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

Stern, Pavao

1972

Akademija nauka i umjetnosti Bosne i Hercegovine

https://bastina.anubih.ba/items/05bee08e-28ec-4b12-b10e-5c9e89b50461

AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE

POSEBNA IZDANJA
KNJIGA XVII

ODJELJENJE MEDICINSKIH NAUKA
KNJIGA 4.

MEĐUNARODNI SIMPOZIJUM

O EKSPERIMENTALNOM TREMORU

1 — 3. APRIL 1971. GODINE

Urednik
PAVEL ŠTERN,
redovni član Akademije nauka i umjetnosti
Bosne i Hercegovine

SARAJEVO
1972

W. OELSZNER, H.-D. FISCHER,
K. H. WESTERMANN AND A. SCHERBER

CHOLINERGIC INDUCED TREMOR AND BRAIN ACETYLCHOLINE LEVELS

Tremor inducing doses of muscarine-like central cholinomimetics give rise to an increased brain acetylcholine content (13, 14, 21). On the other hand tremor antagonizing doses of atropinic agents prevent this increase and given alone decrease brain acetylcholine levels (10, 13, 14). From this finding the question raises, wether these cholinomimetics act by the increased amount of acetylcholine or by direct actions on muscarinic receptors (5, 6, 13).

Initially, we have investigated the correlation between arecoline induced tremor and brain acetylcholine levels in young chicks and rats (25, 26).

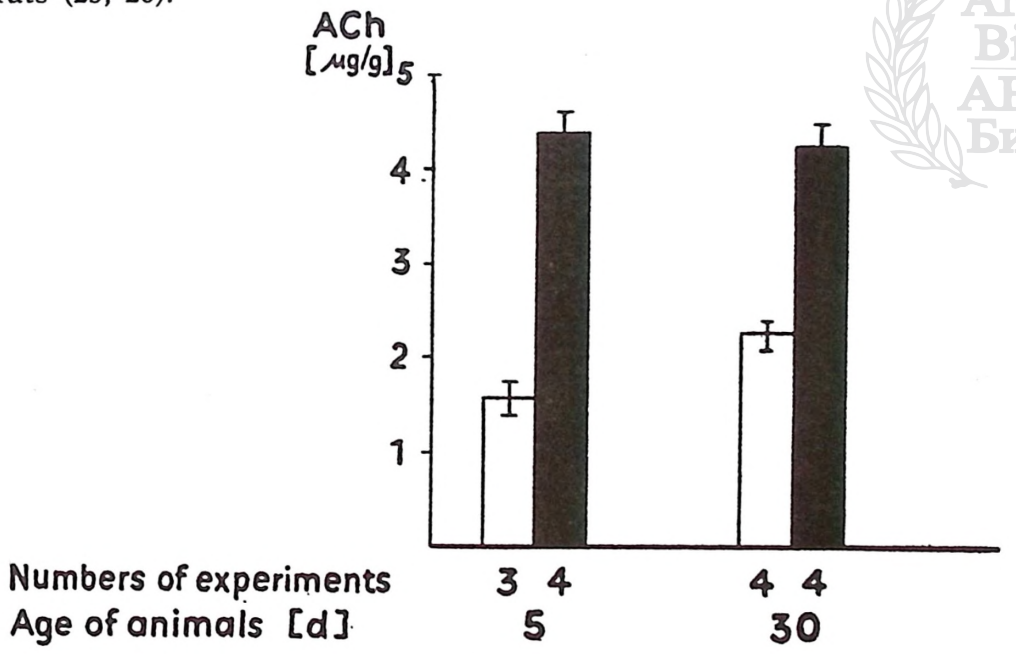

Fig. 1
Effect of arecoline on total brain acetylcholine (without cerebellum) of chicks aged 5 and 30 days.
Open columns: control animals
Dark columns: 5 min after 6 mg/kg arecoline i. p.

In chicks (Leghorn) aged 5 and 30 days arecoline in doses of 3 and 6 mg/kg i. p. produced a marked but short-lasting tremor for about 10 minutes. With oxotremorine in dosage levels up to 1 mg/kg the same symptoms only longer in duration could be obtaines. In all experiments the intensity of tremor was related to dosage but differences due to age never were found. The effect of arecoline on total brain acetylcholine is demonstrated in Fig. 1. The low values of acetylcholine in chicks aged 5 days reached the same elevated amounts as in 30-days old animals. An effect of this magnitude, i. e. an increase by about 180%, has not been described in adult animals.

In rats (Wistar) aged 10 — 300 days 10 mg/kg arecoline i. p. induced a pronounced short lasting tremor without differences in intensity or duration due to age. When the same dose of arecoline was injected into 5-days old animals no tremor response could be obtained. The brain acetylcholine levels in control rats of different age and in rats pre-treated

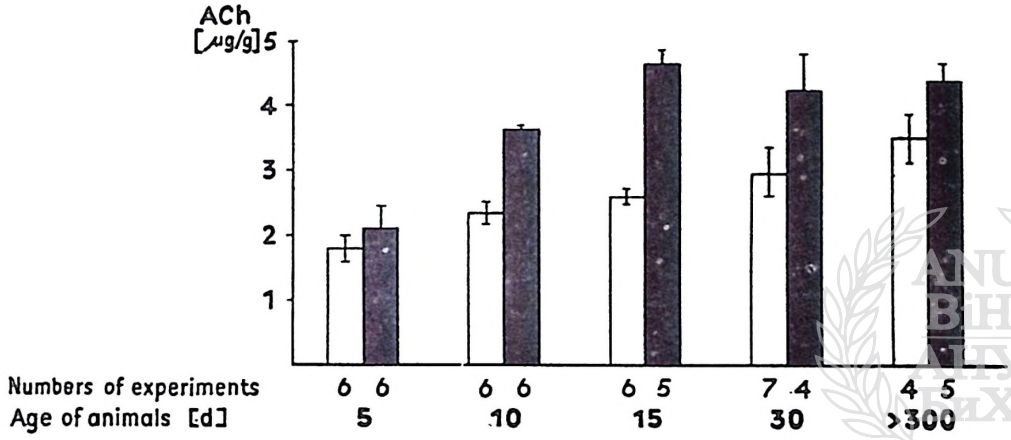

Fig. 2

Effect of arecoline on total brain acetylcholine (without cerebellum) of rats aged 5 — 300 days.
Open columns: control animals
Dark columns: 5 min after 10 mg/kg arecoline i. p.

with arecoline are presented in Fig. 2. On a wet weight basis there was a marked augmentation in the normal acetylcholine levels with increasing age. Arecoline produced a significant increase in the brain level of acetylcholine only in rats aged 10 days or more. In 5-days old animals there was a small increase of about 15%, but this effect was not significant.

Till now only few papers dealing with the different influences of cholinomimetics and cholinolytics on the acetylcholine content of various regions of rat brain are available (11, 18). For this study we used a dissection technique described by P o p o v et al. (24) and estimated the levels of total acetylcholine in eight areas of rat brain before and after injection of arecoline, RS-86, eserine and scopolamine (8). The normal amounts in certain regions varied considerably with lowest values in cortex and pons, higher levels in hippocampus, thalamus and hypotha-

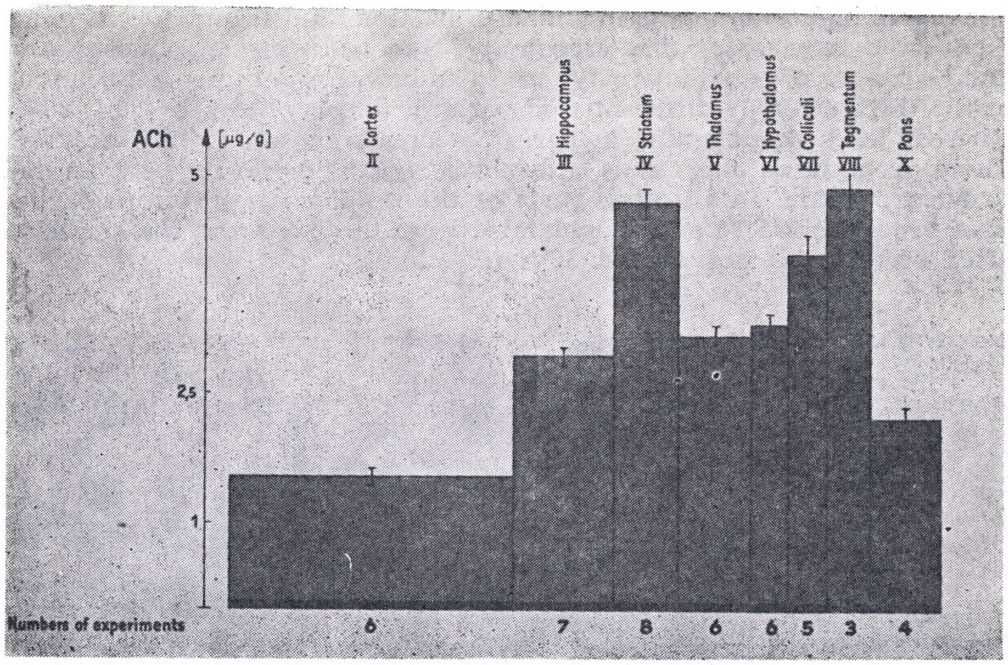

Fig. 3

Acetylcholine content of different regions of rat brain.

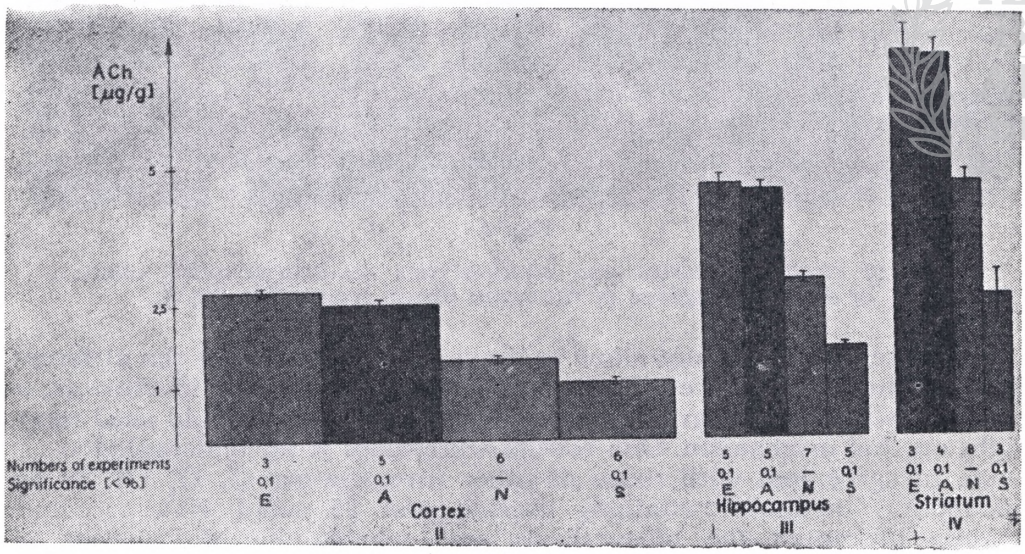

Fig. 4

Effect of cholinomimetics and cholinolytics on acetylcholine content of different regions of rat brain)telencephalon). Calculation of significance by Duncan's test.

N: control animals
E: 15 min after 0,5 mg/kg eserine i. p.
A: 9 min after 20 mg/kg arecoline i. p.
S: 30 min after 0,5 mg/kg scopolamine i. p.

lamus and highest amounts in colliculi, striatum and tegmentum (Fig. 3). Figure 4 demonstrates that arecoline, RS-86 and eserine in doses producing tremor of equal intensity caused an increase in brain acetylcholine in the three strongly differentiated parts of telencephalon by 50 — 80%, whereas after scopolamine a marked decrease by about 45% occured. These pronounced alterations in brain acetylcholine levels were limited to telencephalon. In all other parts of the brain no significant changes after arecoline, RS-86 and scopolamine were observed and the elevation after eserine did not exceed 30% (Fig. 5).

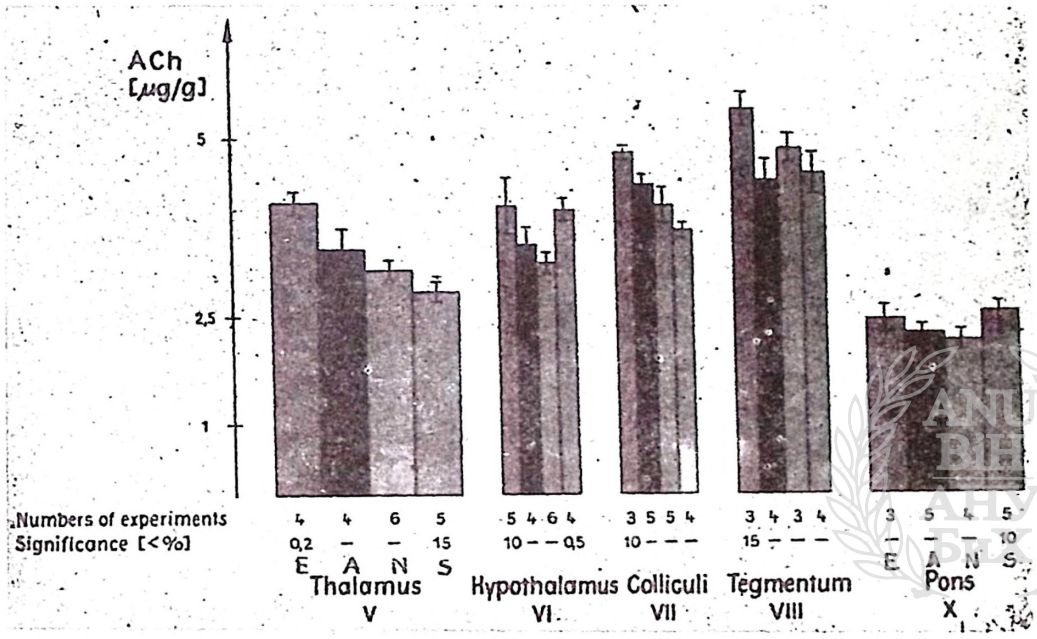

Fig. 5

Effect of cholinomimetics and cholinolytics on acetylcholine content of different regions of rat brain (brain stem). See Fig. 4.

These results did not allow to draw conclusions about the different steps of turnover of acetylcholine. It was suspected that in vitro experiments with slices from telencephalon and brain stem would shed more light on this mechanism. Our results with slices from telencephalon (7) were in agreement with those of other investigators (3, 13, 17). In an incubation medium containing eserine and 25 mmol KCl arecoline mainly promoted the biosynthesis and scopolamine the output of acetylcholine. In our experiments with slices from brain stem no significant changes could be obtained (Fig. 6).

In the presence of an anticholinesterase acetylcholine is released from the cerebral cortex and other parts of the brain (19, 22). Stimulation of afferent pathways or injections of atropinic agents increase the output. But till now only the acetylcholine release from higher parts of the brain, i. e. cerebral cortex, caudate nucleus and thalamus has

been investigated. We have repeated and confirmed these results mainly obtained with cats and rabbits using push pull technique in rats. The spontaneous release and also the evoked output after 1 mg/kg scopo-

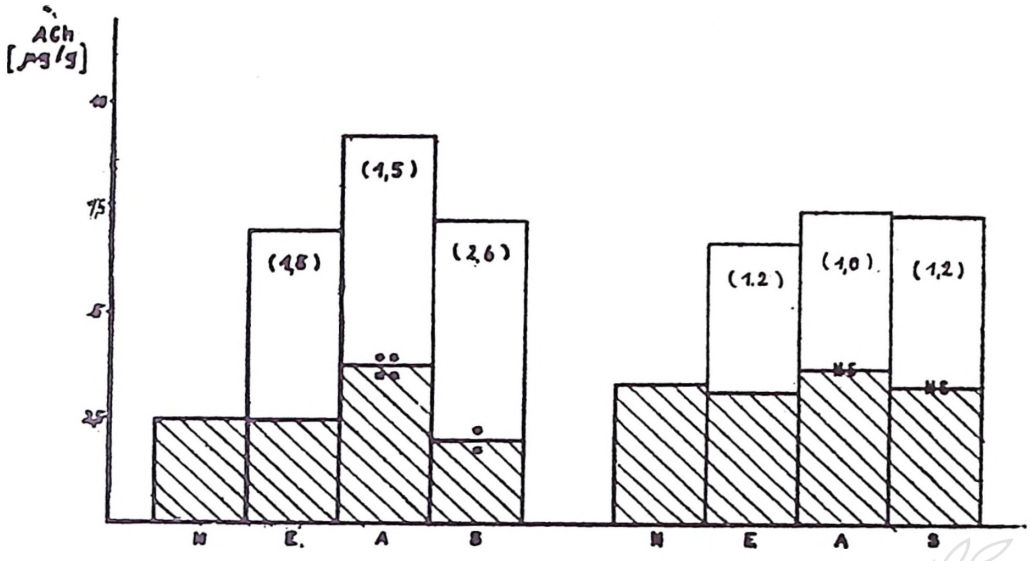

Fig. 6

Effecct of arecoline and scopolamine on the content (shaded) and output (whitte) of acetylcholine (ACh) from slices of telencephalon (left) and brain stem (right) of rats during incubation.

N: tissue ACh-content before incubation.

E: ACh-content and released ACh after 120 min (incubation medium modified from K a l a n t and G r o s e, 25 mM KCl, 10^{-1} mM eserine).

A: like E in presence of 6×10^{-3} mM arecoline.

S: like E in presence of 2×10^{-3} mM scopolamine.

Calculation of significance by S t u d e n t's t-test: $p < 0.01$, $p < 0.05$, NS = not significant. Ratio (medium-ACh: tissue-ACh) in parenthesis.

lamine from caudate nucleus and thalamus was very similar. The spontaneous release from substantia nigra however was much lower and the effect of scopolamine did not exceed 10% (Fig. 7). Oxotremorine also caused an increased release of acetylcholine from caudate nucleus, the output from substantia nigra was influenced only to a much lower degree.

From these findings and further results of other investigators it seemed possible to draw some conclusions about the open questions mentioned above. In our studies on chicks with arecoline and oxotremorine we could not confirm a fall-off of response with age described by B o w m a n and O s u i d e (4) using tremorine. There is some evidence that the developing resistance to tremorine in chicks but not in mammals depends on the existence of two different liver microsomal enzymes metabolizing tremorine to oxotremorine and oxotremorine to inactive metabolites respectively (16, 27). More important is our observation that in chicks and expecially in rats there was a close correlation between

elevation of total brain acetylcholine and production of tremor. These data suggest that the cholinergic induced tremor may be related to the increased amounts and — according to our push pull experiments — also to an increased output of acetylcholine. But our further results do not support this assumption. In accordance with Cox and Potkonjak (5, 6) we believe that there is no causal relationship between these two effects and the inability of arecoline in increasing brain acetylcholine and in producing tremor in 5-days old rats seems to be related do the low stage of brain development.

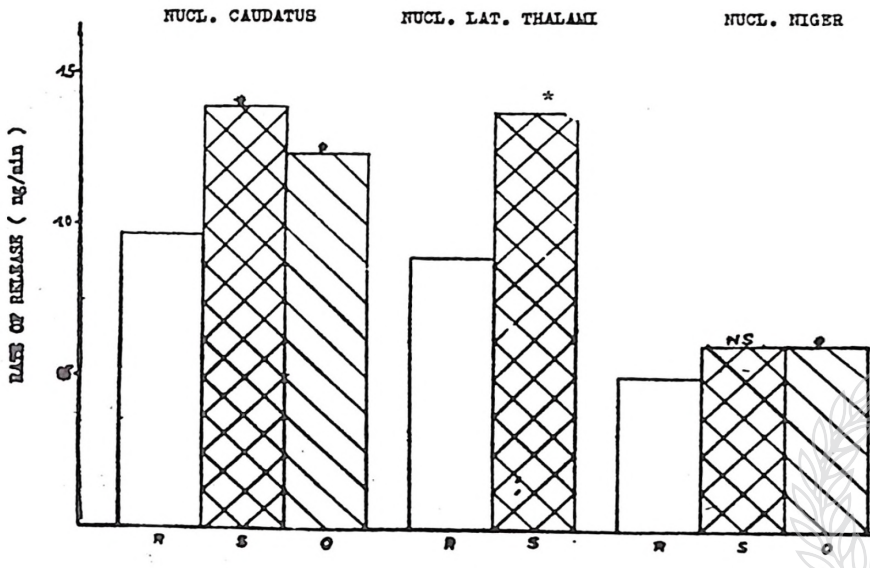

Fig. 7

Acetylcholine release from different regions of rat brain (1,25 g/kg urethane i.p.).
R = spontaneous release
S = scopolamine 1,0 mg/kg i. p.
O = oxotremorine 0,25 mg/kg i. p.
NS = not significant
= $p < 0,01$

In our experiments with intact animals pronounced differences in the reactions of various regions of the brain could be demonstrated. A significant rise or decrease in brain acetylcholine levels was limited to cortex, hippocampus and striatum, i. e. parts of telencephalon. These alterations in acetylcholine content induced by central cholinomimetics and cholinolytics were related to distinct changes in biosynthesis and depletion of acetylcholine as has been shown in our in vitro experiments confirming the results of other investigators. But in former studies only slices from cortex or homogenates of whole brain have been used and informations about the reactions of lower parts of the brain are not available (3, 23). In our present studies in slices from brain stem no significant alteration in turnover or content of acetylcholine could be detected and these results are in agreement with our findings in intact

animals. It is also possible to explain the obtained differences in the spontaneous and evoked release of acetylcholine from caudate nucleus and substantia nigra with these results in vivo and in vitro.

At present the mechanism by which cholinomimetics and cholinolytics influence the turnover and amount of brain acetylcholine is uncertain. The results of our in vitro studies provide good evidence of a similar mode of action of arecoline and high concentrations of potassium (7). In both cases the effects are restricted to telencephalon and closely connected with a generally increased brain metabolism. This rise in respiration and glycolysis shows marked differences in the different brain areas and is especially pronounced in telencephalon (12). These data suggest that cholinergic induced changes in brain acetylcholine levels and brain metabolism are causally related and differences in various brain areas can be explained on this basis.

As Cox and Potkonjak (5, 6) have already pointed out it does not seem likely that the tremorgenic actions of muscarine-like central cholinomimetics are related to the increase in brain acetylcholine levels. The finding in the present work confirms these conclusions especially taking into consideration some observations published by George, Haslett and Jenden (9). These authors have reported blocking of oxotremorine induced tremor in rats only with transections passing through the caudal midbrain or anterior pons. After transsections in a higher level rostral to the red nucleus tremor remained unchanged. These data provide good evidence that the tremor originates in parts of the brain, in which we did not find significant changes in vivo and in vitro. The effects of cholinomimetics and cholinolytics are limited to telencephalon and from these results one may conclude that this area is not necessary for production of cholinergic induced tremor. These and further observations especially the corresponding peripheral and central muscarinic activity of tertiary cholinomimetics and cholinolytics (1, 2, 15, 20) provide good evidence that the tremorgenic actions of cholinomimetics and the tremor antagonizing actions of cholinolytics are related to a direct action on postsynaptic muscarinic receptors.

These investigations were supported by grant of the Ministry of Science and Technic of German Democratic Republic.

W. OELSZNER, H. D. FISCHER, K. H. WESTERMAN I A. SCHERBER

TREMOR IZAZVAN HOLINERGICIMA I MOŽDANI ACETILHOLINSKI NIVOI

KRATAK SADRŽAJ

Poznato je da je tremor izazvan centralnim holinomimeticima praćen povišenjem totalnog acetilholina mozga sa istim vremenskim tokom. Mi smo istraživali vezu između ta dva efekta.

Kod pilića (Leghorn) starih 5 do 30 dana nismo mogli naći razlike u intenzitetu arekolinom izazvanog tremora koji ovisi o starosti. Kod mladih pilića niske vrijednosti moždanog acetilholina dostigle su iste uvećane količine kao i kod životinja starih 30 dana. Kod štakora starih

5 dana arekolin ne izaziva tremor, a primijećeno je samo malo i statički neznačajno povećanje u nivou acetilholina. Kod životinja starih 10 i više dana tremor i znatno povećanje u acetilholinskom sadržaju bili su izazvani slučajno. Kod odraslih štakora povećanje u moždanom acetilholinu prouzrokovano arekolinom i RS-86 i povećanje nakon skopolamina bilo je ograničeno na telencefalon (cortex, hipocampus, striatum). U svim drugim moždanim zonama nisu primijećene uočljivije promjene. Iste razlike mogu se demonstrirati i u in vitro eksperimentima sa režnjevima iz telencefalona i moždanog stabla. Alternacija u biosintezi i deplecija acetilholina pomoću arekolina, skopolamina i visoke koncentracije kalijuma našle su se samo u režnjevima iz telencefalona. Količine acetilholina iz moždanog stabla ostale su nepromijenjene. Ovi rezultati se slažu sa eksperimentalnim proučavanjem spontanog i skopolaminom izazvanog oslobađanja acetilholina iz raznih dijelova mozga štakora, upotrijebivši puskpull-tehniku. Spontano oslobađanje je bilo mnogo manje iz zone substantia nigra i zone retikulata nego iz nucleus caudatus putamena, nucleus lateralis thalami ili cerbralnog cortexa. Skopolamin je izazvao pojačano oslobađanje acetilcholina samo u višim dijelovima mozga. Efekt u supstanciji nigri nije prelazio 10%.

Ovi su rezultati razmotreni u odnosu na mogući način djelovanja tremorgeničkih holinomimetika.

REFERENCES

1. Ahmed, A., Marshall, P. B.: Brit. J. Pharmacol. **18**: 247, 1962.
2. Bebbington, A., Brimblecombe, R. W., Shakeshaft, D.: Brit. J. Pharmacol. **26**: 56, 1966.
3. Bertels-Meeuws, M. M., Polak, R. L.: Brit. J. Pharmacol. **33**: 368, 1968.
4. Bowman, W. C., Osuide, G.: Europ. J. Pharmacol. **1**: 71, 1967.
5. Cox, B., Potkonjak, D.: Brit. J. Pharmacol. **35**: 295, 1969.
6. Cox, B., Potkonjak, D.: Brit. J. Pharmacol. **35**: 521, 1969.
7. Fischer, H.-D., Oelszner, W., unter zeitweiliger Mitarbeit von Westermann, K., v. Littrow, C., v. Schwarzenfeld, I.: in preparation.
8. Fischer, H. -D.., Westermann, K., Oelszner, W.: Acta biol. med. germ. **23**: 181, 1969.
9. George, R., Haslett, W. L., Jenden, D. J.: Int. J. Neuropharmacol. **5**: 27, 1966.
10. Giarman, N. J., Pepeu, G.: Brit. J. Pharmacol. **19**: 226, 1962.
11. Giarman, N. J., Pepeu, G.: Brit. J. Pharmacol. **23**: 123, 1964.
12. Hertz, L., Clausen, T.: Biochem. J. **89**: 526, 1963.
13. Holmstedt, B., Lundgren, G.: Ann. N. Y. Acad. Sci. **142**: 126, 1967.
14. Holmstedt, B., Lundgren, G., Sundwall, A.: Life Sci. 2: 731, 1963.
15. Jenden, D.: Biochemistry and Pharmacology of the Basal Ganglia. p. 159. Raven Press, Hewlett, New York, 1966.

16. Klinger, J., Zimmerman, B., Oelszner, W., Fischer, H.-D., Westermann, K.: Acta biol. med. germ. **26:** in press.
17. Lundgren, G., Malberg, M.: Biochem. Pharmacol. **17:** 2051, 1968.
18. Megazzini, P., Bernardi, G., Ballotti, P. L.: Experientia (Basel) **21:** 406, 1965.
19. Mitchell, J. F.: Mechanism of Release of Biogenic Amines. Ed. by U. S. v. EULER, S. ROSELL. p. 425. Pergamon Press, Oxford, 1966.
20. Oelszner, W., Walther, H., Wagner, U.: Acta biol. med. germ. in press.
21. Pepeu, G.: Nature (Lond.) **200:** 895, 1963.
22. Phillis, J. W., Tebecis, A. K., York, D. H.: J. Pharm. Pharmac. **20:** 476, 1968.
23. Polak, R. L.: J. Physiol. **191:** 34 P, 1967.
24. Popov, N., Pohle, W., Rösler, V., Mathies, H.: Acta biol. med. germ. **18:** 695, 1967.
25. Westerman, K., Fischer, H.-D., Oelszner, W.: Acta biol. med. **22:** 677, 1969.
26. Westerman, K., Fischer, H.-D., Oelszner, W.: Acta biol. med. germ. **25:** 855, 1970.
27. Zimmermann, B., Klinger, J., Oelszner, W., Fischer, H.-D., Westermann, K., Meyer, F. P.: Acta biol. med. germ. **25:** 161, 1970.

DISCUSSION

Schnieden: Could you give me some information about the drug RS 86.

Oelszner: RS-86 is a central cholinomimetic like arecoline and oxotremorine and possesses similar effects (increase of acetylcholine in brain tremor, inhibition of nocizeptive reactions). It is produced by SANDOZ — Basel.

György: 1) How long lasts the effects of arecoline on the brain ACh-level? 2) Have you examined also the activity of OT on the amine level of the different parts of the brain?

Oelszner: Total brain acetylcholine in rats increased after ———— 25 mg/kg i. p. with maximum effect between 5 and 10 min after injection and then decreased (H. — D. Fischer and W. Oelszner, Acta biol. med. germ. 18, 443, 1967).

We have not used oxotremorine, but we believe this drug may act in a similar way.

Cox: Could you, please, tell me about the location of the increase of acetylcholine in brain slices by oxotremorine? Do you think that this acetylcholine is predominantly intracellualar and not released into the eätracellualar fluid? In the acetylcholine coming out of damaged tissue in slices. It is interesting that the highest output of acetylcholine into the incubating medium comes in the scopolamine experiments this drug brain an anti-tremor agent.

Oelszner: We have investigated acetylcholine content in whole brain slices and with this method no conclusions about the location of the increase of acetylcholine are possible. Arecoline predominantly increases biosynthesis and the increased output into the medium may be an overflow, the increased output in vitro and in vivo has already been described by other authors.

Vizi: May I have your opinion on the role of actual content of ACh in producing tremor by oxotremorine or by arecoline. Tremorgenic agents with muscarinic activity increase the ACh content of brain (Holmstedt and Lundgren, Ann. N. Y. Acad. Sciences 142, 126—142. 1967; Oelszner et al. Acta medica Germanica; Cox and Potkonjak, Brit. J. Pharmacol. 35, 295 — 303, 1967, ect) but did not increase the acetylcholin release from the nerve terminals of Auerbach plexus and the cortex of the rat (See: Vizi and Knoll, this Symposium). Dount you think that these fact favour the direct effect of oxotremorine resulting in tremor? Which is the crucial point in tremor: the release of ACh or the content? Oxotremorine mimicks the effect of ACh on muscarinic receptor and does not release it.

Oelszner: We believe that neither the increased acetylcholine content nor an increased output of acetylcholine in the brain after central cholinomimetics is responsible for cholinergic induced tremor. These agents act directly on postsynaptic muscarinic receptors and on this basis induce tremor or other central effects.

Župančič: Doctor Oelszner, you found significant differences in acetylcholine level, e. g., in the corpus striatum after administration of arecoline. One can imagine that these differences might be even greater, if breacing down your area wich you determined acetylcholine, say in nucleus caudatus instaed of in the whole striatum.

Oelszner: It is nearly impossible to dissect the little rat brain to a further degree because this needs time in which acetylcholine is destroyed by acetylcholinesterase.

Štern: Is it possible to inhibit other kind of tremor with glycin?

Oelszner: We have some observations that it is also possible to inhibit. Arecoline or oxotremorine induced tremor in rats with high doses of glycine.