Antispasmodic Effect of Piper nigrum Fruit Hot Water Extract on Rat Ileum

Mohammad Kazem Gharib Naseri and Hoda Yahyavi
Department of Physiology, School of Medicine, Physiology Research Center, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran

Abstract: The aim of this study was to investigate the effect of black pepper fruit hot water extract (BPE) on rat ileum contractility and the mechanism(s) of its action. The extract was prepared by adding black pepper powder to boiling distilled water followed by evaporated the solvent. Ileum was dissected from male adult rat (Wistar) and in Tyrode solution the tissue contractions were recorded by an isotonic transducer under 1 g tension. The cumulative concentrations of the BPE (0.0625-1 mg mL⁻¹) reduced the ileum contractions induced by KCl (60 mM) or carbachol (10 μM) concentration dependently (p<0.001). In Ca²⁺-free Tyrode solution with high potassium (60 mM), BPE, (0.0625-1 mg mL⁻¹) attenuated the contractions induced by cumulative concentrations of CaCl₂ (0.225-2.7 mM) concentration dependently (ANOVA, p<0.05). The incubation of the tissue preparation (20 or 30 min) with L-NAME (100 μM), naloxone (1 μM) or propranolol (1 μM) did not reduce the extract antispasmodic effect on KCl-induced ileum contraction. The extract spasmylytic effect was attenuated neither by glibenclamide (10 μM) nor by tetraethylammonium (1 mM). Present results suggest that the spasmylytic effect of the extract on rat ileum was possibly mediated via Ca²⁺ influx.

Key words: Piper nigrum, rat, ileum, spasmylytic

INTRODUCTION

Black pepper (Piper nigrum) from Piperaceae is the main spice food stuff and piperine is a pungent alkaloid of black pepper (Zargari, 1993). Black pepper stimulates rat gastric acid secretion which is weaker than red pepper effect (Vasudevan et al., 2000) and piperine has a same effect (Ononiwu et al., 2002) but piperine reduces mice small intestine secretions stimulated by castor oil (Capasso et al., 2002). Black pepper increases rat pancreatic enzymes activity (Platel et al., 2002) and piperine inhibits gastric emptying and gastrointestinal transit in rats and mice (Bajaj et al., 2001). Black pepper has antimicrobial (Dorman and Dears, 2000), antimitogenic (El-Hamss et al., 2003), antioxidant and radical scavenging properties (Gulein, 2005; Saxena et al., 2007), reduces oxidative stress induced by high fat diet in rat (Vijayakumar et al., 2004) and inhalation of black pepper oil improves the reflexive swallowing movement (Ebihara et al., 2006). In Iranian folk medicine, black pepper is used for some gastrointestinal disorders and to relief menorrhagia in women. The effect of black pepper on ileal smooth muscle contractility has not been investigated. The aim of the present study therefore, was to study the effect of black pepper fruit hot water extract (BPE) on the rat ileum contractions and the possible mechanism(s) involved.

MATERIALS AND METHODS

Plant material and powder preparation: Black pepper fruit was purchased from local herbal shops (in October 2007) in Ahwaz (Khuzeastan Province, Iran) and identified by botanists in Ahwaz Ramin University of Agriculture and Natural Sources. A voucher specimen was deposited at herbarium of our laboratory for further references. The method of the extract preparation was mainly as same as used traditionally. Therefore, black pepper fruit was powdered by an electrical grinder and the powder (10 g) was mixed with 200 mL boiling distilled water for 15 min. The mixture was then filtered through very fine cloth and thereafter the filtrate was centrifuged at 3500 rpm for 20 min. The supernatant was concentrated in rotary evaporator and then dried at room temperature to obtain a solid mass (1.3 g). The powder of Black Pepper Extract (BPE) was stored at 4°C until being used.

Chemicals and reagents: Propranolol, carbachol, N⁰-nitro-L-arginine methyl ester (L-NAME), glibenclamide and tetraethylammonium (TEA) were purchased from Sigma (USA) and naloxone was purchased from Tolidarlu (Iran). Other chemicals were purchased from Merck (Germany).

Corresponding Author: Mohammad Kazem Gharib Naseri, Department of Physiology, School of Medicine, Physiology Research Center, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran
Animals: All rats used in this study were treated in accordance with principals and guidelines on animals care of Ahwaz Jundishapur University of Medical Sciences (AJUMS). Male adult Wistar rats (213±3.6 g) were obtained from AJUMS animal house and kept at 12 h light/dark cycle and at 20-24°C with free access to food and water. Rats were starved of food but not water for 24 h before experiment.

Ileum preparation: On the day of experiment a sharp blow on the head sacrificed the rats. Two or three segments (2 cm) were dissected out from the terminal ileum (taken within a distance of 2-3 cm from the caecum) and mounted in an organ bath containing Tyrode solution (10 mL) between two stainless steel hooks vertically. The lower hook was fixed at the bottom of the organ bath and upper one was connected to an isotonie transducer (Harvard transducer, UK) connected to a recorder (Harvard Universal Oscillograph, UK). The Tyrode solution composition (pH 7.4 and 37°C) was (in mM): NaCl (136), KCl (5), CaCl₂ (2), NaHCO₃ (11.9), MgCl₂ (0.98) NaH₂PO₄ (0.36) and glucose, 5.55 which continuously was bubbled with air. The initial tension was 1 g throughout the experiment and equilibrium period was 60 min in which the bath solution was refreshed every 15 min. After equilibrium period, the ileum was contracted by 60 mM of KCl (Madeira et al., 2002) and once the plateau was achieved, the extract (0.0625-1 mg mL⁻¹) was added cumulatively to the organ bath. The extract spasmolytic effect was also studied in separate tissues after 30 min incubations with 1 μM of propranolol (Storr et al., 2000) or naloxone (Kaneda et al., 2003) as a non-selective β-adrenoceptors and opioid receptors antagonists, respectively. The extract spasmolytic effect was also studied after 20 min tissue incubation with 100 μM of L-NAME (Ekblad and Sundler, 1997) as a nitric oxide synthase inhibitor. To evaluate the extract effect on CaCl₂-induced ileum contraction, in Ca²⁺-free and rich KCl (60 mM) Tyrode solution, CaCl₂ was applied cumulatively (0.225-2.7 mM) before and after tissue incubation (3 min) with extract (0.0625-1 mg mL⁻¹). In addition, the extract antispasmodic effect on carbachol (CCh, 10 μM)-induced contraction was evaluated after ileum incubation (5 min) with glibenclamide (10 μM) or TEA (1 mM) as ATP-dependent and non-selective potassium channel blocker respectively (Franck et al., 1998). Separate ileum preparations were used for each spasmogen, antagonist or potassium channel blocker. The extract and all chemicals were dissolved in the Tyrode solution and the total volume of all solutions, which were added to the organ bath, did not exceed more than 5% of the bath volume.

Statistical analysis: The plateau of ileal contraction induced by KCl or CCh was regarded as 100% and percentage of relaxation was calculated from changes in the contraction. Results were expressed as means±SEM. Statistical analysis was made by one- and two-way ANOVA. Data was further subjected to LSD post-hoc test and a value of p<0.05 was considered significant.

RESULTS

Effect of BPE on KCl and CCh-induced ileum contractions: Black pepper fruit hot water extract (BPE) attenuated the ileal contractions induced by KCl (60 mM, n = 10) or carbachol (CCh, 10 μM, n = 9) significantly (ANOVA, p<0.001) and in a concentration-dependent manner. The two-way ANOVA indicated that these spasmyloytic effects were not significantly different as shown in Fig. 1.

Effect of the BPE on CaCl₂-induced ileum contractions: In Ca²⁺-free with high K⁺ (60 mM) Tyrode solution, applying cumulative concentrations of CaCl₂ (0.225 to 2.7 mM) induced ileum contractions in a concentration dependent manner (p<0.001) as shown in Fig. 2. Three minutes incubation of tissue preparation with BPE (0.0625-1 mg mL⁻¹) reduced the contractions concentration-dependently. The CaCl₂-induced contractions in the absence and in the presence of BPE (0.0625 mg mL⁻¹) were significantly different (two-way ANOVA, p<0.05, n = 8).

Fig. 1: The spasmolytic effect of black pepper fruit extract on the ileum contractions induced by KCl (60 mM, n = 10) or carbachol (10 μM, n = 9). The responses are different only at 0.25 mg mL⁻¹ (**p<0.05). However, two-way ANOVA indicated that these concentration-response curves are not different.
Fig. 2: The ileal contractions evoked by CaCl₂ before (0.0 mg mL⁻¹) and after incubation with black pepper fruit extract at different concentrations. The Tyrode solution was Ca²⁺-free but with high K⁺ (60 mM). Two-way ANOVA indicated that concentration-response curves of 0.0 and 0.0625 mg mL⁻¹ are different (p<0.05, n = 8). The significance differences between spasmogenic effect of each CaCl₂ concentration in the absence and in the presence of extract (0.0625 mg mL⁻¹) are also demonstrated (*p<0.01, **p<0.001, ***p<0.0001)

Fig. 3: The spasmylytolic effect of black pepper fruit extract on the rat ileum contractions evoked by KCl (60 mM, n = 10) before (Cont.) and after 30 min tissue incubation with propranolol (1 μM, n = 9), naloxone (1 μM, n = 7) or 20 min incubation with L-NAME (100 μM, n = 7). L-NAME potentiated the extract spasmylytic effect (two-way ANOVA, p<0.05). The differences in the extract spasmylytic effects in the absence and in the presence of L-NAME are shown (*p<0.05, **p<0.01)

Fig. 4: Antispasmodic activity of black pepper fruit extract on the rat ileum precontracted by CCh (10 μM, n = 9) before (Cont.) and after tissue incubation (5 min) with glibenclamide (10 μM, n = 9) or with tetraethylammonium (1 mM, n = 9). The spasmylytic effects of the extract have been potentiated by glibenclamide (two-way ANOVA, p<0.05). The differences in the extract spasmylytic effects in the absence and in the presence of glibenclamide are shown (*p<0.05, **p<0.01)

Effect of BPE after ileum incubation with propranolol, naloxone or L-NAME: Ileum incubation (30 min) with propranolol (1 μM, n = 8), naloxone (1 μM, n = 7), or 20 min with L-NAME (100 μM, n = 7) did not reduce the spasmylytic effect of BPE on KCl-induced ileum contractions rather, in the presence of L-NAME, the BPE activity was potentiated (two-way ANOVA, p<0.05) as shown in Fig. 3.

Spasmylytic effect of BPE in the presence of the potassium channel blockers: As Fig. 4 shows, incubation (5 min) of the ileum preparations with glibenclamide (10 μM, n = 9) or with TEA (1 mM, n = 9) as ATP-operated and non-selective potassium channel blockers, respectively did not attenuate the BPE spasmylytic effect on CCh (10 μM)-induced ileum contraction rather, glibenclamide potentiated the BPE activity (two-way ANOVA, p<0.05).

DISCUSSION

The results of present study show that KCl-induced non-receptor-mediated and CCh-induced receptor-mediated ileal contractions are significantly reduced by black pepper fruit hot water extract (BPE). The extract used in this study has been prepared in same way as described in the Iranian folk medicine. The BPE
spasmolytic effect was reversible since tissue responsiveness to spasmyogens was almost restored after refreshing the organ bath solution.

It is well known that the KCl-induced contraction in smooth muscle is due to an increase in Ca\(^{2+}\) influx through voltage-operated Ca\(^{2+}\) channels. On the other hand, CCh-induced contractile responses following receptor activation requires an increase intracellular Ca\(^{2+}\) which is provided by both Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels and Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores (Tanovic et al., 2000; Zhang et al., 2005). The similar reduction by the BPE in CCh- and KCl-induced contractions suggests that the BPE does not affect specific receptor-dependent mechanisms. It has been suggested that those substances that inhibit the KCl-induced contractions act through blocking the VDCCs (Gilani et al., 2001). The BPE spasmolytic effect on the CaCl\(_2\)-induced ileal contractions supported the possible involvement of Ca\(^{2+}\) influx (Fujimoto and Mori, 2004; Zhang et al., 2005). The anticholinergic effect of BPE is unlikely since BPE should only inhibit the CCh-induced contraction.

The \(\beta\)-adrenoreceptor activation relaxes ileum (Brown and Summers, 2001) but the ineffectiveness of propranolol indicated that the extract effect has not mediated via these receptors. Since a Nitric Oxide Synthase (NOS) is present in the myenteric plexus, Nitric Oxide (NO) has become a most likely candidate for mediating nonadrenergic-noncholinergic smooth muscle relaxation through the gastrointestinal tract (Takahashi, 2003). In our study, the extract activity was not reduced by L-NAME, indicates that the extract effect has not been mediated via the NO synthesis. Opioid receptors activation relaxes ileum (Gray et al., 2005) and since naloxone did not reduce the extract activity therefore, opioid receptors has not been involved. The BPE spasmolytic effect neither reduced by glibenclamide nor by TEA, as an ATP-dependent and calcium-operated potassium channel blocker respectively (Nishida and Satch, 2003; Kafali et al., 2002). Therefore, the extract has not induced spasmolytic effect through activated these channels and hyperpolarization. The ileal contractility was unaffected by tissue incubation with applied antagonists or inhibitor. It has been demonstrated that black pepper reduces gastric emptying, gastrointestinal transit (Bajad et al., 2001) and uterine contractions (Gharib Naseri and Yahyavi, 2007) and piperine inhibits the twitch response in guinea-pig ileum (Takaki et al., 1990), which are consistent with the present results. The observed inhibitory effect probably is due to the piperine action.

**CONCLUSION**

Black pepper fruit extract contains spasmolytic constituents mediating their effect through blockade of Ca\(^{2+}\) influx, which may explain its traditional use in the treatment of some gastrointestinal disorders.

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