Antispasmodic and hypotensive effects of Ferula asafoetida gum extract

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Abstract

The effects of Ferula asafoetida gum extract on the contractile responses of the isolated guinea-pig ileum induced by acetylcholine, histamine and KCl, and on the mean arterial blood pressure of rat were investigated. In the presence of extract (3 mg/ml), the average amplitude of spontaneous contractions of the isolated guinea-pig ileum was decreased to 54 ± 7% of control. Exposure of the precontracted ileum by acetylcholine (10−6M) to Ferula asafoetida gum extract caused relaxation in a concentration-dependent manner. Similar relaxatory effect of the extract was observed on the precontracted ileum by histamine (10−6M) and KCl (28 mM). However, when the preparations were preincubated with indomethacin (100 nM) and different antagonists, such as propranolol (1 μM), atropine (100 nM), chlorpheniramine (25 nM) then were contracted with KCl, exposure to the extract (3 mg/ml) did not cause any relaxation. Furthermore, Ferula asafoetida gum extract (0.3–2.2 mg/100 g body weight) significantly reduced the mean arterial blood pressure in anaesthetised rats. It might be concluded that the relaxant compounds in Ferula asafoetida gum extract interfere with a variety of muscarinic, adrenergic and histaminic receptor activities or with the mobilisation of calcium ions required for smooth muscle contraction non-specifically.

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Abbreviations: Ferula asafoetida; Gum extract; Ileum; Blood pressure; Relaxation; Hypotension

1. Introduction

Plants have been a constant source of drugs and recently, much emphasis has been placed on finding novel therapeutic agents from medicinal plants. Today many people prefer to use medicinal plants rather than chemical drugs. The Ferula genus from the family of Umbelliferae has been found to be a rich source of gum-resin (Fernch, 1971). Ferula asafoetida (Ferula assa-foetida L.) is a herbaceous wild plant native to Iran. In Iranian traditional medicine Ferula asafoetida gum extract has been used as a remedy for abdominal pain, constipation, diarrhea and as an antihelminthic. Several fractions such as gum fraction (25%, including glucose, galactose, l-arabinose, rhamnose and glucuronic acid), resin (40–64%, which contains ferulic acid esters (60%), free ferulic acid (1.3%), coumarin derivatives (e.g. umbelliferone), volatile oils (3–17%) including sulphur-containing compounds, and various monoterpenes have been isolated from this plant (Kajimoto, 1989).

Although there is some evidence for anticoagulant action of Ferula asafoetida gum extract (Leung, 1980) its pharmacological effects on intestinal smooth muscles and blood pressure have not been established yet. The present study was performed as a starting point for examining the folkloric claims regarding the beneficial effects of gum obtained from Ferula asafoetida in gastrointestinal and haemodynamic disorders. Here, we report our observations on the effects of Ferula asafoetida gum extract on the contractile responses of guinea-pig ileum induced by various stimuli (in vitro), and on blood pressure recorded from the anaesthetised rats (in vivo).

2. Material and methods

2.1. Plant gum extract

Ferula asafoetida gum was collected from Gonabad region (South of Khorasan province) during the summer. The plant was identified at the Botany Department, Faculty of Science, Mashhad University, Mashhad, Iran. A voucher specimen number was kept in record (293-0606-2) at the Department of Pharmacognosy, Faculty of Pharmacy, Mashhad University of Medical Sciences. The powdered dried gum
Acetylcholine chloride, histamine, propranolol, chlorpheniramine, indomethacin and atropine sulphate were purchased from Sigma. Thiopental was obtained from Biochemie GmbH (Austria). All drugs except indomethacin were dissolved in distilled water and added in a volume less than 1 ml to the organ bath. Indomethacin was dissolved in ethanol. At the final bath concentration (0.01%, v/v), ethanol had no effect on the ileum responses.

2.3. Experimental protocol

To perform in vitro studies, guinea-pigs were killed by a blow to the head and exsanguination. The ileum was exteriorised, the ileo-caecal junction located and approximately 30 cm removed. A 8–10 cm segment of the terminal portion was discarded before the contents in the remaining ileum were flushed out with modified Krebs–Henseleit solution (mM): NaCl 118.4, KCl 4.7, MgSO4 7H2O 1.4, KH2PO4 1.2, CaCl2 2.5, NaHCO3 25 and glucose 11.1. Segments of ileum (2 cm) were set up under 1 g of tension in 10 ml tissue baths, ready for isometric recording using an isometric transducer connected to an Oscillograph 400 MD/2. Preparations were incubated in Krebs–Henseleit solution gassed (95% O2/5% CO2) at 37°C, and allowed for about 1 h to equilibrate prior to testing with a priming concentration of the gum extract or any other drugs.

For in vivo studies, experimental protocol was according to the ‘Guiding Principles in the Use of Animals’, adopted by the WHO. Sprague-Dawley rats (250–300 g) and guinea-pigs (400–500 g) of either sex were anaesthetised with thiopental (15 mg/kg, i.p.). The trachea was cannulated and animals were artificially respired with room air using a Harvard small animal ventilation pump (at a rate of 54 strokes per minute, volume of 1 ml/100 g body weight). Body temperature was maintained at 37 ± 0.5°C by using an incandescent lamp placed over the abdomen and coupled to a rectal thermistor probe. The right common carotid artery and right jugular vein were cannulated using a polythene cannula for the measurement of arterial blood pressure and administration of the extract, respectively. Blood pressure was recorded continuously using an elecromatic EM751 pressure transducer on a Physiograph (Harvard Universal Oscillograph).

2.4. Analysis of data

Values in the text refer to mean ± S.E.M. Changes in MABP were compared using Student’s t-test. Differences between groups have been compared using one-way ANOVA followed by a Tukey-Kramer multiple comparison test. A P-value of less than 0.05 was considered as significant.

3. Results

3.1. Effect of the extract on spontaneous contractions of the isolated guinea-pig ileum

The isolated guinea-pig ileum preparations had spontaneous contractions when they were mounted in the tissue bath under 1 g tension. Exposure of the preparations to 1, 2, 3.5 and 7 mg/ml of the extract reduced the average amplitude of the spontaneous contractions to 83 ± 6%, 68 ± 5%, 54 ± 7%, 21 ± 9% and 9 ± 3% of control, respectively (n = 11).

3.2. Effect of the extract on evoked contractions of the isolated guinea-pig ileum

The contractions were elicited by acetylcholine (Ach), histamine and KCl. Addition of these agents produced rapidly developing increase in tension with minimal increase in rhythmic activity. Returning to resting tension was rapid on washout of the organ bath.

In tissues precontracted with different agents, Ferula asafoetida gum extract induced concentration-dependent relaxations (Fig. 1A). The relaxatory effect of the extract in the preparations precontracted with histamine (25 μM) was more pronounced than those precontracted with Ach (25 μM), and or KCl (28 mM).

3.3. No relaxatory effect in the presence of indomethacin and different antagonists

When preparations were preincubated with indomethacin (100 μM), propranolol (1 μM), atropine (100 μM) and chlorpheniramine (25 nM), then contracted by KCl (28 mM), the extract had no relaxatory effect (Fig. 1B). In the presence of indomethacin, propranolol, atropine and chlorpheniramine, addition of the extract increased tension of the preparations contracted by KCl. As shown in Fig. 1B, contractile responses of the preparations preincubated with indomethacin, propranolol, atropine and chlorpheniramine to the KCl (28 mM) and the extract were 156 ± 17%, 157 ± 2%, 126 ± 9%, 178 ± 28% of response to 1 μM acetylcholine, respectively. When the preparations were incubated with indomethacin and the antagonists simultaneously and then exposed to the extract a contraction (167 ± 5% of response to acetylcholine) was observed (Fig. 1B).

3.4. Effects of the extract on rat blood pressure

Basal blood pressure (109 ± 7 mmHg) and heart rate (464 ± 17) of five anaesthetised rats used in this study remained unchanged over the experimental period. Sev-
Fig. 1. (A) The relaxatory effect of Ferula asafoetida gum extract (1–7 mg/ml) on guinea-pig isolated ileum precontracted with KCl (28 mM, \( n = 6 \), white bars), acetylcholine (20 nM, \( n = 6 \), black bars), and histamine (20 \( \mu \)M, \( n = 5 \), gray bars). The category axis (the bar clusters) of 1–5 represent concentrations of 1, 2, 3, 5 and 7 mg/ml of the extract, respectively. Relaxations induced by the extract (3–7 mg/ml) were significant compared to equivalent volume of normal saline (\( P < 0.05 \)). (B) Effects of different antagonists and a cyclooxygenase inhibitor on responses to Ferula asafoetida gum extract (3 mg/ml) responses in guinea-pig isolated ileum precontracted with KCl (28 mM). Prior to addition of Ferula asafoetida gum extract (Fa), the preparations were incubated with indomethacin (Ind, 100 nM; \( n = 4 \)), propranolol (Pr, 1 \( \mu \)M; \( n = 4 \)), atropine (At, 100 nM; \( n = 4 \)) and chlorpheniramine (Ch, 25 nM; \( n = 4 \)), then preparations were contracted with KCl (28 mM). This procedure completely inhibited the relaxatory effect of Fa and a contractile response was observed. * \( P < 0.05 \) vs. KCl, # \( P < 0.01 \) vs. KCl + Fa.

Fig. 2. (A) Representative tracing of the effect of increasing doses of Ferula asafoetida gum extract (0.3–2.2 mg/100 g body weight) on the arterial blood pressure of an anaesthetised rat. (B) Effects of different doses of Ferula asafoetida gum extract on the mean arterial blood pressure of anaesthetised rats.

4. Discussion

In the present study, Ferula asafoetida gum extract was found to reduce the spontaneous contraction of the isolated guinea-pig ileum. It has been established that the spontaneous contractions of the intestinal smooth muscle are regulated by cycles of depolarisation and repolarisation. Action potentials are generated at the peak of depolarisation and constitute a fast influx of calcium ions through the voltage-activated calcium channels (Walsh and Singer, 1980; Brading, 1981). Therefore, it is possible that the extract contains some compounds which, interfere with the calcium channels activity. The extract also decreases contractions induced by acetylcholine, histamine and KCl in the isolated guinea-pig ileum. This effect was concentration-dependent and reversible after washing. Similar inhibitory effects of Ferula sinaica (another species from Ferula genus) root extract on rat and guinea-pig uterine smooth muscle contractions was reported by Aqel et al. (1991). However, the exact mechanism of action of Ferula sinaica extract remained unknown. Acetylcholine and histamine cause depolarisation and tonic contractions of intestinal smooth muscles. It is generally accepted that an increase in concentration of cytoplasmic-free calcium ions is indispensable for smooth muscle contraction. The activation of muscarinic receptors of longitudinal smooth muscle of guinea-pig small intestine produces an increased frequency of action potential discharge and depolarisation which results in a contraction (Reddy et al., 1995). The acetylcholine-evoked contraction is generally regarded as mediated via M3 subtype of muscarinic receptor although the muscle has a preponderance of M2 subtype muscarinic binding sites. Whereas, histamine-induced contraction happens via H1 receptor ac-
tivation (Zavecz and Yellin, 1982), and contraction induced
by KCl is due to an increase in $K^+$ and depolarisation
of smooth muscle fibers, leading to increased influx of calcium
through L-type voltage-operated channels (Gilani et al.,
1994). In short, calcium ions gain access to the cytoplasm
through voltage-activated or receptor-operated calcium
channels (Triggle, 1985). According to our observations,
when the isolated guinea-pig ileum preparations were pre-
contracted with histamine, the relaxation induced by the
extract was very much higher than that in the presence
of Ach. However, the spasmolytic activity of the extract
could not be attributed solely to any pure antagonistic ef-
fact, since the tissue contracted by KCl was also relaxed
after exposure to the extract. Considering lack of the re-
laxatory effect of the extract in the presence of atropin,
chlorpheniramine and propranolol, one might suggest that
these antagonists competed with the relaxant compounds
of the extract for binding to their acceptors. Therefore,
there is no good reason to exclude any interaction be-
tween some compounds of the extract and cholinergic,
histaminergic and adrenergic receptors. On the other hand,
smooth muscle contractile tone can be relaxed by increased
levels of adenosine 3',5'-cyclic monophosphate (cAMP)
(Berridge, 1975). Therefore, the extract may have its re-
laxatory effect through an increase in cAMP independent
of any specific receptor activity, then a reduction in $Ca^{2+}$
levels.

Furthermore, we observed that indomethacin (a cyclooxy-
genase inhibitor) significantly inhibited the relaxatory effect
of Ferula asafoetida gum extract in isolated guinea-pig
ileum, suggesting that cyclooxygenase metabolites (e.g.
prostaglandins such as PGE2, PGD2 and PGI2) may be
involved in this relaxation.

The present study also demonstrates that Ferula
asafoetida gum extract is effective in reducing blood pres-
sure in anaesthetised normotensive rats. This effect is shown
to be dose-related and rapid in onset. At higher doses, the
duration of the depressor response to Ferula asafoetida
gum extract was long-lasting. Taken together, the relaxatory
effects of the Ferula asafoetida gum extract on vascular
smooth muscle as well as on ileum smooth muscle may
suggest that, this natural product reduce the cytosolic $Ca^{2+}$
in a non-specific manner.

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