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Original Article

The Relaxant Effect of Carvacrol on Acetylcholine-induced Contraction in Isolated Rat's Ileum

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A B S T R A C T

Background and Aims: The antioxidant and anti-inflammatory effects of carvacrol were reported. The aim of the current study was an investigation the relaxant effects of carvacrol on acetylcholine-induced contraction in isolated rat's ileum.

Materials and Methods: In this study, the tissues separated from ileum were fixed on the organ bath containing Tyrode's solution. The amplitude of contractions has been recorded using isotonic transducer. Before and after subjecting the tissues with saline and different concentrations of carvacrol, the procedure was elicited by cumulative logarithmic concentrations of acetylcholine (Ach).

Results: Our results indicated that carvacrol at 10^{-2}, 10^{-3} and 10^{-4}, but not 10^{-5} M decreased acetylcholine (10^{-4} M)-induced contraction (10^{-2} M at $p<0.001$; 10^{-3} and 10^{-4} at $p<0.05$ vs. Ach+saline). The antispasmodic effect of carvacrol was examined and showed that in concentrations of 10^{-5}, 10^{-4} and 10^{-3} M, but not 10^{-6} M, the contractive effect of acetylcholine10^{-3}M was prevented significantly. The concentrations of 1, 2 and 4 µM of verapamil were also tested and indicated that 4 µM of verapamil reduced contraction by 22.1±.5% ($p<0.01$ vs. saline). In samples treated with combined carvacrol (10^{-5} M) and verapamil (4 µM) contraction percentage was decreased by 35±14%, which was significantly different compared to the Ach+saline group as well as the single treated groups ($p<0.01$ and $p<0.05$, respectively).

Conclusions: According to our findings, due to the effect of carvacrol and verapamil, the relaxant mechanism of carvacrol may be mediated by Ca^{+2} release from the sarcoplasmic reticulum and the response of the contractile system.

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Introduction

Carvacrol is a monoterpenoid phenol and is rich in thymus [1] and satureja [2]. There are some evidences that show carvacrol causes anti-histaminic [3] and anti cholinergic [4] property. Vasodilatory effect of carvacrol through Ca^{+2}-activated K^{+} channels has been reported. Carvacrol can also relax arteries by activating transient receptor potential channels in the endothelium [5]. The anticholinesterase, antioxidant and anti-inflammatory effects of carvacrol were also reported [6, 7]. Thymol and carvacrol causes endothelium relaxation in rat's aorta and this effect may be mediated through some mechanisms such as regulation of Ca^{+2}release from sarcoplasmic reticulum and response of the contractile system [8]. Another experimental study designated the role of carvacrol by evaluation of stimulatory effect of Zataria multiflora Boiss extract on β_2-adrenoceptors [9]. It has been suggested that carvacrol exerts its anticholinergic action via inhibition of central nervous cholinergic system [4]. Since the acetylcholine is a major stimulatory transmitter for gastrointestinal motility and due to the controversial suggestions about the effect of carvacrol on cholinergic system, the first aim of this study was to appraise the relaxant effects of carvacrol on acetylcholine-induced contraction in isolated rat's ileum. Regarding the importance of Ca^{2+} homeostasis in muscle contraction was also evaluated.

Materials and methods

Animals

Thirty six male Wister rats (200-250 grams) were kept on a 12-h light-12-h dark cycle in a temperature-controlled room. All of the experimental routines were followed through by approval of the animal ethics Committee of Shahid Sadoughi university of Medical Sciences (Yazd, Iran), which was in conformity with the internationally accepted principles for laboratory animal use and care brought up by the European Community.

Protocols

In this study, the examination of 10^{-5} up to 10^{-2} M concentrations of carvacrol was done for their spasmolytic and antispasmodic action. Acetylcholine chloride was purchased from Sigma Aldrich ChemieGmbh, Germany; as a standard excitant of gastrointestinal smooth muscle. Experiments were executed as reported in our previous study. Concisely, adult male rats were casualtied by cervical dislocation. Sections of ileum (2 cm in length) were excised, purged of their contents, and trimmed of their mesentery.

The samples were saved in Tyrode's solution until the start of the experimental process. The tissue sample was put at the end of the internal chamber of an organ bath holding 50 ml Tyrode's solution in the axis of its longitudinal muscle and its other end was firmly tied to the isotonic transducer lever with a part of a thread; the chamber was fixed at 37°C and bubbled with 95% O_2 and 5% CO_2. The recordings of isotonic responses were held by an isotonic transducer (T2) and an Oscillograph recording system (the Bioscience 400 Series Washington Oscillograph).Then, it was possible for it to

stabalize for 15 minutes prior to the addition of the drug, and drained out in 30-minute intervals by a fresh Tyrode's solution.

Relaxant action

To investigate the spasmolytic action

In this stage, spasmolytic effect of different concentrations of carvacrol and verapamil on the maximum contraction of ileum smooth muscle after using10^{-4} M of acetylcholine was examined.

To investigate the antispasmolytic action

In this experiment, the different concentrations of carvacrol and verapamil were applied 7 min. before acetylcholine (10^{-9} up to 10^{-2} M)-induced smooth muscle contraction.

Statistical analysis

The results of different observational solutions were declared as Mean ±SD of the percentage decrease of contraction amplitude and compared to maximum effect brought on by acetylcholine. All of the statistical analyses and comparisons were made using the ANOVA followed by Tukey's test. The statistical significance was advised as $p<0.05$.

Results

Spasmolytic action of Carvacrol

To investigate the spasmolytic effect of carvacrol, the effective dose of acetylcholine (10^{-4} M) significantly elevated the baseline in all assessments and then the effect of carvacrol and saline on isotonic contraction was tested. Our results indicated that all concentrations of carvacrol 10^{-2}, 10^{-3} and 10^{-4} M except 10^{-5} M reduced acetylcholine (10^{-4} M)-induced contractions significantly by 64.8±10.1% ($p<0.05$), 46.7±8% ($p<0.05$) and 16.2±5.2% ($p<0.001$), respectively vs. Ach+saline group (Fig. 1).

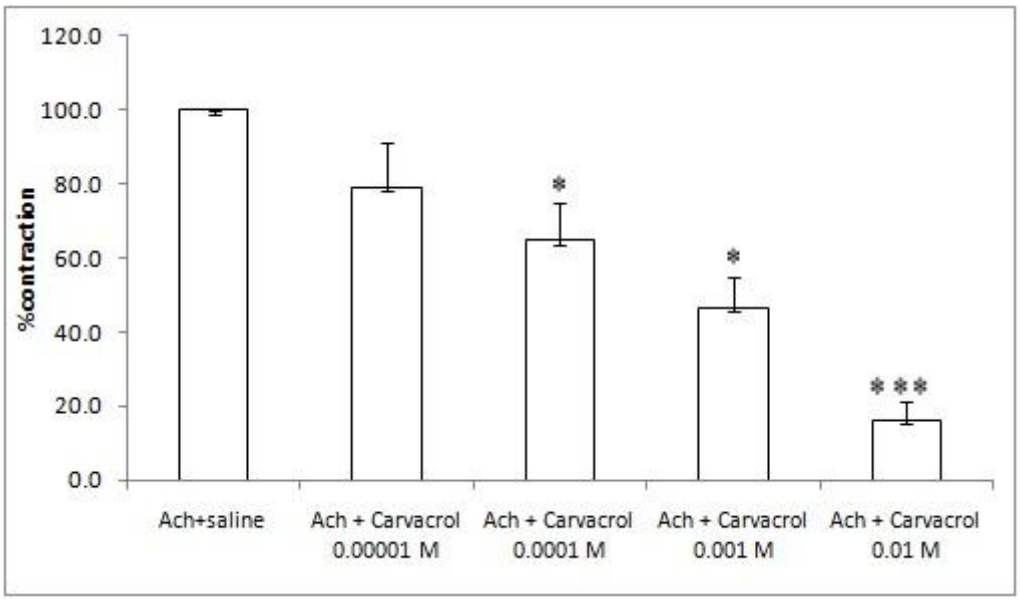

Fig. 1. Spasmolytic response of carvacrol on acetylcholine (0.0001 M)-induced contraction of separated rat's ileum (n=6).*$p<0.05$ and ***$p<0.001$ shows the significant difference as equated to the Ach-saline group on the base of the one-way ANOVA through Tukey's post-test. Data are presented as Mean±SD.

Antispasmodic action of Carvacrol

To assessment the antispasmodic effect of carvacrol, different doses of the solutions (10^{-2}, 10^{-3}, $10^{-4}$and 10^{-5} M) were added to the organ bath before Ach with concentration of 10^{-9} up to 10^{-2} M. Then, isotonic contraction of ileum was recorded. Our findings showed that carvacrol in concentrations of 10^{-5}, 10^{-4} and 10^{-3} M, but not 10^{-6} M prevented from the contractive effect of acetylcholine (10^{-3} M) (Fig. 2).

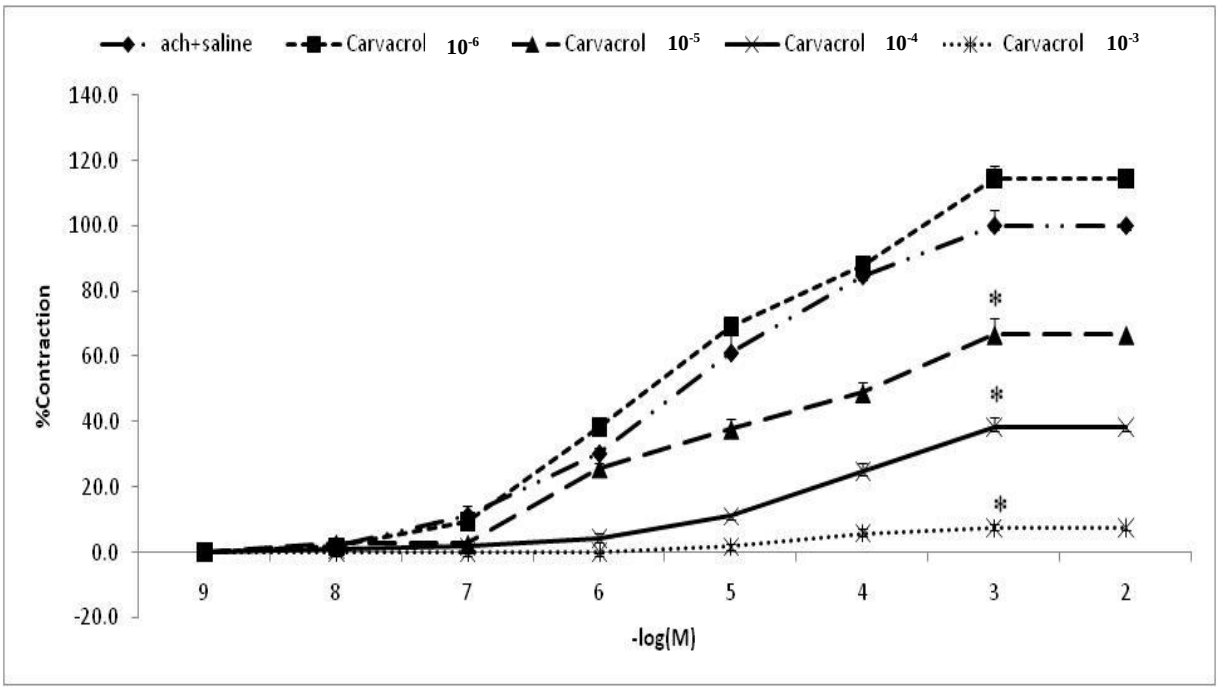

Fig. 2. Antispasmodic response of carvacrol. Different concentrations of carvacrol were applied before acetylcholine (10^{-9} up to 10^{-2}M) –induced ileum smooth muscle contraction. Each point signals the means of 6 tests. *p<0.05 and *** p<0.001 shows the significant difference as equated to the Ach-saline group at the base of the one-way ANOVA through Tukey's post-test.
Data are presented as Mean±SD.

Spasmolytic action of Verapamil

For studying the spasmolytic effect of verapamil as a positive control, the effective dose of acetylcholine (10^{-4} M) which significantly elevated the baseline in all assessments was used and then the effect of verapamil and saline on isotonic contraction was tested. Our results indicated that concentrations of 4 µM of verapamil reduced acetylcholine-induced contraction by 22.1±.5% (P< 0.01 vs. Ach+saline group; Fig. 3).

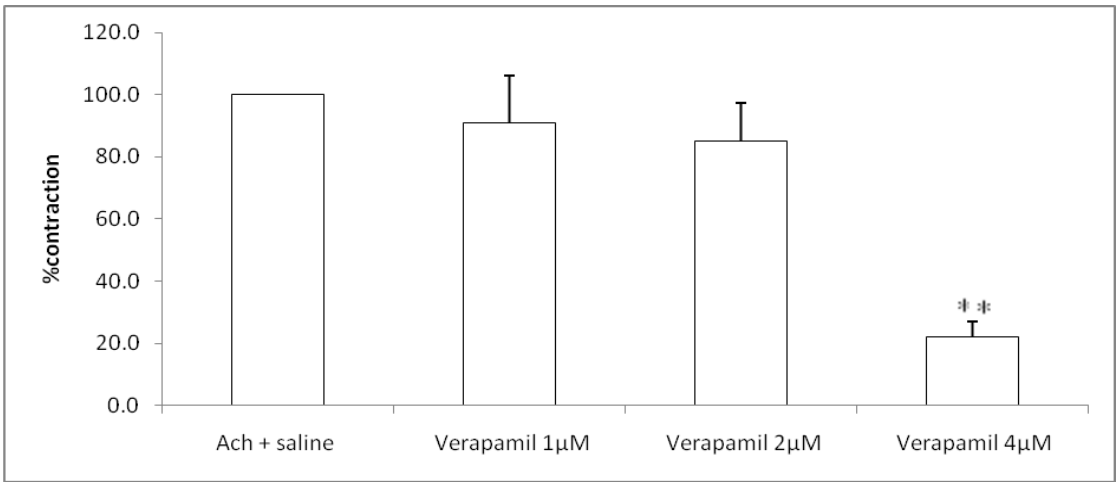

Fig. 3. Spasmolytic effect of Verapamil on contraction of rat's ileum, which was produced by acetylcholine (10^{-4} M). Each point signals the means of 6 tests and the vertical bars represent the SD. **shows the significant difference ($p<0.01$) as equated to the acetylcholine-induced contraction on the base of the one-way ANOVA through Tukey's post-test.

Discussion

According to our findings, carvacrol induced a relaxant effect on ileal smooth muscle. Therelaxation of carvacrol in gastrointestinal smooth muscle induced by many ways, including muscarinic M3, histaminic H1 and 5-HT receptors blocking [11, 12]. The relaxant effect may also be induced by stimulating the nitric oxide [13], purinergic [14], adrenergic [15], or gamma-aminobutyric acid ergic modulatory systems [16]. There are some evidences that show carvacrol causes anti cholinergic [4] and anti-histaminic [3] property on tracheal smooth muscle. Modulation of transient receptor potential (TRP) channels, inhibition of the mammalian TRPM7 [17], hyperpolarization of smooth muscle and reduction of intracellular calcium in arterial myocytes are the major suggested mechanisms for relaxant effects of carvacrol [5]. Our findings indicate that carvacrol inhibited the intestinal motility contrast this suggestion since the cholinergic system is the most important excitatory transmitter regarding the intestinal smooth muscle contraction [18]. However, other studies reported the effects of carvacrol on inflammation [6] and contraction by Ca^{+2} release from sarcoplasmic reticulum and the response of the contractile system [7]. These actions are through inhibition of the central nervous cholinergic system and its muscarinic [4] receptors, which are in accordance with our findings, indicating the inhibitory effect of carvacrol on Ach-induced contractions in a peripheral tissue. This action may be due to the blockade of muscarinic receptors on the ileal smooth muscle cells or via a competitive inhibitory effect on Ca^{+2} release from sarcoplasmic reticulum. On the other hand, it has been demonstrated that carvacrol attenuated allergic responses via its inhibitory action on the release of histamine [3].

Therefore, part of the inhibitory action on ileal smooth muscle contraction expressed by the carvacrol may be due to its anti-cholinergic activity and another probable mechanism, which may inhibit the contraction of gastrointestinal smooth muscle is via activating and modifications in Ryanod in receptors that affect sarcoplasmic reticulum Calcium regulation and excitation-contraction coupling [19]. All of the above mechanisms of carvacrol may be affected by activation of the TRP cation channels, which need further investigations for more details.

Conclusions

According to our findings, carvacrol poses a potent relaxant action on acetylcholine induced contraction in isolated rat's ileum. Verapamil also induced contraction, which is significantly different with maximum contraction of acetylcholine in the presence of saline.

Conflict of Interest

Authors declare that there is no conflict of interest.

Acknowledgments

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