COMPARATIVE STUDIES ON ANTI-NICOTINIC ACTION OF HEXAMETHONIUM, MECAMYLAMINE AND ADENOSINE IN THE GUINEA PIG ISOLATED ILEUM

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Accepted May 25, 1977

Abstract—The mechanism of anti-nicotinic actions of hexamethonium, mecamylamine and adenosine was investigated in guinea pig isolated ileum. Mecamylamine shifted the dose-response curves for nicotine to the right with a gradual depression. On the other hand, hexamethonium shifted the curves to the right without a depression and adenosine made only a gradual depression, suggesting the different modes of their anti-nicotinic actions. The transmurally-stimulated twitch response was unaffected, partially inhibited and abolished by hexamethonium, mecamylamine and adenosine, respectively. These three compounds also had little effect on direct muscle response to acetylcholine and on the acetylcholinesterase activity of the ileum. From these results, it is suggested that the antagonism to the effect of nicotine shown by mecamylamine does not appear to be a simple competitive blockade of ganglionic receptors as is the case with hexamethonium and that adenosine may antagonize the effect of nicotine non-competitively. The mechanism by which mecamylamine and adenosine showed anti-nicotinic action is discussed.

Stone et al. (1) found that a secondary amine, 3-methylaminoisocamphane hydrochloride (mecamylamine) had a potent ganglion-blocking action with a long duration and suggested the similarity of its action to a quarternary ammonium ion, hexamethonium. On the other hand, Bennet et al. (2) reported that the mode of action of mecamylamine completely differed from that of hexamethonium and pentolinium in that mecamylamine, a substance readily diffusible into cells, was not competitive with acetylcholine at the autonomic ganglia and neuromuscular junction. In the guinea pig isolated intestine, mecamylamine reportedly exerts non-competitively or competitively-non-competitive antagonism on the dose-response curves for nicotinic stimulants (3–6).

Adenosine, a potent coronary vasodilator, reduces acetylcholine release from post-ganglionic nerves and exerts an anti-nicotinic action (7–10). However, the mechanism by which mecamylamine and adenosine show anti-nicotinic action has not been fully characterized.

In an attempt to determine the mechanism of the anti-nicotinic action, effects on the contractile responses of the guinea pig isolated ileum to agonists such as nicotine, acetylcholine and 5-hydroxytryptamine, and to transmural stimulation were examined using hexamethonium as the reference compound.
MATERIALS AND METHODS

Construction of the dose-response curves for agonists

Male guinea pigs weighing 300–500 g were sacrificed by a blow on the head and a segment of the ileum was dissected at least 8 cm from the ileocaecal junction. A preparation (2–3 cm, unstretched) was suspended in Tyrode solution in a thermostatically controlled organ bath (10 ml capacity) at 37°C and gassed with oxygen. Responses of the ileum to drugs were recorded on a smoked drum with an isotonic frontal writing lever producing an approximately six-fold magnification and exerting a tension of 1 g. The preparation was allowed to stabilize for 40 to 50 min before addition of drugs. Acetylcholine was added by means of the cumulative dose method described by van Rossum et al. (11, 12) and nicotine and 5-hydroxytryptamine by the single dose method. In each experiment, the control dose-response curves were constructed for the agonist with five to six doses before and after the construction of the dose-response curves in the presence of antagonists, the second control curve being obtained 20 min after washing out the antagonists. The time of contact with the agonist was 30 sec and the interval between doses was 10 min. The antagonists were added to the organ bath 3–5 min before an addition of the agonist at 3–5 dose levels. The dose-response curves for the agonists were obtained by plotting the log concentration of the agonist used against the contraction expressed as a percentage of the control maximal contraction.

Transmural stimulation

Transmural stimulation was carried out by a technique essentially similar to described by Paton (13, 14). The electrodes were made of platinum and the intraluminal electrode was the anode. Rectangular pulses were used of 0.4 msec duration at a frequency of 0.1 Hz and strength sufficient to give a maximal response. The responses were recorded isometrically.

Measurement of the activity of acetylcholinesterase

The activity of the acetylcholinesterase of the guinea pig isolated ileum was determined by the method of Guenther and Klaus (15).

The drugs used were obtained from the following sources: acetylcholine chloride (Daiichi), nicotine tartrate (Wako), 5-hydroxytryptamine (Wako), hexamethonium chloride (Wako), mecamylamine hydrochloride (Sigma), adenosine (Kohjin), phenoxybenzamine hydrochloride (Tokyo Kasei), morphine hydrochloride (Takeda), tetrodotoxin (Sankyo), atropine sulphate (Merck), physostigmine sulphate (Merck). The doses refer to the weights of the salts.

RESULTS

Effects on the dose-response curves for nicotine

Complete dose-response curves for nicotine (2 × 10^-6–2 × 10^-3 M) were determined in the presence of hexamethonium, mecamylamine and adenosine. The curves were bell-shaped as described by Trendelenburg (3) and van Rossum (16). Fig. 1 shows the inhibitory
effects of hexamethonium, mecamylamine and adenosine on the dose-response curves for nicotine. The curves for nicotine were shifted to the right and depressed gradually as the concentration of mecamylamine increased ($5 \times 10^{-7} - 5 \times 10^{-6}$ M) (Fig. 1c). On the other hand, hexamethonium ($3.7 \times 10^{-6} - 3.7 \times 10^{-5}$ M) shifted the curves for nicotine to the right without the depression (Fig. 1a), and adenosine ($3 \times 10^{-6} - 3 \times 10^{-5}$ M) made only a gradual depression (Fig. 1b). These results indicate that their modes of anti-nicotinic action differ from each other.

$pA_2$ and $pD'_2$ values for these blockers were calculated from the shift and the depression of the dose-response curves for nicotine as described by previous workers (17, 18). $pA_2$ values for hexamethonium and mecamylamine were $5.95 \pm 0.04$ ($n=3$) and $6.68 \pm 0.09$ ($n=3$) respectively. $pD'_2$ values for mecamylamine and adenosine were $5.70 \pm 0.05$ ($n=3$) and $4.89 \pm 0.09$ ($n=3$) (mean $\pm$ s.e.). These values for hexamethonium and mecamylamine in the present experiment were reasonably close to those (hexamethonium: $pA_2=5.8$, mecamylamine: $pA_2=6.6$, $pD'_2=5.5$) obtained with the guinea pig intestine by van Rossum (16).

**Effects on the dose-response curves for acetylcholine**

The effects of hexamethonium, mecamylamine and adenosine on the dose-response
curves for acetylcholine are shown in Fig. 2. Mecamylamine did not affect the dose-response curves for acetylcholine at concentrations less than $10^{-5}$ M, whereas it depressed the maximal response by 10–20% at the concentration of $3 \times 10^{-5}$ M (Fig. 2c). The curves were unaffected by the presence of hexamethonium ($10^{-6}$–$10^{-4}$ M) (Fig. 2a). Adenosine did not affect the curves for acetylcholine at the concentrations less than $3 \times 10^{-5}$ M, while 10–30% inhibition of the maximal effect was observed at higher concentrations ($10^{-4}$–$3 \times 10^{-4}$ M) (Fig. 2b).

Effects on the dose-response curves for 5-hydroxytryptamine

Hexamethonium ($10^{-4}$ M) did not show any effect on the contractile response of the ileum to 5-hydroxytryptamine ($2 \times 10^{-6}$ M), whereas mecamylamine ($10^{-5}$ M) and adenosine ($10^{-5}$ M) reduced the response by 20–30% and by 40–50%, respectively (Fig. 3). In the presence of phenoxybenzamine ($10^{-6}$ M for 30 min), a D-receptor blocker of 5-hydroxytryptamine (19), mecamylamine ($10^{-5}$ M) significantly inhibited the contraction by 5-hydroxytryptamine ($2 \times 10^{-6}$ M) (inhibition rate; 45.1% without and 78.2% with mecamylamine, n=2), while in the presence of morphine ($3 \times 10^{-6}$ M), a M-receptor blocker (19), mecamylamine ($10^{-5}$ M) scarcely inhibited the morphine-resistant component of the contraction (inhibition rate; 75.6% without and 79.0% with mecamylamine, n=2). The phenoxybenzamine-resistant component was also blocked by atropine ($10^{-7}$ M). Adenosine ($10^{-5}$ M) showed a similar effect on the contraction by 5-hydroxytryptamine ($2 \times 10^{-6}$ M) in the presence of phenoxybenzamine ($10^{-6}$ M) or morphine ($3 \times 10^{-6}$ M) (inhibition rate; phenoxybenzamine: 56.1% without and 82.7% with adenosine, n=2, morphine: 66.3% without and 62.5% with adenosine, n=2).

Effects on the twitch response to transmural stimulation

Contraction of the ileum induced by transmural stimulation was blocked by morphine ($8 \times 10^{-6}$ M), tetrodotoxin ($3.1 \times 10^{-7}$ M) and atropine ($1.4 \times 10^{-7}$ M), and potentiated by eserine ($1.5 \times 10^{-7}$ M). Hexamethonium had little effect on the response at concentrations less than $10^{-4}$ M (Fig. 4). These results are in accord with previous findings (13, 14, 20) that contractions of the guinea pig isolated ileum by transmural stimulation involved the excitation of postganglionic cholinergic nerves and the consequent release of acetylcholine. Fig. 4 illustrates the effects of hexamethonium, mecamylamine and adenosine on the twitch response to transmural stimulation in the guinea pig isolated ileum. The dose-inhibition relationships of mecamylamine and adenosine are shown in Fig. 5. Mecamylamine reversibly
inhibited the twitch response by approximately 20\% at $10^{-6}$ M and by 30–40\% at $3 \times 10^{-6}$–$3 \times 10^{-5}$ M, but the remaining contraction was not abolished even at higher concentrations. On the other hand, adenosine showed a greater inhibitory effect than mecamylamine on the contraction as shown in Figs. 4 and 5. The inhibitory effect due to mecamylamine and adenosine disappeared with wash out.

**Effects on the acetylcholinesterase activity of the ileum**

Since inhibition of the twitch response by mecamylamine and adenosine in the guinea pig isolated ileum may be mediated by an activation of the acetylcholinesterase activity, effects on the activity were examined. Mecamylamine and adenosine had no effect on the acetylcholinesterase activity of the ileum at concentrations less than $10^{-5}$ and $3 \times 10^{-5}$ M, respectively.

**DISCUSSION**

The mechanism of the anti-nicotinic actions of mecamylamine and adenosine was investigated in the guinea pig isolated ileum and compared with that of hexamethonium.

Hexamethonium appears to produce a simple competitive blockade of ganglionic receptors since it shifted the dose-response curves for nicotine in parallel to the right.

Mecamylamine ($5 \times 10^{-7}$–$5 \times 10^{-6}$ M) shifted the dose-response curves for nicotine
to the right with the gradual depression. This is consistent with results reported by van Rossum (16) in which mecamylamine ($10^{-6}$-$10^{-5}$ M) caused a depression as well as a shift of the dose-response curves for nicotine in the guinea pig intestine. On the other hand, Sethi and Gulati (5) have shown that low concentrations ($6.0 \times 10^{-7}$, $1.9 \times 10^{-6}$ M) of mecamylamine produced only parallel shifts of the dose-response curves for nicotine and DMPP in guinea pig ileum. One possible explanation for this discrepancy is that they used a narrow concentration range of the nicotinic stimulants ($1.5 \times 10^{-6}$-$3 \times 10^{-5}$ M) for constructing the dose-response curves, whereas we used a wide range of nicotine ($2 \times 10^{-6}$-$2 \times 10^{-5}$ M) as did van Rossum (16). In fact, the dose-response curves for a narrow range of nicotine ($2 \times 10^{-6}$-$6 \times 10^{-5}$ M) seem to be shifted in parallel in the presence of mecamylamine ($5 \times 10^{-7}$-$1.5 \times 10^{-6}$ M) as shown in Fig. 1c.

In the present study, mecamylamine appears to act preferentially upon the intrinsic nerve of the ileum rather than on the smooth muscle since it antagonized the effects of nicotine without affecting direct muscle response to acetylcholine. This antagonism to nicotine, however, does not appear to be a simple competitive blockade of ganglionic receptors such as is seen with hexamethonium. The maximum response was depressed, perhaps resulting from an inhibitory effect upon the postganglionic nerves per se in addition to the blockade of ganglionic receptors. This concept is supported by the findings that mecamylamine produces (a) the partial depression of the twitch response to transmural stimulation, (b) considerable inhibition of the phenoxybenzamine-resistant component of the response to 5-hydroxytryptamine, attributable to intramural nerve stimulation (19), (c) no atropine-like effect and (d) no stimulation in acetylcholinesterase activity. In conclusion, it appears likely that mecamylamine may exert the anti-nicotinic action primarily through the blockade of the nicotinic receptor in intrinsic cholinergic ganglia and possibly partly through an inhibition of acetylcholine liberation from the intrinsic postganglionic nerves of the guinea pig ileum. In this connection, it is of interest that mecamylamine inhibits ganglionic transmission at both the presynaptic (acetylcholine release) and postsynaptic (ganglionic receptor) sites in the rabbit superior cervical ganglia, while hexamethonium inhibits it solely at the postsynaptic site (21).

Adenosine, on the other hand, seems to act preferentially upon the postganglionic nerves per se, reducing acetylcholine release since it abolished the transmurally induced twitch response, depressed the maximal response of the dose-response curves for nicotine (non-competitive antagonism) and had neither an atropine-like effect nor a stimulatory effect on acetylcholinesterase activity. In other words, these results suggest that adenosine antagonizes the effects of nicotine non-competitively by reducing acetylcholine liberation from the intrinsic postganglionic nerves of the guinea pig ileum. The proposal sites for the inhibition by these agents are shown schematically in Fig. 6.

The dose-response curves for agonists are commonly assumed to be modified by their antagonists in three different ways depending on the type of antagonism; competitive, non-competitive and competitively-non-competitive antagonism (4, 12, 22, 23). Competitive antagonists shift the dose-response curves in parallel to the right by competing with agonists...
for the same receptor site, while non-competitive antagonists depress the maximal effect to agonists by interfering with the allosteric site or the intermediate step so called "stimulus transfer" behind the agonist-receptor interaction. Competitively-non-competitive antagonist is the combined type of competitive and non-competitive antagonism, characterized by a parallel shift of the dose-response curves to the right with a concomitant depression of the maximal effect. Our results obtained with hexamethonium, adenosine and mecamylamine in the present study may also support this theory of modification in dose-response curves with the inhibitors.

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