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E. S. VIZI AND J. KNOLL

THE MECHANISM OF ACETYLCHOLINE RELEASE BY NICOTINE

The distinction between »nicotinic« and »muscarinic« effects of acetylcholine, as proposed by Dale (16) more than fifty years ago, has proved its validity both for peripheral and central nervous system. Nicotine, like ACh, acts on postsynaptic cholinoceptive sites and produces excitatory postsynaptic potentials (EPSP). This action can be blocked by excess of nicotine and by antinicotinic agents, such as hexamethonium and tubocurarine. The nicotine block at ganglia is associated with an initial depolarization superseded by a phase without depolarization (36).

So far, it has been accepted that nicotine acts through a cholinergic mechanism. A crucial question emerging in this problem is whether nicotine acts by mimicking acetylcholine or by releasing it or by both.

Tremor is a typical action of nicotine in both man and different species of animals. The first time in 1885 van Praag observed, that nicotine possesses this action.

Nicotine gives rise to a very shortlasting tremor, and when a second dose is administered, no tremorgenic activity can be observed (25). Also a fast tachyphylaxis was observed to the effect of nicotine in intestine.

The finding that in the longitudinal muscle strip of guinea-pig ileum all the ACh is contained in the nerve network (37), provides an opportunity for studying the release of ACh of nervous origin. Since the Auerbach plexus of ileum also contains a huge number of ganglia we studied the effect of nicotine on ACh release. Subcortex-cortex and cortex slice preparations were also used to study the site of action of nicotine and that of oxotremorine.

Methods. Longitudinal muscle strip of guinea-pig ileum was prepared as it was described by Paton and Vizi (39). Field stimulation was used. The strips were incubated at 35°C in Krebs solution containing eserine sulphate in a concentration of 2×10^{-6} g/ml. 5 strips weighing each 50 — 60 mg were mounted together in an organ bath of 3,5 ml capacity.

Subcortex-cortex preparation. Rats weighing 150 — 200 g were used. The animal was stunned by a blow on the neck and immediately decapitated. The brain was removed as rapidly as possible (6 — 10 sec) and placed in a moist atmosphere. Wedge-shaped slice from the brain

were cut with a blade. The slices weighing 200 — 250 mg contained some part of the thalamus, reticular system and cortex (Fig. 1). Two slices were incubated at 35°C in Krebs solution of 2 ml gassed with a mixture of 95% O_2 and 5% CO_2. Eserine sulphate (2×10^{-6} g/ml) was present throughout the experiments. However, when the action of oxotremorine was studied the slices in eserinized (10^{-5} g/ml) Ringer-Locke solution were incubated and bubbled with oxygen.

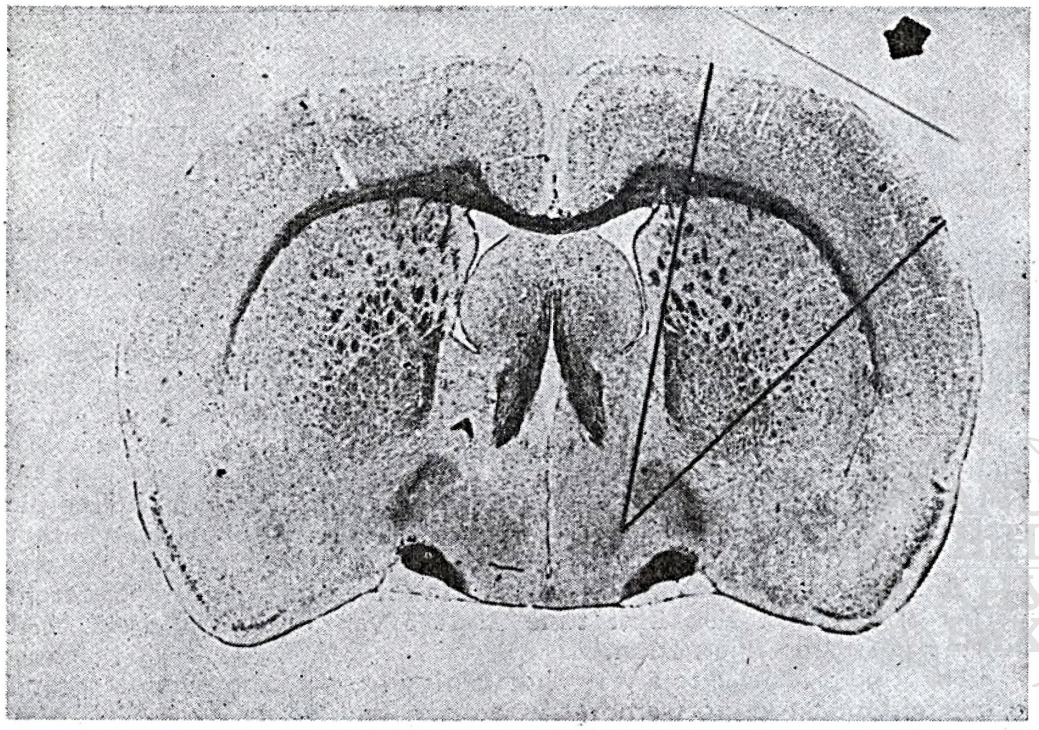


Fig. 1

Subcortex-cortex preparation. Scheme shows how the slices were prepared.

Cortical brain slice. Slices (1,5 — 2 mm thickness) were cut from the cortical surface of the rat brain.

Assay of acetylcholine. To assay ACh a length of ileum was removed from the guinea-pig. The whole ileum was used, a thread being tied at the oral end for attachment to the isometric transducer system via phosphor-bronze spring (see Fig. 2a) and another to the aboral end connecting to an open-ended polythene tube which projected through the bottom of the organ bath to allow the luminal contents to be excruded from the ileum without contaminating the bath. The contractions were recorded by means of an isometric recording system. A transistor differential amplifier was used to overcome the drift problem and to get a high gain for driving pen recorder (Servogor). The Fig. 2a shows the block scheme of the set up for recording contractions of the guinea-pig ileum. Since prolonged isometric contraction tended to reveal spon-

taneous movements of the ileum a soft spring was used eliminate isometrical condition. In this way the sensitivity of ileum is higher and the spontaneous movement is negligable.

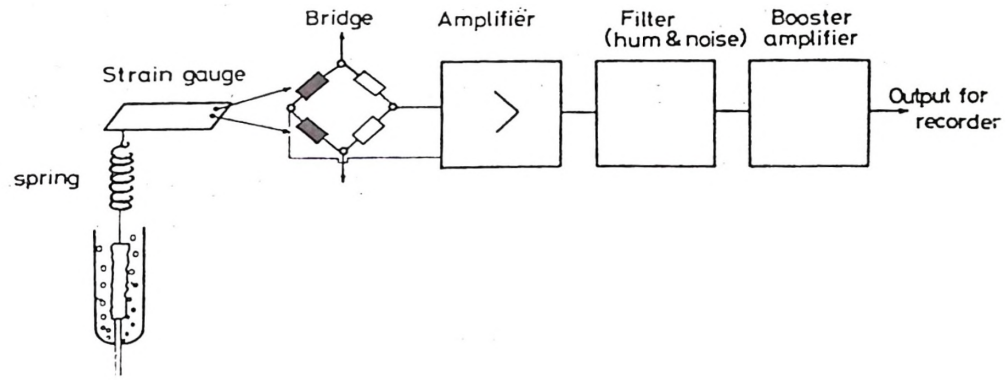
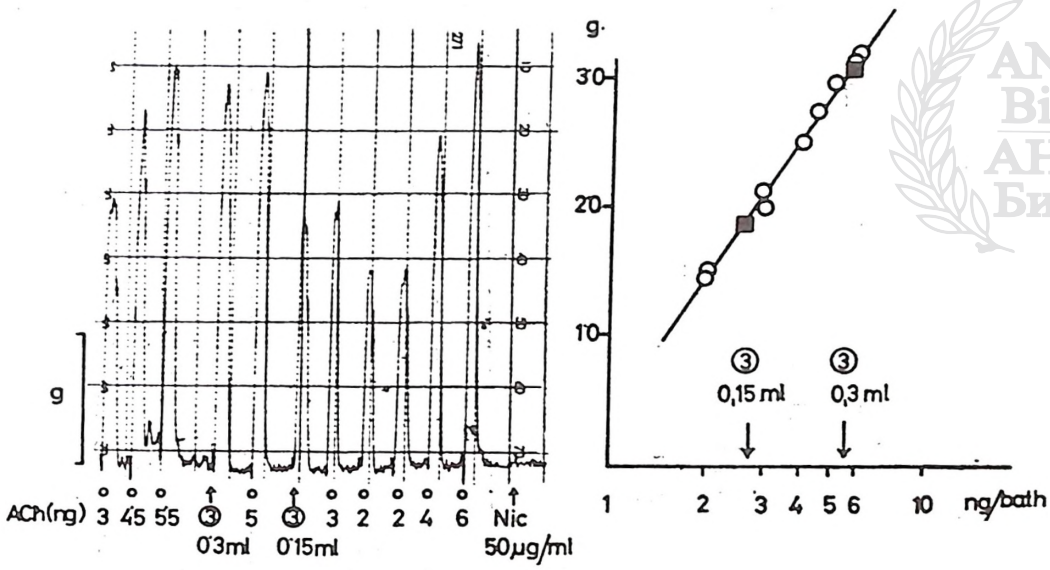


Fig. 2

a) Block-scheme of the set-up for recording the contractions of guinea-pig ileum.



b) Assay of ACh released from eserinized longitudinal a muscle strip of guinea-pig ileum. Donor bath volume 3.5 ml. In the assay bath (3.5 ml (C_6 was ased in a concentration of 3×10^{-4} g/ml.

In order to avoid difficulties in assaying samples contaminated by the drug used (nicotine) to release ACh, hexamethonium (C_6 3×10^{-4} g/ml) was added to the assay bath. In this way any interference from nicotine carried over into the test assays was countered, although, the sensitivity to ACh reduced to 1 — 10 ngAChI (3,5 ml bath) (see Fig. 2b.)

In the presence of oxotremorine the ACh release has been measured in leech muscle. The leech muscle was suspended in Ringer-locke solution diluted by destilled water in a ratio of 5:7. The bath contained 10^{-5} g/ml physostigmine sulphate and 10^{-5} g/ml Morphine HCl. The sensitivity of the preparation to ACh was 2 — 8 ng/ml. The contractions were recorded isometrically.

Drugs used were: Acetylcholine iodide (BDH), Nicotine hydrogen tartrate (BDH), Hexamethonium bromide, /—/-noradrenaline bitartrate (Fluka), Yohimbine HCl (Smith Kline and French), adrenaline HCl (Fluka), Morphinum sulphate (Alkaloida, Tiszavasvár), Tetrodotoxin (Sankyo), Mecamylamine (Erypharm Italiana), Oxotremorine esquifumarat (Loba-Chemie). The doses quoted being given in terms of their salts.

RESULTS

The effect of nicotine. It is generally accepted, that nicotine and nicotine-like drugs elicite contractions of the innervated guinea-pig ileum and of the intestine of other species by acting indirectly through the postganglionic parasympathetic nerve pathways (19, 37, 17, 1, 20). In addition, Paton and Zar (37) presented evidence for the indirect action

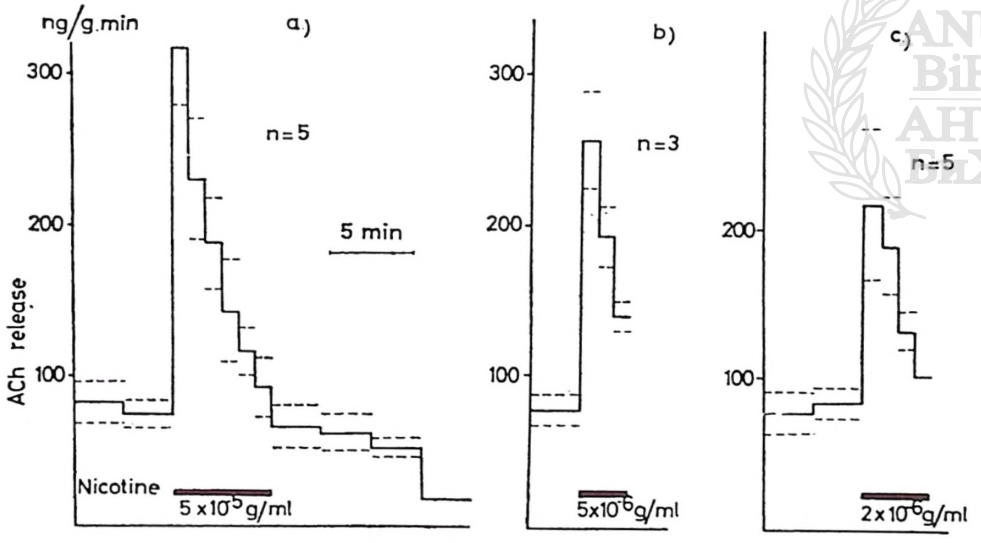


Fig. 3

ACh-releasing effect of nicotine. Long. muscle strip of guinea-pig ileum (3—5 strips, total weight 200—350 mg). The accumalated sample during the collection period (1 min when the strip was exposed to nicotine) was withdrawn entirely from the bath and the bath refilled immediately with fresh eserinized Krebs-solution. Total ACh release is expressed in ng/g. min, and the standard error of the mean is indicated by dotted lines. In the figures a, b and c n indicates the number of experiments.
Note the fast tachyphylaxis to nicotine AChI and stricotine hydrogen tartrate were used.

of nicotine, showing that nicotine and DMPP failed to produce contrations in denervated smooth muscle. Their actions are also inhibited by local anaesthetic in concentrations which selectively block axonal conduction, by tetrodotoxin (20) or by ganglion blocking agents.

Fig. 3. shows the effect of nicotine added to the organ bath on the release of ACh from the nerve elements of longitudinal muscle strip of guinea-pig ileum. A concentration of 5×10^{-5} g/ml increased the resting release of ACh from 74.2 ± 10.2 to 318.5 ± 38.8 ng/g. min ($n = 5$; $p < 0.01$). The increases by concentrations of 5×10^{-6} and 2×10^{-6} g/ml, respectively, were also significant ($p < 0.01$ in both cases). There was a very fast tachyphylaxis to the ACh-releasing action of nicotine (Fig. 3 and 4). The half time of decrease was 3,5 min in all of the three cases. If tachyphylaxis produced by nicotine represents the lack of ACh relea-

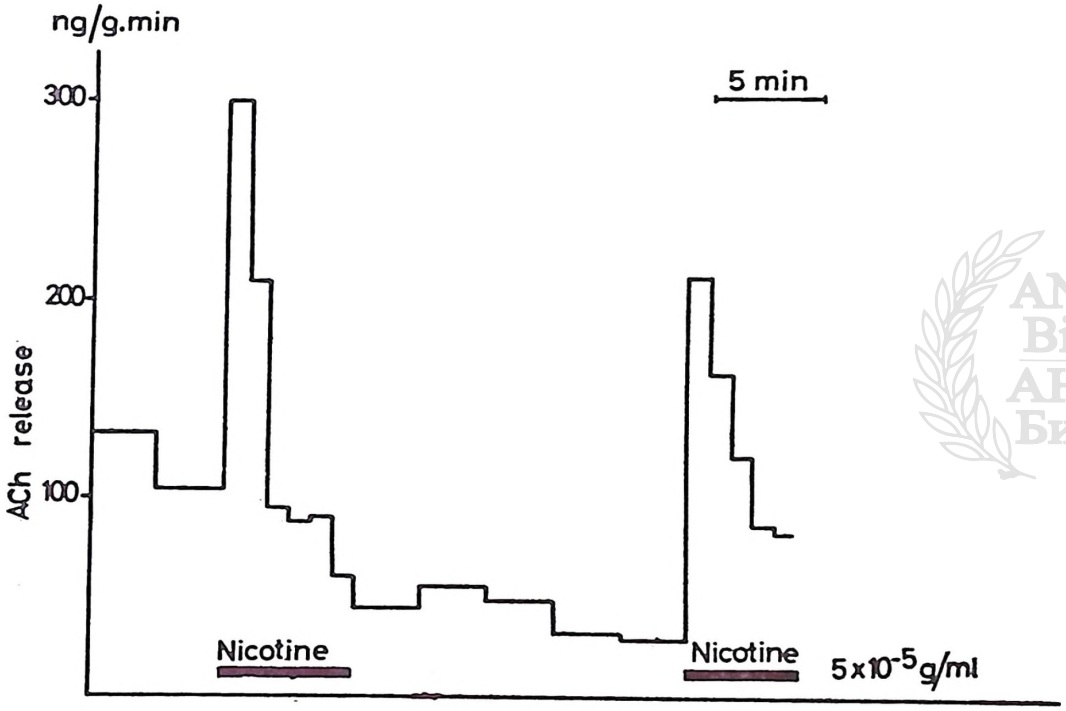


Fig. 4

ACh-releasing action of nicotine. Long. m. strip of guinea-pig ileum (5 strips in the donor bath). Average of two experiments with identical schedule. Otherwise see fig. 3.

sable from the nerve terminals, then our experiments do not exclude the possibility that nicotine releases ACh from the nerve terminals by a presynaptic action, as was suggested by Chiou et al. (7, 8). However, it seems more likely that this reduction in ACh release represents a ganglion blocking action of nicotine on ganglion cells, competitively inhibiting its own effect, since direct depolarization by high $(K^+)_o$ of nerve terminals still releases ACh (Fig. 5). When the effect of nicotine

was reduced i. e. still tachyphylaxis developed, postganglionic stimulation was effective (Fig. 6). These facts indicate, that during nicotine tachyphylaxis ACh from the nerve terminals is still releasable, and the stores are not exhausted. The tachyphylaxis already developed to nicotine disappeared within 5 — 10 min after washing out the tissue (Fig. 4). It is worth mentioning that the ACh output per minute de-

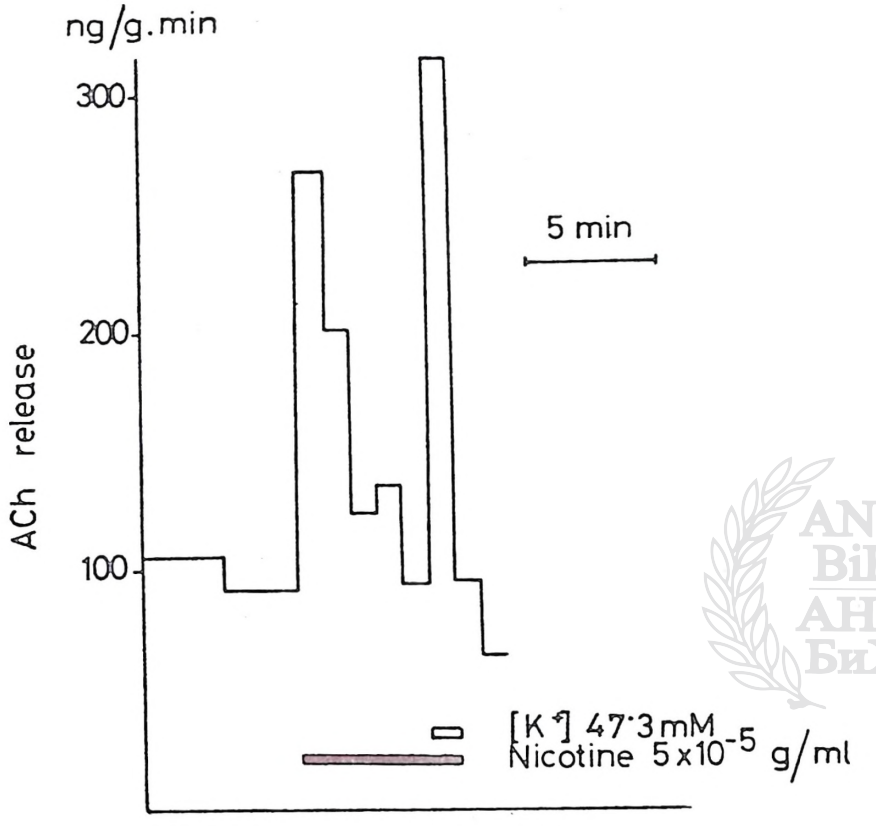


Fig. 5
The effectiveness of high $K^+/_o$ (47.3 mM) in releasing ACh during nicotine tachyphylaxis. Long. m. strip of guinea-pig ileum. When KCl was increased the NaCl was decreased to maintain isoosmolarity. Average of two experiments with identical schedule.

pends on the exposure time, since nicotine is able to build up tachyphylaxis. The ACh releases by longitudinal muscle strips of guinea-pigs were compared when 5 collection periode of 1 min and when 1 collection period of 5 min were used (Fig. 3 and 7a). The average of ACh output per minute did not differ; they were 198,9 (Fig. 3a) and 204,5 ng/g min (Fig. 7a) respectively.

Tetrodotoxin (5×10^{-7} g/ml), adrenaline (5×10^{-7} g/ml), noradrenaline (10^{-6} g/ml) and hexamethonium (3×10^{-4} g/ml) completely prevented the action of nicotine administered in a concentration of 5×10^{-5} g/ml (Fig. 7. 8). Morphine 10^{-6} g/ml) also reduced the effect of nicotine on ACh release, from 318,8 to 146,2 ng/g min (Fig. 8).

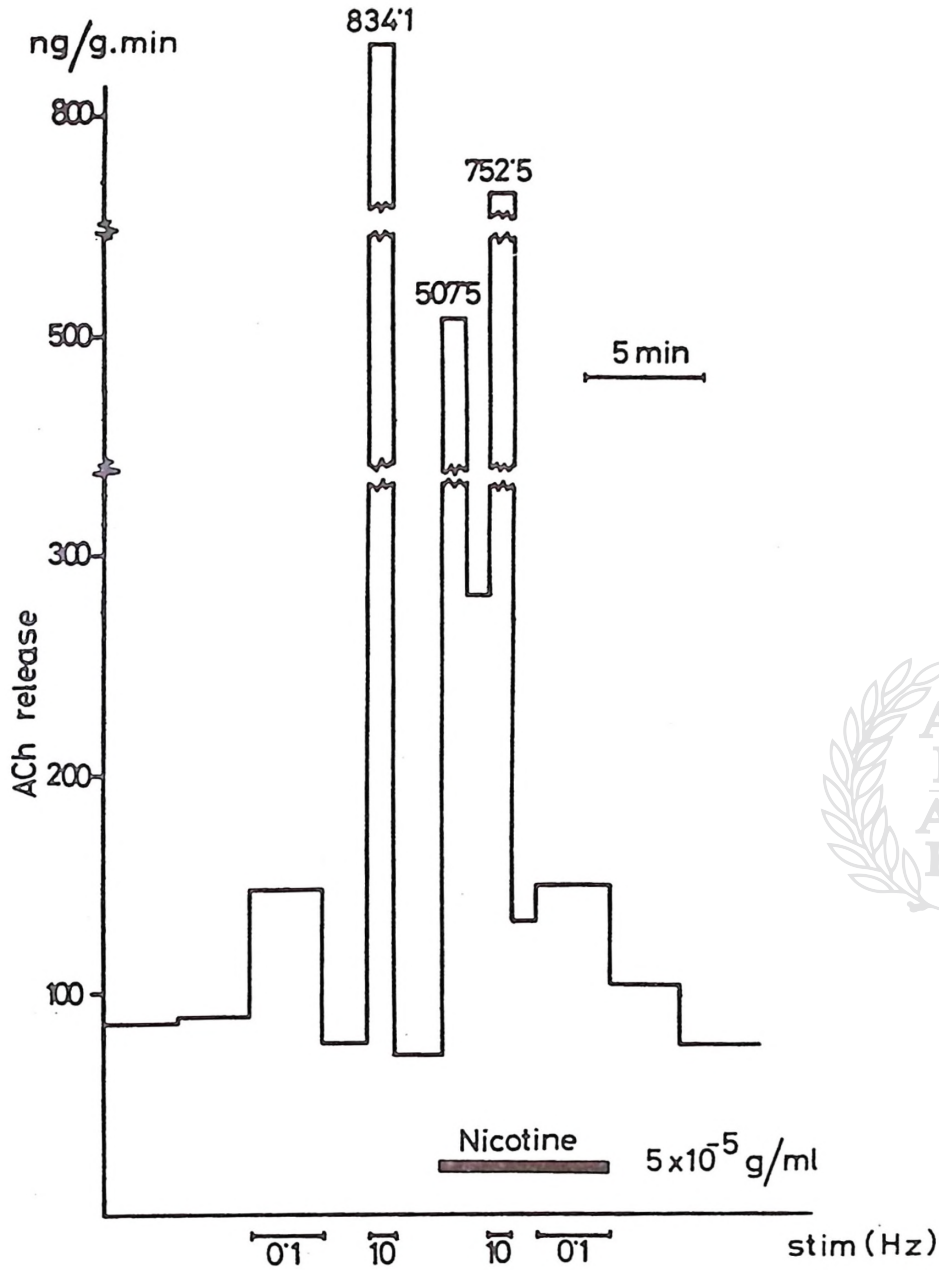


Fig. 6

ACh release by nicotine and electrical stimulation from the Auerbach plexus of long. m. strip of guinea-pig ileum. Supramaximal field stimulation was delivered using DISA Biostim at frequencies of 0.1 and 10 Hz. The length of train is indicated. Impulse duration is 1 msec.

It is interesting to note, that C_6 which reduces the ACh resting release from longitudinal muscle strip preparation during long applica-

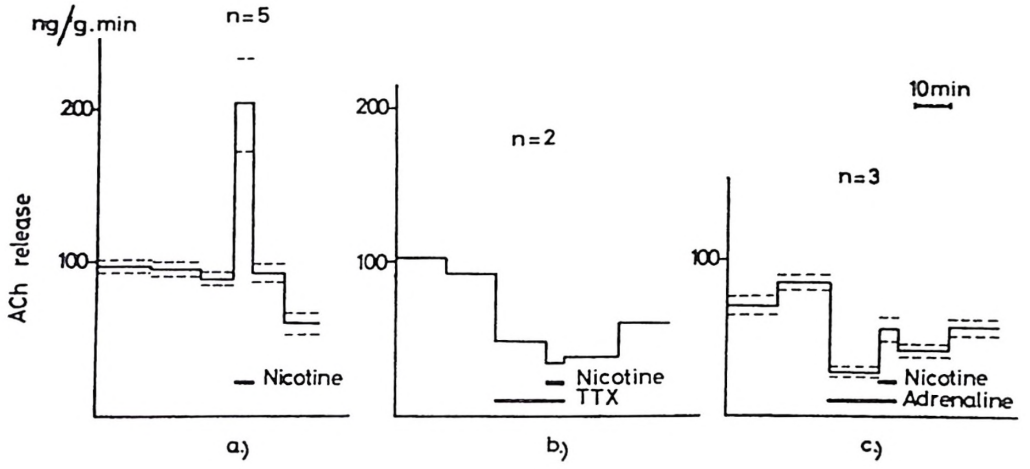


Fig. 7

The inhibition by tetrodotoxin (5x10⁻⁷ g/ml) or by adrenaline (5x10⁻⁷ g/ml) of ACh release enhanced by nicotine (5x10⁻⁵ g/ml). Long. m. strip of guinea-pig ileum.

tion (Paton, Vizi and Zar, unpublished) failed to reduce the ACh release, when the exposure time was as short as 3 min. However, in the next 3 min collection period there was an inhibition of 50%. When C_6 and nicotin were added together to the bath for one min, there was no reduction in ACh release. However, pretreatment by C_6 completely abolished the action of nicotine (Fig. 8).

Nicotine (5×10^{-6} g/ml) added to the bath containing cortex preparation of the rat, failed to increase the ACh release. However, when it was applied to the subcortex-cortex preparation, the ACh release was increased from $3{,}3 \pm 0{,}46$ to $6{,}7 \pm 0{,}6$ ng/g. min (Fig. 9). Since there are no true nicotinic receptors in the cortex (32) and in our experiments nicotine did not enhance the ACh release, the site of action of nicotine appears to be in the subcortex. The cell bodies of the great majority gf corticocholinergic fibres are situated subcortically (23). Thus, subcortical stimulation of nicotinic receptors by nicotine resulted in the increase of ACh release. It is difficult to say whether the ascending cholinergic nonspecific reticulocortical system (28, 9, 10) and/or thalamocortical pathways (9, 10) are totally involved in our preparation, but at least some parts of them must be involved. In addition, Armitage et al. (2) have observed that nicotine applied topically to the parietal cortex had no effect on the EEG in concentrations greater than those likely to reach the same site following repeated i. v. administration. However, on its. iv. administration there was a desynchronization. This experiment also indicates that there are no nicotine sensitive receptors in the cortex. In further series of experiments we have found that the increased ACh relase by nicotine from subcortex-cortex preparation was inhibited by TTX (6×10^{-7} g/ml), by mecamylamine (10^{-5} g/ml) and by adrenaline (10^{-6} g/ml). The inhibition by noradre-

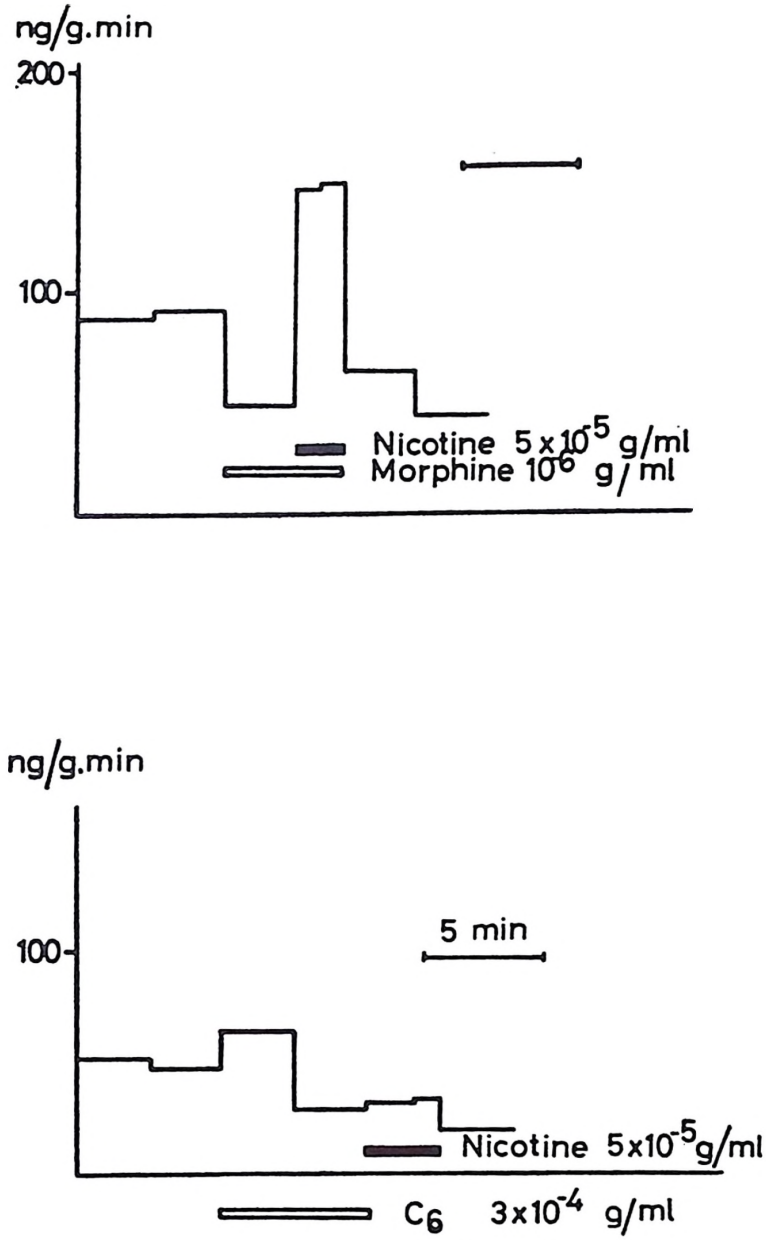


Fig. 8

The inhibition by morphine (10—6 g/ml) or by hexamethonium (C_6; 3x10—5 g/ml) of ACh release enhanced by nicotine (5 x 10—5 g/ml). Long. m. strip of guinea-pig ileum. Morphine sulphate, hexamethonium bromide and nicotine hydrogen tartrate were used.

naline of ACh release induced by nicotine was prevented by yohimbine (Table I). The ACh release augmented by nicotine (from 65.5±7.1 to 245.4±26.0 ng/g. min) was inhibited when noradrenaline in a concen-

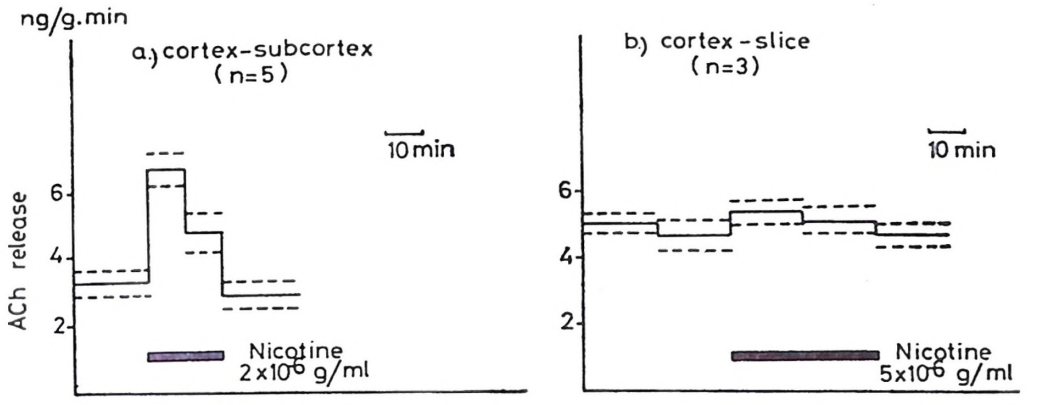


Fig. 9

The effect of nicotine on ACh release from subcortex-cortex and from cortex slice preparation of the at. Eserinized Krebs solution, 2 ml.

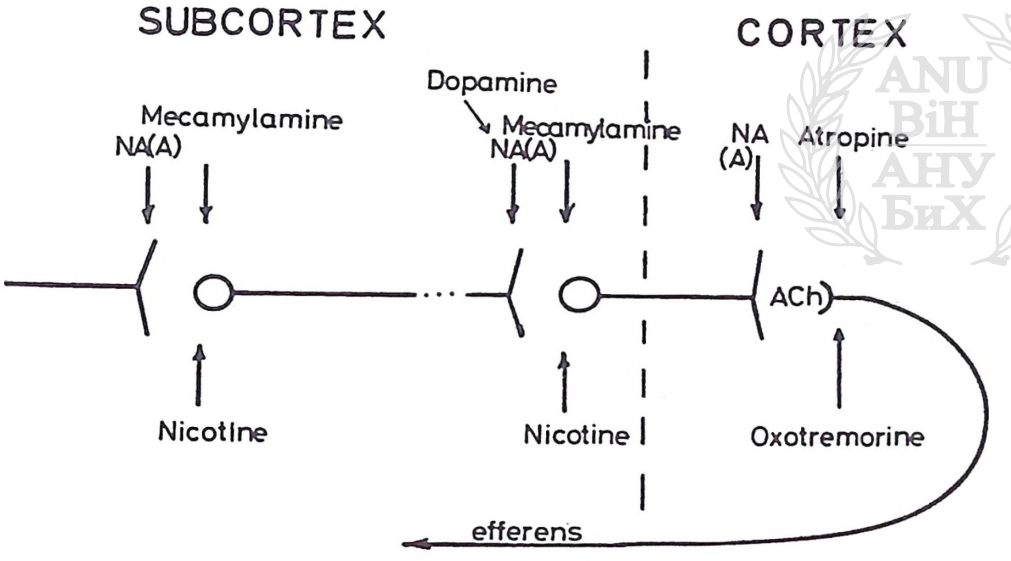


Fig. 10

The site of action of nicotine in the central nervous system.

tration of 2×10^{-6} g/ml was added to the bath; 84.2 ng/g. min was the release of ACh. However, when the strips were exposed to yohimbine (3×10^{-6} g/ml) for 20 min, there was a complete prevention of inhibition by noradrenaline (Table I). Our experiments indicate the role of α-receptor in the inhibitory action of noradrenaline on ACh release.

Table I

THE ROLE OF α-RECEPTOR IN THE INHIBITION BY NORADRENALINE OF NICOTINE EFFECT ON ACETYLCHOLINE RELEASE. LONGITUDINAL MUSCLE STRIP OF GUINEA-PIG ILEUM

conditions	ACh release ng/g. min	signif.	
1. Control (5)	65.5 ± 7.1		
2. Nicotine (4) 5×10^{-6} g/ml	245.4 ± 26.0	2:1	$p<0.01$
3. (—)-noradrenaline (3) 2×10^{-6} g/ml	16.1 ± 3.4	3:1	$p<0.01$
4. (—)-noradrenaline 2×10^{-6} g/ml + nicotine 5×10^{-6} g/ml (4)	60.5 ± 21.1	4:6	$p<0.01$
5. Yohimbine 30×10^{-6} g/ml + (—)-noradrenaline 2×10^{-6} g/ml (5)	84.2 ± 14.5	5:3 5:1	$p<0.01$ $p>0.05$
6. (—)-noradrenaline (3) 2×10^{-6} g/ml + Yohimbine 30×10^{-6} g/ml + nicotine 5×10^{-6} g/ml	191.5 ± 14.8	6:4 6:1	$p<0.01$ $p<0.01$

The exposure time to nicotine is 1 min, at Yohimbine administration 20 min. Collection time during control = 3 min. Yohimbine HCl, (—)-noradrenaline bitartrate and nicotine hydrogen tartrate were used.

The effect of oxotremorine

It has been suggested that the tremorgenic action of tremorine is due to its capability of releasing ACh (24). The indirect effect of oxotremorine was also suggested by Crossland and Slater (15) and György et al. (21). Cox and Potkonjak (12), however, found that after oxotremorine, the time course of the tremor and the time course of the increase in whole brain ACh level differed markedly. Futhermore they also observed that the tremor induced by oxotremorine was suspended by atropine in a dose which did not prevent the oxotremorine-induced increase in whole ACh brain level. Dyflos, a strong cholinesterase inhibitor, failed to potentiate the tremorgenic action of oxotremorine (13). Keranen et al. (29) observed only summation of the tremorgenic action of tremorine and physostigmine, and some antagonism between neostigmine and tremorine. Also physostigmine failed to increase the effect of tremorine on temperature.

If the indirect action of oxotremorine were the case, one would have to assume, that the oxotremorine increases the ACh release. However, direct measurement of ACh release from Auerbach plexus and from cortex slice of the rat failed to support this hypothesis (26). Oxotremorine (10^{-4} M) did not increase the ACh release from the Auerbach plexus of guinea-pig intestine, even decreased from 21.8±3.8 ng/g. min to 14.1±4.1 ng/g. min ($p<0.05$). Oxotremorine also failed to increase

Table II
THE EFFECT OF OXOTREMORINE ON ACh RELEASE

	ACh release* ng/g. min	signif	
Control (7)	21.8 ± 3.8	p<0,05	long. m. strip of. g. p. ileum
Oxotremorine (7) 10^{-4} M	14.1 ± 4.1		
Control (3)	4.44 ± 1.3	n. s.	cortex slice of the rat
Oxotremorine (3) 10^{-5} M	4.86 ± 0.5		
Control (3)	4.01 ± 0.25	n. s.	
Oxotremorine (3) 10^{-4} M	3.53 ± 0.15		

* ACh release during the collection period of 60 min. In brackets are indicated the number of experiments.

the ACh release from cortex-slice of the rat (Table II). Our findings are in agreement with those of Cox and Hecker (54) who also presented evidence that oxotremorine acts directly on the muscarinic receptors and did not release ACh from the guinea-pig ileum. The fast tolerance to some of the effects of oxotremorine was also attributed to catecholamines (22).

DISCUSSION

Until recently the evidence for the acetylcholine releasing action of nicotine was indirect, and was based upon the fact that drugs like atropine, hexamethonium, tetrodotoxin etc. can inhibit its pharmacological actions.

In these experiments a highly significant increase by nicotine of ACh release was observed. The ACh release was measured directly. Noradrenaline, adrenaline, tetrodotoxin, hexamethonium prevented and morphine reduced the effect of nicotine on ACh release. Atropine has already been shown to have an inhibitory effect against physostigmine-induced tremors, while generally inhibiting nicotine-induced tremors only in much greater doses.

Taking into account our results presented in this paper it is highly probable, that the »muscarinic« tremor (e. g. produced by physostigmine or oxotremorine) and the »nicotinic« tremor is similar in mechanism. Both are produced by increased saturation of muscarinic receptors by ACh, but in the case of nicotine it is caused by increased ACh release *in loco*. However, there is a great difference in antagonism. The nicotine depolarizes the cell bodies which in turn releases a high amount of ACh *in loco* at receptor areas, resulting in tremor. This can more easily be inhibited by antinicotinic agents than by atropine since the concen-

tration of agonist released is very high in a minute time unit. The depolarization by nicotine can be more easily inhibited, than to prevent the effect of already released ACh at receptor sites. The problem is similar to that of antagonism existing between endogenous (released), exogenous noradrenaline and alpha-blocking agents. The effect of exogenous noradrenaline can be more easily blocked than that of NA released to at nerve terminals.

It appears likely that the fast tolerance to nicotine-induced tremor (25, 6) is strongly related to the tachyphylaxis observed with ACh release.

Holmstedt and Lundgren (24, 25) have demonstrated that the tremorgenic agents with muscarin-like activity increase the ACh content of the total brain, while nicotine fails to change the content (25). Although, oxotremorine is considered an indirect acting tremorgenic agent (24), in our experiments it failed to release ACh measured directly.

Noradrenaline (adrenaline) was capable of inhibiting the effect of nicotine to increase ACh release in the Auerbach plexus of the longitudinal muscle strip and in cortex-subcortex preparation. The action of NA (A) to reduce ACh release induced by stimulation is situated presynaptically, as was shown by Paton and Vizi, (39); Vizi, (43, 44); Knoll and Vizi, (30, 31).

In addition, the effectiveness of dopamine and adrenaline (11, 24) in supressing tremor following intracaudate injection of ChE-inhibition or carbachol can be attributed to the inhibitory effect of catecholamines on ACh release (39, 43, 44, 30, 31).

The inhibitory action of noradrenaline on ACh release induced by nicotine was prevented by Yohimbine, inhicating that the α-receptor plays some role.

The role of α-receptor was also established by Paton and Vizi (39) showing that the inhibition by noradrenaline of ACh output induced by nerve stimulation was prevented by α-blocking agents. Our results with nicotine can probably explain the observations of McDougal and West (35), who found that the contraction of longitudinal smooth muscle produced by nicotine can be reduced by doses of noradrenaline and adrenaline which have little or no effect on the contraction produced by histamine and acetylcholine. Furthermore, they found that sympatholytic agents prevented the effect of adrenaline in antagonizing the nicotine contraction. I. e. nicotine exerts its effect through ACh release and noradrenaline can inhibit the release.

The failure of nicotine to increase the ACh release in the presence of C_6 also favoures the postsynaptic action of nicotine on nicotinic receptors (Fig. 8). Brown (4, 5) presented evidences that ACh and even nicotinic stimulants (carbachol, TMA, nicotine) are not capable of releasing ACh from the presynaptic nerve terminals of the superior cervical ganglion of the cat. The slight inhibitory action of C_6 on Nicotine uptake by the ganglion (3) cannot be actived inhibitory effect on the nicotine induced depolarization. Chiou and his coworkers (8) have shown, that nicotinic agents such as nicotine, TMA, carbachol release ACh efficiently from the nerve terminals and from rat cerebral cortex synaptic vesicles. These results suggest that the nicotinic agents are taken to an intracel-

luar site of the nerve tissues and release ACh from the synaptic vesicles, i. e. a presynaptic site of action of nicotine and nicotinic agents is concluded. However in order to observe any significant release of ACh by nicotine from isolated vesicles, Chiou had to use nicotine in a concentration of at least 100 ug/ml.

However, in our experiments nicotine was effective in releasing ACh as low as 2—5 x 10^{-6} g/ml concentrations. Feldberg and Vartiainen (18) perfused the superior cervical ganglion of the cat in situ with a solution containing nicotine. The perfusate was found to be free from ACh on bioassay. TTX prevented the effect of nicotine in releasing ACh. Gershon (20) also observed that TTX blockes the contraction caused by nicotine in intestine. These facts also indicate that nicotine itself do not release ACh from the presynaptic (prejunctional) nerve terminals, i. e. the site of action is not on the nerve terminals.

Nicotine was able to release ACh from the subcortex-cortex preparation and the Auerbach plexus which is rich in ganglia. One can therefore reasonably conclude that nicotine excites the cell bodies of subcortical afferent neurons which in turn leads to an ACh release (see Fig. 10) from the cortex nerve terminals. The increased release of ACh can evoke (via muscarinic receptors and via efferent neurons) symptoms which are characteristic of enhanced cholinergic function (tremor, convulsions etc.) It can be concluded, therefore, that the tremor, convulsions, behavioural actions and the peripheral actions induced by nicotine is highly probably due to the released ACh acting on muscarinic receptors. As regards its complex action, its releasing effect on other transmitters, can not be excluded.

The fact that noradrenaline and adrenaline could inhibit the ACh release augmented by nicotine gives a further evidence that the adrenergic system plays a very important role in cholinergic system by inhibiting the ACh release presynaptically as was suggested by Paton and Vizi (39) and Knoll and Vizi (30, 31). Since the imbalance between adrenergic and cholinergic system by reserpine or by phenoxybenzamine pretreatment led to an increased ACh release (39, 44) the Parkinson--syndrome induced by drugs which depress adrenergic function either by reducing the output of NA, such as reserpine, tetrabenazine or by antagonizing its action, as for instance phenoxybenzamine, haloperidol, chlorpromazine, can be attributed to the augmented ACh release. The reduction of adrenergic ontflow has to be central in origin since Jelicic and Stern (27) have shown, that the peripheral sympathetic nervous system has no effect on the tremor.

Nicotine proved to be a very useful tool for studying the action of different drugs on the ACh release from nerve terminals. Nicotine mimicks the physiological transmission process depolarizing the nerve cells which in turn leads to an increase of ACh release.

Acknowledgment. We are particularly indebted to Miss Maria Simon for highly skilled technical assistance and for an unusual devotion to this research.

E. S. VIZI I J. KNOLL

O MEHANIZMU OSLOBAĐANJA ACETILHOLINA POMOĆU NIKOTINA

KRATAK SADRŽAJ

Proučavano je djejstvo nikotina, jednog od najšire upotrebljavanog tremorgeničkog agensa, na oslobađanje acetilholina (ACh). Oslobođeni acetilholin je prikupljen u Krebsovoj otopini na 37°C i sadržavao je ezerin sulfat (2×10^{-6} g/ml), a zatim je isproban na ileumu zamorčeta uz prisustvo hexametoniuma (C_6; 3×10^{-4} g/ml). Ostatak oslobođenog ACh iz Auerbach plexusa longitudinalne mišićne niti ileuma zamorčeta bio je 74.2 ng/g. min. Ako je bio dodan nikotin (5×10^{-5} g/ml), oslobađanje se povećalo na 318.5 ng/min. Ipak, tetrodotoxin (5×10^{-7} g/ml), adrenalin (5×10^{-7} g/ml), noradrenalin (10^{-6} g/ml), morfin (10^{-6} g/ml) i C_6 (10^{-4} g/ml) zaustavili su djejstvo nikotina na povećanje oslobađanja ACh.

Nikotin (5×10^{-5} g/ml) nije uspio da poveća oslobađanje ACh iz korteksa štakora. Ipak, nikotin je udvostručio oslobađanje (5.6 ng/g. min) iz korteks-subkorteks preparata in vitro. Tetrodotoxin (3×10^{-7} g/ml) i adrenalin (10^{-6} g/ml) inhibirali su djejstvo nikotina. Razvila se brza tolerancija na djejstvo nikotina da oslobađa ACh dok se davao u dozama 5×10^{-5} g/ml. Ovo djejstvo nikotina na oslobađanje ACh može se pripisati njegovom depolarizirajućem djejstvu na ganglijske ili nervne ćelije, što u turnusu dovodi do oslobađanja ACh iz nervnih završetaka. Budući da je aktivizacija muskarinskih ACh receptora neizvjesna u pogledu na stvaranje tremora, ispitaće se uloga oslobađanja ACh u tremogeničkom djejstvu nikotina. Vrijedi napomenuti da oksotremorin nije uspio povećati oslobađanje ACh.

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DISCUSSION

Štern. I think that nicotin does'nt produce tremor. However, I have never seen it.

Vizi: Well, one thing is sure, that nicotine does not produce a long-lasting tremor. But it does evoke a tremor which lasts for a short time. The short-lasting tremor by nicotine is probable correlated with the fact tachyphylaxis to nicotine observed in ACh-release. I am agreeing with Professor Stern that nicotine not the best drug in producing tremor nevertheless, it causes tremor but short in time (Holmstedt and Lundgern, Ann. N. Y. Acad. Sci. 142: 126—142, 1967).

Župančič: Doctor Vizi, you said that hexamethonium prevents nicotine to release acetylcholine, right? Next, I understood you found this result unexpected, and I wonder just why would you like to comment on this point.

Vizi: I have a feeling this was misunderstood. Not the inhibition is unexpected the time course.

Barbeau: I would like to confirm two important remarks made by Prof. Vizi: 1) Dopamine does inhibit release of ACh, especially in the striatum and this is one of the most important control mechanisms. 2) You rightly emphasize the importance of muscarinic receptor site saturation for the production of tremor. The receptor has been the forgotten part of this mechanism. Lastly a question: instead of mimicking ACh could it be that oxotremorine changes the sensitivity of the receptor site or possibly of the »Target« cell?

Vizi: I am glad that Prof. Barbeau is agreeing with our results and suggestions. Yes, in one experiment Dopamine reduced by 50% the volley output (ACh output due stimulation) of ACh from the nerve terminals of Auerbach plexus. In subcortex-cortex preparation the nicotin-induced increase of ACh release was also reduced by Dopamine. This confirms your work on the role of Dopamine in Parkinson syndrome. I do not think that oxotremorine could change the sensitivity of the receptor site. The fact that there is a competitive type of antagonism between Atropine and Oxotremorine, indicates that Oxotremorine acts on the receptor. We have studied this problem. Otherwise the $pA_2 - pA_{10}$ value which (Schield) was calculed also a little piece of evidence for believing the effect of Oxotremorine on muscarinic receptors. We do think that Oxotremorine mimicks the action of acetylcholine.

Schäfer: During the reserpin induced tremor increase of the alpha-activity was noticed, but tremorin induced tremor showed gentel gamma-activity. On

a base of different changes of sensibility of the motor system, a different forme of the tremor after inducing the drugs must be expected. Does the tremors in both case differ? Because the impulses of the alpha-motor neuron on spinal level because activity in both case can bee different too. Have you researched for this?

Vizi: We did not make any attempts to distinguish reserpine and oxotremorine tremor by frequency analysis. Concerning your question on reflex excitability of alpha motoneurons, it was tested by stimulation of the dorsal roots.

Huković: Have you seen similar effects on other organs? Do you have explanation for different results with nicotine and oxotremorine considering biphasic effect on release and effect of nerve stimulation?

Vizi: If you think on ACh release I can say, yes. We have studied the ACh release in other organs, as well. We have found that e. g. was deferens of the rat contains ACh in a concentration of 2 microgr/ml (Kroll, Somogyi and Vizi, in press) and using fiels stimulation there was on increase of ACh release depending on the frequency used (Knoll et al. in press). But the action of nicotine or of oxotremorine on ACh release from the was deferens we did not study. I do not think that would be any correlation between, tachyphylaxis (to the ACh releasing action of nicotine) and »biphasic« effect of oxotremorine on the contraction of vas deferens in your experiments, since at the moment it is generally accepted that in vas deferens the NA is the transmitter. However we also believe that in vas deferens ACh might have some role in maintaining excitability of smooth muscle cells. In addition, in our Department Dr Somogyi also observed that oxotremorine (10^{-7} g/ml) increase contraction by stimulation of was deferens and that this action can be prevented by Atropine.

Bernard: It was mentioned that dopamine reduces acetylcholine release. Has L-DOPA a similar action?

Vizi: I can not tell you, because we have not tried.

Cox: Dr Vizi may I raise a few points some of which lend support to your hypothesis. Firstly oxotremorine on guinea-pig ileum is competetively antagonized by atropine when tested by the method of Arunlakshana and Schild. It is also twice as potent as ACh on this preparation. Therefore one might except it to be a potent muscarinic agonist in the CNS such as at the site you postulate. Mayi also point out the problem of measuring ACh release — »so colled« resting release differs depending on the cholinesterase inhibitor used to protect ACh. Thus release rates for eserine are much higher than release rates for mipafox (an organophosphorus inhibitor). It is quite possible that eserine may itself induce ACh release and this could affect results obtained. Finally there is the problem of facilitated or non action potential mediated relaese of ACh. The agents used tetrodotoxin and morphine will only prevent action potential released ACh. Nevertheless it seems to me that the weight of evidence favours a post-synaptic effect of oxotremorine on a central muscarinic receptor.

Vizi: I should like to make clear asfar as the »resting« release is considered. Resting release from long. m strip of guinea-pig ileum can be reduced by about 50 — 70% by TTX, Hexamethonium and by NA (A) (Paton and Vizi, Brit. J. Pharmacol. 35, 10 — 28, 1969 and Paton, Vizi and Zar, in press). This indicates that one part of the release is maintained by the activity nervous and the conduction is involved in the release. I think it is the high time to discuss about Mipafox. Paton and Zar (personal communication) found that Mipafox somehow interferes with the release mechanism. Furthermore, if I am correct, in your last paper (Cox and Hecker, Brit. J. Pharmacol. 41, 19—25, 1971) you presented evidence that the output to stimulation was very low when Mipafox was applied. These fact seem to me to strengthen the

feeling that Mipafox is not the appropriate CHe-I, when one wants to measure the ACh release by action potential. The physostigmine is not only the most frequently used ChE, but it seems to be the drug which does not influence the conduction, and the release of ACh due to nervous activity can be measured reliable. We could not found for instance difference in the action of NA/A (in reducing ACh release) either in the presence or in the absence of physostigmine. There was also a good correlation between the reduction by TTX of ACh release in the presence of physostigmine and the reduction of size of contraction induced by stimulation in long. m. strip of guinea-pig ileum.