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Relaxant effects of carvacrol on guinea pig tracheal chains and its possible mechanisms

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The bronchodilatory effects of three cumulative volumes (0.02, 0.04, and 0.08 ml) of 1/100 diluted carvacrol were examined by their relaxant effects on tracheal chains of guinea pigs precontracted by 60 mM KCl (group 1) and 10 μM methacholine in two different conditions including: non-incubated tissues (group 2) and incubated tissues with 1 μM propranolol and 1 μM chlorpheniramine (group 3), (for each group, $n = 5$). The relaxant effects of all three volumes of carvacrol were significantly higher than those of ethanol and even theophylline in all three groups of experiments ($p < 0.05$ to $p < 0.001$). There were positive correlations between the relaxant effect of carvacrol and incremental volume ($p < 0.05$ to $p < 0.001$). These results indicated that carvacrol has a potent relaxant effect on tracheal chains of guinea pigs which is not due to β_2-adrenergic stimulatory, histamine H_1, and muscarinic blocking effect.

1. Introduction

Carum copticum is a grassy, annual plant which grows in the east of India, Iran and Egypt with white flowers and small brownish seeds. The seeds of this plant have an odour similar to thymol, and its essential oil contains γ-terpinene, p. cymene, α-pinene, β-pinene and other substances such as thymol and carvacrol [1]. The seeds of *Carum copticum* have several therapeutic effects including diuretic, anti-vomiting, analgesic, anti-asthma and anti-dyspnea effects [2]. They also have a therapeutic effect on some cutaneous disorders, neural disorders and urinary tract disorders. *Carum copticum* is therefore used in household remedies.

Although a muscarinic effect of roasted *Carum copticum* has been shown [3], our previous studies have demonstrated bronchodilatory and anticholinergic effects [4], inhibitory effects on histamine (H_1) receptors [5], and stimulatory effects on β-adrenergic [6] for this plant. However, in another study when muscarinic, histamine H_1 and β-adernergic receptors were blocked, the bronchodilatory effect of essential oil from this plant was not reduced and a xanthine-like effect has been suggested for essential oil from this plant [7]. In the present study the relaxant effect of three increment of volumes of carvacrol on guinea pig tracheal chains was examined.

2. Investigations and results

2.1. Relaxant (bronchodilatory) effect

In all groups of experiments different volumes of carvacrol and theophylline showed significant relaxant effects compared with those of ethanol ($p < 0.05$ to $p < 0.001$). The relaxant effect of the first volume of carvacrol only in group 1 was not significantly different from that of ethanol.

Table 1: Relaxant effect of carvacrol in comparison with negative control (ethanol) and positive control (theophylline) in three groups of experiments

Experimental Groups	Different volumes	Ethanol	Theophyline	Carvacrol
1	1	-5.22 ± 1.8	8.85 ± 12.4 NS	49 ± 15.50* ns
	2	-18.55 ± 3.1	42.26 ± 8.9**	80.83 ± 9.95*** +
	3	-32.61 ± 4.0	74.50 ± 9.0***	100.0 ± 0.0*** +
2	1	-1.37 ± 2.1	10.46 ± 3.9 NS	6.60 ± 2.71* ns
	2	6.15 ± 2.25	31.25 ± 7.0*	86.02 ± 17.74* +
	3	17.33 ± 4.8	67.78 ± 7.0*	137.60 ± 35.72*** +
3	1	3.03 ± 0.92	9.40 ± 0.96***	59.89 ± 14.60* +
	2	11.26 ± 2.10	34.09 ± 3.0***	109.20 ± 10.31*** ++
	3	26.16 ± 4.5	68.18 ± 5.6**	114.60 ± 10.20*** +

Values are presented as mean ± SEM. Differences between ethanol with carvacrol and theophylline: NS; nonsignificant difference, *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$. Differences between theophylline and carvacrol: ns; nonsignificant difference, +; $p < 0.05$, ++; $p < 0.001$. Volumes of ethanol tested were 0.1, 0.2, and 0.4 ml. For assessing relaxant effect of carvacrol; 0.02, 0.04, and 0.08 ml were used and for theophylline 0.25, 0.5 and 1 ml. Group 1; contracted tracheal chains by 60 mM KCl, group 2; non incubated tracheal chains contracted by 10 μM methacholine, and group 3 experiments; tracheal chains incubated with propranolol and chlorpheniramine and contracted by 10 μM methacholine, (for each group, $n = 5$)

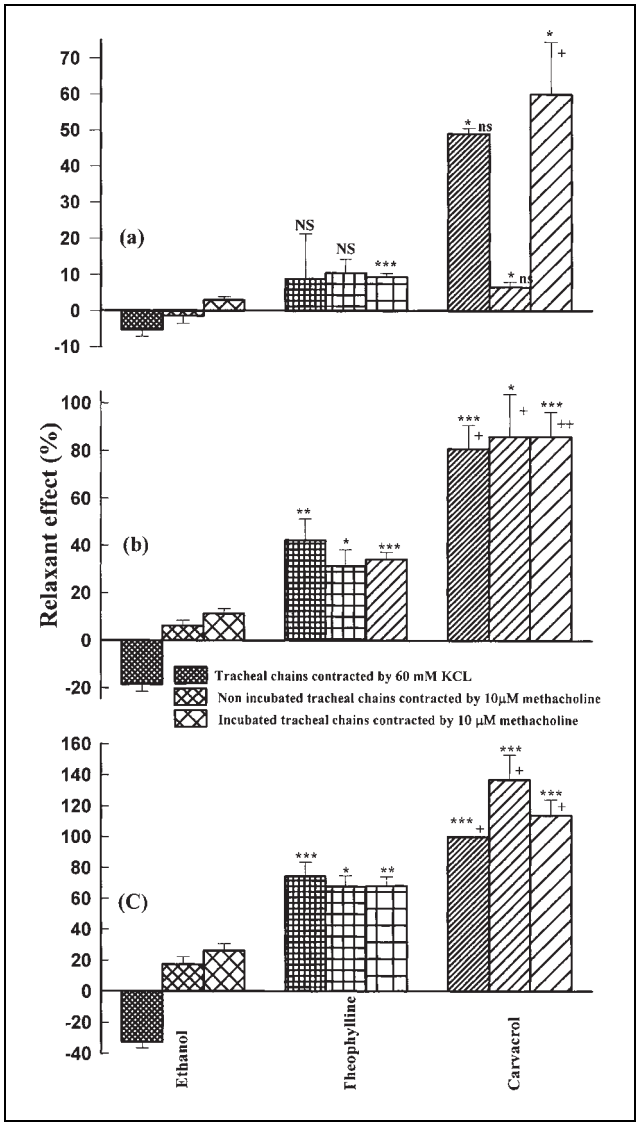

Fig. 1: Relaxant effect of three cumulative volumes of carvacrol in group 1 (tracheal chains contracted by 60 mM KCl, fine filled bar), group 2 (non incubated tracheal chains contracted by 10 μM methacholine, medium filled bar), and group 3 experiments (incubated tracheal chains with propranolol and chlorpheniramine contracted by 10 μM methacholine, coarse filled bar), (for each group, n = 5) (a) with low volume (b) with medium volume and (c) with high volume. Volumes of ethanol tested were 0.1, 0.2, and 0.4 ml. For assessing relaxant effects of carvacrol; 0.02, 0.04, and 0.08 ml were used and for theophylline 0.25, 0.5 and 1 ml. The effect of only the first volume of carvacrol was significantly greater than that of group 2 ($p < 0.05$). There was no significant difference between the relaxant effects obtained in three groups of experiments for other volumes of carvacrol. Differences between ethanol and carvacrol; NS: nonsignificant difference, *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$. Differences between theophylline and carvacrol; ns: nonsignificant difference, +; $p < 0.05$, ++; $p < 0.001$.

In addition the relaxant effects of volumes 2 and 3 of carvacrol in group 1 and 2 and the effects of all volumes of carvacrol in group 3 were significantly greater than those of theophylline ($p < 0.05$ to $p < 0.001$), (Table 1, Fig.).

2.2. Comparison of the relaxant effect of carvacrol between the three groups of experiments

There were no significant differences between the relaxant effects of most volumes of carvacrol found in the three groups of experiments. The relaxant effect of the first volume only of carvacrol found in group 3 was significantly greater than that in group 2 ($p < 0.05$) (Table 2).

Table 2: Comparison of the relaxant effects of carvacrol in three different groups of experiment

Different Volumes	Group 1	Group 2	Group 3
1	49.00 ± 15.5	6.60 ± 2.71 NS	59.89 ± 14.60 NS +
2	80.83 ± 9.95	86.02 ± 17.74 NS	109.20 ± 10.31 NS ns
3	100.0 ± 0.0	137.60 ± 15.97 NS	114.60 ± 10.20 NS ns

Values are presented as mean ± SEM. Difference between the results of groups 2 and 3 and those of group 1: NS; nonsignificant difference. Difference between the results of group 3 and those of group 2: ns; nonsignificant difference, +; $p < 0.05$.

Table 3: Correlation between the relaxant effects of carvacrol and the effects of theophylline and volume in three different groups of experiments

Group 1		Group 2		Group 3	
Cor. with volume	Cor. with theo.	Cor. with volume	Cor. with theo.	Cor. with volume	Cor. with theo.
0.665**	0.396 NS	0.673**	0.465 NS	0.584*	0.682***

Cor.; corelation, theo.; theophylline, significance of differences; NS; nonsignificant difference, *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$.

2.3. Correlation between the volume of carvacrol and its relaxant effect

There were significant positive correlations between the relaxant effects of carvacrol and increasing volume in all groups of experiments ($p < 0.05$ to $p < 0.001$). Only in group 3 was the correlation between the relaxant effect of carvacrol and theophylline significantly positive ($p < 0.001$) (Table 3).

3. Discussion

In this study the relaxant (bronchodilatory) effects of cumulative volumes of carvacrol were studied in comparison with ethanol as a negative control and theophylline as a positive control. All volumes of carvacrol in all three experimental conditions showed potent and volume dependent relaxant effects. In addition, the relaxant effects of most volumes of carvacrol were significantly greater than those of theophylline.

The bronchodilatory effect of carvacrol might be due to several different mechanisms including stimulation of β-adrenergic receptors, inhibition of histamine H_1 receptors or an anticholinergic property of this substance. In fact, our previous studies demonstrated anticholinergic [4], histamine (H_1) inhibitory [5] and β-adrenergic stimulatory [6] effects of *Carum copticum*. In addition a previous study (unpublished) suggests that the constituent of the plant mainly responsible for its bronchodilatory effect is carvacrol. To evaluate the contribution of β-adrenergic stimulatory and/or H_1 histamine blocking effects of carvacrol on its bronchodilatory effect, the effects of carvacrol, theophylline, and ethanol were re-examined as in group 2 but with β adrenergic and H_1 histamine receptors inhibited by propranolol and chlorpheniramine. Only the smallest volume of carvacrol showed a relaxant effect in the group 3 experiments significantly greater than in group 2. These

findings may suggest a stimulatory effect of carvacrol on the histamine H_1 receptor. However, this suggestion is not supported by the effect of two larger volumes of carvacrol. Therefore, the β_2-adrenergic stimulatory and/or histamine H_1 blocking properties of carvacrol may not contribute to the bronchodilatory effect of this substance.

To investigate the relaxant effect of carvacrol on contraction of tracheal chains by a non selective substance, we examined the effects of carvacrol, theophylline and ethanol on contraction induced by KCl. The relaxant effects of different volumes of carvacrol obtained in the group 1 experiments were not significantly different from those in group 2 and 3. These findings suggest that the relaxant effect of carvacrol is not due to the anticholinergic property of this substance.

The relaxant effect of carvacrol was volume (concentration) dependent. There were positive correlations between increasing volumes of carvacrol and its relaxant effect. The relaxant effects of increasing volumes of carvacrol were also positively correlated with those of theophylline, but only in group 3.

Although the volumes of carvacrol were much less than those of theophylline (less than one-twelfth), the relaxant effects of various volumes of carvacrol on guinea pig tracheal chains in all groups of experiments were significantly greater than those of theophylline. Therefore, it is difficult to suggest that the relaxant effect of carvacrol is due to a xanthine like activity.

The other possible mechanisms causing the bronchodilatory effect of carvacrol include: stimulation of inhibitory non-adrenergic non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC [8], methylxanthine activity [9], inhibition of phosphodiestrase [10] and calcium antagonism [11, 12]. The contributions of these mechanisms on bronchodilatory effect of carvacrol should be clarified in further studies. In conclusion, the results of this study showed a relatively potent relaxant (bronchodilatory) effect of carvacrol on guinea pig tracheal chains. The β_2-adrenergic stimulatory, histamine H_1, and muscarinic blocking effects do not contribute to the bronchodilatory effect of this substance on tracheal chains.

4. Experimental

4.1. Tissue preparations

Male guinea pigs (400–700 g) were killed by a blow on the neck and trachea were removed. Each trachea was cut into 10 rings (each containing 2–3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle, and sutured together to form a tracheal chain [13]. Tissue was then suspended in a 10 ml organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent U.K.) containing Krebs-Henseliet solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11.

The Krebs solution was maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

4.2. Protocols

The relaxant effects of three cumulative volumes of carvacrol (0.02, 0.04, and 0.08 ml), ethanol as negative control (0.1, 0.2, and 0.4 ml), and anhydrous theophylline (Sigma Chemical Ltd UK) as positive control (0.25, 0.5, and 1 ml) were assessed as follows: In each experiment the effect of three cumulative volumes of one of the solutions (ethanol, theophylline, or carvacrol) on contracted tracheal smooth muscle was measured after exposing tissue to the solution for 10 min. A decrease in tone was considered as a relaxant (bronchodilatory) effect and expressed as a positive percentage change in proportion to the maximum contraction obtained due to contractile substance; and an increase in tone was considered as a contractile (bronchoconstrictory) effect which was expressed as a negative percentage change [14].

The relaxant effect of different solutions was tested with three different experimental designs as follows:

1. On tracheal chains contracted by 60 mM Kcl (group 1 experiments).
2. On non-incubated tracheal chains contracted by 10 μM methacholine hydrochloride (Sigma Chemical Ltd UK), (group 2 experiments).
3. On incubated tracheal chains 30 min prior to and during experimental relaxation with 1 μM propranolol hydrochloride (Sigma Chemical Ltd UK) and 1 μM chlorpheniramine maleate (Sigma Chemical Ltd UK) after contraction by 10 μM methacholine hydrochloride (group 3 experiments).

The relaxant effects in three groups of experiments were examined in three different series of tracheal chains (for each group, $n = 5$). All of the experiments were performed randomly with a 1 h resting period for the tracheal chains between each two experiments while washing the tissues every 15 min with Krebs solution. In all experiments responses were recorded on a kymograph (ET8 G-Boulitt, Paris) and were measured after fixation.

4.3. Statistical analysis

The data on the bronchodilatory effects of different experiments were expressed as mean ± SEM. The data for bronchodilatory effects of different volumes of carvacrol were compared with the results with negative and positive controls using the unpaired "t" test. The data on bronchodilatory effects obtained in the three groups of experiments were compared using a one-way analysis of variance (ANOVA) test. The relaxant effect of carvacrol was correlated with both volume of carvacrol and the effect of theophylline, using least squares regression. Significance was taken as $p < 0.05$.

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