Arecoline Excites the Colonic Smooth Muscle Motility *via* M_3 Receptor in Rabbits

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Abstract

Arecoline is an effective component of areca (betel nuts, a Chinese medicine named pinang or bing-lang). The purpose of this study was to investigate the effect of arecoline on the motility of distal colon in rabbits and its mechanisms involved. Strips of colonic smooth muscle were suspended in organ baths containing Krebs solution, and their isometric contractions were examined. The response of smooth muscle to arecoline in colonic strips was recorded. The effects of atropine, gallamine and 1, 1 - dimethyl - 4 - diphenylacetoxypiperidiniumiodide (4 - DAMP) on arecoline - induced contraction were also observed. Arecoline (1 nM - 1 μM) produced a concentration - dependent contraction in both the longitudinal and the circular smooth muscle of rabbit colon. Atropine (10 μM) abolished the arecoline (80 nM) - induced contraction. M_3 receptor antagonist, 4 - DAMP (0.4 μM), abolished the arecoline (80 nM) - related response, whereas M_2 receptor antagonist, gallamine (0.4 μM), did not affect the effect of arecoline. These results suggest that arecoline excites the colonic motility *via* M_3 receptor in rabbits.

Key Words: arecoline, colon, antagonist, intestinal motility

Introduction

The functional dyspepsia, irritable bowel syndrome, constipation and disorders of the biliary tract are related with the functional gastrointestinal motility, but the therapeutic medicine in the functional gastrointestinal disorders is still not satisfactory. Areca (*Areca catechu* L., betel nuts; a Chinese herbal medicine named pinang or bing-lang) had been shown to relieve indigestion. Areca had been used to treat abdominal distention and constipation caused by stagnation of the circulation (12). Our previous study showed that areca stimulated the colonic smooth muscle strips in rats (25). Since arecoline is one of the most important effective components of areca, we therefore expect that the effect of areca on colonic motility be mainly caused by arecoline.

Arecoline is usually used as non-selective muscarinic agonist. It has been reported that arecoline antagonizes the leu-enkephalin – caused peristaltic block (2), but not tetramethylammonium – caused peristaltic block (1). Arecoline dose – dependently increase the contraction of the guinea pig ileum and the gastrointestinal transit of the mouse (24). However, the effect of arecoline on rabbit colonic motility is still unknown.

Smooth-muscle contraction in the gastrointestinal tract is mediated primarily by muscarinic (M) receptors. Muscarinic acetylcholine receptors (mAChRs) comprise five different families of receptors (M_1-M_5) that expresses in different tissues (5). M_1 receptors are involved in synaptic transmission

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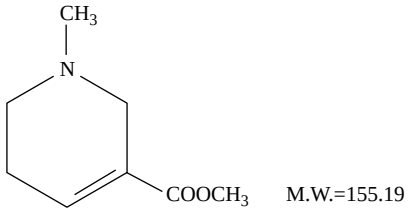

Fig. 1. The chemical structure and molecular weight (M.W.) of arecoline.

within intramural plexuses, at interneuronal synapses in the parasympathetic excitatory pathway to colonic smooth muscle (4). Coexistence of M_3 and M_2 receptors in gastrointestinal smooth muscle had been reported (11, 28). Although the M_2 receptor is predominant in most smooth muscles, in most cases, it is the M_3 receptor that mediates the contractile response (15, 20).

The purpose of this study was to investigate [1] the effect of arecoline on the distal colonic smooth muscle in rabbits, and [2] the involvement of M receptor and selective M receptor subtype in the action of arecoline on rabbit colon.

Materials and Methods

Animals and Preparation

New Zealand White rabbits (2.0 - 2.5 kg) of both sexes were fasted for 24-hour and decapitated. Segment of the distal colon (5 cm proximal to the anus) was quickly removed (26). The segment of the colon was opened along the mesentery. Muscle strips (8×2 mm) were cut, parallel to the longitudinal fibers or the circular fibers, and named longitudinal muscle (LM) and circular muscle (CM), respectively. The mucosa on each strip was carefully removed. The muscle strip was suspended in a tissue chamber containing 5 ml Krebs solution (37°C) and bubbled continuously with 95% O_2 and 5% CO_2. The composition (mM) of the Krebs solution of the following was used: NaCl 119, KCl 4.75, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.5, $CaCl_2$ 2.5 and glucose 11.

One end of the strip was fixed on a hook at the bottom of the chamber and placed in organ bath. Another end was connected to an external isometric force transducer (JH-2B, Beijing, China). The transducer was connected to the amplifier (SMUP-PC, Shanghai, China). Spontaneous contractile activity of colonic strips (under an initial tension of 1 g) was simultaneously recorded using MFlab system (Fudan University, Shanghai, China). All experiments started after a minimal 60 min equilibration period. After stable level had been recorded, spontaneous motility was observed in the presence of drugs.

Chemicals such as arecoline (Fig. 1), atropine

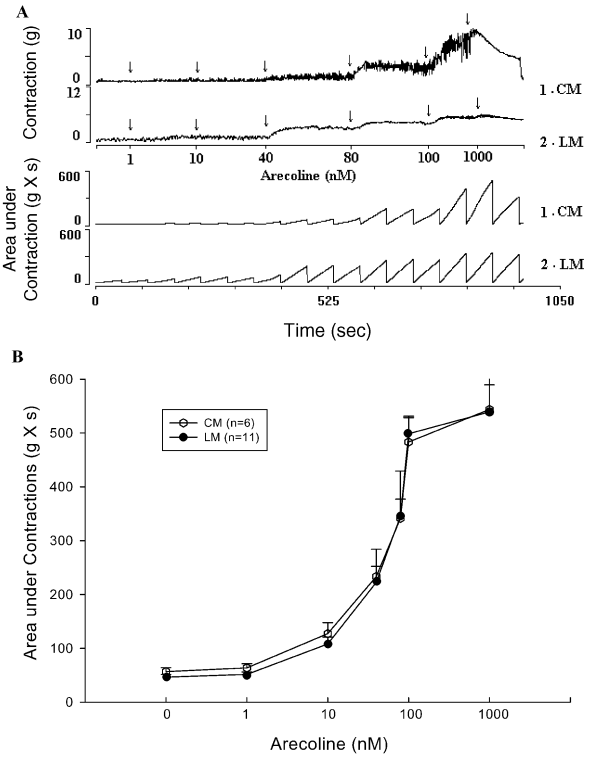

Fig. 2. Dose - dependent response induced by arecoline (1 - 1000 nM) on the contraction of circular muscle (CM) and longitudinal muscle (LM) of rabbit distal colon. (A). Representatives of the recording of the colonic motility. 1, channel 1; 2, channel 2; ↓, add the drugs. (B). Dose - dependent response curves induced by arecoline on the contraction of colonic CM and LM. Con, the data prior to arecoline administration. Each point represents the mean±S.E.M.

sulfate salt hydrate, gallamine triethiodide and 4 - diphenylacetoxy - N - methylpiperidine methiodide (4 - DAMP) were obtained from Sigma Co. Ltd. (St. Louis, MO, USA). Atropine was first dissolved in 75% ethanol, and then diluted with water. Other chemicals or drugs were dissolved in water.

Effect of Arecoline on Distal Colonic Motility in Rabbits

The distal colonic strips were stabilized in the organ baths for 60 min. Preliminary experiments showed that arecoline produced maximal effect within 3 min. Dose-response curves of arecoline (1 nM, 10 nM, 40 nM, 80 nM, 100 nM, and 1000 nM, Fig. 2) were constructed by applying chemicals at 5 min intervals.

Effect of Atropine on Arecoline - Induced Colonic Motility

To determine if arecoline acts *via* muscarinic receptor, the effects of atropine (10 μM) on arecoline - induced response were examined. After stable level had been recorded, spontaneous motility was observed

in the presence of atropine. Arecoline (80 nM) was added 10 min after addition of atropine.

Effects of M_2 Antagonist and M_3 Antagonist on Arecoline - Induced Colonic Motility

To determine if arecoline acts *via* M_2 or M_3 receptor, the effects of selective M_2 receptor antagonist, gallamine (0.4 μM), and selective M_3 receptor antagonist, 4 – DAMP (0.4 μM), on arecoline - induced response were examined (27). After stable level had been recorded, spontaneous motility was observed in the presence of gallamine or 4 – DAMP. Arecoline (80 nM) was added 10 min after applying of gallamine or 4 – DAMP.

Statistical Analysis

The contractile response of colonic motility was quantified as the area under contractions using the MFlab software (Fudan University, Shanghai, China). In each experiment, the values of basic contractions and antagonist – induced contractions were evaluated 3 min. The values of arecoline – induced responses were also evaluated for 3 min after it was added. The results were expressed as means± SEM. Significant differences between different means were determined using one – way analysis of variance (ANOVA) followed by Dunnett's test. The difference were judged to be significant when $P < 0.05$. The number of replications of rabbit colonic specimens in each experiment was indicated by *n*.

Results

Effects of Arecoline on Colonic Motility in Strips

Arecoline (1 - 1000 nM) dose-dependently enhanced the contraction of CM and LM of rabbit distal colon (Fig. 2A). The dose – response curves of the arecoline are shown in Fig. 2B, in which the contractions were expressed as the area under contractions.

Effects of Atropine on Arecoline - Induced Contraction

Atropine (10 μM) itself had no effect on the contraction of CM and LM of rabbit distal colon ($P > 0.05$, data not shown). When given 10 min before the administration of arecoline (80 nM), atropine completely blocked the arecoline - induced contraction of both CM and LM (Fig. 3, $n = 8$).

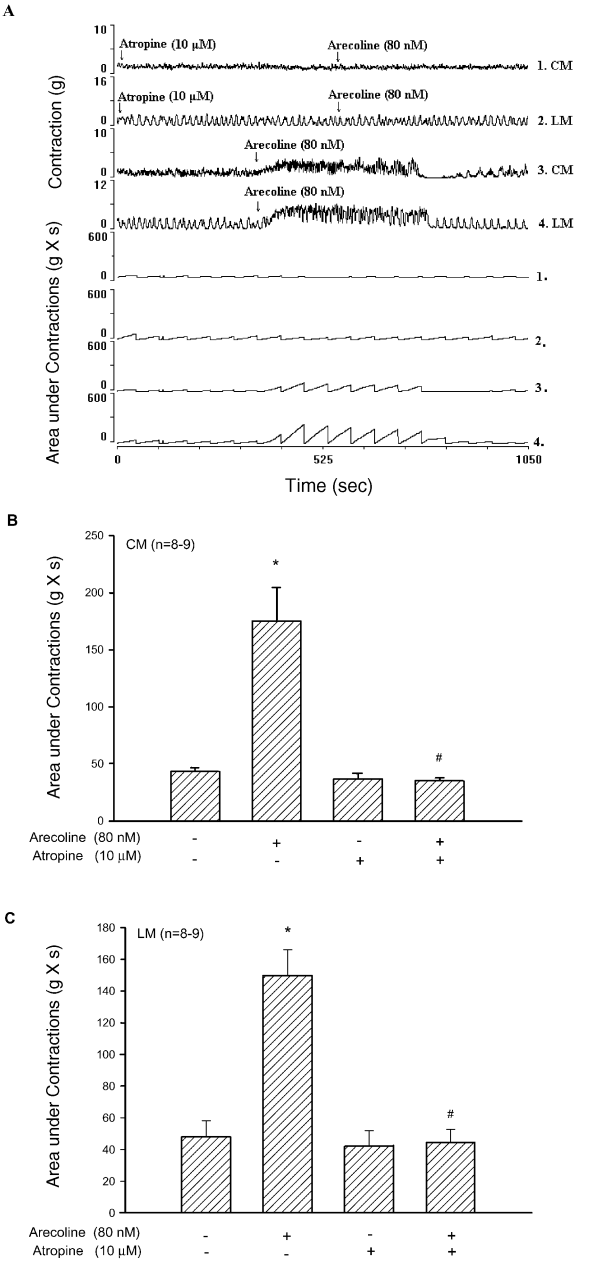

Fig. 3. Effects of atropine (10 μM) on arecoline (80 nM) - induced contraction of circular muscle (CM) and longitudinal muscle (LM) of rabbit distal colon. (A). Representative of the recording of the colonic motility. 1, channel 1; 2, channel 2; 3, channel 3; 4, channel 4; ↓, add the drugs. (B). Statistic analysis of the effect of atropine on arecoline - induced contraction in CM. (C). Statistic analysis of the effect of atropine on arecoline - induced contraction in LM. Each column represents the mean±S.E.M. $^{*}P < 0.01$ compared with the data prior to arecoline administration; $^{\#}P < 0.01$ compared with the data after arecoline administration.

Effect of M_2 Antagonist on Arecoline - Induced Colonic Motility

Administration of gallamine (0.4 μM), a selective M_2 receptor antagonist, had no effect on the contraction of CM and LM of rabbit distal colon ($P > 0.05$, data not shown). When given 10 min before the administration of arecoline (80 nM), gallamine did not affect the arecoline - induced contraction of both CM and LM (Fig. 4, $n = 9$).

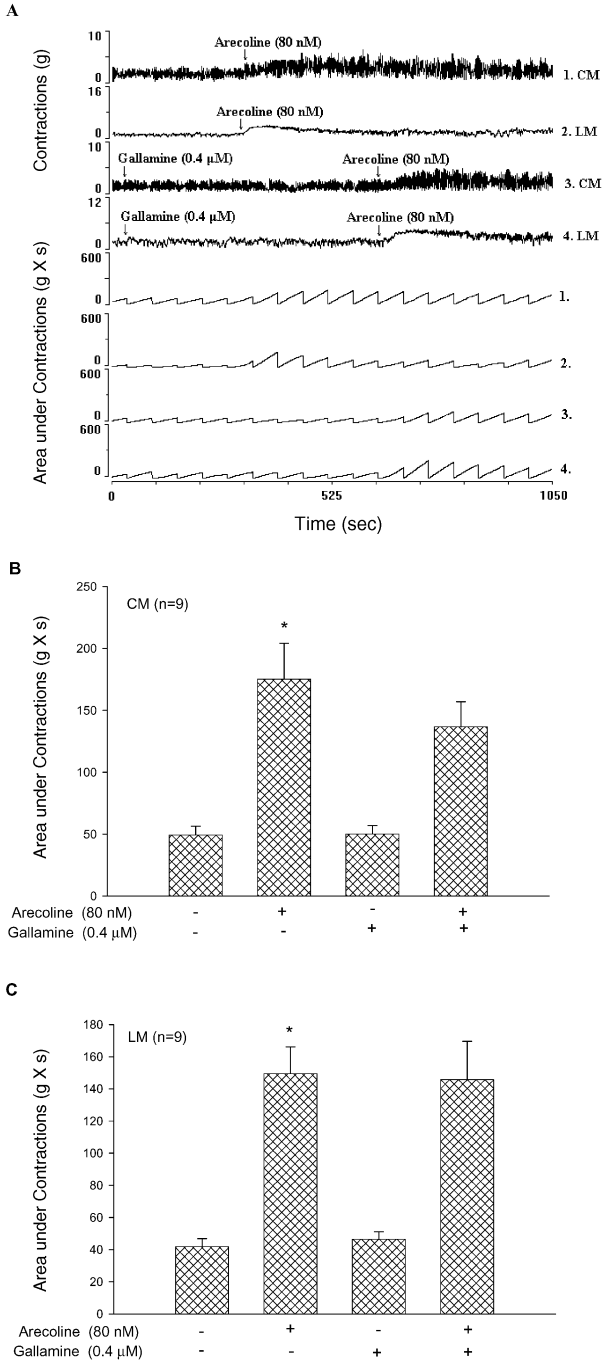

Fig. 4. Effects of gallamine (0.4 μM) on arecoline (80 nM) - induced contraction of circular muscle (CM) and longitudinal muscle (LM) of rabbit distal colon. A. Representative of the recording of the colonic motility. 1, channel 1; 2, channel 2; 3, channel 3; 4, channel 4; ↓, add the drugs. B. Statistic analysis of the effect of gallamine on arecoline - induced contraction in CM. C. Statistic analysis of the effect of gallamine on arecoline - induced contraction in LM. Each column represents the mean±S.E.M. **P* < 0.01 compared with the data prior to arecoline administration.

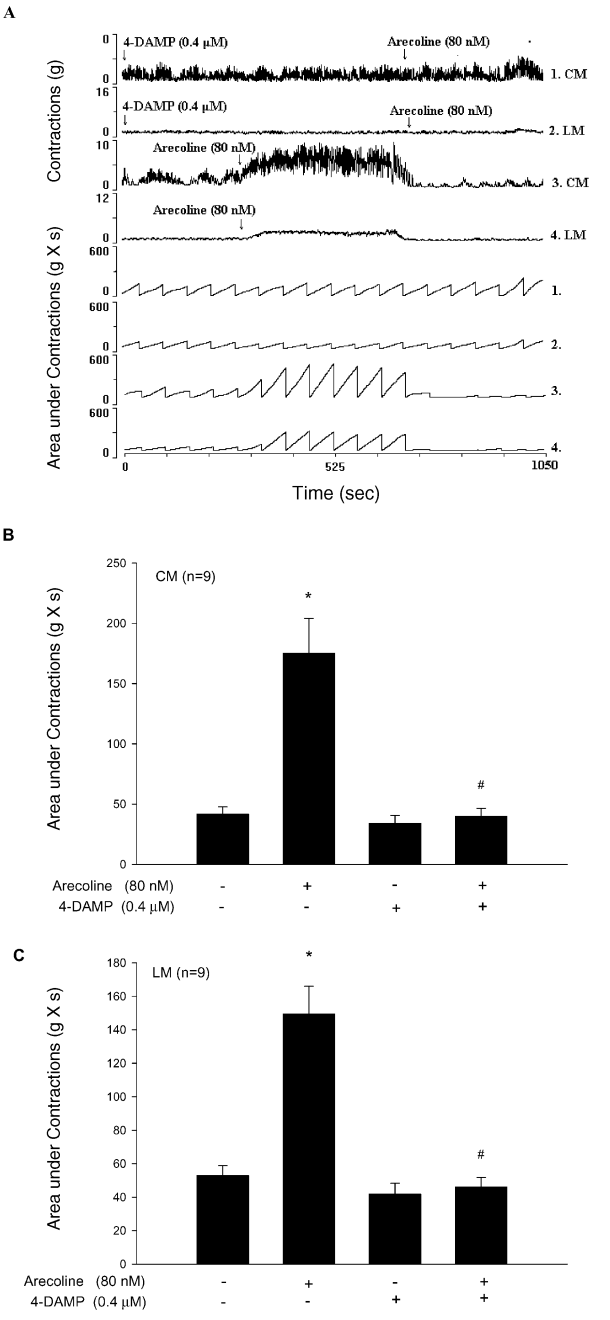

Fig. 5. Effects of 4 - DAMP (0.4 μM) on arecoline (80 nM) - induced contraction of circular muscle (CM) and longitudinal muscle (LM) of rabbit distal colon. (A). Representative of the recording of the colonic motility. 1, channel 1; 2, channel 2; 3, channel 3; 4, channel 4; ↓, add the drugs. (B). Statistic analysis of the effect of 4 - DAMP on arecoline - induced contraction in CM. (C). Statistic analysis of the effect of 4 - DAMP on arecoline - induced contraction in LM. Each column represents the mean±S.E.M. **P* < 0.01 compared with the data prior to arecoline administration; #*P* < 0. 01 compared with the data after arecoline administration.

Effects of 4 - DAMP on Arecoline - Induced Contraction

Administration of 4 - DAMP (0.4 μM) had no effect on the contraction of CM and LM of rabbit distal colon ($P > 0.05$, data not shown). When given 10 min before the administration of arecoline (80

nM), 4 - DAMP completely blocked the arecoline - induced contraction of both CM and LM (Fig. 5, n = 9).

Discussion

Our previous study showed that traditional Chinese herbal medicine areca stimulated the colonic contractions (25). In the present study, we found that arecoline stimulated the spontaneous contractions of distal colon in rabbits. Arecoline is one of the most important effective components of areca. Therefore, the effect of areca on colonic motility might result mainly from arecoline. These results also indicated that the relief of abdominal distention and the constipation after the treatments of areca is likely caused by arecoline (12).

The response to arecoline was blocked by atropine, indicating that arecoline stimulated the rabbit distal colonic motility *via* M receptors. This finding is consistent with the previous study reported by Ennes *et al.* (10) in which the M receptors present in very high levels (600,000 receptors/cell) in freshly isolated rabbit colonic smooth muscle cells.

Muscarinic receptors are expressed abundantly in smooth muscle throughout the gastrointestinal tract in a manner that approximates a three-to-one mixture of the M_2 and M_3 subtypes (9). Radioligand binding studies and Northern blot analysis have determined that M_2 and M_3 muscarinic receptors coexisted (82% M_2, 18% M_3) in the circular smooth muscle of canine proximal colon (30). Gomez *et al.* (11) characterized the muscarinic receptor subtypes in human and rat colon smooth muscle homogenates by ligand binding studies, and suggested that the first subtype of muscarinic receptor was M_2 and the second subtype was M_3. Human colon samples showed the major densities of subtype M_2 (76% of the total receptors). The relative densities of the receptor subtypes are significantly different in both species. Zhang (28) further demonstrated the coexistence of muscarinic M_3 (45%) and M_2 (55%) receptors in adult rat colon. Blanquet *et al.* (3) investigated the action of methoctramine (a selective M_2 antagonist) and 4 - DAMP (a selective M_3 antagonist) on excitatory junction potentials (EJPs) and inhibitory junction potentials (IJPs) in the smooth muscle cells of the rabbit proximal colon. The results indicated that 4 - DAMP decreased EJPs, whereas methoctramine decreased the amplitude of the IJPs. These results suggested the coexistence of muscarinic M_3 and M_2 receptors in rabbit colon.

Our results showed that 4 – DAMP but not gallamine abolished the arecoline – induced contractions in rabbit distal colon. This suggested that arecoline stimulate the contraction of rabbit distal colon *via* M_3 receptors.

Abundant data on smooth muscle demonstrated that M_3 is the primarily receptor that mediates contraction to M receptor agonists in the absence of other contractile and relaxant agents (6-9). This condition occurs in a variety of smooth muscle types including the circular muscle of canine proximal colon and longitudinal muscles of guinea pig colon (18, 29). Our present data are also consistent with this hypothesis: the contractile response of gastrointestinal smooth muscle was sensitive to M_3 receptor antagonist and insensitive to M_2 receptor antagonist (19).

M_3 receptor stimulates phospholipase C-β causing inositol-1, 4, 5-trisphosphate accumulation and calcium mobilization (30). The extent to which the contractile response depends on this burst of calcium and how this calcium interacts with other transduction mechanisms in rabbit colon remains to be investigated.

Data abtained from knockout mice, lacking either M_3 or M_2 receptors, reveals a role of gastrointestinal motility in both subtypes (16, 17, 22). A lot of evidence suggests that an interaction between M_2 and M_3 receptors plays a crucial role in mediating the gastrointestinal contraction response (19, 14, 21, 23). Jin *et al.* (13) measured the currents in rabbit colonic smooth muscle cells by patch - clamp technique and found that Ca^{2+} current is stimulated in colonic smooth muscle cells by M_2 receptor coupled to Galphai-G protein and c-src activation. Although in the present study, we did not find the effect of selective M_2 receptor antagonist (gallamine) on arecoline - induced contractions, further studies are needed to investigate whether M_2 and M_3 receptors interact in mediating the colonic contraction response.

In conclusion, arecoline excites the colonic motility *via* M_3 receptor in rabbit distal colon.

Acknowledgments

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