J. Pharm. Pharmacol. 1980, 32: 97–100 Received June 13, 1979

Mechanism of mesaconitine-induced contractile response in guinea-pig ileum*

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Mesaconitine (MA) caused contractions of the guinea-pig isolated ileum in a dose-dependent manner (10^{-8}–10^{-5} g ml^{-1}), slightly potentiated the contractile response to acetylcholine (ACh) and histamine and enhanced responses to electrical stimulation. Repeated application of MA (10^{-5} g ml^{-1}) produced tachyphylaxis. Atropine blocked contractions to MA (3×10^{-7} g ml^{-1}), but only partially those to MA (10^{-5} g ml^{-1}). Morphine, strychnine and hemicholinium-3, but not hexamethonium, also inhibited MA-induced contractions. Contractions produced by both doses of MA were abolished by cocaine, tetrodotoxin, or noradrenaline, or by previous cooling of the ileum. The atropine-resistant contractions produced by MA (10^{-5} g ml^{-1}) were blocked by indomethacin. MA (3×10^{-7}–10^{-5} g ml^{-1}) elicited a dose-dependent release of ACh from the isolated ileum which was blocked by treatment with tetrodotoxin or cocaine, or exclusion of calcium ions from the bath. It is concluded that the contractions induced by lower doses of MA are brought about by the release of ACh from the postganglionic cholinergic nerve and that the contractions by higher doses could also be mediated by release of prostaglandins from the ileum.

We have previously investigated some pharmacological actions of certain *Aconitum* roots (Hikino et al 1979) as well as the aconitine alkaloids (Sato et al 1979b). These drugs induced contractions of the isolated vas deferens and the isolated ileum of the guinea-pig. The mechanism of the contractile response produced by mesaconitine (MA) in the guinea-pig vas deferens has been elucidated (Sato et al 1979a). In the present work we have examined the effect of this alkaloid on the guinea-pig isolated ileum. MA has been chosen as representative of the aconitine alkaloids because it is the main alkaloid of the aconitine analogues contained in the Japanese crude drug "bushi" prepared from the roots of certain species of *Aconitum* (Ranunculaceae) (Hikino et al 1977), its chemical structure (*N*-desethyl-*N*-methylaconitine) closely resembles that of aconitine, and its pharmacological actions are similar to those of aconitine but it is more active (Sato et al 1979b).

MATERIALS AND METHODS

Measurement of contraction of the guinea-pig isolated ileum

Guinea-pigs (Hartley strain, 300–500 g) were stunned, bled, the ileum excised and segments of 3 cm were taken from the middle region. The preparation was suspended in a 10 ml bath containing Tyrode solution of the following composition (mg ml^{-1}): sodium chloride, 8·0; potassium chloride, 0·20; calcium chloride, 0·26; magnesium chloride, 0·22; sodium bicarbonate, 1·0; sodium dihydrogenphosphate, 0·06 and glucose, 1·0. The calcium-free medium was prepared as above but calcium chloride was omitted from the solution.

The longitudinal contraction was recorded on a kymograph by an isotonic lever with an 8–10 magnification. Dose-response curves were by the cumulative technique for the agonists, acetylcholine (ACh) and histamine, and by the single dose technique for MA. Each experiment began with a dose-response curve to ACh. The medium was maintained at 28 °C and bubbled with air.

Electrical stimulation of the ileum was according to Paton (1957). Thus, rectangular current pulses of 1 ms duration and of sufficient strength (10–30 V) to produce a maximal response to a single shock (0·1 Hz) were applied; the intraluminal electrode was the anode. The longitudinal contraction was isometrically recorded on a polygraph through a force displacement transducer. A resting tension of 0·5 g was applied. During these experiments, the isolated preparation was suspended in a 50 ml bath containing Tyrode solution at 32 °C and bubbled with 5% CO_2 in oxygen. In other experiments, atropine, hexamethonium, morphine, strychnine, noradrenaline, cocaine, tetrodotoxin, papaverine, diphenhydramine or neostigmine was added to the bath 5–10 min before the MA, and hemicholinium-3 (5×10^{-5} g ml^{-1}) was added 30 min before the MA. The influence of MA on the ileal response to ACh or histamine was studied by adding MA (10^{-6} g ml^{-1}) to the bath medium 5 min before the agonists. In the

* Pharmaceutical studies on Aconitum roots—Part 6.
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experiment on cooling, the temperature of the bath medium was lowered to about 15 °C for 60–90 min.

Assay of acetylcholine released from the guinea-pig isolated ileum

The assay of ACh released by MA from the ileum into medium containing neostigmine (10^{-9} g ml^{-1}) was conducted on another strip of the ileum treated with neostigmine (10^{-9} g ml^{-1}) and tetrodotoxin (10^{-8} g ml^{-1}).

Statistical analysis of the data

Student's *t*-test for paired data was used to evaluate the results.

Drugs used

Acetylcholine chloride (ACh, Daiichi), histamine dihydrochloride (Wako Pure Chemical), atropine sulphate (Tanabe), hexamethonium bromide (Yamanouchi), diphenhydramine hydrochloride (Tanabe), strychnine nitrate (Hoei), cocaine hydrochloride (Sankyo), morphine hydrochloride (Takeda), hemicholinium-3 (Sigma), neostigmine bromide (Tokyo Kasei), papaverine hydrochloride (Wako Pure Chemical), indomethacin (Merk), (−)-noradrenaline hydrochloride (Sankyo), tetrodotoxin (TTX, donated from Prof. K. Tsuda) and mesaconitine hydrobromide (MA, prepared from aconite roots).

RESULTS

Pattern of contractile responses to mesaconitine

MA caused phasic contractions of the ileum followed, in most cases, by pendular movements. Contractions appeared almost immediately after the addition of MA, taking 0–3 min for full development and were dose-related from 10^{-8} to 10^{-5} g ml^{-1} (Fig. 1).

Tachyphylaxis developed when MA (10^{-5} g ml^{-1}) was administered at 10–15 min intervals (Fig. 2 B). The responses were reproducible after 30 min intervals. In determining the dose-response relationship, only the response to the first administration of MA to each preparation was used (Fig. 2 A), and then adjacent segments of the ileum were used.

Effect of various treatments (antagonists, cooling, calcium-free medium) on mesaconitine-induced contraction

Pretreatment with atropine (10^{-7} g ml^{-1}), strychnine (10^{-5} g ml^{-1}), morphine (10^{-5} g ml^{-1}), or hemicholinium-3 (5×10^{-5} g ml^{-1}) abolished the contractile response to a low dose of MA (3×10^{-7} g ml^{-1}) but did not completely inhibit the contractile

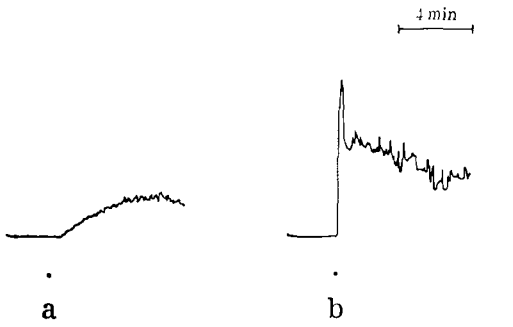

FIG. 1. Isotonic contractions induced by mesaconitine on the guinea-pig isolated ileum. a: mesaconitine (MA) 3×10^{-7} g ml^{-1}, b: MA 10^{-5} g ml^{-1}.

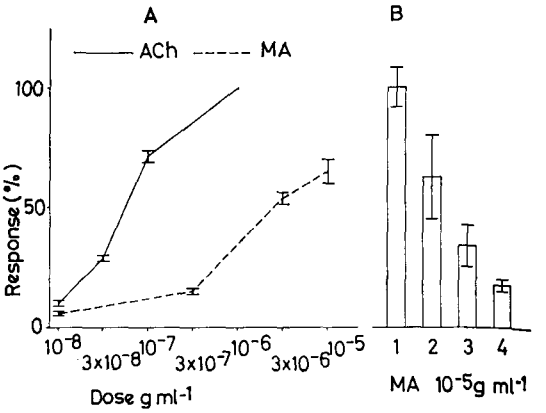

FIG. 2. Dose-dependent responses and tachyphylaxis of contractions induced by mesaconitine on the guinea-pig isolated ileum. A: Responses as % of maximal response to ACh to different doses of MA and ACh. In each preparation, only the response to the first administration of MA was considered. (n = 6, mean ± s.e.m.). B: Responses of the ileum to MA (10^{-5} g ml^{-1}) administered every 10 min and expressed as % of the first response. (n = 5, mean ± s.e.m.).

response to as high a dose of MA (10^{-5} g ml^{-1}) (Fig. 3). Pretreatment with hexamethonium (3×10^{-4} g ml^{-1}) did not modify any response to MA. Pretreatment with neostigmine (10^{-9} g ml^{-1}) for 10 min caused a potentiation of the contractile response to MA (3×10^{-4} and 10^{-5} g ml^{-1}) (Fig 3). Pretreatment with cocaine (10^{-5} g ml^{-1}), noradrenaline (10^{-5} g ml^{-1}), tetrodotoxin (3×10^{-8} g ml^{-1}) or papaverine (10^{-5} g ml^{-1}) completely abolished the response to MA at any dose. In the same way, after previous cooling of the ileum (15 °C, 60–90 min) or in a calcium-free medium, MA (3×10^{-7} or 10^{-5} g ml^{-1}) caused no contractions (Figs 3–4).

The atropine-resistant contractions induced by higher doses of MA (10^{-6}–10^{-5} g ml^{-1}) were prevented by pretreatment with indomethacin (3×10^{-6}

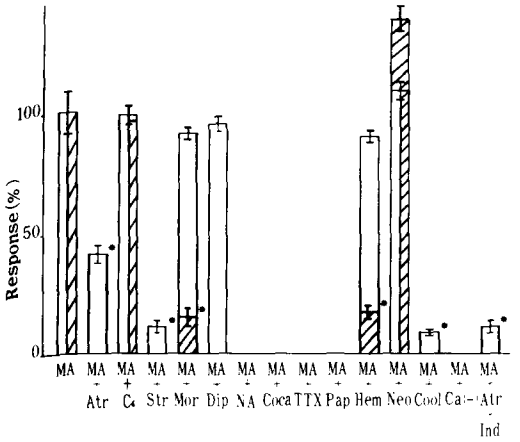

FIG. 3. Effect of various inhibitory agents on the contraction of the guinea-pig isolated ileum induced by mesaconitine at 3×10^{-7} g ml^{-1} (hatched columns) and 10^{-5} g ml^{-1} (open columns). The responses are expressed as % of the first response. The agents and treatments were mesaconitine (MA), atropine (Atr, 10^{-7} g ml^{-1}), hexamethonium (C_6, 3×10^{-4} g ml^{-1}), strychnine (Str, 10^{-5} g ml^{-1}), morphine (Mor, 10^{-5} g ml^{-1}), diphenhydramine (Dip, 2×10^{-6} g ml^{-1}), noradrenaline (NA, 10^{-5} g ml^{-1}), cocaine (Coca, 10^{-5} g ml^{-1}), tetrodotoxin (TTX, 3×10^{-8} g ml^{-1}), papaverine (Pap, 10^{-5} g ml^{-1}), hemicholinium-3 (Hem, 5×10^{-5} g ml^{-1}), neostigmine (Neo, 10^{-9} g ml^{-1}), indomethacin (Ind, 3×10^{-6} g ml^{-1}), previous cooling (Cool), and Ca-free medium (Ca(—)). Inhibitors were added 10–30 min before MA. (n = 5, mean ± s.e.m.). * Significantly different from the reference (MA 10^{-5} g ml^{-1}), $P < 0.01$.

g ml^{-1}) for 15 min in the presence of atropine (10^{-5} g ml^{-1}). In addition, the atropine-resistant contractions were completely blocked by the administration of cocaine, noradrenaline, tetrodotoxin or previous cooling of the ileum at 15 °C for 60–90 min, while they were not affected by diphenhydramine (2×10^{-6} g ml^{-1}) (Fig. 3).

Effect of mesaconitine on contractile responses of the isolated ileum to various stimuli

The effect of MA on the dose-response curve of ACh- and histamine-induced contractions is shown in Fig. 4. Responses to ACh and histamine were not affected significantly by application of MA (10^{-8}–10^{-5} g ml^{-1}).

MA (10^{-8}, 3×10^{-7} and 10^{-6} g ml^{-1}) potentiated the contractions induced by electrical stimulation, the responses being 115 ± 11, 124 ± 20 and 142 ± 16%* ($P < 0.05$), respectively, compared with the control (n = 5). The effects of MA (10^{-8} g ml^{-1}) were short-lasting (Fig. 5). MA (10^{-6} g ml^{-1}) had a dual effect, potentiation followed by progressive inhibition (60 ± 15%*, $P < 0.05$, compared with the

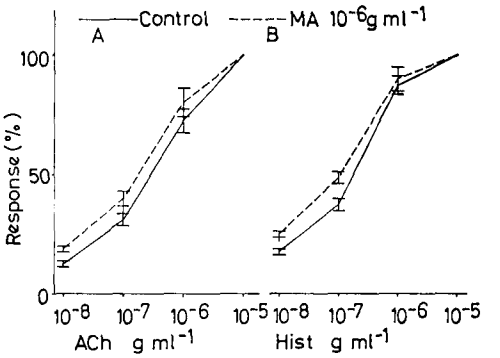

FIG. 4. Effect of mesaconitine on the responses of the guinea-pig isolated ileum to acetylcholine and histamine. The responses are expressed as % of the maximal response of each agonist (10^{-5} g ml^{-1}). (n = 5, mean ± s.e.m.).

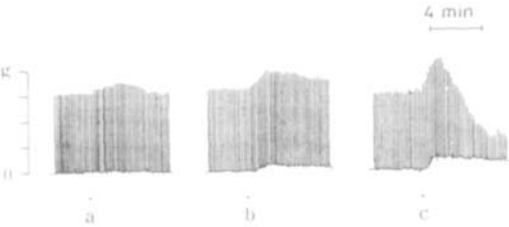

FIG. 5. Effect of mesaconitine on the contraction of the guinea-pig isolated ileum induced by electrical stimulation. a: mesaconitine (MA) 10^{-8} g ml^{-1}. b: MA 3×10^{-7} g ml^{-1}. c: MA 10^{-6} g ml^{-1}.

control, n = 5) after prolonged contact (5 min). This last effect was not easily reversible after washing MA from the bath.

Effect of various agents and procedures on release of ACh from the ileum

MA (3×10^{-7}–10^{-5} g ml^{-1}) increased the release of ACh from the isolated ileum (Table 1). Pretreatment with tetrodotoxin (3×10^{-8} g ml^{-1}) or cocaine (10^{-5} g ml^{-1}), or exclusion of calcium ion from the bath medium, considerably reduced the liberation of ACh by MA (10^{-5} g ml^{-1}) (Table 1).

DISCUSSION

Mesaconitine (MA) caused phasic contractions followed in most cases by pendular movements of the ileum. The action of lower doses of MA (10^{-8}–3×10^{-7} g ml^{-1}) on the ileum differed from that of higher doses of MA (10^{-6}–10^{-5} g ml^{-1}): the contractions induced by the lower doses were inhibited by atropine, whereas those by the higher doses were only partially inhibited.

Table 1. Effect of various treatments on acetylcholine release induced by mesaconitine in the guinea-pig isolated ileum.

Agent and procedure	Number of experiments	Amount of ACh release $\mu g\ g^{-1}$ of tissue (mean ± s.e.m.)
Control	6	4·1 ± 0·6
Mesaconitine (3×10^{-7} g ml^{-1})	6	6·8 ± 0·9*
Mesaconitine (10^{-5} g ml^{-1})	9	9·5 ± 2·2*
Cocaine (10^{-5} g ml^{-1}) + mesaconitine (10^{-5} g ml^{-1})	6	4·2 ± 1·0**
Tetrodotoxin (3×10^{-8} g ml^{-1}) + mesaconitine (10^{-5} g ml^{-1})	6	4·5 ± 0·8**
Ca-free medium + mesaconitine (10^{-5} g ml^{-1})	6	5·3 ± 1·1**

* Significantly different from the control, $P < 0.05$.
** Significantly different from the reference (mesaconitine 10^{-5} g ml^{-1}), $P < 0.05$.

Furthermore, contractions of the ileum induced by MA (3×10^{-7} g ml^{-1}) were blocked by tetrodotoxin or cocaine, previous cooling to 15 °C of the ileum or removal of calcium ion from the bath. This demonstrated that MA acts by stimulating the intramural nerves through a cholinergic calcium-dependent mechanism in the responses observed. Treatment with morphine, strychnine or noradrenaline, which inhibits the ACh release at the endings of postganglionic cholinergic neurons (Schaumann 1957; Takagi & Takayanagi 1966), almost completely abolished the contraction induced by the lower doses of MA, whereas hexamethonium failed to inhibit them. This points to an effect of MA at the postganglionic cholinergic nerves. This is confirmed by the facts that MA increased the ACh release by the ileum and that the effect is prevented by cocaine, tetrodotoxin or in the absence of calcium ions. Furthermore, MA slightly potentiated responses of the ileum to ACh, histamine and electrical stimulation which could be mainly due to excitation of the postsynaptic cell membrane.

The action of higher doses of MA on the ileum is similar to that observed at the lower doses, however, at the higher doses MA caused an atropine-resistant response which was prevented by indomethacin at a dose that not only has an inhibitory effect on prostaglandin synthesis (Vane 1971) but also interferes with ionic movements (Northover 1967, 1971). Its inhibitory effect on the responses of the ileum to indirect agonists has been largely described in the literature and explained by various theories (Lembeck & Juan 1974; Schulz & Cartwright 1976; Famaey et al 1977).

It is thus suggested that the contractile response of MA at the higher doses could be primarily brought about by the release of ACh from the postganglionic cholinergic nerve and partially by the release of prostaglandins from the ileum.

An important additional finding of the present study is the typical tachyphylaxis of the smooth muscle revealed by repeated applications of MA. The most probable mechanism underlying the tachyphylaxis is considered to be the blocking effect of action potentials in the nerve fibres by MA. This postulate may be supported by the present result that MA at the higher doses depressed the contraction of the ileum caused by the coaxial stimulation, and by the observation of Ellis & Bryant (1973) that inactivation of sodium conductance and blockage of action potentials in the frog sartorius muscle occurred when the depolarization induced by aconitine became large enough. Thus, repeated applications of MA might create a situation in which MA is accumulated, ultimately leading to partial or complete nerve block.

Acknowledgement

We wish to express our thanks to Prof. K. Tsuda, The Kyoritsu College of Pharmacy, for kind gift of tetrodotoxin.

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