Br. J. Pharmac. (1973), **47**, 765–777.

Acetylcholine release from guinea-pig ileum by parasympathetic ganglion stimulants and gastrin-like polypeptides

E. S. VIZI*

Institute of Pharmacology, University of Parma, Parma, Italy and Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary

Summary

1. The release of acetylcholine by parasympathetic ganglion stimulants from the nerve terminals of the guinea-pig longitudinal muscle strip was studied. The acetylcholine released was collected in the presence of physostigmine sulphate.

2. Nicotine and 1,1-dimethyl-4′-phenylpiperazinium iodide (DMPP) released acetylcholine. Tetrodotoxin (50 ng/ml) and hexamethonium completely prevented their action on acetylcholine release, indicating that their effect is on nicotinic receptors of cell bodies. Noradrenaline (1 μg/ml), excess magnesium, and calcium withdrawal inhibited the acetylcholine release induced by nicotine or by DMPP.

3. There was rapid tachyphylaxis to the acetylcholine releasing action of both nicotine and DMPP.

4. The octapeptide amide of cholecystokinin and caerulein (10^{-9}M) enhanced the acetylcholine release without producing tachyphylaxis. Tetrodotoxin completely inhibited acetylcholine release. However, hexamethonium or desensitization with 5-hydroxytryptamine did not prevent their action. In the early phase of nicotine action the polypeptides studied failed to increase acetylcholine release. However, a few minutes later the tissue became sensitive to polypeptides despite the fact that the tissue was still exposed to nicotine. These data suggest the presence in the parasympathetic ganglion cells of separate gastrointestinal hormone-sensitive receptors.

5. Noradrenaline inhibited the acetylcholine releasing action of polypeptides. This effect was mediated via α-adrenoceptors since phentolamine prevented its action.

6. Excess magnesium (9·3 mM) also reduced the acetylcholine release in response to the polypeptides.

Introduction

The traditional view that ganglionic transmission involves only nicotinic receptors is based on the observation that stimulation of ganglion cells by nicotine-like drugs can be prevented by drugs related to hexamethonium. However, it is also accepted that drugs such as muscarine are able to stimulate ganglion cells by mechanisms that are sensitive to blockade by atropine. Either electrical recording techniques or

* Present address: Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary.

recording of the response of effector cells have been used for studying the actions of ganglionic stimulants on ganglion cells. For anatomical reasons these studies were carried out mainly on sympathetic ganglia. However, no attempt has so far been made to study in detail the release of transmitter from postganglionic nerve terminals as a result of the firing caused by ganglion cell stimulation. Nicotine and 1,1-dimethyl-4'-phenylpiperazinium iodide (DMPP) were used as ganglion stimulants. Since it has been shown (Vizi, Bertaccini, Impicciatore & Knoll, 1972a, b) that gastrin and cholecystokinin-like polypeptides are also capable of releasing acetylcholine from the nerve elements of the longitudinal muscle strip preparation by stimulating ganglion cells of Auerbach's plexus, the mode of action and kinetics of these polypeptides were also studied.

Methods

Longitudinal muscle strip preparation from guinea-pig ileum

Muscle strips with attached nerve elements of Auerbach's plexus were prepared as described by Paton & Vizi (1969).

Assay of acetylcholine released

Four strips weighing about 40–70 mg each, were set up in an organ bath of 3·5 ml capacity in Krebs solution at 36° C, bubbled with 5% carbon dioxide in oxygen. The acetylcholine released was collected in the presence of physostigmine sulphate (2 μg/ml).

Before the collection of the first samples for assay of the acetylcholine released, strips were allowed to equilibrate for 60 minutes. The acetylcholine was assayed on a length of guinea-pig ileum suspended in 3·5 ml Krebs solution at 36° C. Its oral end was ligated and connected to the isometric transducer system via a phosphor–bronze spring; the aboral end was connected to an open-ended polythene tube which projected through the bottom of the organ bath to allow the luminal contents to be extruded from the ileum without contaminating the bath. Since prolonged isometric contractions tended to reveal spontaneous movements of the ileum, a soft spring was used to eliminate the isometric condition. In order to reduce the release of endogenous acetylcholine, morphine sulphate (5 μg/ml) was also added to the Krebs solution of the assay bath. To eliminate the possibility of interference from traces of nicotine or DMPP carried over in the sample, hexamethonium (300 μg/ml) was added to the Krebs solution for assay. In this way any interference from ganglionic stimulants carried over into test assays was countered although the sensitivity to acetylcholine was reduced to 1–10 ng/bath.

When gastrin-like polypeptides were present in the samples their interference was prevented by the use of tetrodotoxin (0·5 μg/ml). Furthermore, during the assay, control responses to standard solutions of acetylcholine were obtained in the presence of the same concentration of the substance under test as was produced by adding the test sample to the assay bath. Samples or control acetylcholine solutions freshly made up in 0·9% w/v NaCl solution (saline) were added to the assay bath at intervals of 1 min in volumes of 0·1–0·4 ml. The estimate of the acetylcholine content of samples was based on repeated control dose-response curves. Changes of bath fluid were made by overflow. The activity of assay sample was always abolished by pretreatment of the assay organ with atropine (1 μg/ml) or by the addition of

alkali to the samples; moreover, mepyramine had no effect on the contractile responses.

The acetylcholine released and assayed is expressed in (ng/g)/minute.

Preparation of superior cervical ganglion of the cat

Some experiments were performed on cats of either sex, weighing 2·2 to 3 kg. Anaesthesia was induced with chloralose (75 mg/kg). Close intra-arterial injections to the superior cervical ganglion were made into the external carotid artery as described by Trendelenburg (1959).

Injections were made when the external artery was clamped and this type of drug administration is described as 'injection to the ganglion'. For intra-arterial injections the drugs were given in a volume of 0·2 ml, in saline.

The pre- and postganglionic fibres were also prepared and placed on electrodes and covered with warm liquid paraffin. Square-wave impulses of 0·2 ms were applied at a rate of 4 Hz for 5 seconds. Contractions of the nictitating membrane were recorded isometrically on a pen-recorder. Since prolonged isometric contractions tended to exhaust the nictitating membrane a soft phosphor–bronze spring was used to eliminate the isometric condition.

Solution and drugs

The Krebs solution used had the following composition (mM): NaCl, 113; KCl, 4·7; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $NaHCO_3$, 2·5; and glucose, 11·5. Calcium-free solutions were made by omitting $CaCl_2$.

Magnesium-enriched Krebs was prepared by adding $MgCl_2$. Potassium-rich Krebs was made by increasing the KCl concentration up to 47·3 mM potassium at the expense of an equivalent amount of NaCl.

The following drugs were used: acetylcholine iodide (BDH), nicotine hydrogen tartrate (BDH), 1,1-dimethyl-4′-phenylpiperazinium iodide (Fluka), hexamethonium bromide (Fluka), (−)-noradrenaline bitartrate (Fluka), (±)-phentolamine methane sulphonate (CIBA), adrenaline hydrochloride (Fluka), morphine sulphate (Merck AG), tetrodotoxin (Sankyo), yohimbine hydrochloride (Smith, Kline & French), mepyramine maleate (Merck, Sharpe & Dohme), physostigmine sulphate (BDH), synthetic caerulein (compound F.I. 6934; Farmitalia, Milan), cholesystokinin octapeptide (The Squibb Inst.), angiotensin (Hypertensin-Ciba; val^5-Hypertensin II-asp-β-amide).

Results

Release of acetylcholine by nicotine and DMPP

DMPP in concentrations from 0·5 to 20 μg/ml increased the acetylcholine release from nerve elements of the longitudinal muscle strip of guinea-pig ileum. In higher concentrations it was not possible to measure the acetylcholine output because of the atropine-like effect of DMPP (Bennett & Whitney, 1966), which could have contaminated the assay ileum. The action of DMPP was dose-related. As shown in Fig. 1a, DMPP in a concentration of 2 μg/ml augmented the acetylcholine release from 95·1 ± 7·6 (ng/g)/min (344 ± 27·8 (pmol/g)/min) to 428·5 ± 25·4 (ng/g)/min (1,569 ± 93·0 (pmol/g)/min). In spite of the fact that DMPP was

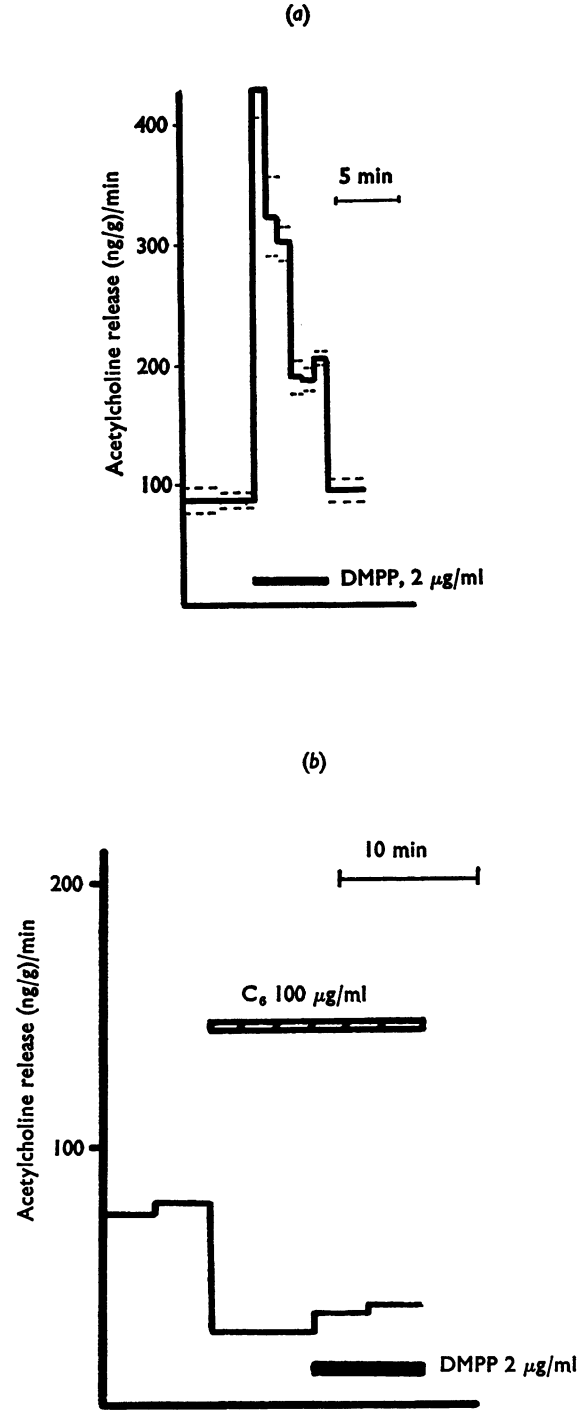

FIG. 1. Release of acetylcholine by DMPP from the nerve elements of longitudinal muscle strip of guinea-pig ileum, and its prevention by hexamethonium. (a) 1,1-Dimethyl-4'-phenylpiperazinium iodide (DMPP). Mean of three experiments with identical treatment schedules; dashed lines indicate the S.E. Time mark, 5 minutes. (b) Hexamethonium bromide (C_6). Mean of two experiments with identical schedules. Time mark, 10 minutes.

still present in the organ bath the acetylcholine output declined with time to a lower level. In one experiment the acetylcholine release in response to DMPP (2 μg/ml) was measured every 15 seconds. The acetylcholine output during the first collection period of 15 s was the highest, 550 (ng/g)/minute. Output declined with a half time of 2·2 minutes. Hexamethonium by itself, as described by Paton & Zar (1968) and Paton, Vizi & Zar (1971), reduced the resting release by about 60%. Hexamethonium, 100 μg/ml, prevented the effect of DMPP on release (Fig. 1b). However, when the tissue was not preincubated with hexamethonium, but was exposed to hexamethonium and DMPP together for a period of 10 min (usually a sufficient exposure time for hexamethonium to inhibit nicotinic receptors), the tissue still remained sensitive to DMPP.

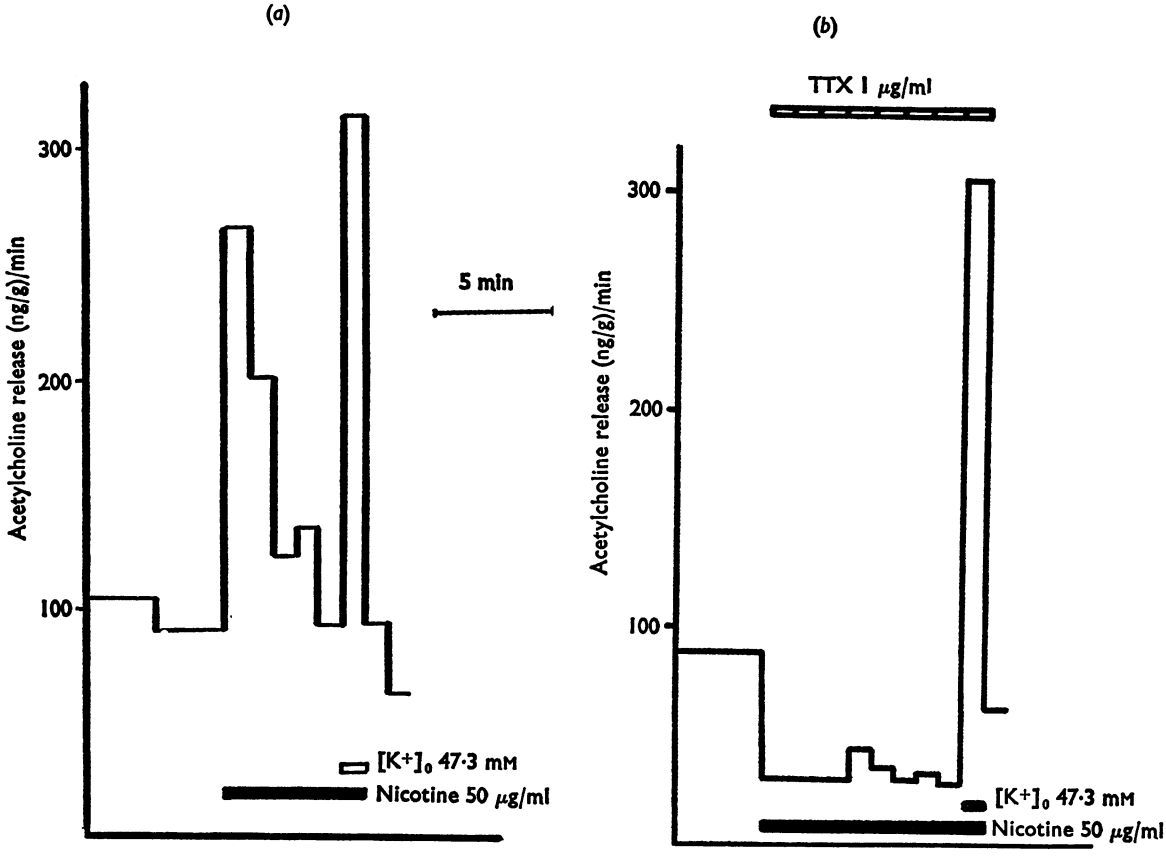

FIG. 2. Release of acetylcholine by nicotine from the nerve elements of longitudinal muscle strip of guinea-pig ileum and its prevention by tetrodotoxin. (a) Mean of two similar experiments. The concentration of potassium in the Krebs solution was increased to 47·3 mM. Note the fast decline of acetylcholine release in the presence of nicotine. Time mark, 5 minutes. (b) Mean of two similar experiments. Tetrodotoxin (1 μg/ml) and nicotine (50 μg/ml) were present as indicated; tetrodotoxin (TTX) was added to the bath 5 s before nicotine. Note the complete inhibition by tetrodotoxin of acetylcholine release in response to nicotine. Time as in Fig. 2a.

The effect of nicotine was similar to that of DMPP in that it increased the resting output of acetylcholine. It differed from DMPP in that tachyphylaxis to its effect on acetylcholine release developed more rapidly. Figure 2a shows the effect of nicotine (50 μg/ml) in two experiments. During tachyphylaxis, when nicotine was

no longer able to increase acetylcholine release, even when administered in a concentration as high as 200 μg/ml, direct depolarization of the nerve terminals by excess potassium still elicited acetylcholine release. These data indicate that the acetylcholine stores are not exhausted. When the concentration of potassium in the Krebs solution was increased to 47·3 mM at the expense of an equivalent amount of NaCl, there was a marked increase in acetylcholine release which ceased immediately after the return to the normal solution. The effect of nicotine also proved to be dose-dependent. The threshold dose for nicotine was as low as 0·5 μg/ml (10^{-6}M). Although the output by this dose of nicotine was only slightly enhanced from 72·5 to 102·3 (ng/g)/min, a tendency for tachyphylaxis to occur was also observed. Tetrodotoxin reduced the resting output by 66%, and totally inhibited the effect of nicotine in increasing the output (Fig. 2b).

A characteristic feature of the actions of nicotine and DMPP on parasympathetic ganglion cells was the occurrence of tachyphylaxis. A comparison of the amount of acetylcholine released by nicotine in the first collection period of 1 min with that of the second and third periods, showed that the tissue became insensitive to nicotine. The acetylcholine release declined to below the control level. DMPP (2 μg/ml) added to the bath in any period of tachyphylaxis produced by nicotine did not enhance the acetylcholine release. These results indicate a cross-tachyphylaxis.

Nicotine failed to enhance the acetylcholine output from tissue which had been exposed to a high dose of DMPP (50 μg/ml) for 2 minutes. DMPP in lower concentration (2 μg/ml) did not produce cross-tachyphylaxis to nicotine to the same extent as nicotine did to DMPP.

Inhibition by nicotine of DMPP-induced acetylcholine release

In the absence of calcium, the acetylcholine output was reduced and was then only slightly increased by DMPP (2 μg/ml) from 19·5 $\pm$ 2·0 to 50·6 $\pm$ 7·1 (ng/g)/

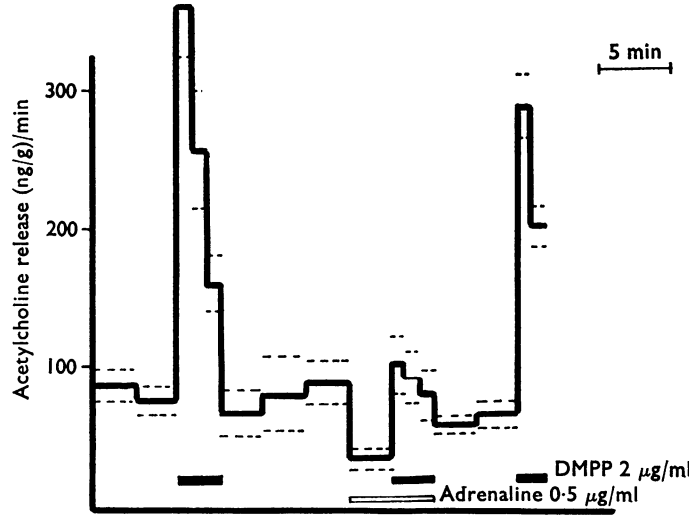

FIG. 3. Inhibitory effect of adrenaline on acetylcholine release induced by 1,1-dimethyl-4′-diphenylpiperazinium iodide (DMPP) (2 μg/ml) from Auerbach's plexus of longitudinal muscle of guinea-pig ileum. Four similar experiments. Dashed lines indicate the S.E. Time mark, 5 minutes.

minute. However, when calcium was present in the solution the output was as high as 445·0 ± 85·5 (ng/g)/minute. Both magnesium excess (9·3 mM) and adrenaline (0·5 μg/ml, Fig. 3) were able to inhibit the acetylcholine released in response to DMPP. In another series of experiments noradrenaline in a concentration of 1 μg/ml completely inhibited the acetylcholine release due to nicotine (5 μg/ml): the control acetylcholine output (52·5 (ng/g)/min) was enhanced by nicotine to 302·8 (ng/g)/minute. However, in the presence of noradrenaline the output was reduced to 36·5 (ng/g)/minute. In two experiments it was observed that when noradrenaline was added to the medium for the first 3 min of a 10 min exposure to nicotine (50 μg/ml) there was no increase in acetylcholine output. A 3 min exposure time was long enough to build up tachyphylaxis to nicotine as shown in Figure 2a. Although the initial increase in output was inhibited by noradrenaline, after withdrawal of noradrenaline there was still no increase in acetylcholine release. These results indicate that the fast reduction in acetylcholine output is not related to an exhaustion of the acetylcholine store but is rather connected with a phenomenon which results from an interaction of nicotine with the effector cells.

Acetylcholine release by the octapeptide amide of cholecystokinin and caerulein

It has been reported (Vizi *et al.*, 1972a, b) that the gastrin-like polypeptides are capable of releasing acetylcholine from the longitudinal muscle strip of guinea-pig ileum. Tetrodotoxin prevented this action but hexamethonium did not. When the tissue was exposed to the octapeptide amide of cholecystokinin in a concentration sufficient to produce the maximal effect, there was no decline in release of acetylcholine as observed with nicotine and DMPP. Tachyphylaxis also failed to develop with caerulein, a polypeptide similar to cholecystokinin in structure and action (Erspamer, 1970; Grossman, 1970; Bertaccini, 1971). However, the highest output was always observed in the first period.

Paton & Perry (1953) have shown that nicotine blocks ganglionic transmission and responses to acetylcholine and to itself nonspecifically and insurmountably by depolarizing the ganglion cell. Even with continued exposure to nicotine, repolarization occurs, and after repolarization a selective block to nicotinic stimulants persists. The effect of the octapeptide amide of cholecystokinin on acetylcholine release during the two types of inhibition produced by nicotine is shown in Figure 4a. In the first phase of nicotinic action there was a transient increase in acetylcholine output from 92·5 ± 9·5 to 325·0 ± 31·0 (ng/g)/min, followed by a reduction in output and an inhibition of the action of the amide of cholecystokinin in enhancing acetylcholine release. However with time, the sensitivity to the amide of cholecystokinin partly recovered: the output in response to the peptide was enhanced from 44·5 ± 6·5 to 170·1 ± 19·5 (ng/g)/min, despite the fact that the tissue was continuously exposed to nicotine. However, when hexamethonium (100 μg/ml) was also present in the medium the effect of nicotine was completely inhibited so that the action of the octapeptide amide of cholecystokinin in increasing the release of acetylcholine was not prevented (Figure 4b).

When the 5-hydroxytryptamine-sensitive receptors of parasympathetic ganglia were desensitized by a 45 min exposure to this drug (Brownlee & Johnson, 1963) caerulein in a concentration of 50 ng/ml still increased the acetylcholine release from

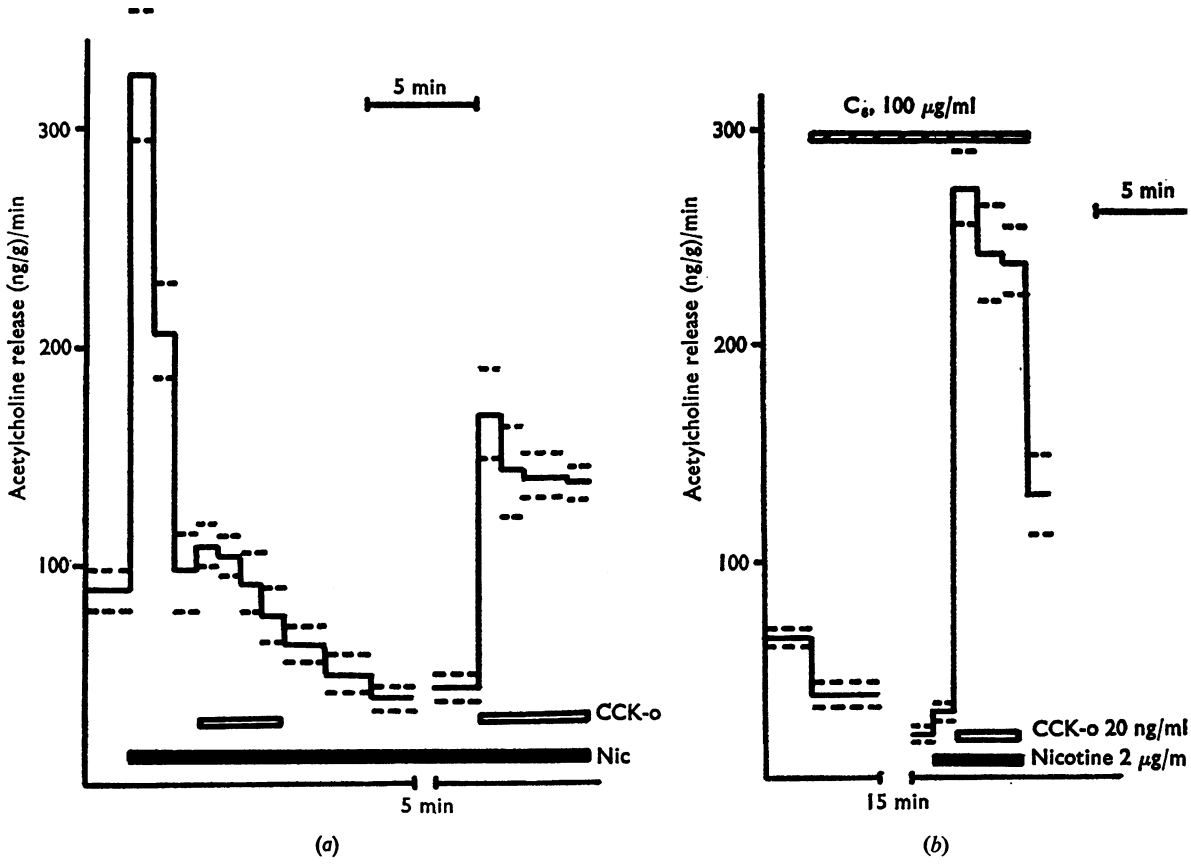

FIG. 4. Evidence for the existence of receptors sensitive to octapeptide amide of cholecystokinin in parasympathetic ganglion cells of Auerbach's plexus. Longitudinal muscle strip of guinea-pig ileum. (a) Inhibition of acetylcholine-releasing action of 20 ng/ml octapeptide amide of cholecystokinin (CCK-o) in the first phase of nicotine action. Nicotine (Nic) is present in concentration of 2 μg/ml, as indicated. Note the release of acetylcholine in response to octapeptide amide of cholecystokinin in the second phase of nicotine action. Three similar experiments. Dashed lines indicate the S.E. Time mark, 5 minutes. The time (5 min) when acetylcholine release was not measured, but the tissue was still exposed to nicotine is indicated. (b) Augmented release of acetylcholine by octapeptide amide of cholecystokinin (CCK-o, 20 ng/ml) in the first phase of nicotine action in the presence of hexamethonium (C_6, 100 μg/ml). The time (15 min) when acetylcholine release was not measured but the tissue was still exposed to hexamethonium is indicated. Time mark, 5 minutes. Mean of three similar experiments; dashed lines indicate the S.E.

49·5 to 221·5 (ng/g)/min, an increase not different from those observed in control experiments.

In two experiments, mepyramine (20 ng/ml) did not influence the acetylcholine output in response to the polypeptide (caerulein, 20 ng/ml). The output was enhanced from 65·5 and 57·2 to 190·1 and 179·6 (ng/g)/min, respectively. The increased acetylcholine output produced by caerulein was also inhibited by noradrenaline (1 μg/ml), and this inhibition was prevented by the α-blocking agent, phentolamine (2 μg/ml; Figs. 5a and b). However, the β-adrenoceptor blocking agent, pindolol (LB-46) (Saameli, 1967) failed to influence the inhibitory action of noradrenaline. In three experiments excess magnesium (9·3 mM) prevented the enhanced acetylcholine output produced by caerulein (20 ng/ml). The output in the

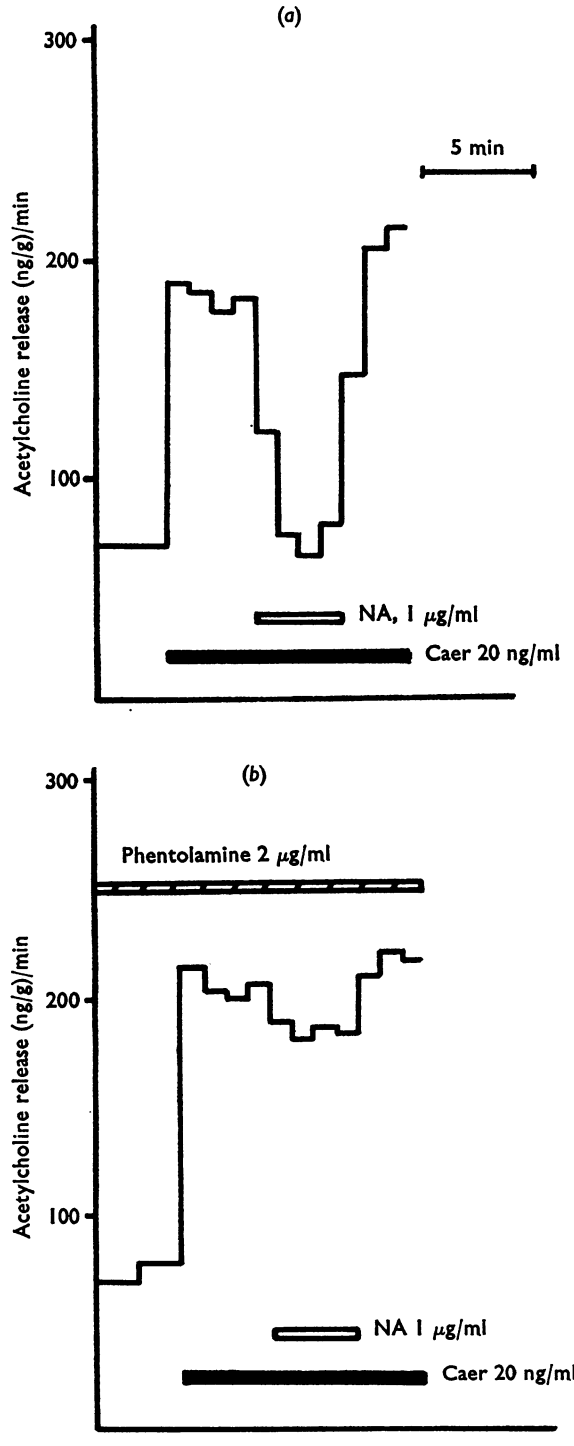

FIG. 5. Inhibitory action of noradrenaline on acetylcholine releasing effect of caerulein and its prevention by phentolamine. (a) Noradrenaline (NA), 1 μg/ml. Caerulein, (Caer), 20 ng/ml. Time mark, 5 minutes. Two similar experiments. (b) Noradrenaline (NA), 1 μg/ml. Caerulein (Caer), 20 ng/ml. Phentolamine, 2 μg/ml, was present as indicated. Two experiments with identical doses and time schedules. Time as indicated in Fig. 5a.

presence of high magnesium concentrations was $65{\cdot}8 \pm 3{\cdot}8$ (ng/g)/minute. However, with caerulein in solutions containing a normal magnesium concentration, it was as high as $201{\cdot}5 \pm 12{\cdot}8$ (ng/g)/minute. The difference is significant, $P<0{\cdot}01$.

Lack of stimulatory action of caerulein on sympathetic ganglia

In six experiments, caerulein was injected into the superior cervical ganglion of the cat. Caerulein in a dose as high as 20 μg failed to cause contraction of the nictitating membrane or to influence the contraction in response to preganglionic stimulation. However, nicotine (100 μg) or angiotensin (5 μg) caused contractions. After the injection of polypeptide, hexamethonium (300 μg) was injected into the ganglion preparation, and it was verified that it blocked the contractions to preganglionic stimulation without influencing the response to postganglionic stimulation.

Discussion

Until recently direct evidence was lacking for the acetylcholine releasing action of nicotine-like substances. However, it was shown that drugs like atropine, hexamethonium, tetrodotoxin, adrenaline or cooling (Ambache, 1951; McDougal & West, 1954; Day & Vane, 1963; Daniel, 1966; Gershon, 1967) can inhibit, and anticholinesterases can potentiate the action of nicotine-like drugs on the intestine, and it was suggested that their action is mediated through acetylcholine release.

Paton & Zar (1968) presented additional evidence for the indirect action of nicotine-like substances; nicotine and DMPP failed to produce contractions in denervated smooth muscle. Although Brownlee & Johnson (1965) showed that DMPP releases acetylcholine from the whole ileum, they did not make any attempt to study in detail the mechanism of release. Experiments presented in this paper show that nicotine and DMPP release acetylcholine from the longitudinal muscle strip of guinea-pig ileum. The acetylcholine releasing action of nicotine and DMPP was totally inhibited by hexamethonium indicating a specific effect of nicotine and DMPP on the hexamethonium sensitive receptors of ganglion cells. These results also show that nicotine and DMPP are not able to release acetylcholine from the presynaptic, pre-effector nerve terminals of the parasympathetic ganglia, as was also shown by Brown, Halliwell, Jones & Quilliam (1970) for presynaptic nerve terminals of sympathetic ganglia. Furthermore, nicotine was also ineffective in releasing acetylcholine in the rat cortex slice preparation (Vizi & Knoll, 1972), a preparation which provided a possibility for studying the action of drugs on nerve endings of cell bodies situated subcortically (Hebb, Krnjević & Silver, 1963). The failure of nicotine to enhance the release of acetylcholine in the presence of tetrodotoxin also supports this view.

Thus, if the parasympathetic ganglion cells in Auerbach's plexus behave in a similar way (and this can be concluded from the experiments of Perry & Talesnik (1953) with parasympathetic ciliary ganglion) then nicotine depolarizes the ganglion cells which in turn leads to firing of postganglionic fibres and results in transmitter release; this probably occurred in the experiments described here. Nicotinic ganglionic stimulants released acetylcholine by stimulating the nicotinic receptors on ganglion cells but rapid tachyphylaxis developed to this effect, and

the acetylcholine release declined quickly. This decline in acetylcholine release is probably due to the depolarizing block.

It has been shown (Vizi *et al.*, 1972a) that the gastrin-like polypeptides (Gastrin-I, octapeptide amide of cholecystokinin, caerulein, pentagastrin) are able to release acetylcholine from Auerbach's plexus in the longitudinal muscle strip of guinea-pig ileum. The kinetic study of the acetylcholine-releasing action of the octapeptide amide of cholecystokinin, which has all the biological properties of the intestinal hormone, cholecystokinin-pancreozymin (Ondetti, Rubin, Engel, Pluscec & Sheehan, 1970) and which includes the active sequence of the whole hormone (Erspamer, 1970), showed that this polypeptide enhanced acetylcholine release. A similar observation was made with caerulein.

The failure of gastrin-like peptides to release acetylcholine in the early phase of nicotine action, when the ganglion cells are depolarized, but not their axons (Paton & Perry, 1953), makes it possible to assume that their action is located on non-nicotinic receptors of ganglion cells. This early phase lasted about 2–7 minutes. However, after this the gastrin-like polypeptides became effective, but the preparation still did not respond to nicotinic stimulants. It might be concluded that the block by depolarization was followed by a competitive block like that produced by hexamethonium (Paton & Perry, 1953). Nevertheless, our results provide strong supporting evidence for the transient inhibitory action of nicotine on parasympathetic ganglion cells which also extends to drugs which do not act on nicotinic receptors. A similar mechanism was also discussed by Trendelenburg (1951 ; 1966) for the superior cervical ganglion which failed to respond to non-nicotinic stimulants (histamine, 5-hydroxytryptamine, angiotensin) when it was exposed to nicotine.

However, there is another possible explanation for this ; nicotine might inhibit the effect of gastrin-like substances, not through depolarization, but by some type of competition at receptors sensitive to gastrin-like polypeptides. This possibility was excluded by our experiments with hexamethonium. When the effect of nicotine on nicotinic receptors was inhibited by hexamethonium, and the depolarization was possibly also prevented (Brown, 1966), the peptides added after nicotine were effective (Fig. 4b). There was even some increase in their effect on acetylcholine release. Furthermore, the fact that during the competitive type of block by nicotine the octapeptide amide of cholecystokinin was effective, also supports this view.

The fact, that tetrodotoxin prevented the acetylcholine releasing effect of polypeptides (Vizi *et al.*, 1972a) provides convincing evidence for excluding direct action on prejunctional nerve endings. Evidence has been given earlier, that tetrodotoxin does not inhibit acetylcholine release elicited by direct depolarization of nerve terminals (Elmquist & Feldman, 1965 ; Katz & Miledi, 1967 ; Paton *et al.*, 1971 ; Vizi, Illés, Rónai & Knoll, 1972 ; Dawes & Vizi, unpublished observations). Hexamethonium did not inhibit the acetylcholine releasing effect of gastrin-like peptides.

The role of 5-hydroxytryptamine receptors was also excluded by the experiment in which a preparation that had been exposed to a high dose of 5-hydroxytryptamine still remained sensitive to peptides and released acetylcholine. A possible effect on the muscarinic receptors is also excluded by the fact that in the presence of tetrodotoxin these polypeptides failed to cause contraction of the longitudinal muscle of guinea-pig ileum, which is very rich in muscarinic receptors.

All these data indicate that there is a receptor which is sensitive to gastrin-like polypeptides and this receptor is located on parasympathetic ganglion cells, but not

on sympathetic cells since the superior cervical ganglion failed to respond to gastrin-like polypeptides. This seems to be the first difference found so far between sympathetic and parasympathetic ganglia.

Noradrenaline and adrenaline which were found to inhibit the acetylcholine release from parasympathetic nerve terminals produced by either low frequency stimulation (Paton & Vizi, 1969 ; Kosterlitz, Lyden & Watt, 1970) or high frequency stimulation of short duration (Knoll & Vizi, 1970, 1971 ; Vizi & Knoll, 1971) or inhibition of (Na^+-K^+-Mg^{2+})-activated ATP-ase (Vizi, 1972), also reduced the release of acetylcholine in response to nicotine or to polypeptides.

It was also found, in support of an earlier finding (Paton & Vizi, 1969), that the presynaptic (prejunctional) inhibitory effect of noradrenaline can be prevented by an α-blocking agent indicating that α-adrenoceptors are involved in the effect of noradrenaline on nerve endings.

REFERENCES

AMBACHE, N. (1951). Unmasking, after cholinergic paralysis by botulinum toxin, of a reversed action of nicotine on the mammalian intestine, revealing the probable presence of local inhibitory ganglion cells in the enteric plexuses. *Br. J. Pharmac. Chemother.*, **6**, 51–67.

BENNETT, A. & WHITNEY, B. (1966). A pharmacological investigation of human isolated stomach *Br. J. Pharmac. Chemother.*, **27**, 286–298.

BERTACCINI, G. (1971). Active polypeptides in amphibian skin. *Arch. Pharmac.*, **269**, 139–152.

BROWN, D. A. (1966). Effects of hexamethonium and hyoscine on the drug-induced depolarization of isolated superior cervical ganglia. *Br. J. Pharmac.*, **26**, 521–537.

BROWN, D. A., HALLIWALL, J. V., JONES, K. B. & QUILLIAM, J. P. (1970). An attempt to determine whether acetylcholine can release acetylcholine from a sympathetic ganglion. *Br. J. Pharmac.*, **38**, 446–449.

BROWNLEE, G. & JOHNSON, E. S. (1963). The site of the 5-hydroxytryptamine receptor on the intramural nervous plexus of the guinea-pig isolated ileum. *Br. J. Pharmac.*, **21**, 306–322.

BROWNLEE, G. & JOHNSON, E. S. (1965). The release of acetylcholine from the isolated ileum of the guinea-pig induced by 5-hydroxytryptamine and dimethylphenylpiperazinium. *Br. J. Pharmac. Chemother.*, **24**, 689–700.

DANIEL, E. E. (1966). Electrical and contractive responses of the pyloric region to adrenergic and cholinergic drugs. *Can. J. Physiol. Pharmac.*, **44**, 951–979.

DAY, M. & VANE, J. R. (1963). An analysis of the direct and indirect actions of drugs on the isolated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **20**, 150–170.

ELMQUIST, D. & FELDMAN, D. S. (1965). Spontaneous activity at a mammalian neuromuscular junction in tetrodotoxin. *Acta physiol. Scand.*, **64**, 475–477.

ERSPAMER, V. (1970). Progress report: Caerulein. *Gut*, **11**, 79–87.

GERSHON, M. D. (1967). Effects of tetrodotoxin on innervated smooth muscle preparation. *Br. J. Pharmac. Chemother.*, **29**, 259–279.

GROSSMAN, M. I. (1970). Gastrin and its activities. *Nature*, **228**, 1147–1150.

HEBB, C. O., KRNEJVIĆ, K. & SILVER, A. (1963). Effect of undercutting on the acetylcholine esterase and choline acetyltransferase activity in the cat's cerebral cortex. *Nature, Lond.*, **198**, 692.

KATZ, B. & MILEDI, R. (1967). Tetrodotoxin and neuromuscular transmission. *Proc. R. Soc. B.*, **167**, 8–22.

KNOLL, J. & VIZI, E. S. (1970). Presynaptic inhibition of acetylcholine release by endogenous and exogenous noradrenaline at high rate of stimulation. *Br. J. Pharmac.*, **40**, 554–555*P*.

KNOLL, J. & VIZI, E. S. (1971). Effect of frequency of stimulation on the inhibition by noradrenaline of the acetylcholine output from parasympathetic nerve terminals. *Br. J. Pharmac.*, **42**, 263–272.

KOSTERLITZ, H. W., LYDEN, R. J. & WATT, A. J. (1970). The effects of adrenaline, noradrenaline and isoprenaline on inhibitory α- and β-adrenoceptors in the longitudinal muscle of the guinea-pig ileum. *Br. J. Pharmac.*, **39**, 398–413.

MCDOUGAL, M. D. & WEST, G. B. (1954). The inhibition of the peristaltic reflex by sympathomimetic amines. *Br. J. Pharmac. Chemother.*, **9**, 131–137.

ONDETTI, M. A., RUBIN, B., ENGEL, S. L., PLUSCEC, J. & SHEEHAN, J. T. (1970). Cholecystokinin-pancreozymin: recent developments. *Digestive Diseases*, **15**, 149–156.

PATON, W. D. M. & PERRY, W. L. M. (1953). The relationship between depolarization and block in the cat's superior ganglion. *J. Physiol., Lond.*, **119**, 43–57.

PATON, W. D. M. & VIZI, E. S. (1969). The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig longitudinal muscle strip. *Br. J. Pharmac.*, **35**, 10–28.

PATON, W. D. M., VIZI, E. S. & ZAR, M. ABOO (1971). The mechanism of acetylcholine release from parasympathetic nerves. *J. Physiol., Lond.*, **215**, 819–848.

PATON, W. D. M. & ZAR, M. ABOO (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. *J. Physiol., Lond.*, **194**, 13–33.

PERRY, W. L. M. & TALESNIK, J. (1953). The role of acetylcholine in synaptic transmission at parasympathetic ganglia. *J. Physiol., Lond.*, **119**, 455–469.

SAAMELI, K. (1967). Untersuchungen mit β-receptorenblockern am isolierten Meerschweichenvorhof. *Helv. Physiol. Acta*, **25**, CR 219–CR 221.

TRENDELENBURG, U. (1951). Reaktion sympathischer Ganglien während der Ganglienblockade durch Nicotine. *Arch. exp. Path. Pharmac.*, **230**, 448–465.

TRENDELENBURG, U. (1959). Non-nicotinic ganglion-stimulating substances. *Fedn Proc.*, **18**, 1001–1005.

TRENDELENGURG, U. (1966). Observations on the ganglion-stimulating action of angiotensin and bradykinin. *J. Pharmac. exp. Ther.*, **154**, 418–425.

VIZI, E. S. (1972). Stimulation, by inhibition of (Na^+-K^+-Mg^{2+})-activated ATP-ase, of acetylcholine release in cortical slices from rat brain. *J. Physiol., Lond.*, **226**, 95–117.

VIZI, E. S., BERTACCINI, G., IMPICCIATORE, M. & KNOLL, J. (1972a). Acetylcholine-releasing effect of gastrin and related polypeptides. *Eur. J. Pharmac.*, **17**, 175–178.

VIZI, E. S., BERTACCINI, G., IMPICCIATORE, M. & KNOLL, J. (1972b). Evidence that acetylcholine released by gastrin and related polypeptides contributes to their effect on gastrointestinal motility. *Gastroenterology*. (In press).

VIZI, E. S., ILLÉS, P., RÓNAI, A. & KNOLL, J. (1972). The effect of lithium on acetylcholine release and synthesis. *Neuropharmac.*, **11**, 521–530.

VIZI, E. S. & KNOLL, J. (1971). The effects of sympathetic nerve stimulation and quanethidine on parasympathetic neuroeffector transmission; the inhibition of acetylcholine release. *J. Pharmac. Pharm.*, **23**, 918–925.

VIZI, E. S. & KNOLL, J. (1972). The mechanism of acetylcholine release by nicotine. In Symposium on *Experimental Tremor*. Special Publ. Vol. **17**, 131–150. Sarajevo.

(*Received August* 15, 1972)