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EFFECTS OF ANCISTROTECTORINE ON THE CONTRACTION OF THE ISOLATED STOMACH OF RATS AND MICE

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ABSTRACT

The antispasmodic activity of ancistrotectorine on the isolated rat stomach strip and whole mice stomach was studied. Ancistrotectorine (1×10^{-5}, 2×10^{-5} and 3×10^{-5} M) produced a dose-dependent reduction of cumulative maximum dose-response contraction of rat stomach strip induced by acetylcholine, serotonin, barium chloride, potassium chloride and by calcium-freed solution. Ancistrotectorine also reduced the contraction of the whole isolated mice stomach induced by barium chloride (2×10^{-4} M) and carbachol (11×10^{-7} M). The inhibitory effect of ancistrotectorine on contraction of the rat stomach strip induced by various agonists was similar to that of papaverine and verapamil. The results suggest that the antispasmodic activity of ancistrotectorine is not mediated via any specific receptors.

Key words : Ancistrotectorine, isolated stomach, stomach strip, antispasmodic activity

INTRODUCTION

Ancistrocladus tectorius (Lour) Merr. is known in Thai as "Korn-ma-deang". Its leaves have been used in Thailand for treatment of edema and dermatitis (Na Songkla, 1982). Its roots have been used for treatment of malaria and dysentery in Burma and Malaysia (Burkill, 1935). Ruangrungsi et al. (1985) had isolated two types of alkaloids from the leaves known as ancistrocladidine and ancistrotectorine. Ancistrotectorine was found in large amount and has 7-3'-linked napthalene-isoquinoline in its structure (Fig. 1) (Ruangrungsi et al., 1985).

Previous studies have demonstrated that ancistrotectorine has antispasmodic effects on intestinal contraction (Pasupat, 1985) and contractions of vas deferens (Ketkosol, 1986) and smooth muscles of blood vessel (Osathanukul, 1986). It was suggested that ancistrotectorine had a direct effect on smooth muscle and caused a non-specific relaxation. Whether this compound has a similar action on the smooth muscle of stomach is not known. The objectives of this study are to investigate the antispasmodic activity and the mode of action of ancistrotectorine on the isolated stomach preparations.

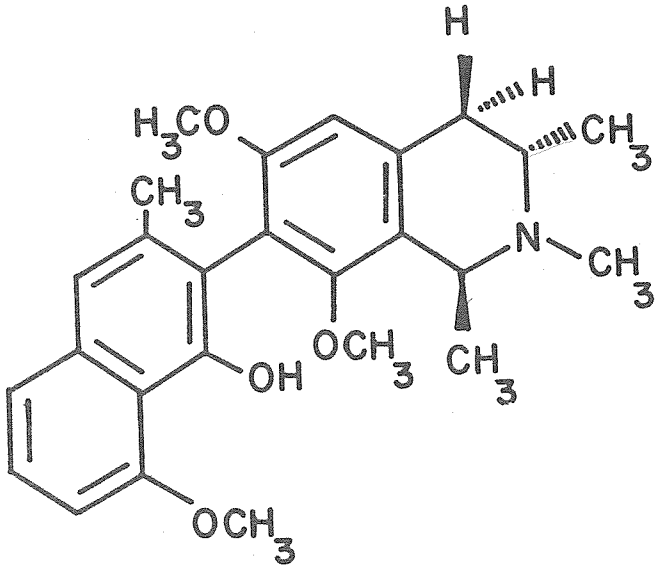

Figure 1. The structure of ancistrotectorine isolated from the leaves of *Ancistrocladus tectorius (Lour) Merr.*

Materials and Methods

Animals

Fifty male albino rats of Wistar strain weighing 280-320 g and fifty male mice weighing 23-26 g were used throughout. The animals were allowed free access to water and food for 1-2 weeks prior to the experiments.

Drugs

For studying the mechanisms of action of ancistrotectorine, various drugs of known mechanisms of action were used such as acetylcholine chloride (Sigma), atropine sulfate (Sigma), barium chloride (Sigma), calcium chloride (Merck), carbachol (Sigma), potassium chloride (Merck), serotonin (5-hydroxytryptamine, 5HT, Sigma), methylsergide (Sigma), verapamil (Ludwigshafen) and papaverine hydrochloride (Sigma). All these drugs were prepared in Tyrode's solution. The concentration of ancistrotectorine were expressed in molar concentration of the base.

Isolated rat stomach strip preparation

The rats were killed by a blow on the head. The stomach was excised and placed in a petri-dish containing oxygenated Tyrode's solution. The fundus strip was isolated according to the method of Vane (1957). Briefly, the stomach was dissected out and the pink pyloric end cut away from the grey fundal end. The fundal end was splited open so as to form a sheet. Cuts were made in this sheet of muscle to produce a strip to which a thread was attached at each end. The fundus strip was allowed to equilibrate in oxygenated Tyrode's solution in an isolated organ bath until the rate and amplitude of spontaneous contraction were stable, before experiments began. The contraction was recorded by an isometric force transducer connecting to a Beckman Dynograph recorder.

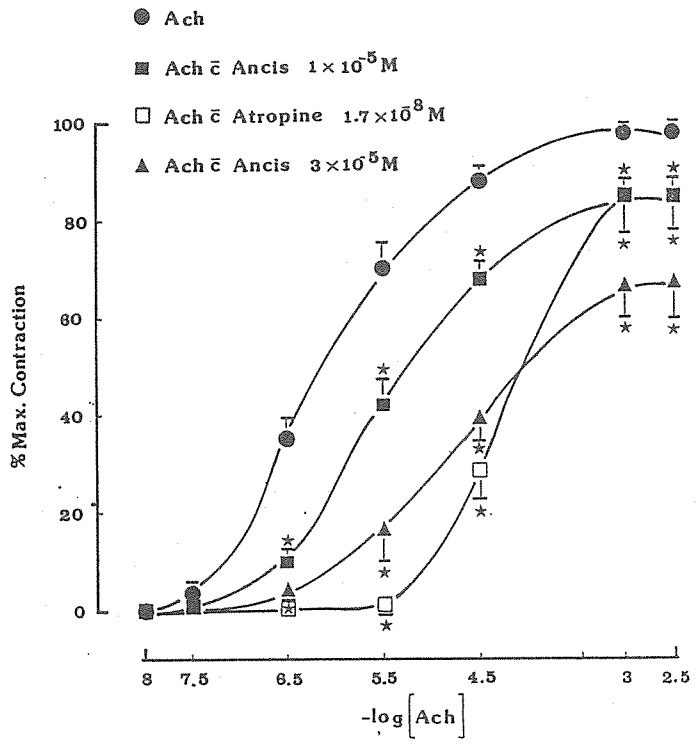

Figure 2. Cumulative log dose-response curve of the rat stomach strip in response to various concentrations of acetylcholine (Ach, n = 18), acetylcholine and ancistrotectorine at 1 × 10^{-5} M (Ancis, n = 7), acetylcholine and ancistrotectorine at 3 × 10^{-5} M (n = 6). Symbols with bar graphs represent mean ± SEM. * $P < 0.05$ when compared to the reponse to acetylcholine alone.

Whole isolated mice stomach preparation

The mice were anaesthetized and the whole stomach was isolated and placed in a petri-dish. The content was washed out by oxygenated Tyrode's solution and then filled with Tyrode's solution. A polyethylene tubing (0.032 inch, I.D.) was inserted in to the pyloric opening. The other opening was ligated with thread. The other end of the polyethylene tubing was connected to a pressure transducer and a Beckman Dynograph recorder. The stomach was then immersed in an organ bath filled with oxygenated Tyrode solution and maintained at 37°C. Contraction of the whole stomach was induced by barium chloride (2x.0^{-4}M) or cabachol (1 × 10^{-7}M) added to the bath.

Analysis of data

Experimental data were expressed as mean ± S.E.M. Statistical significance was tested according to Student's t-test for paired or unpaired variates.

RESULTS

Isolated rat stomach strip preparation

Acetylcholine-induced contraction

Acetylcholine caused contraction of the stomach strip at a concentration of 5.5×10^{-9}M, and the maximal contraction was reached at the dose of 4.8×10^{-3} M. Ancistrotectorine at doses of 1×10^{-5} and 3×10^{-5} M significantly decreased the contraction induced by acetylcholine. At the maximal dose of acetylcholine, the contraction was decreased by $12.98 \pm 2.40\%$ and $31.52 \pm 2.10\%$, respectively (Fig. 2). In the presence of atropine (1.7×10^{-9}M), which is a competitive antagonist of acetylcholine, a different dose response curve was noted (Fig. 2). However, at the maximal dose of acetylcholine, significant inhibition by atropine was still observed. This is similar to that occurred in the presence of a low dose of ancistrotectorine.

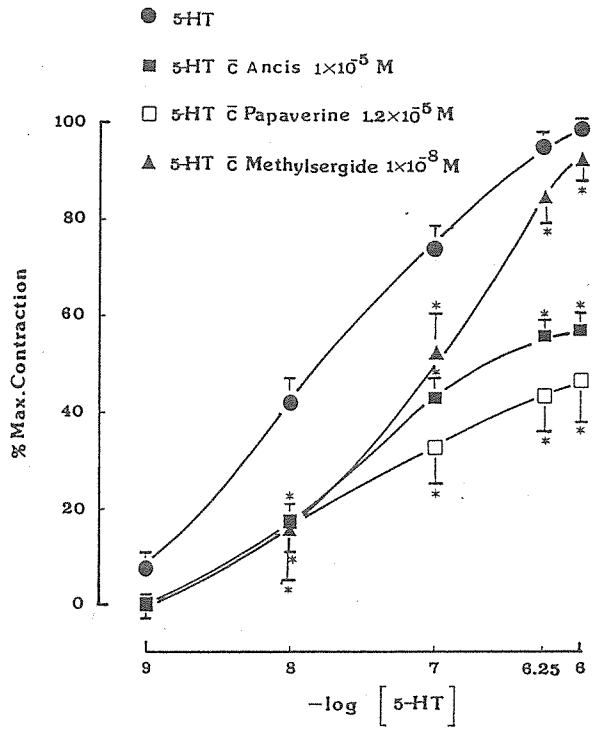

Figure 3. Cumlative log dose-response curve of the rat stomach strip in response to various concentrations of serotonin (5-HT, n = 18), serotonin and ancistrotectorine at 1 x 10^{-5} M (n = 7), serotonin and paraverine at 1.2 x 10^{-5} M (n = 6), serotonin and methylsergide at 1 x 10^{-8} M (n = 5). Symbol with bar graphs represent mean ± SEM. *P < 0.05 when compared to the response to serotonin alone.

Serotonin-induced contraction

Serotonin caused contraction of the stomach strip at a concentration of 5×10^{-6}M, and the maximal contraction occurred at the dose of 1×10^{-5}M. Ancistrotectorine (1×10^{-5}M) significantly reduced ($P<0.05$) the maximal contraction

induced by serotonin by 41.86 ± 2.65% (Fig. 3). The dose-response curve of serotonin-induced contraction in the presence of ancistrotectorine was similar to that in the presence of papaverine, 1.2×10^{-5} M, (Fig. 3). On the contrary, methylsergide (1×10^{-8} M) produced a different dose-response curve, which resembled that of a competitive antagonist of serotonin (Fig. 3).

$BaCl_2$-induced contraction

Barium chloride produced contraction of the stomach strip at a concentration of 4.9×10^{-6} M, and the maximal contraction occurred at a concentration of 5×10^{-3} M (Fig. 4). Ancistrotectorine (1×10^{-5} and 2×10^{-5} M) caused significant decreases in the maximal contraction induced by barium chloride by 33.62 ± 5.09% and 58.97 ± 3.59%, respectively ($P<0.05$). The dose-response curves were similar to that in the presence of verapamil at a concentration of 2.6×10^{-7} M (Fig. 4).

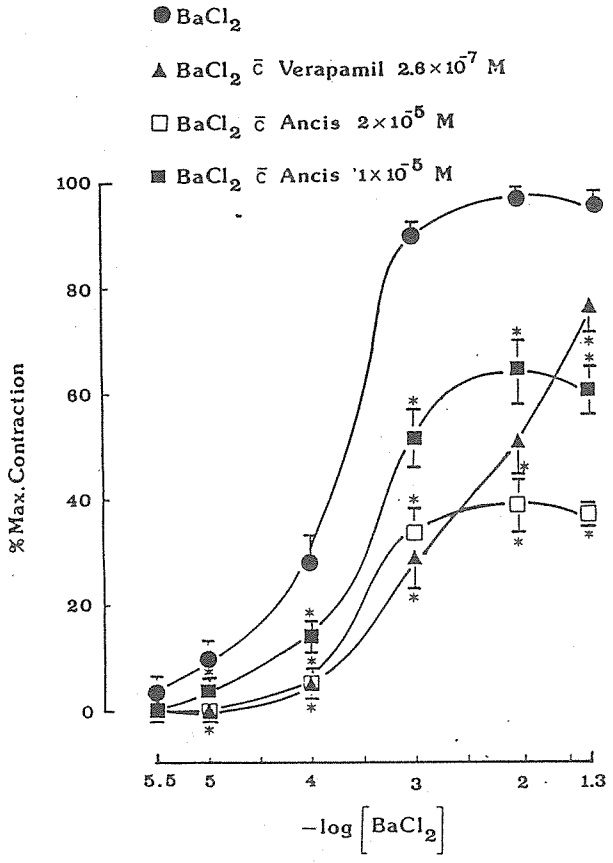

Figure 4. Cumulative log dose-response curve of the rat stomach strip in response to various concentrations of barium chloride ($BaCl_2$, n=20), barium chloride and verapamil at 2.6×10^{-7} M (n=6), barium chloride and ancistrotectorine at 1×10^{-5} (n=7) and 2×10^{-5} M (n=7). Symbols with bar graphs represent mean ± SEM, $^{*}P<0.05$ when compared to the response to barium chloride alone.

KCl-induced contraction

Potassium chloride (1×10^{-1} M) produced a maximal contraction of the stomach strip. Ancistrotectorine at concentrations of 1×10^{-5} M and 3×10^{-5} M caused significant decreases in the maximal contraction induced by potassium chloride by $28.83 \pm 4.84\%$ and $48.23 \pm 7.37\%$, respectively ($P<0.05$). The dose-response curve revealed a similar pattern as that in the presence of 2.5×10^{-5} M papaverine (Fig. 5).

CaCl₂-induced contraction

The stomach strip was incubated in a high potassium and calcium-freed depolarizing solution for 1 h before the experiment. The maximal contraction of the stomach strip was produced by a dose of 9.0×10^{-4} M calcium chloride. Ancistrotectrorine at the doses of 2.4×10^{-6} and 1×10^{-5} M caused significant decreases in the maximal contraction induced by calcium chloride by $33.90 \pm 5.93\%$ and $69.59 \pm 4.48\%$, respectively ($P<0.05$). The dose-response curves showed non-competitive antagonistic effects of ancistrotectorine which were similar to that produced by verapamil at 3.5×10^{-9} M (Fig. 6).

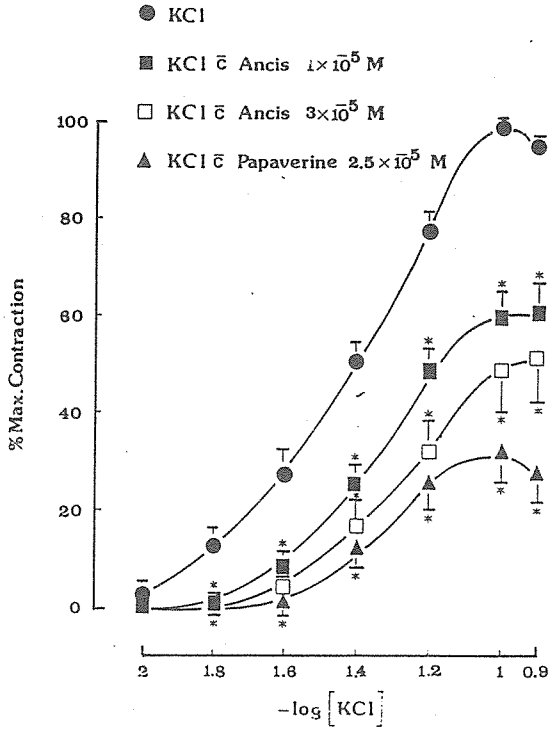

Figure 5. Cumulative log dose-response curve of the rat stomach strip in response to various concentrations of potassium chloride (KCl, n=20), potassium chloride and ancistrotectorine at 1 x 10^{-5} M (n=7), potassium chloride and ancistrotectorine at 3 x 10^{-5} M (n=6), potassium chloride and papaverine at 2.5 x 10^{-5} M (n=7). Symbols with bar graphs represent mean ± SEM, $^{*}P<0.05$ when compared to the response to potassium chloride alone.

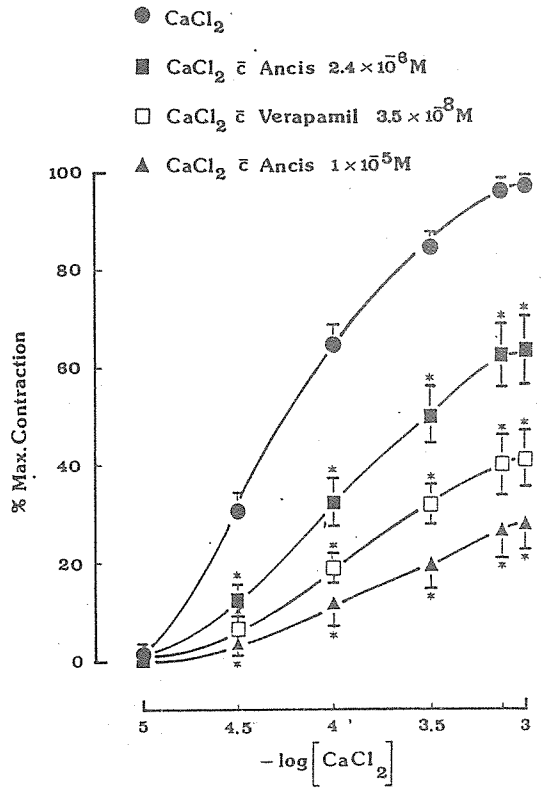

Figure 6. Cumulative log dose-response curve of the rat stomach strip in response to various concentrations of calcium chloride ($CaCl_2$, n=25), calcium chloride and ancistrotectorine at 2.4×20^{-5} M (n=8), calcium chloride and verapamil at 3.5×10^{-8} M (n=9), calcium ahloride and ancistrotectorine at 1×10^{-5} M (n=8). Symbol with bar graphs represent mean ± SEM, $^{*}P < 0.05$ when compared to the response to calcium chloride alone.

Whole isolated mice stomach preparation

$BaCl_2$-induced contraction

As shown in Figure 7, administration of barium chloride at a concentration of 2×10^{-4} M produced marked contractions of the whole isolated mice stomach. Both tonic and phasic contractions increased. Ancistrotectorine given at a concentration of 6×10^{-5} M caused a significant decrease of the phasic contraction. Similarly, when ancistrotectorine was administered prior to barium chloride, a significant suppression of phasic contraction occurred (Fig. 7). It should be noted that ancistrotectorine had virtually no effect on the barium chloride-induced tonic contraction.

Carbachol-induced contraction

As shown in Figure 8, administration of carbachol at a concentration of 1×10^{-7} M produced marked contractions of the whole isolated mice stomach. Addition of 6×10^{-5} M ancistrotectorine to the tissue bath either prior to or after

carbachol caused a significant inhibition of carbachol-induced contractions (Fig. 8). It appeared that both phasic and tonic contractions were suppressed in this preparation.

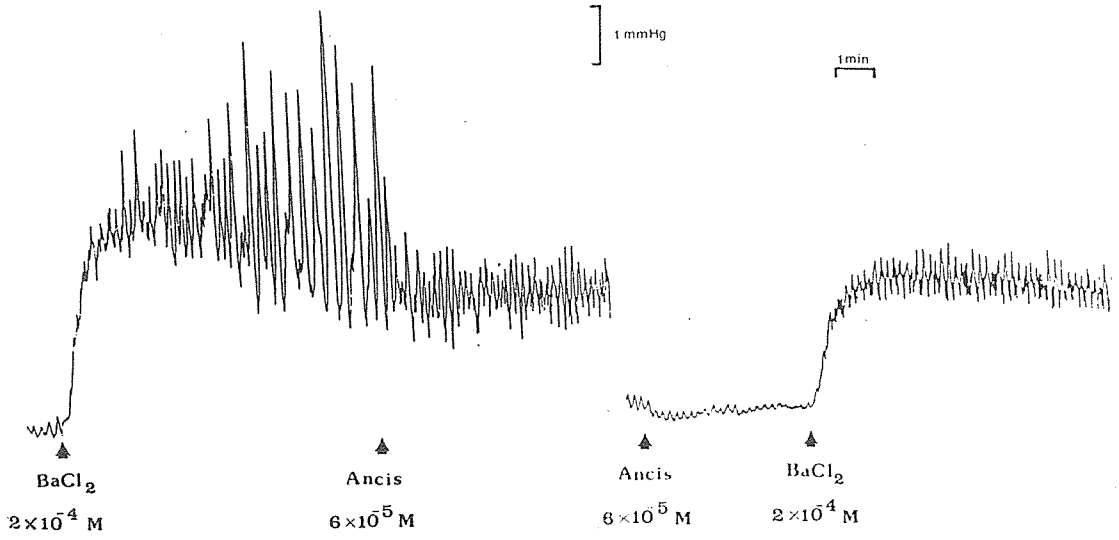

Figure 7. Representative records showing the effect of ancistrotectorine on contractions of the whole isolated mice stomach induced by barium chloride.

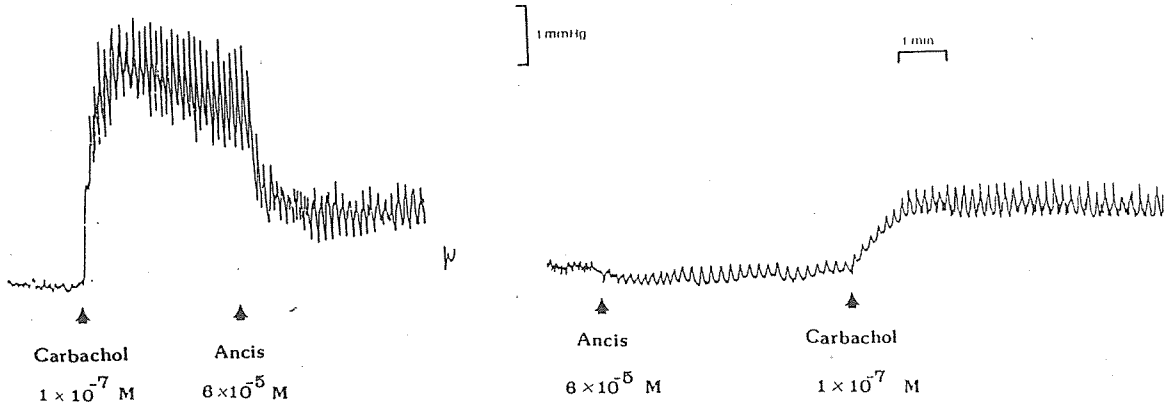

Figure 8. Representative records showing the effect of ancistrotectorine on contractions of the whole isolated mice stomach induced by carbachol.

DISCUSSION

The results in the present study demonstrated that ancistrotectorine produced a dose-dependent reduction of maximal contraction of the rat stomach strip induced by several agents. The effect of ancistrotectorine was compared with other specific

and non-specific antagonists. It should be pointed out that the contractions of the rat fundus strip is a complex response of both longitudinal and circular smooth muscles which may be induced by a direct action of an agonist on smooth muscle cells or an indirect action through intramural nerve terminals. Acetylcholine has been shown to act directly on muscarinic receptors (Day & Vane, 1963; Paton & Zar, 1968) and indirectly through the nicotinic receptors of the intestinal smooth muscle (Day & Vane, 1963; Chiou, 1973). Atropine is a competitive antagonist of acetylcholine at the muscarinic cholinergic receptors (Shutt & Bowes, 1979). However, the results in this study failed to show the typical dose-response curve for competitive inhibition (Fig. 2). Nevertheless, ancistrotectorine caused a shift in the dose-response curve which was different from atropine (Fig. 2). This suggests that the action of ancistrotectorine is not similar to that of atropine in blocking of acetylcholine.

It has been reported that serotonin acts directly on D-receptor (musculotropic receptor) of rat stomach and opens the receptor-operated calcium channels (Day & Vane, 1963; Costa & Furness, 1979) resulting in an increase of muscle contraction (Mukai & Kubota, 1980). Again, ancistrotectorine demonstrated a different dose-response curve of the serotonin-induced contraction when compared to that produced by the specific antagonist methylsergide (Fig. 3). In contrast, it caused a similar dose-response curve to that induced by papaverine, a non-specific antispasmodic agent.

Stimulation of smooth muscle contraction by barium chloride could be due to several mechanisms. Susuki et al. (1964) suggested that barium chloride increased the prolongation of depolarization whereas Sakamoto (1970) explained in terms of low potassium conductance which, in turn, resulted in prolonged repolarization of membrane potentials. Karaki et al. (1986) found that barium opened voltage-dependent calcium channels leading to calcium influxes into muscle cells and contraction. In the fundus strip preparation, the inhibitory effect of ancistrotectorine on barium-induced contraction was similar to the effect of verapamil (Fig. 4).

Stimulation of the stomach strip by potassium chloride or calcium chloride in high potassium and calcium-freed depolarizing solution produced similar effects. High potassium concentration reduced potassium gradient between the cell membrane resulting in depolarization and opening of the potential sensitive calcium channels (Bolton, 1979). Early phasic contractions induced by high external potassium were due to the release of bound intracellular calcium store whereas the late tonic contractions were ascribed to the calcium influx (Hay & Wadsworth, 1982). Ancistrotectorine caused significant decreases in the stomach strip contraction induced either by potassium chloride (Fig. 5) or calcium chloride (Fig. 6). These effects were similar to those produced by papaverine and verapamil, respectively.

Taken together, the antispasmodic activity of ancistrotectorine appears to be nonspecific, which is similar to papaverine. It is known that papaverine relaxes smooth muscle by various mechanisms such as (1) inhibition of receptor-operated calcium channels and voltage-dependent calcium channels (Bolton, 1979),

(2) stimulation of the beta-adrenergic receptors at the smooth muscle membrane resulting in the inhibition of phosphodiesterase activity and hence increases in cAMP and calcium storage (Inatomi et al., 1979), and (3) inhibition of mitochondrial respiration in the process of electron transport chain (Bolton, 1979). Whether the action of ancistrotectorine could be accounted for by all these mechanisms remains to be investigated.

When a whole mice stomach preparation was used, ancistrotectorine caused significant decreases of phasic contraction induced by barium chloride (Fig. 7), and both phasic and tonic contractions induced by carbachol (Fig. 8). The results corroborate with those in the fundus strip preparation. It is not known how ancistrotectorine caused a reduction in the tonic contraction induced by carbachol but not by barium chloride.

In conclusion, we have shown that ancistrotectorine possesses an antispasmodic activity on smooth muscle of the stomach strip and its inhibitory effect is dose-dependent. This effect is possibly not mediated via a specific receptor.

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บทคัดย่อ

ได้ศึกษาฤทธิ์ของแอนซีสโตรเทคโตรีนต่อการบีบตัวของกล้ามเนื้อกระเพาะอาหารของหนูขาวที่แยกออกมา และต่อการบีบตัวของกระเพาะอาหารทั้งกระเพาะของหนูถีบจักรที่แยกออกมา พบว่าแอนซีสโตร-เทคโตรีน (ขนาดความเข้มข้น 1×10^{-5}, 2×10^{-5} และ 3×10^{-5} โมลาร์) สามารถลดการหดเกร็งสูงสุดของกล้ามเนื้อกระเพาะอาหารหนูขาวที่เกิดจากการกระตุ้นแบบ cumulative dose-response โดยสารอะซิทิลโคลีน ซีโรโทนิน แบเรียมคลอไรด์ โปแตสเซี่ยมคลอไรด์ และแคลเซี่ยมคลอไรด์ในสารละลายดีโพลาไรด์ด้วยโปแตส-เซี่ยมที่ปราศจากแคลเซี่ยม นอกจากนี้ยังมีฤทธิ์ลดแรงบีบตัวของกระเพาะอาหารของหนูถีบจักรทั้งกระเพาะซึ่งกระตุ้นด้วยแบเรียมคลอไรด์ (2×10^{-4} โมลาร์) และคาร์บาคอล (1×10^{-7} โมลาร์) อย่างมีนัยสำคัญทางสถิติเช่นเดียวกัน จากผลการทดลองอาจสรุปได้ว่า แอนซีสโตรเทคโตรีนมีฤทธิ์ลดการหดเกร็งของกล้ามเนื้อกระเพาะอาหารได้ และฤทธิ์ยับยั้งนี้ไม่น่าจะมีความจำเพาะเจาะจงต่อ receptor แต่เป็นแบบ non specific antagonism