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Analysis of the mechanism of action of some ganglion-blocking drugs in the rabbit superior cervical ganglion

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Summary

1. Mechanisms of action of hexamethonium, mecamylamine and (+)-tubocurarine on the rabbit superior cervical ganglion were investigated by intracellular recording techniques.

2. In concentrations up to 1 mM, none of these drugs affected the resting membrane potential nor altered the excitability of the postganglionic neurone to direct or antidromic stimulation.

3. Post-tetanic potentiation of the excitatory postsynaptic potential (e.p.s.p.) was inhibited by mecamylamine (10–100 μM) but not affected by either hexamethonium (5–100 μM) or (+)-tubocurarine (10–50 μM).

4. The decline in amplitude of successive e.p.s.ps in a train (40 Hz) was not influenced by hexamethonium or (+)-tubocurarine but was greatly exaggerated in the presence of mecamylamine; desensitization of the receptors for acetylcholine was excluded as a possible explanation for this latter finding.

5. Mecamylamine depressed the quantal content of e.p.s.ps in a train, with the exception of the first e.p.s.p. which had an increased quantal content.

6. Reduction in quantal content was attributed to a substantial fall in the size of the store of quanta of transmitter immediately available for release and to a reduction in the rate of mobilization of acetylcholine into that store; mecamylamine also caused a simultaneous increase in the fractional release.

7. Hexamethonium and (+)-tubocurarine had no effect on transmitter release.

8. The time-course of presynaptic effects of mecamylamine was similar to the duration of its postsynaptic blocking action.

9. It is concluded that inhibition of ganglionic transmission by mecamylamine is due to both presynaptic and postsynaptic inhibitory actions; in contrast, hexamethonium and (+)-tubocurarine reduce transmission solely by their postsynaptic actions.

Introduction

Indirect measurements of ganglionic responses have often been used in analyses of the mechanisms of action of ganglion-blocking agents (e.g. Paton & Zaimis,

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1951; Trendelenburg, 1956; McIsaac & Millerschoen, 1963). Results obtained from such experiments and even with extracellular recording of electrical activity of ganglion cells (Eccles, 1952; Paton & Perry, 1953; Volle, 1962; Kosterlitz, Lees & Wallis, 1970) give little information as to the mechanisms and sites of action of drugs affecting ganglionic transmission. A more direct approach would be to estimate changes in the spontaneous and evoked output of acetylcholine caused by these drugs. This method, however, is not suited to the determination of the output per pulse in a train because the amounts released are so small that a value for only the average output per pulse can be derived (Birks & MacIntosh, 1961; Matthews, 1963; Lees, 1968). Of ganglion-blocking drugs which are commonly used, only tetraethylammonium has been shown to have a presynaptic action in addition to its blocking action on nicotinic receptors in mammalian autonomic ganglia (Mason & Wien, 1955; Corne & Edge, 1958; Douglas & Lywood, 1961; Matthews & Quilliam, 1964; Matthews, 1966). In order to investigate more fully the mechanisms by which hexamethonium, (+)-tubocurarine and mecamylamine inhibit ganglionic transmission, we have used conventional intracellular recording techniques and analysed the data by the method of Elmqvist & Quastel (1965); Christ & Nishi (1971a and b) have shown recently that this method may be applied successfully to investigate possible presynaptic actions of drugs in a sympathetic ganglion. Our preliminary findings of a presynaptic action of mecamylamine have been reported elsewhere (Lees & Nishi, 1971).

Methods

Young adult New Zealand White rabbits of either sex were killed by air embolism. The superior cervical ganglion, together with the pre-ganglionic nerve and the two main postganglionic trunks (external and internal carotid nerves) was rapidly excised and transferred to the recording chamber. The ganglion was superfused with a solution of the following composition (mM): NaCl 136·9, KCl 5·0, $CaCl_2$ 2·0, $MgCl_2$ 0·5, NaH_2PO_4 1·0, $NaHCO_3$ 12·0 and glucose 11·1; it was equilibrated with 95% O_2 and 5% CO_2 (pH 7·19) or, in some experiments, with 100% O_2 (pH 7·55). The solution was maintained at a constant temperature between 36 and 38° C. Recordings of intracellular potentials were made with 3 M KCl microelectrodes, with resistances greater than 30 MΩ, inserted into the body of the postganglionic neurone, as previously described (Nishi & Koketsu, 1960; Christ & Nishi, 1971a).

Possible presynaptic actions of the ganglion-blocking drugs were investigated on post-tetanic potentiation. Conditioning tetani were delivered to the preganglionic nerve at a frequency at 40 Hz for 10 s, with an interval of 5 min or more between tetani; this frequency was chosen since it produced the most consistent post-tetanic potentiation. Before and after the tetanus, test stimuli eliciting a maximal excitatory postsynaptic potential (e.p.s.p.) were given at a frequency of 0·3 or 0·5 Hz; neither of these frequencies of stimulation by itself caused a demonstrable facilitation of transmission. In experiments on post-tetanic potentiation, choline chloride (10 μM) was added to the bathing solution in order to ensure an adequate supply of substrate for acetylcholine synthesis during frequent repetitive stimulation (Birks & MacIntosh, 1961; Matthews, 1963; Lees, 1968). For each investigation, only those cells were used which had an e.p.s.p. with a rapid rising phase; this type of cell response implied that the presynaptic input was well localized and near the

soma of the cell impaled. When necessary, the cell was hyperpolarized by passing current through the recording electrode until the generation of a ganglionic action potential was suppressed (Fig. 1).

For the analysis of effects of drugs on the release of acetylcholine, the following measurements and calculations were made on each train of stimuli, containing at least 50 e.p.s.ps, at a frequency of 40 Hz, the interval between trains being 2–5 minutes. The amplitude (V) of each e.p.s.p. corrected for non-linear summation (Martin, 1955) was derived from observed values, assuming an equilibrium potential of −10 mV for the e.p.s.p. A train was considered as consisting of a head (initial ten e.p.s.ps) and a tail, which was subdivided into groups of five consecutive e.p.s.ps. In the calculation of the variance of e.p.s.p. amplitude of each group, the corrected values of the amplitudes of e.p.s.ps in the tail were used. The quantal size, which is a measure of the response of the subsynaptic membrane to a single quantum of the transmitter, was derived by dividing the variance of each group by the mean e.p.s.p. amplitude of that group; the mean quantal size (q) was obtained by dividing the sum of quantal sizes for all groups by the number of groups. The quantal content (m) of each e.p.s.p. in the head and of each group in the tail of the train was obtained (V/q). All values were calculated with the aid of a CDC 6400 computer. The drugs used were hexamethonium bromide (City Chemicals), mecamylamine hydrochloride (Merck) and (+)-tubocurarine chloride (Calbiochem).

Results

Effect of drugs on membrane properties of post-ganglionic neurone

The minimum effective concentrations of hexamethonium, mecamylamine and (+)-tubocurarine were about 1 μM but for consistent reductions in amplitude of

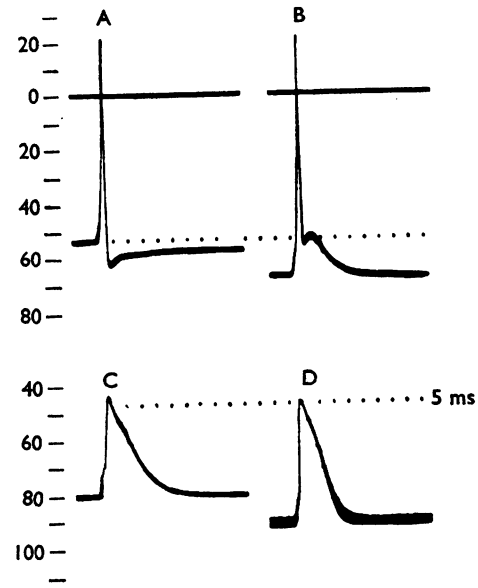

FIG. 1. Suppression of generation of ganglionic action potential by hyperpolarization. Calibrations: vertical, membrane potential (mV); horizontal, intervals of 5 ms, also indicating original membrane potential. A, action potential at resting membrane potential; B, C and D, progressive hyperpolarization with loss of action potential in C and D. D, two superimposed excitatory postsynaptic potentials.

excitatory postsynaptic potentials (e.p.s.ps) higher concentrations (3–30 μM) were required. In concentrations as high as 1 mM, none of the drugs caused consistent changes in the resting potential; there were no effects on the amplitude and duration of the ganglionic actions potential elicited by direct stimulation of the soma, nor on the threshold for its generation. There was no reduction in the amplitude of the antidromically-conducted action potential. The time-course for recovery of cell membrane excitability after single antidromic or direct responses was also not affected.

Post-tetanic potentiation

Unlike hexamethonium (10–100 μM) and (+)-tubocurarine (10–50 μM), mecamylamine (10–1,000 μM) had an effect on post-tetanic potentiation (Figs. 2 and 3). This inhibitory effect was observable at a time when the e.p.s.ps were only slightly reduced in amplitude (Fig. 2); recovery of post-tetanic potentiation

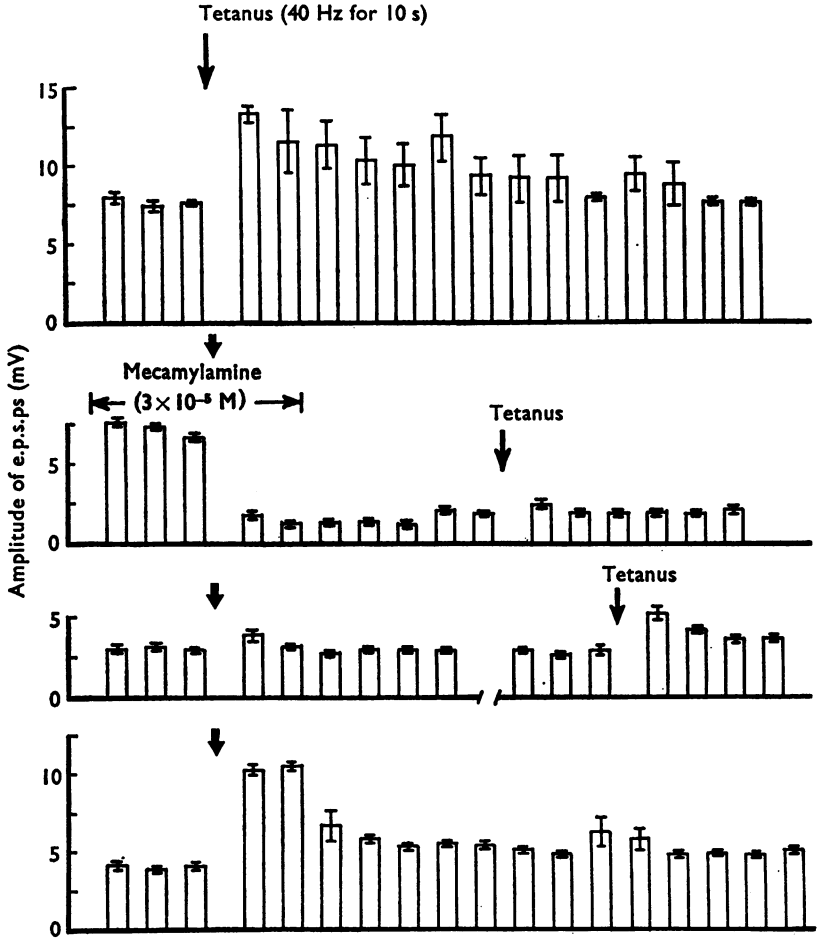

FIG. 2. Inhibition by mecamylamine of post-tetanic potentiation of excitatory postsynaptic potentials (e.p.s.ps.). Ordinates amplitude of e.p.s.ps. (mV). Except during periods of tetanic stimulation at 40 Hz for 10 s (arrows), e.p.s.ps were elicited at a frequency of 0·3 Hz and the bars show that mean amplitude (±S.E.M.) of consecutive groups of 5 e.p.s.ps. After cessation of exposure to mecamylamine (30 μM for 2 min) tetani given at 105 s, 5, 20 and 35 minutes. All records were taken from the same cell. Note that e.p.s.ps immediately after tetanus in presence of mecamylamine were of greatly reduced amplitude.

followed the time-course of recovery of the amplitude of e.p.s.ps. Thus it was not possible to separate clearly the time-course of pre- and postsynaptic actions of mecamylamine. Since recovery of the amplitude of e.p.s.ps was not observed in cells exposed to mecamylamine in concentrations of 100 μM or greater, concentrations of 10 and 30 μM were used in the majority of experiments with mecamylamine. An additional and consistent finding with mecamylamine was a rapid and progressive decline in amplitude of e.p.s.ps during the conditioning tetanus (Fig. 4); this was not observed with (+)-tubocurarine (Fig. 5) or hexamethonium. In order to exclude the possibility that the cause of the reduction was a desensitization of the subsynaptic membrane to acetylcholine, two types of experiments were performed. In the first, the effect of the conditioning tetanus was tested on the postsynaptic response to acetylcholine applied iontophoretically. As can be seen in Fig. 6, the amplitude of the acetylcholine potential in the presence of mecamylamine (10 μM) was not reduced when the potential was elicited im-

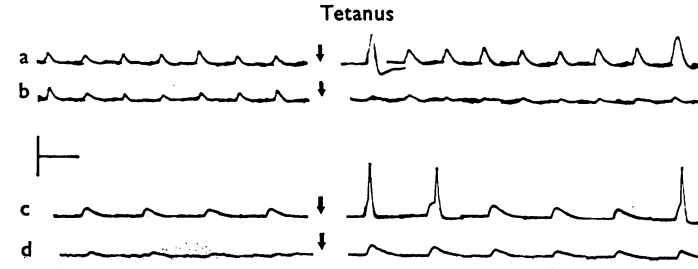

FIG. 3. Comparison of effect of mecamylamine and hexamethonium on post-tetanic potentiation. (a) Control test stimuli (0·5 Hz) before and immediately following conditioning tetanus (40 Hz). (b) Test stimuli (0·5 Hz) in presence of mecamylamine (10 μM for 4 min) before and after tetanus. Note depression of excitatory postsynaptic potentials after tetanus. In a different cell (c) control test stimuli (0·3 Hz) before and immediately following conditioning tetanus (40 Hz). (d) Test stimuli (0·3 Hz) in presence of hexamethonium (10 μM for 10 min) before and after tetanus. Both sets of records obtained on continuously moving film; slightly retouched in editing. Calibrations: (a), (b) 20 mV and 10 ms; (c) 40 mV and 5 ms; (d) 20 mV and 5 ms.

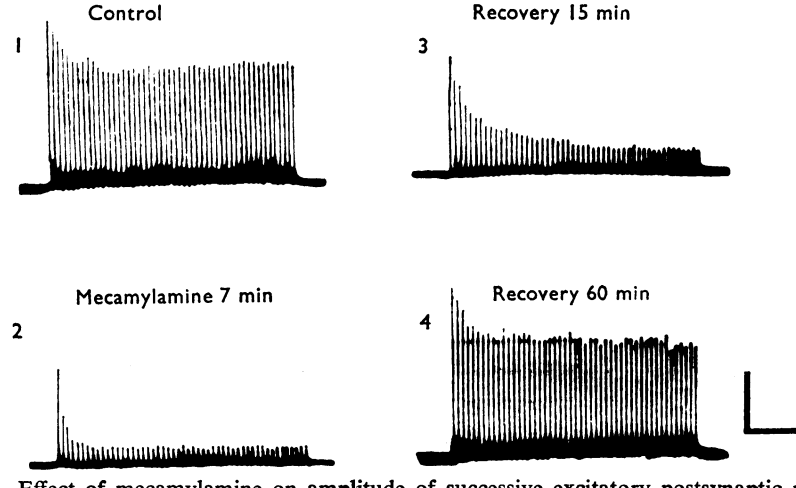

FIG. 4. Effect of mecamylamine on amplitude of successive excitatory postsynaptic potentials in a train (40 Hz). 1, Control; 2, after exposure to mecamylamine (10 μM) for 7 min; 3 and 4, recovery at 15 and 60 min, respectively, after cessation of exposure to mecamylamine. All records were taken from the same cell. Calibrations 20 mV and 300 ms.

mediately after the early tetanic run-down. In the second type, the membrane response to acetylcholine given iontophoretically at a rate of 2 Hz was tested. There was no reduction in the amplitude of the acetylcholine potential with the repeated application of acetylcholine in the presence of mecamylamine (10 μM) (Fig. 7).

Effect on quantal content of excitatory postsynaptic potentials of tetanus

Since, in the presence of mecamylamine, the excessive reduction in amplitude of e.p.s.ps of the conditioning tetanus must have been due to a reduction in amount of acetylcholine released, it was necessary to explore the possible reasons for its

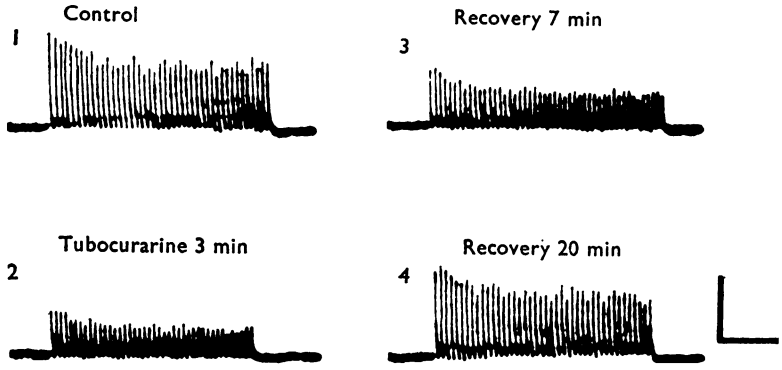

FIG. 5. Lack of effect of (+)-tubocurarine on amplitude of successive excitatory postsynaptic potentials in a train (40 Hz). 1, Control; 2, after exposure to (+)-tubocurarine (10 μM) for 3 min; 3 and 4, recovery at 7 and 20 min, respectively, after cessation of exposure to (+)-tubocurarine. All records were taken from the same cell. Calibrations 20 mV and 300 ms.

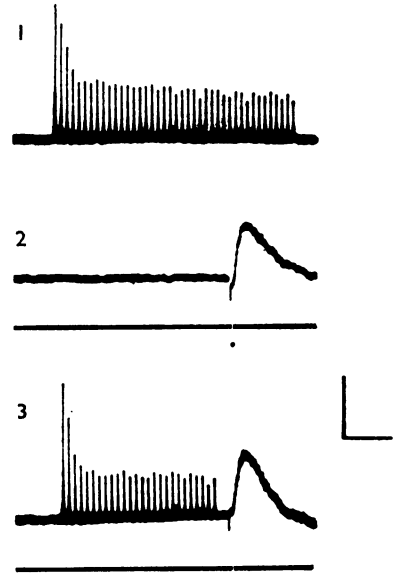

FIG. 6. Response of ganglion cell to acetylcholine in presence of mecamylamine (10 μM). 1, Tetanus at 40 Hz; 2, control cell response to iontophoretic application of acetylcholine; 3, tetanus at 40 Hz followed by iontophoretic application of acetylcholine at time of maximal depression of amplitude of excitatory postsynaptic potentials. Lower trace in records 2 and 3 shows the current pulse (55 nA for 10 ms) for acetylcholine ejection. All records were taken from the same cell. Calibrations 10 mV and 200 ms.

occurrence. The quantal content (m) of e.p.s.ps reflects the amount of transmitter released with each presynaptic action potential. In order to calculate the quantal content of each e.p.s.p. in a train according to the method of Elmqvist & Quastel (1965) (see **Methods**), we have made use of the finding of Christ & Nishi (1971a and b) that the quantal size of e.p.s.ps calculated statistically by the variance method (q), corresponds closely to the mean amplitude of spontaneously occurring miniature e.p.s.ps. It can be seen that, under control conditions, the quantal content falls rapidly, but only to a small extent, before the value is maintained relatively constant, probably because mobilization of acetylcholine then matches release (Fig. 8). A surprising, but consistent finding was that, in the presence of mecamylamine, the quantal content of the first e.p.s.p. in a train was greatly increased. Thereafter, m was less than in the corresponding control e.p.s.p. and declined much more rapidly (Fig. 8). The increase in m of the first e.p.s.p. of a train was not usually recognizable with intervals of less than 2 min between trains.

The information can be expressed in another form. The quantal content (m) of e.p.s.ps is dependent on the number of quanta which are immediately available for release (n) and the proportion of that number which can be released (p), according to the relationship $m=np$. When m of each e.p.s.p. in the head of the train is plotted against the cumulative output, i.e. the sum of previous m in the train (Σm), it is found that there is a close approximation to a linear relationship between m and Σm for the initial few e.p.s.ps (Fig. 9). This is what should be expected if p is constant and if the release is occurring at a rate too great for mobilization of acetylcholine from a main store to make a significant contribution to n, the amount immediately available for release. If these assumptions are correct, the intercept on the x-axis may give a good estimate of n. As can be seen from Fig. 8 and Table 1, mecamylamine depressed n to about 40% of its control value; since this depression, together with a reduction in the rate of

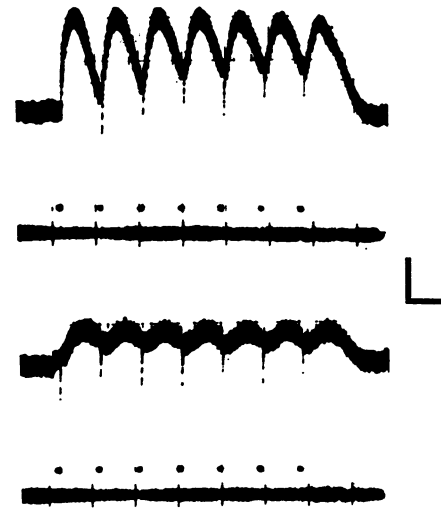

FIG. 7. Membrane responses to rapid repetitive application of acetylcholine. Acetylcholine potentials (upper traces) induced at 2 Hz by applying current pulses (lower traces) of 61 nA for 20 ms through the acetylcholine electrode. 1, Before, and 2, 10 min after, exposure of ganglion to mecamylamine (10 μM). Calibrations 10 mV and 200 ms.

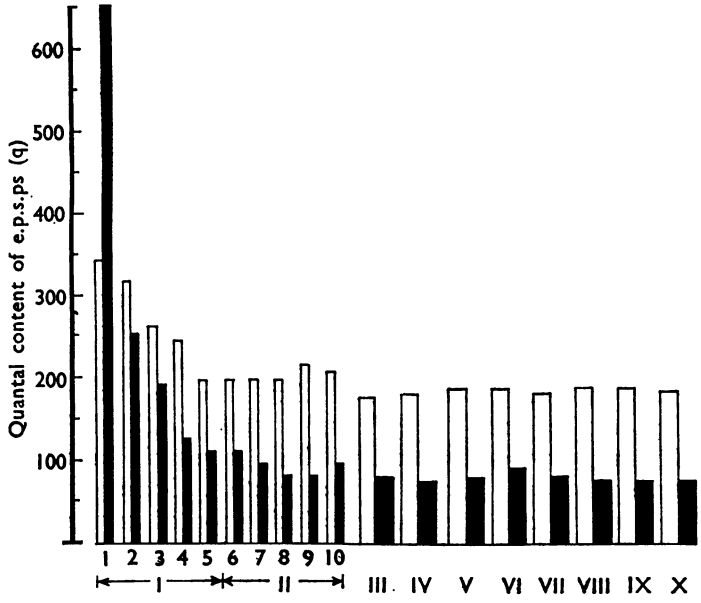

FIG. 8. Quantal content of excitatory postsynaptic potentials (e.p.s.ps) in a train (40 Hz). Ordinates, quantal content of e.p.s.ps (quanta). Abscissae, 50 e.p.s.ps in groups for analysis (for details see **Methods**). Histogram, quantal content or mean quantal content. Open columns, control; black columns, mecamylamine (10 μM).

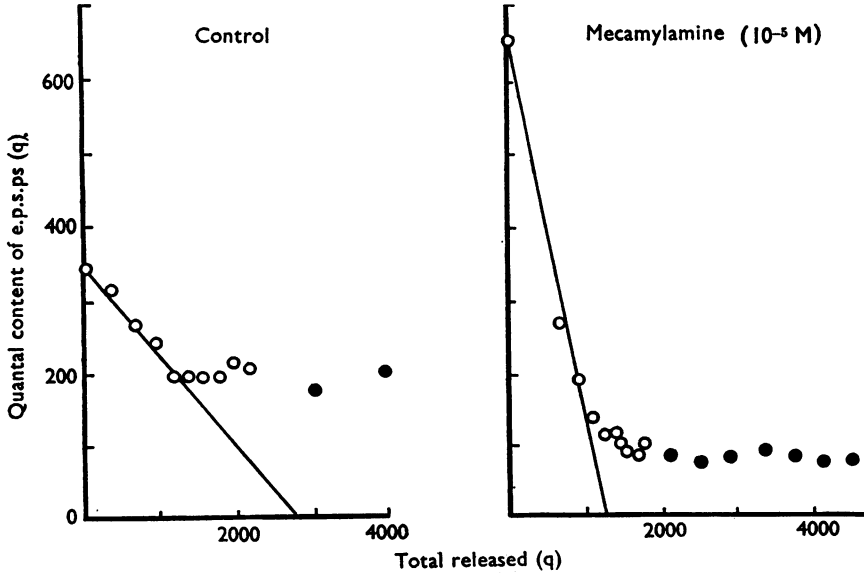

FIG. 9. Effect of mecamylamine (10 μM) on quantal content of excitatory postsynaptic potentials (e.p.s.ps) in a train (40 Hz). Ordinates, quantal content of e.p.s.ps (quanta). Abscissae, sum of quantal contents of all previous e.p.s.ps (quanta). Open circles, initial ten e.p.s.ps of train; closed circles, groups of five consecutive e.p.s.ps (for details see **Methods**).

mobilization (quantal content/stimulus interval), is greater than the simultaneous opposing increase in p, the fractional release, the net effect is a reduction in the quantal content of e.p.s.ps other than the first in a train. Similar experiments in other preparations demonstrated that (+)-tubocurarine and hexamethonium did not alter n or p, and therefore, did not affect m (Tables 2 and 3).

Discussion

Since hexamethonium and (+)-tubocurarine had no effect on the resting membrane potential, on the threshold for generation of the ganglionic action potential elicited by direct stimulation of the cell, on the amplitude of the direct spike or of the antidromically-conducted action potential nor on the recovery of excitability of the postganglionic neurone, it may be safely concluded that the principal postsynaptic mechanism by which these drugs inhibit ganglionic transmission is a non-depolarizing type of occupation of nicotinic receptors. Since post-tetanic potentiation is predominantly due to a presynaptic facilitation of transmission, it may be used as a screening test for possible presynaptic actions of drugs. In confirmation of the idea that hexamethonium and (+)-tubocurarine do not have a presynaptic blocking effect, it was found that there was no reduction in post-tetanic potentiation; furthermore, there was no inhibition of release of neurotransmitter substance during repetitive stimulation in the presence of concentrations of hexamethonium and (+)-tubocurarine which depressed the amplitude of e.p.s.ps.

TABLE 1. *Effects of mecamylamine on quantal content of excitatory postsynaptic potentials (e.p.s.ps) in rabbit superior cervical ganglion (mean values of 6 experiments)*

	Quantal content (m) of first e.p.s.p. quanta	Available store (n) quanta	Fractional release (p)	Quantal size (q) mV	Mobilization rate quanta/ms
Control	340	3,144	0·11	0·15	4·68
Mecamylamine 10 μM (10 min)	494† (+46%)	1,854* (−41%)	0·28* (+157%)	0·04† (−72%)	2·65* (−43%)
Recovery (30 min)	414† (+22%)	2,494* (−21%)	0·20 (+85%)	0·09 (−40%)	3·47* (−27%)

* Difference is significant at $P=0·01$ as compared to control. † Difference is significant at $P=0·05$ as compared to control.

TABLE 2. *Effects of hexamethonium on quantal content of excitatory postsynaptic potentials (e.p.s.ps) in rabbit superior cervical ganglion (mean values of 4 experiments)*

	Quantal content (m) of first e.p.s.p. quanta	Available store (n) quanta	Fractional release (p)	Quantal size (q) mV	Mobilization rate quanta/ms
Control	256	3,139	0·09	0·10	3·88
Hexamethonium 50 μM (10 min)	269	3,561	0·08	0·05*	3·93
Recovery (20 min)	272	3,486	0·08	0·09†	3·86

* Difference is significant at $P=0·01$ as compared to control. † Difference is significant at $P=0·05$ as compared to control.

Mecamylamine, however, has a more complex mode of action in inhibiting ganglionic transmission. In common with hexamethonium and (+)-tubocurarine, mecamylamine did not affect the postganglionic neurone except to depress its response to acetylcholine. When the amplitude of e.p.s.ps was depressed, even minimally, post-tetanic potentiation was inhibited by mecamylamine. It was shown that a desensitization of the subsynaptic membrane to acetylcholine cannot be a possible explanation of the depression of post-tetanic potentiation or of the marked tetanic run-down in the presence of mecamylamine, since the depolarization by acetylcholine applied iontophoretically was not reduced at a time when the e.p.s.p. amplitude had declined. In this connexion, it is of interest that mecamylamine does not inhibit post-tetanic potentiation of the neuromuscular junction (Bennett, Tyler & Zaimis, 1957).

TABLE 3. *Effects of (+)-tubocurarine on quantal content of excitatory postsynaptic potentials (e.p.s.ps) in rabbit superior cervical ganglion (mean values of 4 experiments)*

	Quantal content (m) of first e.p.s.p. quanta	Available store (n) quanta	Fractional release (p)	Quantal size (q) mV	Mobilization rate quanta/ms
Control	222	2,940	0·08	0·09	4·02
(+)-Tubocurarine 10 μM (10 min)	252	2,924	0·08	0·05*	3·58
Recovery (20 min)	234	2,970	0·08	0·08†	4·06

* Difference is significant at $P=0·01$ as compared to control. † Difference is significant at $P=0·05$ as compared to control.

For detailed analysis of possible presynaptic actions of these drugs, it has been assumed that the release of acetylcholine continues to follow a Poisson distribution in the presence of the drugs; under normal circumstances, release of acetylcholine is Poisson distributed in the superior cervical ganglion of the rabbit (Christ & Nishi, 1971a). Since mecamylamine affects both the amount of acetylcholine immediately available for release and the fraction of that store which is released, and alters them in opposite ways, it is unlikely that these effects have a common, simple causation. At present, there is insufficient information at other junctions regarding the means by which drugs alter the amount of transmitter immediately available for release to comment on the possible mechanisms by which mecamylamine reduced n so readily in a sympathetic ganglion. Although it is known that mecamylamine can penetrate membranes easily by virtue of being a secondary amine, it cannot be decided on the basis of our results whether the location of the presynaptic action is intracellular or extracellular. Clues to the location of the presynaptic blocking effect may be obtained from a comparison of the actions of other non-quaternary ammonium compounds on ganglionic transmission. It would appear, however, from the results of the present investigation that inhibition of release of acetylcholine is not an essential property of 'ganglion-blocking' drugs. On the other hand, mecamylamine acts both pre- and postsynaptically in concentrations which inhibit ganglionic transmission.

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REFERENCES

BENNETT, G., TYLER, C. & ZAIMIS, E. (1957). Mecamylamine and its mode of action. *Lancet*, **2**, 218–222.

BIRKS, R. I. & MACINTOSH, F. C. (1961). Acetylcholine metabolism of a sympathetic ganglion. *Can. J. Biochem. Physiol.*, **39**, 787–827.

CHRIST, D. D. & NISHI, S. (1971a). Site of adrenaline blockade in the superior cervical ganglion of the rabbit. *J. Physiol., Lond.*, **213**, 107–117.

CHRIST, D. D. & NISHI, S. (1971b). Effects of adrenaline on nerve terminals in the superior cervical ganglion of the rabbit. *Br. J. Pharmac.*, **41**, 331–338.

CORNE, S. J. & EDGE, N. D. (1958). Pharmacological properties of pempidine (1:2:2:6:6-pentamethylpiperidine), a new ganglion blocking compound. *Br. J. Pharmac. Chemother.*, **13**, 339–349.

DOUGLAS, W. W. & LYWOOD, D. W. (1961). The stimulant effect of TEA on acetylcholine output from the superior cervical ganglion: comparison with barium. *Fedn Proc.*, **20**, 324.

ECCLES, R. M. (1952). Responses of isolated curarized sympathetic ganglia. *J. Physiol., Lond.*, **117**, 196–217.

ELMQVIST, D. & QUASTEL, D. M. J. (1965). A quantitative study of end-plate potentials in isolated human muscle. *J. Physiol., Lond.*, **178**, 505–529.

KOSTERLITZ, H. W., LEES, G. M. & WALLIS, D. I. (1970). Synaptic potentials recorded by the sucrose-gap method from the rabbit superior cervical ganglion. *Br. J. Pharmac.*, **40**, 275–293.

LEES, G. M. (1968). Synaptic transmission in mammalian sympathetic ganglia: a physiological and pharmacological investigation. Ph.D. Thesis, University of Aberdeen.

LEES, G. M. & NISHI, S. (1971). Electrophysiological evidence for a presynaptic action of a ganglion-blocking agent. *Proc. XXV Int. Congr. physiol. Sci.*, Munich, IX, 339.

MCISAAC, R. J. & MILLERSCHOEN, N. R. (1963). A comparison of the effects of mecamylamine and hexamethonium on transmission in the superior cervical ganglion of the cat. *J. Pharmac. exp. Ther.*, **139**, 18–24.

MARTIN, A. R. (1955). A further study of the statistical composition of the end-plate potential. *J. Physiol., Lond.*, **130**, 114–122.

MASON, D. F. J. & WIEN, R. (1955). The actions of heterocyclic bisquaternary compounds especially of a pyrrolidinium series. *Br. J. Pharmac. Chemother.*, **10**, 124–132.

MATTHEWS, E. K. (1963). The effects of choline and other factors on the release of acetylcholine from the stimulated perfused superior cervical ganglion of the cat. *Br. J. Pharmac. Chemother.*, **21**, 244–249.

MATTHEWS, E. K. (1966). The presynaptic effects of quaternary ammonium compounds on the acetylcholine metabolism of a sympathetic ganglion. *Br. J. Pharmac. Chemother.*, **26**, 552–566.

MATTHEWS, E. K. & QUILLIAM, J. P. (1964). Effects of central depressant drugs upon acetylcholine release. *Br. J. Pharmac. Chemother.*, **22**, 415–440.

NISHI, S. & KOKETSU, K. (1960). Electrical properties and activities of single sympathetic neurons in frogs. *J. cell. comp. Physiol.*, **55**, 15–30.

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

PATON, W. D. M. & ZAIMIS, E. J. (1951). Paralysis of autonomic ganglia by methonium salts. *Br. J. Pharmac. Chemother.*, **6**, 155–168.

TRENDELENBURG, U. (1956). The action of 5-hydroxytryptamine on the nictitating membrane and on the superior cervical ganglion of the cat. *Br. J. Pharmac. Chemother.*, **11**, 74–80.

VOLLE, R. L. (1962). The responses to ganglion stimulating and blocking drugs of cell groups within a sympathetic ganglion. *J. Pharmac. exp. Ther.*, **135**, 54–61.

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