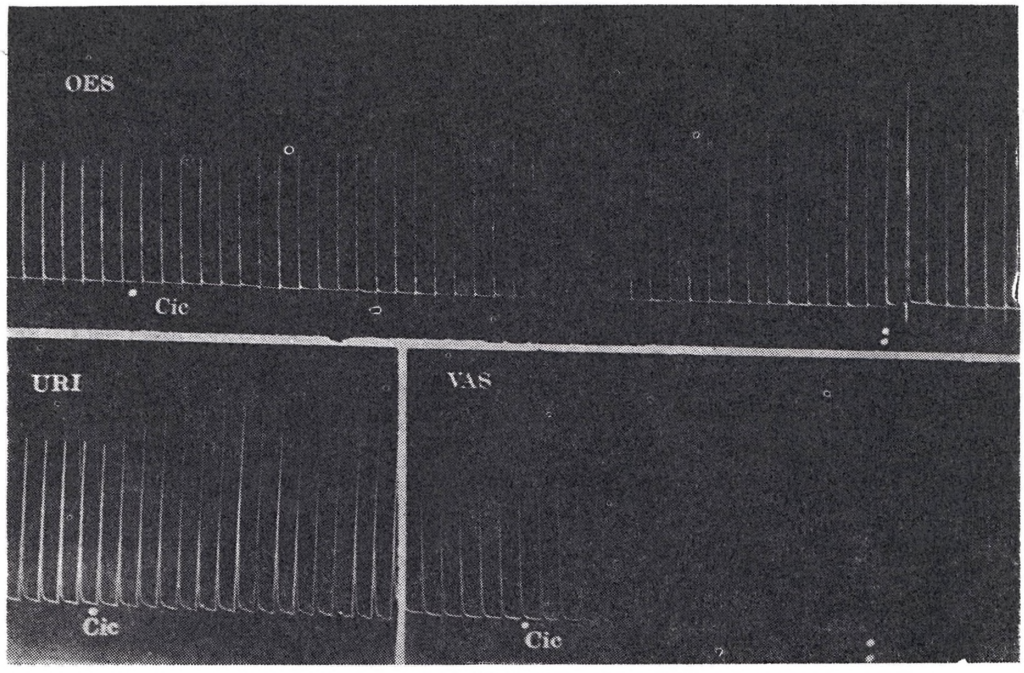

Fig. 3

The contractions of rat's isolated organs induced by the electrical nerve stimulation (5 V, 1 mSec, 40 Hz, every min. for 2 Sec). At the point CIC infusion of cyclopium $10^{-6,3}$M/ml/min. Oes is oesophagus, Uri is urinary bladder, Vas is ductus deferens.

DISCUSSION

Oxotremorine, harmine and cyclopium increase the effect of nerve stimulation of great part of preparations used in this work. The excitatory effect of oxotremorine was followed by decrease of the height of contractions. The difference $pB_{75} - pS_m$ was of low range of value 0,5 approximately. Harmine induced biphasic reactions, exceptions are rat's oesophagus and mouse's urinary bladder. Nicotine was not effective on rat's oesophagus. It stimulating effect is not frequent except on ductus deference.

The effect of oxotremorine at the indirectly stimulated junction was reported by Ganguly and Chaudhury (8). Theire resultes may explain oxotremorine induced tremor and rigidity involving its effect on periphery. Oxotremorine induced excitatory effect by releasing of acetylcholine (13), or by depolarisation at the muscle endplate (7). It is suggested that oxotremorine does not act p e r s e but by mobilization of acetylcholine (10). Oxotremorine is substance with peripheral muscarinic properties which penetrate into the central nervous system and produces tremor in mice and rats (9). It has no nicotinic action but might be producing its effects (18) by increasing activity in the efferent sympathetic

nervous system. The peripheral effect of oxotremorine seen in this work could be explained by mobilizing effect on acetylcholine in the cholinergic and adrenergic nerves. Cyclopium induced smooth muscle contraction (19).

The concentration of oxotremorine used in vitro and expressed as negative logarithm are of the 4 range of value and that used in vivo for tremor are 5 and more. The difference is 1 to 2 range of value. It means that the central site of action is predominant and if periphery was involved the excitatory effect would be the most important. The same could be said for cyclopium, in vivo produce the effect in the range of value 6 or more. In vitro only at 5. Harmine and nicotine induced tremor at about 4,5 or 5,0 and was effective in vitro at 5,5 or 6,0 range of value. The difference was more than one range of value on account periphery that might suggest prevalent involvment of peripheral nervous system and muscle in tremorgenezis. The first significant effect of nicotine and harmine was depression of the nerve stimulation on striated muscle and theire tremorgenic effect might be connected with depression and not excitation.

SUMMARY

Oxotremorine, harmine, nicotine and cyclopium exerts an action on the contraction induced in vitro by nerve stimulation of oesophagus, urinary bladder and ductus deferens of rats and mice. For different extent of the effect of drugs the dimensions were expressed as negative logarithm of molar concentration pS_m, pB_{75} and pB_{50}.

Oxotremorine and harmine induced biphasic reaction. Increase of contraction of muscle followed by decrease of the effect of nerve stimulation. The nerves were cholinergic and adrenergic and muscle were striated and smooth ones. Nicotine decreased the effect of cholinergic nerve stimulation on both kind of muscle but increased the effect of adrenergic nerv stimulation.

Comparing the concentration of oxotremorine for inducing the effect in vitro with the same in vivo it could be seen that the difference is one range of value in favour of in vivo effect. It is opposit for harmine and nicotine. The conclusion was drown that the site of tremogenic effect of oxotremorine is in central nervous system but the site of action of harmine and nicotine should be in periphery. If the peripheral effect of oxotremorine and cyclopium contributed to the tremogenezis it contributed by excitatory effect, vice versa, if harmine and nicotine contributed to tremogenezis they contribute with theire depressive effect.

ELVEDINA ZVIZDIĆ I SEID HUKOVIĆ

UTICAJ TREMOGENIH SUPSTANCI NA NEUROMIŠIĆNU TRANSMISIJU IN VITRO

KRATAK SADRŽAJ

Oksotremorin i druge tremogene supstance imaju mjesto djelovanja u sinapsama centralnog nervnog sistema, a posebno u mezencefaličkoj retikularnoj formaciji. Osim ovog mjesta djelovanja, poznato je da tremogene supstance mogu djelovati na periferiji, pa je cilj ovog rada da se dobiju kvantitativni podaci o djelovanju na neuromišićnoj transmisiji. Upotrijebljena su tri tipa nerava i dva tipa muskulature. Uzeti su autonomni adrenergični i holinergični nervi i somatski holinergični nerv, te glatka i poprečnoprugasta muskulatura. Izazvane su kontrakcije mišićnih organa in vitro submaksimalnom električnom stimulacijom nerava i aplicirane tremogene supstance.

Izolirani su ezofagus, mokraćni mjehur i duktus deferens štakora i miša sa pripadajućim nervima. Poslije adaptacije i kontrolnih kontrakcija infundirani su oksotremorin, harmin, nikotin i ciklopium tačno određene koncentracije i brzine. Efekt pomenutih supstanci je bio izražen farmakološkim konstantama pS_m, pB_{75} i pB_{50}. Oksotremorin, harmin i ciklopium izazivaju bifazičku reakciju, tj. prvo povećanje, iza čega uslijedi smanjenje efekta stimulacije nerava. Razlika između pB75 do pS_m je malog reda veličina oko 0,5. Nikotin je bez efekta na ezofagusu štakora, a stimulira samo efekt adrenergične nervne stimulacije na duktus deferensu. Koncentracija oksotremorina upotrijebljena u ovom radu in vitro u uporedbi sa efektivnom dozom in vivo veća je za jedan i više redova veličina, što znači da je centralno mjesto djelovanja u CNS-u predominantno. Obratno, manje je supstance potrebno in vitro za vidljiv efekt harmina i nikotina, što ukazuje da oni djeluju na periferiji. Ako oksotremorin i ciklopium doprinose tremogenezi, onda djeluju ekscitirajući, vice versa, ako harmin i nikotin doprinose tremogenezi, doprinose depresivnim efektom.

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DISCUSSION

Bošković: 1) How one can explain the inhibitory effect of oxotremorine on the striated muscle? 2) Does Harmine, under certain experimental conditions has an atropine-like action.

Huković: 2) H depends on experimental conditions. The explantation of the effect of Harmine, which induced decreased sensitivity toward transmittors, is not contradictory wtih explantation of the efect of atropine. Harmine and atropine are unexpected kinds of antagonist sometmies, because Hamine decreased and Atopine mercased the effect of oxotremorine could be of three kinds dependent on species and isolated organs. I think that the effect could be direct on recoptor, indirect throgh the nerve and inhibition of cholinesterase, the resultant of these mechanism induced reaction.