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Ginger facilitates cholinergic activity possibly due to blockade of muscarinic autoreceptors in rat stomach fundus

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ORIGINAL ARTICLE

GINGER FACILITATES CHOLINERGIC ACTIVITY POSSIBLY DUE TO BLOCKADE OF MUSCARINIC AUTORECEPTORS IN RAT STOMACH FUNDUS

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ABSTRACT

Ginger (*Zingiber officinale*) is a universally known food plant reputed for its medicinal use in gastrointestinal disorders as a prokinetic and laxative. We recently showed that 70% aqueous-methanolic extract of ginger (Zo.Cr) exhibits prokinetic activity in rats via activation of post-synaptic muscarinic M_3 receptor in rat stomach fundus. In view of the physiological significance of pre-synaptic muscarinic M_1 and M_2 autoreceptors, this study was undertaken to further look into the possible mode of action of the prokinetic effect of ginger through inhibition of pre-synaptic muscarinic receptors. Isolated tissue bath experiments were performed with Sprague-Dawley rat stomach fundus strip preparations immersed in Kreb's solution at 37°C. Carbachol (CCh) maximum responses (1 μM) were obtained in rat stomach fundus. Zo.Cr, given in multiple increasing bolus concentrations (0.01-0.1 mg/ml) 10 min prior to administration of CCh, potentiated the CCh peak responses showing that it is possibly inhibiting the pre-synaptic muscarinic receptors. Like wise, increasing bolus concentrations of pirenzepine (0.03-0.3 μM) and himbacine (0.01-0.03 μM), standard muscarinic M_1 and M_2 antagonists respectively, also potentiated the CCh responses. These results show that ginger, in addition to having a direct cholinergic agonistic effect on the post-synaptic M_3 receptors, also has a possible inhibitory effect on pre-synaptic muscarinic autoreceptors, similar to standard muscarinic antagonists, thus reiterating the gastric stimulant effect of this age-old plant.

Keywords: *Zingiber officinale*, ginger, muscarinic, autoreceptors, rat stomach fundus.

INTRODUCTION

Ginger (*Zingiber officinale*, family Zingiberaceae) is a universally known food plant reputed for its medicinal uses. Ginger finds immense usage in many of the world's different traditional medicine systems. It has popularly been used in different gastrointestinal disorders as stimulant, antiemetic, stomachic, laxative, prokinetic, digestive, appetizer and sialogogue (Kapoor, 1990; Gilani and Ghayur, 2005). Ginger contains a number of chemicals and pungent principles. The volatile oil portion of the rhizome has a variety of sesquiterpene hydrocarbons such as curcumene, geranyl acetate, terpineol, terpenes, geraniol, alpha pinene, limonene, linalool, zingiberene, beta-besabolene, and alpha-farnesene. The pungent constituents of ginger are the gingerols, shogaols, zingerone and paradol (Langner *et al.*, 1998; Gilani and Ghayur, 2005). 6-Gingerol and 6-shogaol are the major gingerol and shogaol present in the rhizome. Two recent research articles (Jolad *et al.*, 2004, 2005) have shown the isolation of a number of new phenolic pungent principles from fresh and dried ginger extracts with anti-inflammatory activity indicating the still vast amount of interest left in this already extensively studied plant.

Recently, we reported that an aqueous-methanolic extract of ginger exhibits gastrointestinal stimulatory activity via activity on the post-synaptic muscarinic receptors (Ghayur and Gilani, 2005). In this investigation, we report an additional mechanism for the gastric stimulatory activity of the ginger extract mediated possibly via inhibition of the pre-synaptic muscarinic autoreceptors thus providing further insight into the possible mode of gastric prokinetic effect of this very ancient and popular food plant.

MATERIALS AND METHODS

Drugs and standards

The following reference chemicals namely carbachol chloride (CCh), pirenzepine dihydrochloride and himbacine were obtained from Sigma Chemical Company, St. Louis, MO, USA. The following chemicals

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were used to make the physiological salt solutions: potassium chloride (Sigma Chemical Company, St. Louis, MO, USA), calcium chloride, glucose, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate and sodium chloride (E. Merck, Darmstadt, Germany).

Animals

Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. Sprague-Dawley rats (170-200 g) of either sex used in the study were housed in the animal house of The Aga Khan University under a controlled environment (23-25°C). Animals were fasted for 24 h before the experiment but were given tap water *ad libitum* and a standard diet.

Plant material and extraction procedure

A total of 1 kg of fresh ginger rhizome (*Zingiber officinale* Roscoe; family Zingiberaceae) was bought from the central vegetable market in Karachi. A sample of the rhizome was deposited at the Herbarium of Department of Biological and Biomedical Sciences, Aga Khan University, Karachi with the voucher # ZO-RH-06-02-46. The ginger rhizomes were washed and then sliced to expose the inner part and soaked in 2 L of 70% aqueous methanol for a total of 3 days (at 20°C). Afterwards, the plant material was again soaked for 3 days, twice. The combined filtrate was filtered through Whatman qualitative grade 1 filter paper and later concentrated in a rotary evaporator to obtain the crude extract (Zo.Cr) with a yield of 4.2% (w/w).

Isolated rat stomach fundus

Experiments with rat stomach fundus were performed as described earlier (Ghayur and Gilani, 2005). Rats were sacrificed by cervical dislocation. The stomach was removed and placed in Kreb's solution. The fundus was cut open along the lesser curvature and divided into two longitudinal strips of 2 mm width and 15 mm length. Each strip preparation was mounted in a 10 ml tissue bath with Kreb's solution at 37°C and aerated with carbogen. The composition of Kreb's solution was (mM): NaCl 118.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KCl 4.7, KH_2PO_4 1.3, $MgSO_4$ 1.2 and glucose 11.7 (pH 7.4). Basal tension of 1 g was applied to each tissue and the responses recorded following an equilibrium period of 60 min. A sub-maximal concentration of CCh 0.3 μM was tested repeatedly to stabilize the preparations while the isometric responses were recorded through isotonic Harvard force transducers (cat # BS4 50-6360) coupled with Harvard student oscillographs (cat # BS4 50-8168).

To test for a possible muscarinic autoreceptor inhibitory effect, the protocol of Ogishima *et al.* (2000) was followed with some modifications. Maximum responses from CCh (obtained from a bolus concentration of 1 μM) were pretreated (for 10 min) with single bolus concentrations of the crude extract given separately in an increasing fashion starting from 0.01 mg/l up to 3 mg/ml to observe for any potentiation in control responses of CCh (1 μM). Thus the effect of CCh was noted in the absence and then in the presence of increasing concentrations of Zo.Cr. Any potential enhancement in the CCh control response would indicate possible inhibition of muscarinic pre-synaptic autoreceptors (M_1 and/or M_2) resulting in facilitation of the cholinergic response. Likewise, the effect of pirenzepine (given in bolus from 0.03 to 10 μM) and himbacine (given in bolus from 0.01 to 0.1 μM), selective muscarinic M_1 (Caulfield and Birdsall, 1998) and M_2 (Gilani and Cobbin, 1986; Takadoi *et al.*, 2003) antagonists, was also determined on CCh (1 μM) control responses to compare with activity of the extract. Similar to the protocol adopted with the extract, the effect of CCh (1 μM) was noted in the absence and then in the presence of increasing bolus concentrations of pirenzepine and himbacine.

Statistical analysis

All the data expressed are mean ± standard error of mean (SEM, n=number of experiments). The statistical parameter applied is the Student's *t*-test with $P < 0.05$ noted as significantly different (GraphPAD program, GraphPAD, San Diego, CA, USA).

RESULTS AND DISCUSSION

Ginger is a universally known food plant not only for its culinary but also for its medicinal uses. It has been used in a variety of disorders especially in the motility disorders of the gastrointestinal tract. Ginger is traditionally regarded as a stimulant and prokinetic because of its ability to enhance gastric emptying and an anti-emetic effect believed to be due to its local activity in the gut as a gastric stimulating agent (Kapoor, 1990; Gilani and Ghayur, 2005). In continuation of our efforts to unravel the pharmacological activities of ginger rhizome, we recently reported that the aqueous-methanolic extract of ginger exhibits gut prokinetic activity, as evident by its contractile effects *in vitro* on isolated rat and mouse stomach fundus tissues and *in vivo* on intestinal transit of charcoal meal in mice (Ghayur and Gilani, 2005). Based on the observation that pre-treatment of gastric tissues with atropine blocked the stimulatory effect of ginger extract, it was speculated that this stimulatory effect was mediated via interaction with the post-synaptic muscarinic M_3 receptors which are the main muscarinic receptor sub-type mediating contraction in gut smooth muscle cells (Uchiyama and Chess-Williams, 2004). But the M_3 receptors are not the only muscarinic receptor sub-types in gut smooth muscles as M_1 and M_2 receptor sub-types are also shown to be present as autoreceptors in the pre-synaptic terminals modulating the release of acetylcholine from cholinergic nerves via a negative feedback

mechanism (Starke *et al.*, 1989). Once these M_1 (Kilbinger *et al.*, 1993) and M_2 (Ren and Harty, 1994) autoreceptors are activated, they inhibit the release of acetylcholine from cholinergic neurons while conversely if these are inhibited, they lead to facilitation of cholinergic transmission. This led us to examine whether ginger, apart form having a direct agonistic effect on M_3 receptors, has any inhibitory activity on muscarinic autoreceptors (M_1 and M_2) in its gastric stimulant effect on rat stomach fundus.

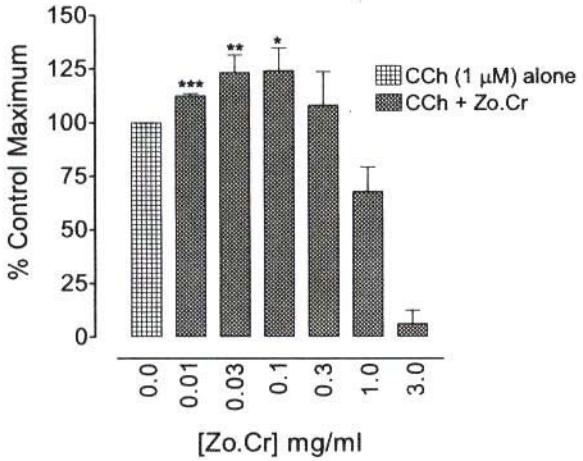

Fig. 1: Figure showing effect of increasing concentrations of ginger crude extract (Zo.Cr) on carbachol (CCh) control peak responses (1 μM) in isolated rat stomach fundus (*$P < 0.05$, **$P < 0.02$ and ***$P < 0.001$ vs. CCh control response, Student's *t*-test; values shown are mean ± SEM, n=7).

Zo.Cr (0.01-0.1 mg/ml, n=7) exhibited potentiation of control CCh (1 μM) responses (Fig. 1). The CCh peaks were potentiated to 112.6 ± 0.9% ($P < 0.05$), 123.3 ± 8.1% ($P < 0.02$) and 124.2 ± 10.1% ($P < 0.001$) by the corresponding three concentrations of the extract. Higher concentrations (0.3-3.0 mg/ml) of the extract led to complete suppression of CCh responses (Fig. 1), possibly due to its antispasmodic behaviour mediated via Ca^{++} antagonism reported earlier (Ghayur and Gilani, 2005). The potentiation of cholinergic response by the ginger extract can be explained either due to inhibition of muscarinic M_1 and/or M_2 autoreceptors or inhibition of acetylcholinesterase (AChE) enzyme which is responsible for degrading acetylcholine in the synaptic cleft and thus its inhibition also leads to facilitation of cholinergic responses (Gilani *et al.*, 2004, 2005). However, the latter speculation may not be valid because the agonist used in this study (CCh) is not subjected to hydrolysis by the AChE (Trevor *et al.*, 2005). Moreover, when ginger extract was tested for a possible AChE inhibitory effect, it did not exhibit any such effect at the concentration of up to 1 mg/ml (n=3, data not shown) indicating that the potentiation of CCh peaks is possibly due to muscarinic autoreceptor inhibition. Our earlier results (Ghayur and Gilani, 2005) and the one observed here in this study support the idea for ginger extract of a mixed muscarinic activity with agonistic effect at the post-synaptic M_3 while antagonist-like effect at pre-synaptic M_1 and/or M_2 autoreceptors. Due to limitations, we could not determine whether ginger extract had an inhibitory effect on both M_1 and M_2 receptor subtypes or on one of them. This mixed profile from ginger is not surprising as different serotonergic ligands have been reported to possess similar mixed profiles simultaneously on pre- and post-synaptic 5-HT receptor subtypes such as the compound MDL 72832 hydrochloride with agonistic activity at post-synaptic $5HT_{1A}$ and antagonistic activity at the pre-synaptic $5HT_{1A}$ receptors (Mir *et al.*, 1988), anpirtoline hydrochloride with agonistic at $5HT_1$ and antagonistic activity at $5HT_3$ receptors (Gothert *et al.*, 1995) and another compound L-694,247 with agonistic behaviour at post-synaptic $5HT_{1D}$ and inhibitory activity at $5HT_{1D\beta}$ autoreceptors (Buhlen *et al.*, 1996). This is the first report for such a facilatatory effect of ginger on cholinergic transmission in stomach tissues.

In order to compare this M_1 and/or M_2 antagonist effect of Zo.Cr, specific muscarinic M_1 and M_2 sub-type antagonists (pirenzepine and himbacine) were used. Both pirenzepine (0.03-0.3 μM, n=5) and himbacine (0.01-0.03 μM, n=5) enhanced the CCh peak responses (Fig. 2) indicating blockade of pre-synaptic M_1 and M_2 autoreceptors respectively. Otherwise, both pirenzepine and himbacine have no stimulatory effect if they are given on resting baseline of the tissue, thus the potentiating effect seen here with their increasing concentrations on CCh points to their ability to block the pre-synaptic receptors and potentiate the CCh response. The results show that both the antagonists were able to exhibit around an additional 30% potentiation of the CCh response. If we look back to the potentiation shown by the extract, also around 30%, which means that had Zo.Cr been inhibiting both M_1 and M_2 sub-types, then the potentiation would have been the double at around 60% rather than 30%. This indicates that the extract is possibly inhibiting only one of the muscarinic receptor sub-types, either M_1 or M_2 and not both. With higher concentrations of both the antagonists, suppression of the CCh peaks was seen, possibly because of a generalized anticholinergic effect from the compounds at the higher concentrations. Literature search revealed that pirenzepine is known to augment gastric contractility (Stacher *et al.*, 1982) while is also reported to increase gastrointestinal transit in humans (Jaup *et al.*, 1985) thus reiterating the significance of this mechanism of gastric muscarinic autoreceptor inhibition for gut motility stimulation.

The results show that ginger possibly exhibits muscarinic autoreceptor inhibitory activities in rat stomach fundus,

but further studies are needed to confirm this effect. Although we were able to rule out a cholinesterase inhibitory effect of the extract that also could have lead to similar results as shown in this study, there are some other possibilities and confirmatory tests that need to be performed before any definitive conclusion is reached, such as: ruling our release of other excitatory neurotransmitters that are present in the enteric nerves, attenuation of inhibitory transmitters that can lead to potentiation of CCh response, receptor binding/ligand displacement studies, demonstration of affinity of extract for muscarinic receptors and evidence for neural mediation of this potentiation. Even with a lot to be done, this possible mode of gastric stimulatory action of ginger adds up to the already reported stimulant effect of ginger, mediated via activation of muscarinic M_3 receptors, thus further reiterating the usefulness of this plant as a gastric prokinetic agent.

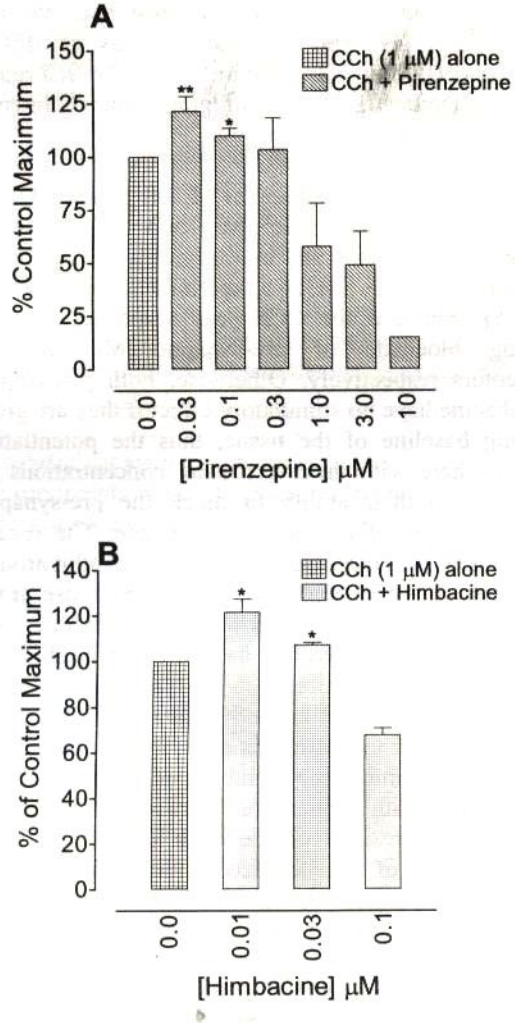

Fig. 2: Bar diagrams showing effect of [A] pirenzepine and [B] himbacine on carbachol (CCh) control peak responses (1 μM) in rat stomach fundus preparations (*P < 0.05 and **P < 0.02 vs. CCh control response, Student's t-test; values shown are mean ± SEM, n=5).

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ORIGINAL ARTICLE

IN VITRO AVAILABILITY STUDIES OF ENOXACIN IN PRESENCE OF H_2 RECEPTOR ANTAGONISTS

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ABSTRACT

Enoxacin is a second-generation quinolone with increased antibacterial activity both in potency as well as in terms of broad spectrum against a wide range of clinically important pathogens over the first generation quinolones and produces its effect by inhibiting bacterial enzyme DNA gyrase. There are a number of drug interactions reported for enoxacin. On the other hand H_2-receptor antagonists block gastric acid secretion and some cardiovascular effects of histamine. As the later drugs are used for a long-term therapy, they may be coadministered with other drugs. In present study *in vitro* release of enoxacin in presence of cimetidine, ranitidine and famotidine has been studied on a B.P. 2003 dissolution test apparatus and compared with the availability of enoxacin and H_2-receptor antagonists alone. The interacting drugs were analyzed spectrophotometrically. These studies were carried out in simulated gastric juice, simulating empty stomach, simulated intestinal juice (pH 9) and buffers of pH 7.4 simulating blood pH at 37°C. In order to support these interaction studies, the effect of H_2-receptor antagonists on the antibacterial efficacy (MIC) of enoxacin was also studied by turbidity method and compared with parent drug against *Staphylococcus aureus, Streptococcus pyogens, Streptococcus pneumoniae, Enterococcus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis* and *Bacillus subtilis.*

On the basis of these results, it is suggested that enoxacin should be coadministered with care along with H_2-receptor antagonists especially in case of ranitidine; although chances of adverse reactions are rare but decrease in MIC of enoxacin may result in delayed effect or require prolonged use of the drug.

Keywords: Enoxacin, drug interactions, antagonists, antibacterial studies.

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