

Full Length Research Paper

Vanillin reduces intestinal smooth muscle contractility

Abuirmeileh, A.* , Talhouni, A. , Alsalahat, I. and Wadie Abed

Department of Clinical Pharmacy and Applied Pharmaceutical Sciences, Faculty of Pharmacy,
Al Isra University Amman – Jordan.

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Vanillin consumption is common within the global population, often as a flavoring agent. Many vanillin-containing drinks claim a soothing effect on the body as a whole with some reports on their benefit in reducing gastrointestinal (GI) upset. In this study, vanillin was investigated for its ability to reduce rat ileal smooth muscle contractility induced by acetylcholine (ACh) and potassium chloride (KCl). A section of rat ileum was suspended in an organ bath containing Tyrode's solution. The tissue was stimulated using ACh or KCl and kept under 1 g tension at 37°C while continuously gassing with oxygen. Ileal smooth muscle contractility was studied in the absence and presence of vanillin. The results illustrated that vanillin (1.4×10^{-6} and 2.2×10^{-6} M) hindered ileal smooth muscle contractility induced by both ACh and KCl. Vanillin (1.4×10^{-6} and 2.2×10^{-6} M) also caused a rightward shift of the ACh concentration response curve and brought about a decrease (21.6 and 38.3%, respectively) in the maximum response. It also produced a rightward shift in the KCl dose response curve but without affecting the maximum response. These results indicate that vanillin counteracts ACh and KCl induced smooth muscle contractility in rat ileum. The results also suggest that vanillin prevented ACh induced contractility via non competitive inhibition kinetics. The reduction in KCl induced contractility also indicates that vanillin, at least partially, conveyed its effect through acting on ileal smooth muscle calcium channels.

Key words: Vanillin, acetylcholine, KCl, rat Ileum, smooth muscle contraction.

INTRODUCTION

Gastro intestinal (GI) motility results from complex interaction between the enteric nervous system, hormones and ileal smooth muscles (Andersson and Hedlund, 2002). Any imbalance between the interplay of these contributors will result in a loss of the normal physiologic rhythm, and in many cases, manifest clinically as a GI symptom (Kim et al., 2008). These symptoms

include diarrhea, constipation, GI spasms and general GI discomfort (Samuels, 2009). In particular, GI spasms usually present as an unpleasant feeling of GI pain. Therapeutically, there are many remedies capable of relieving this pain, with the majority achieving their effect through inhibiting smooth muscle contractility (Kim et al., 2008; Samuels, 2009). Cellularly, smooth muscle

*Corresponding author. E-mail: amjad.abuirmeileh@iu.edu.jo, mj11a@hotmail.com. Tel: +962 6 4711710 Ext: 2733. Fax: +962 6 4711505.

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contractions occur secondary to extra cellular stimulation when hormones or neurotransmitters bind membrane bound receptors. After initial receptor stimulation, the signal is relayed inside the cell where it transpires as an increase in free intracellular calcium ion (Ca^{+2}) leading to consequent activation of the actin and myosin fibers and hence muscle contraction (Andersson and Hedlund, 2002; Kim et al., 2008).

Vanillin is the primary component of the vanilla bean extract; it has a distinct flavour and aroma and is thus very often used as a flavouring agent. Therapeutically, vanillin has demonstrated an antioxidant and an antimicrobial effect (Burri et al., 1989). Another possible therapeutic benefit for vanillin is its use as an anti spasmodic remedy where individual reports have credited vanilla flavored drinks to possess such properties (Sinha et al., 2008). And while vanillin is not the only constituent in vanilla beans, it does present a suspect for this effect (Scharer and Mosandl, 2001). One approach in determining whether plant extracts or chemical agents possess antispasmodic activity is to examine their effect on isolated ileal smooth muscle tissue. In this study, the clinical viability of vanillin as an antispasmodic remedy was investigated through examining its *in vitro* effect on rat ileum.

MATERIALS AND METHODS

Chemicals

Ach, KCl, vanillin and all tyrode solution constituents; NaCl, KCl, CaCl_2 , NaHCO_3 , NaH_2PO_4 , MgCl_2 and glucose were obtained from Sigma-Aldrich.

Animals

Adult male Sprague Dawley rats (190 ± 10 g) were purchased from the animal facility at Jordan Applied University and housed at 20 to 24°C with free access to food and water. The rats were deprived of food (not water) for 24 h before the experiment to minimize ileal contents. All procedures concerning animals were carried out in accordance with Jordanian regulations for animal experimentation and care, and approved by the committee of institutional animal care and use (Protocol and Ethical approval memo number HS/KC/949 dated 7th of November, 2012). The study commenced on 1st of December, 2012 and lasted for a duration of 6 months. All experimentation was carried out at the Pharmacology research lab in the Faculty of Pharmacy at Isra University, Amman – Jordan.

Ileum preparation

On the day of experiment, rats were sacrificed by cervical displacement and one or two segments (1.5 to 2 cm) of ileum were dissected and freed of adhering mesentery. The pieces of ileum were cleaned from their luminal contents by flushing gently with a stream of Tyrode's solution using a 5 ml pipette. Tyrode solution contained 136.8 mM NaCl, 2.7 mM KCl, 1.3 mM CaCl_2 , 0.14 mM

NaH_2PO_4 , 12 mM NaHCO_3 , 0.5 mM MgCl_2 and 5.5 mM glucose. Ileal tissue was then mounted between two stainless steel hooks in a 40 ml tissue bath containing Tyrode solution being continuously gassed with oxygen. Temperature and pH were maintained at 37°C and 7.4. The lower hook was fixed at the bottom of the tissue bath and the upper one was connected to an isotonic transducer to measure forced smooth muscle contraction from base line (Harvard Transducer, UK). Each piece of tissue was placed under 1 g resting tension and equilibrated for 60 min prior to the execution of experimental protocols. During this period, the tissue was washed with Tyrode solution every 15 min. Ileum contractions were displayed and recorded on a Universal Harvard Oscillograph, (UK). The ileal contractions were initially induced by 15 mM of Ach or 10 mM of KCl. Dose response curves were then established in the presence of either Ach (0.1 to 2 mM) or KCl (1 mM to 1 M) non-cumulatively with a tissue contact time of one minute. Tissue was washed 3 times between each treatment. Further dose response curves were obtained after vanillin (1.4×10^{-6} and 2.2×10^{-6}) was added to the tissue bath.

Statistical analysis

All data were expressed as mean \pm standard error of the mean (SEM). Results were analyzed using non linear regression (curve fit) with an extra sum-of squares F test comparison method for $\log\text{EC}_{50}$ and hillslope values using GraphPad Prism 5[®] software.

RESULTS

Effect of vanillin on ileal contraction induced by Ach

The dose response curve for Ach was evaluated in the presence and absence of vanillin. Here, two concentrations of the vanillin were used, 1.4×10^{-6} and 2.2×10^{-6} M. The results clearly demonstrated that there was a concentration dependent rightward shift of the Ach dose response curve. Also, vanillin brought about a significant concentration dependent reduction in the maximal response (21.6 and 38.3% respectively; Figure 1).

Effect of vanillin on ileum's contraction induced by KCl

The dose response curve induced by KCl was also evaluated in the presence and absence of vanillin (1.4×10^{-6} and 2.2×10^{-6} M). Again, vanillin caused a concentration dependent rightward shift of the KCl dose response curve but this time without affecting the maximal response (Figure 2).

DISCUSSION

The results clearly showed that vanillin counteracted ileal smooth muscle contractility induced by both Ach and KCl, and thus indicated that vanillin could influence GI motility.

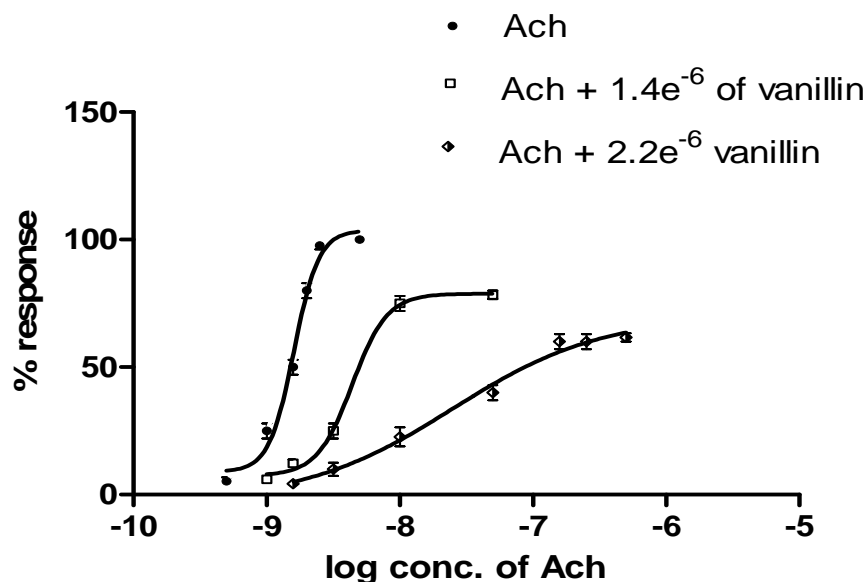


Figure 1. Effect of vanillin on rat ileum contraction induced ACh. Each point represents mean \pm SEM of 3 observations. LogEC₅₀ for ACh = -8.840 for ACh alone, -8.221 in the presence of 1.4×10^{-6} vanillin and -6.927 in the presence of 2.2×10^{-6} vanillin. Hill Slope for ACh = 3.851 for ACh alone, 1.420 in the presence of 1.4×10^{-6} vanillin and -6.927 in the presence of 2.2×10^{-6} vanillin. LogEC₅₀ was significantly different for each data set ($P < 0.0001$). HillSlope was also significantly different for each data set ($P < 0.0001$).

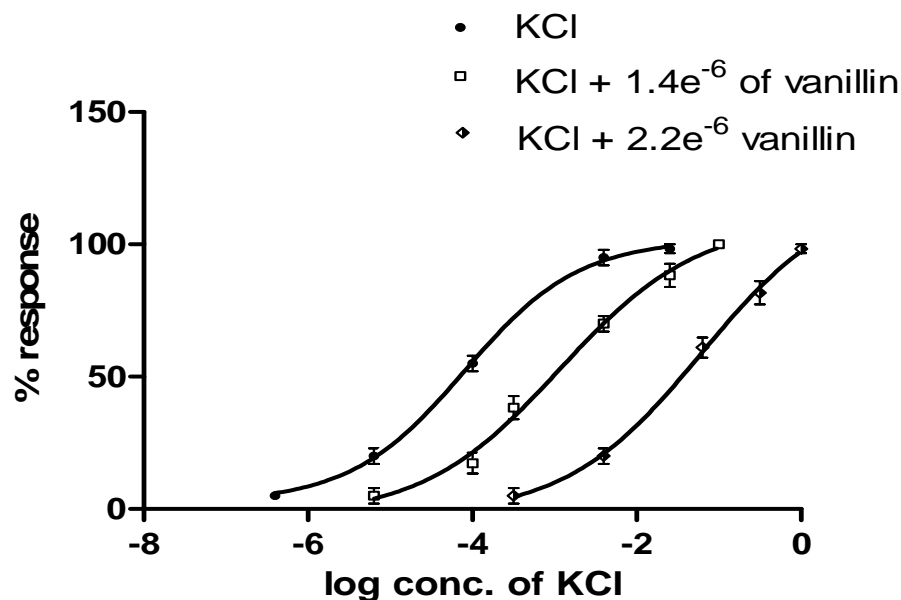


Figure 2. Effect of vanillin on rat ileum contraction induced by KCl. Each point represents mean \pm SEM of 3 observations. LogEC₅₀ for KCl = -4.181 for KCl alone, -3.064 in the presence of 1.4×10^{-6} vanillin and -1.511 in the presence of 2.2×10^{-6} vanillin. LogEC₅₀ was significantly different for each data set ($P < 0.0001$). HillSlope was not significantly different for any of the data sets ($P > 0.05$).

To better place vanillin as an agent used for the treatment of gastro intestinal tract (GIT) disorders its exact mode of action needed to be determined. Vanillin's capacity to reduce ileal smooth muscle contractility displayed reversibility where washing allowed ileal tissue to regain normal responsiveness to the inducers (Gharib et al., 2007). This could be relevant to clinical application where reversibility allows obtaining desired antispasmodic effects without causing a sustained inhibition of contraction which may result in side effects. Also, the ability of vanillin to cause a rightward shift of the Ach dose response curve accompanied by a dose dependent decrease in the maximum response strongly suggests that it non-competitively inhibited Ach stimulation. This may perhaps occur through either indirectly blocking GI muscarinic receptors or through interfering with another down stream consequence of stimulating these receptors. Ach binds to M_2 and M_3 receptors in ileal tissue causing smooth muscle contraction through promoting calcium influx via receptor-operated calcium channels (Zhang et al., 2005). Ach also promotes inositol trisphosphate (IP_3) synthesis via phospholipase C activation which in turn increases calcium release from the sarcoplasmic reticulum (Coulson et al., 2004; Pacaud et al., 1996). Any step in this latter signal transduction pathway poses as a potential target for vanillin.

To further investigate how vanillin counteracted this ileal contraction, its effect was examined on KCl induced ileal smooth muscle contractility. Here, vanillin produced a rightward shift in the KCl dose response curve without affecting the maximum response. This indicated that vanillin was somehow hindering the normal response of voltage gated Ca^{+2} channels where high potassium concentrations depolarizes smooth muscle cells with a resultant activation of the voltage dependent calcium channels present in rat ileum (Schneider et al., 2004). The opening of these channels in turn increases intracellular Ca^{+2} influx and causes smooth muscle contraction (Sadraei et al., 2013a; Godfraind et al., 1986; Sadraei et al., 2013b). It has been suggested that substances which inhibit KCl-induced smooth muscle contractility produce their effect via blocking voltage-gated Ca^{+2} channels (Gilani et al., 2001). Also, when high KCl concentration causes membrane depolarizing (Fujimoto and Mori, 2004), only sufficient intracellular Ca^{+2} levels will initiate muscle contraction (Zhang et al., 2005), which indicates that vanillin maybe interfering with final intracellular Ca^{+2} levels rather than preventing initial cell depolarizing. This notion is supported by the reversible non competitive inhibition displayed by vanillin on Ach induced ileal smooth muscle contractility. This suggests the involvement of voltage gated Ca^{+2} channels with the distant possibility of IP_3 and sarcoplasmic reticulum contribution.

Our findings are supported by Sadraei et al. (2013a),

where they demonstrated that isovanillin, an isomer of vanillin, inhibited ileal contraction induced by both Ach and KCl and it was concluded that the mechanism of action of isovanillin was somehow different from that of simple muscarinic antagonism (Sadraei et al., 2013a). Although vanillin and isovanillin possess structural similarities as phenolic aldehydes, they may not necessarily behave similarly on ileal smooth muscles. This is verified by the fact that these isomers possess varying metabolic pathways where vanillin is metabolized by aldehyde oxidase and isovanillin is not, and rather metabolized by aldehyde dehydrogenase (Panoutsopoulos and Beedham, 2005). And although isovanillin may possess similar GI effects, vanillin remains the molecule of interest because of its wide use as a flavouring agent.

Conclusion

Taken together, our findings suggest that vanillin hinders ileal smooth muscle contractility. Vanillin is possibly causing this effect through interfering with the natural response of voltage gated Ca^{2+} channels and not via directly blocking muscarinic receptors. These results demonstrate that the potential usefulness of vanillin in the pharmaceutical industry is not limited to a flavouring agent but possibly as an antispasmodic remedy.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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