Journal of Research in Pharmacy

Research Article

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# Relaxing actions of carvacrol on isolated rat duodenum smooth muscles: Evidence for the role of potassium ion channels

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Received: 9 July 2024 / Revised: 27 July 2024 / Accepted: 27 July 2024

**ABSTRACT**: Mammals have approximately 80 genes that encode potassium ($K^+$) channels. They have a wide range of physiological functions, such as modulation of action potential duration and relaxation of smooth muscles. Carvacrol, a compound found in various aromatic plants, has been used traditionally for treating asthma, menstrual spasms, and gastrointestinal disorders. However, its mechanism of action on smooth muscles remains insufficiently understood. This study investigates the role of potassium ion channels in the relaxing effects of carvacrol on isolated rat duodenum smooth muscles. Rats were euthanized and duodenal segments were prepared and placed in an isolated organ bath with Krebs' solution. The segments were equilibrated for 1 h and then treated with chemicals to obtain concentration-response curves. The data were evaluated using one-way Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (Tukey's HSD) test for multiple comparisons. Our findings demonstrate that carvacrol induces a dose-dependent relaxation of these muscles. The relaxation effects were significantly reduced in the presence of specific potassium channel inhibitors, including paxilline, UCL1684, linopirdine, barium chloride, and 4-aminopyridine. They were completely blocked by the combination of barium chloride and tetraethylammonium. However, glibenclamide, ruthenium red, and nitroarginine did not alter the relaxing effects of carvacrol. In conclusion: carvacrol relaxes duodenum smooth muscles by opening barium chloride-sensitive Kir2.1 and Kir3.1, as well as tetraethylammonium-sensitive KCa1.1, KCa2.1, KCa2.2, KCa2.3, Kv1.2, Kv1.4, Kv1.5, Kv2.1, Kv2.2, Kv4.1, Kv4.2, Kv4.3, Kv7.1, Kv7.2, Kv7.3, Kv7.4, and Kv7.5 potassium channels.

**KEYWORDS**: Carvacrol; Ion channel; Transient receptor potential; Potassium; Duodenum; Smooth muscle.

## 1. INTRODUCTION

Changes in the contractility of smooth muscles play an important role in the physiopathology of various diseases such as irritable bowel syndrome, achalasia, and hyperactive bladder [1-3]. Mammals have approximately 80 genes that encode potassium ($K^+$) channels [4]. The four primary types of human $K^+$ channels are inward-rectifying (Kir), calcium-activated (KCa), two-pore (K2P), and voltage-gated (Kv) channels [5]. They are expressed in numerous tissues and have a wide range of physiological functions, such as modulation of action potential duration and relaxation of smooth muscle cells [6]. Among the potassium channels that are distributed and play a role in the relaxation of smooth muscles are KCa1.1, KCa2.1, KCa2.2, KCa2.3, Kv2.1, Kv2.2, Kv4.1, Kv4.2, Kv4.3, Kv7.1, Kv7.2, Kv7.3, Kv7.4, Kv7.5, Kv1.2, Kv1.5, Kv1.4, Kir2.1, Kir3.1, Kir6.1, and Kir6.2 [4,7].

Carvacrol (2-methyl-5-(1-methyl ethyl) phenol) is a synthetic chemical compound that can also be naturally found in various aromatic plants [8]. Since ancient times, carvacrol-containing plants have been used ethnomedically for various purposes, especially against asthma, menstrual spasms, and gastrointestinal disorders [8]. In studies on smooth muscles, it has been reported that carvacrol relaxes the smooth muscles of the trachea independently of beta-adrenergic, histaminergic, and muscarinic receptor interactions [9]. However, subsequent research has demonstrated that carvacrol exhibits antimuscarinic and antihistaminic effects [10,11] and acts as an adrenergic agonist on the trachea [12]. On the other hand, it has been reported that carvacrol is ineffective on the aorta [13]. However, other studies have shown that it relaxes the aorta and

**How to cite this article:** Yusuf M, Aydin S. Relaxing actions of carvacrol on isolated rat duodenum smooth muscles: evidence for the role of potassium ion channels. J Res Pharm. 2024; 28(5): 1501-1507.



ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.828

mesenteric artery, likely by reducing calcium release from the sarcoplasmic reticulum and calcium entry from the cell membrane and transient receptor potential (TRP) ion channels [14,15].

Considering the studies conducted to date with carvacrol on smooth muscles and the diversity of potassium ion channels involved in smooth muscle relaxation, the mechanism of action of carvacrol remains insufficiently clarified. Therefore, this study was conducted to elucidate the role of potassium channels in the mechanisms mediating carvacrol's effect on the smooth muscles of the rat duodenum.

## 2. RESULTS

In our study, carvacrol showed relaxant effects on the smooth muscles of the duodenum in a dose-dependent manner (Figures 1, 2, 3). The relaxing effect of carvacrol decreased in the presence of paxilline, UCL1684, linopirdine (Figure 1) (Table 1), barium chloride (BaCl2) (Figure 2) (Table 2), and 4-aminopyridine (4-AP). (Figure 3) (Table 3). The relaxant effect of carvacrol was completely blocked in the presence of BaCl2 + tetraethylammonium (TEA) (Figure 2) (Table 2). In contrast, in the presence of glibenclamide (Figure 2) (Table 2), ruthenium red, and nitroarginine (L-NOARG) (Figure 3) (Table 3), there was no change in the relaxant effect of carvacrol.

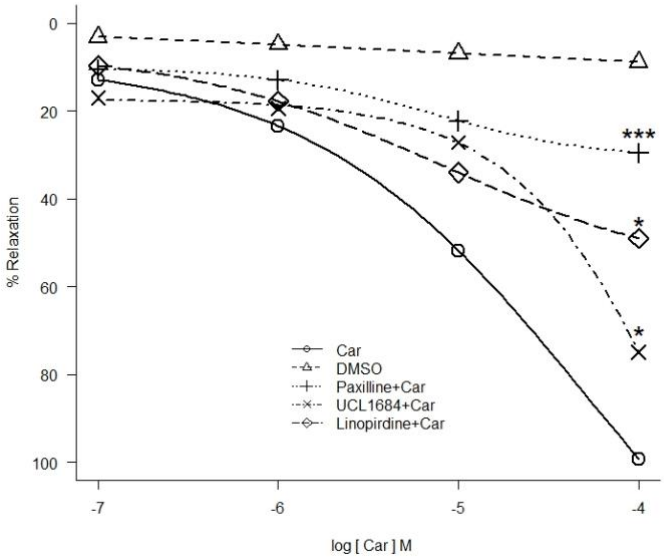


**Figure 1.** Effect of paxilline, UCL1684, and linopirdine on the relaxant effect of carvacrol on isolated rat duodenal smooth muscles. Maximum relaxation by carvacrol was reduced in the presence of paxilline, UCL1684, and linopirdine. (n=7). *p<0.05 (*), 0.01 (**),0.001 (***).*

**Table 1.** Comparison of the $IC_{50}$ values for the effects of paxillin, UCL1684, and linopirdine on the relaxing effect of carvacrol on rat duodenum smooth muscles.

| Groups | $IC_{50}$ (estimate) | $IC_{50}$ (SEM) | p |
|---|---|---|---|
| Carvacrol / DMSO | 1.6284e+00 | 1.0054e+01 | 0.95029 *** |
| Carvacrol / Paxilline+Carvacrol | 2.9720e+03 | 2.5179e+03 | 0.24080 *** |
| Carvacrol / UCL1684+Carvacrol | 1.7390e+02 | 1.2160e+03 | 0.88722 * |
| Carvacrol / Linopirdine+Carvacrol | 1.8041e-01 | 4.7643e-02 | < 2e-16 * |
| DMSO / Paxilline+Carvacrol | 1.8251e+03 | 1.1360e+04 | 0.87275 |
| DMSO / UCL1684+Carvacrol | 1.0680e+02 | 9.9565e+02 | 0.91559 |
| DMSO / Linopirdine+Carvacrol | 1.1079e-01 | 6.8377e-01 | 0.19644 |
| Paxilline+Carvacrol / UCL1684+Carvacrol | 5.8513e-02 | 4.1173e-01 | -0.02433 * |
| Paxilline+Carvacrol / Linopirdine+Carvacrol | 6.0702e-05 | 5.0345e-05 | < 2e-16 *** |
| UCL1684+Carvacrol / Linopirdine+Carvacrol | 1.0374e-03 | 7.2518e-03 | < 2e-16 *** |

*SEM: standard error of mean; p<0.05 (*), 0.01 (**), 0.001 (***).*

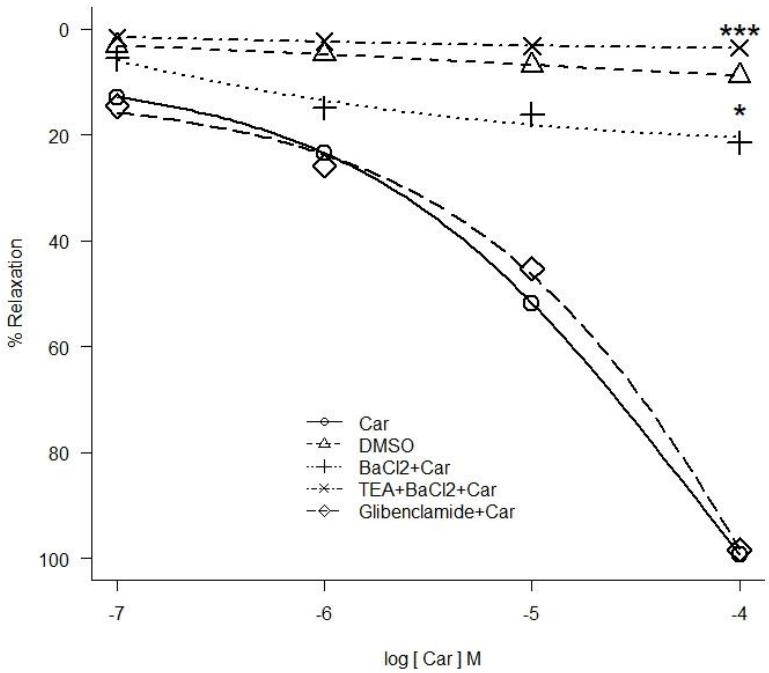


**Figure 2.** Effect of BaCl2, TEA + BaCl2, and glibenclamide on the relaxant effect of carvacrol on isolated rat duodenal smooth muscles. Maximum relaxation by carvacrol was reduced in the presence of BaCl2 and was completely blocked in the presence of BaCl2 + TEA, in the presence of glibenclamide there was no change in the relaxant effect of carvacrol (n=7). *p<0.05 (*), 0.01 (**),0.001 (***).*

**Table 2.** Comparison of the $IC_{50}$ values for the effects of BaCl2, TEA +BaCl2, and glibenclamide on the relaxing effect of carvacrol on rat duodenum smooth muscles

| Groups | $IC_{50}$ (estimate) | $IC_{50}$ (SEM) | p |
|---|---|---|---|
| Carvacrol / DMSO | 1.6284e+00 | 1.0054e+01 | 0.95029 *** |
| Carvacrol / BaCl2+Carvacrol | 2.9720e+03 | 2.5179e+03 | 0.24080 * |
| Carvacrol / TEA+BaCl2+Carvacrol | 1.7390e+02 | 1.2160e+03 | 0.88722 *** |
| Carvacrol / Glibenclamide+Carvacrol | 1.8041e-01 | 4.7643e-02 | < 2e-16 |
| DMSO / BaCl2+Carvacrol | 1.8251e+03 | 1.1360e+04 | 0.87275 |
| DMSO / TEA+BaCl2+Carvacrol | 1.0680e+02 | 9.9565e+02 | 0.91559 |
| DMSO / Glibenclamide+Carvacrol | 1.1079e-01 | 6.8377e-01 | 0.19644 * * |
| BaCl2+Carvacrol / TEA+BaCl2+Carvacrol | 5.8513e-02 | 4.1173e-01 | 0.02433 * |
| BaCl2+Carvacrol / Glibenclamide+Carvacrol | 6.0702e-05 | 5.0345e-05 | < 2e-16 * |
| TEA+BaCl2+Carvacrol / Glibenclamide+Carvacrol | 1.0374e-03 | 7.2518e-03 | < 2e-16 *** |

*SEM: standard error of mean; p<0.05 (*), 0.01 (**), 0.001 (***).*

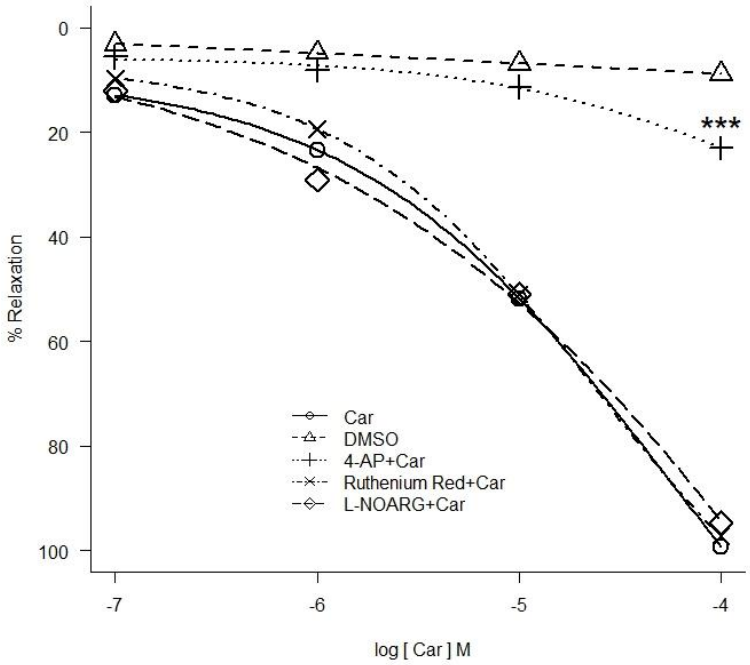


**Figure 3.** Effect of 4-AP, ruthenium red, and L-NOARG on the relaxant effect of carvacrol on isolated rat duodenal smooth muscles. Maximum relaxation by carvacrol was reduced in the presence of 4-AP, in the presence of ruthenium red, and L-NOARG there was no change in the relaxant effect of carvacrol (n=7). *p<0.05 (*), 0.01 (**), 0.001 (***).*

**Table 3.** Comparison of the $IC_{50}$ values for the effects of 4-AP, ruthenium red, and L-NOARG on the relaxing effect of carvacrol on rat duodenum smooth muscles.

| Groups | $IC_{50}$ (estimate) | $IC_{50}$ (SEM) | p |
|---|---|---|---|
| Carvacrol/ DMASO | 1.093057 | 9.381999 | 0.9921 *** |
| Carvacrol / 4-AP+Carvacrol | 0.323375 | 0.527365 | <2e-16 *** |
| Carvacrol / Ruthenium Red+Carvacrol | 2.254434 | 1.205454 | 0.3006 |
| Carvacrol / L-NOARG+Carvacrol | 0.089918 | 0.071585 | 0.2024 |
| DMASO / 4-AP+Carvacrol | 0.295845 | 2.576608 | 0.7852 |
| DMASO / Ruthenium Red+Carvacrol | 2.062504 | 17.679743 | 0.9522 *** |
| DMASO / L-NOARG+Carvacrol | 0.082263 | 0.706822 | 0.1971 *** |
| 4-AP+Carvacrol / Ruthenium Red+Carvacrol | 6.971580 | 10.947604 | 0.5866 ** |
| 4-AP+Carvacrol / L-NOARG+Carvacrol | 0.278061 | 0.466429 | 0.1248 ** |
| Ruthenium Red+Carvacrol /L-NOARG+Carvacrol | 0.039885 | -36.2845 | 0.6896 |

*SEM: standard error of mean; p<0.05 (*), 0.01 (**), 0.001 (***).*

## 3. DISCUSSION

It has been reported that carvacrol affected potassium channels in the substantia gelatinosa of the spinal medulla, which were not sensitive to barium and tetraethylammonium [16]. However, in our studies, the relaxing effect of carvacrol was diminished in the presence of various channel inhibitors; BaCl2 (Kir2.1 and Kir3.1) (Figure 2), 4-AP (Kv1.2, Kv1.4, Kv1.5, Kv2.1, Kv2.2, Kv4.1, Kv4.2, and Kv4.3) (Figure 3), linopirdine (Kv7.1, Kv7.2, Kv7.3, Kv7.4, and Kv7.5) (Figure 1), paxillin (KCa1.1) (Figure 1), and UCL1684 (KCa2.1, KCa2.2, and KCa2.3) (Figure 1); and was completely blocked in the presence of BaCl2 (Kir2.1 and Kir3.1) + TEA (Kv1.2, Kv1.4, Kv1.5, Kv2.1, Kv2.2, Kv4.1, Kv4.2, Kv4.3, Kv7.1, Kv7.2, Kv7.3, Kv7.4, Kv7.5, KCa1.1, KCa2.1, KCa2.2, and KCa2.3) (Figure 2). Our findings indicate that carvacrol interacts with barium chloride -sensitive; Kir2.1, Kir3.1, and tetraethylammonium-sensitive; KCa1.1, KCa2.1, KCa2.2, KCa2.3, Kv1.2, Kv1.4, Kv1.5, Kv2.1, Kv2.2, Kv4.1, Kv4.2, Kv4.3, Kv7.1, Kv7.2, Kv7.3, Kv7.4, Kv7.5 potassium channels. Compared to previously reported findings, differences between neurons and smooth muscles may lead to different and even contradictory experimental results. These results suggest that carvacrol does not exhibit the same effects on every tissue and cell, and its effects vary on different tissues and cell types.

Previous studies reported that carvacrol protects against oxidative damage via Kir6.2 channels [17]. However, in our studies, it was observed that the relaxing effect of carvacrol did not change in the presence of glibenclamide, an inhibitor of Kir6.1 and Kir6.2 channels (Figure 2). Contrary to previously reported findings, our findings indicate that Kir6.1 and Kir6.2 channels do not play any role in the relaxing effect of carvacrol observed on the gastrointestinal system's smooth muscles, and the effect of carvacrol in the oxidative damage mechanism may differ from its effect on smooth muscles.

The non-adrenergic non-cholinergic (NANC) system, which uses nitric oxide (NO) as a transmitter, is a part of the autonomic nervous system with a key role in gastrointestinal relaxations [18]. Some studies reported that the gastroprotective effect of carvacrol is mediated by the activation of nitric oxide synthase [19]. In our study, the lack of blocking effect through the nitric oxide synthase inhibitor L-NOARG (Figure 3), indicates no significant role for nitric oxide in the relaxant effect of carvacrol on the duodenum smooth muscles.

Transient Receptor Potential ion channels include at least 28 subtypes in mammals [20]; and they are vital in regulating gastrointestinal (GI) smooth muscle relaxation and contraction. The key TRP channels involved include TRPC, TRPV, and TRPM [21, 22]. Some of them play an important role in transferring calcium and sodium ions into the cell [23, 24]. Different studies have reported the role of TRP channels in the mechanism of action of carvacrol; and while some of them are activated (TRPV3 and TRPA1) [25, 26], others are inhibited by carvacrol (TRPC1 and TRPM7) [27, 28]. The lack of effect of ruthenium red (Figure 3), indicates no significant role for ruthenium red-sensitive TRP ion channels in the relaxant effect of carvacrol on the duodenum smooth muscles.

Potassium channels are not just an ion channel family belonging to humans and mammals. They are an important ion channel family that appeared very early in the evolutionary development of life on Earth and are found in almost all living organisms, from protozoa to single-celled organisms [29]. Our findings suggest that KCa1.1, KCa2.1, KCa2.2, KCa2.3, Kv1.2, Kv1.4, Kv1.5, Kv2.1, Kv2.2, Kv4.1, Kv4.2, Kv4.3, Kv7.1, Kv7.2, Kv7.3, Kv7.4, Kv7.5, Kir2.1, and Kir3.1 channels play a major role in the relaxing effect of carvacrol on isolated duodenum smooth muscles. Furthermore, a review of the UniProt protein database as of December 2023 (version 2023_05) revealed 90 potassium channel proteins and 92 isoforms in humans (http://ftp.ebi.ac.uk/). Currently, there are no specific agonists or antagonists available for each of these ion channels; and due to the large number and variety of these channels and the lack of sufficiently specific agonists and/or antagonists for each of these channels, further research is needed on the effects of carvacrol on potassium channels.

## 4. CONCLUSION

Carvacrol relaxes duodenum smooth muscles by opening barium chloride and tetraethylammonium-sensitive potassium channels. Our research highlights its effects on a broad range of potassium channels and suggests it is a noteworthy subject for further study.

## 5. MATERIALS AND METHODS

### 5.1. Chemicals

Carvacrol, paxilline, ruthenium red, L-NOARG, UCL1684, linopirdine, TEA, 4-aminopyridine, glibenclamide, $BaCl_2$, dimethyl sulfoxide (DMSO), ketamine hydrochloride, KCl, NaCl, $NaHCO_3$, d-glucose, $KH_2PO_4$, $MgSO_4.7H_2O$, and $CaCl_2.2H_2O$ were purchased from Sigma-Aldrich (Germany).

### 5.2. Animals

Ethical approval for the study was obtained from the ethics committee of Anadolu University on 16.06.2021 (Decision no: 2021-36). The study utilized male Sprague-Dawley rats, each weighing 220-250 g, obtained from the Experimental Animals Research Center at Anadolu University. These rats were maintained in a controlled environment with a 12-h light/dark cycle, 55%-60% humidity, and a constant temperature of 22 °C. They had ad libitum access to food and water.

### 5.3. Isolated duodenum experiments

After anesthetizing the rats with ketamine hydrochloride, they were euthanized by cervical dislocation. The duodenal organs were excised and placed in Krebs' solution with the following composition (in mM): NaCl, 118,4; KCl, 4,7; $MgSO_4.7H_2O$, 1.2; $CaCl_2.2H_2O$, 2.5; $NaHCO_3$, 25; $KH_2PO_4$, 1.2; and d-glucose 11. The organs were cleaned of fat and connective tissue, and 1-1.5 cm segments were prepared. These

segments were placed in isolated organ baths (Ugo Basile, Varese, Italy) with 20 ml of Krebs' solution (pH 7.4), continuously aerated with 5% $CO_2$ and 95% $O_2$ at 37°C. The segments were connected to isotonic transducers which were connected to a two-channel pen recorder. The tissues were equilibrated for 1 h, during this period the solution was replaced every 15 minutes while maintaining a resting tension of 1 g. Then, chemicals were applied to the tissues, and cumulative concentration-response curves were obtained. In the experiments, the following substances were used: carvacrol ($10^{-7}$, $10^{-6}$, $10^{-5}$, $10^{-4}$ M), dissolved in water and DMSO (1:1) [13]; and paxilline ($10^{-6}$ M) [30], UCL1684 ($10^{-7}$ M) [31], linopirdine ($10^{-6}$ M) [32], tetraethylammonium ($10^{-2}$ M) [33], 4-aminopyridine ($10^{-3}$ M) [33], glibenclamide ($10^{-6}$ M) [34], BaCl2 ($10^{-3}$ M) [35], ruthenium red ($10^{-5}$ M) [36], and L-NOARG ($10^{-4}$ M) [37], all dissolved in water.

### 5.4. Statistical analysis of data

The R programming language and related packages were used for statistical evaluation of experimental data, nonlinear regression, and graphics drawing (R core team, 2019). The data were evaluated using one-way analysis of variance (ANOVA) and Tukey's HSD test for multiple comparisons. The IC50 was determined by non-linear regression analysis of log-dose vs. response curves. A p value <0.05 was considered as statistically significant. Data is expressed as the estimate ± standard error of mean [SEM].

**Acknowledgments:** This article is a study produced from the doctoral thesis of Muhammed YUSUF (Anadolu University, Institute of Graduate Studies).

**Author contributions:** Concept – M.Y., S.A.; Design – M.Y., S.A.; Supervision – S.A.; Resources – M.Y.; Materials – M.Y.; Data Collection and/or Processing – M.Y.; Analysis and/or Interpretation – M.Y., S.A.; Literature Search – M.Y., S.A.; Writing – M.Y.; Critical Reviews – M.Y., S.A.

**Conflict of interest statement:** The authors declared no conflict of interest.

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