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Inhibition of the Mechanical Activity of Mouse Ileum by Cactus Pear (*Opuntia Ficus Indica*, L, Mill.) Fruit Extract and Its Pigment Indicaxanthin

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We investigated, using an organ bath technique, the effects of a hydrophilic extract from *Opuntia ficus indica* fruit pulp (cactus fruit extract, CFE) on the motility of mouse ileum, and researched the extract component(s) responsible for the observed responses. CFE (10−320 mg of fresh fruit pulp equivalents/mL of organ bath) reduced dose-dependently the spontaneous contractions. This effect was unaffected by tetrodotoxin, a neuronal blocker, N_ω-nitro-L-arginine methyl ester, a nitric oxide synthase blocker, tetraethylammonium, a potassium channel blocker, or atropine, a muscarinic receptor antagonist. CFE also reduced the contractions evoked by carbachol, without affecting the contractions evoked by high extracellular potassium. Indicaxanthin, but not ascorbic acid, assayed at concentrations comparable with their content in CFE, mimicked the CFE effects. The data show that CFE is able to exert direct antispasmodic effects on the intestinal motility. The CFE inhibitory effects do not involve potassium channels or voltage-dependent calcium channels but rather pathways of calcium intracellular release. The fruit pigment indicaxanthin appears to be the main component responsible for the CFE-induced effects.

KEYWORDS: ***Opuntia ficus indica*; indicaxanthin; intestinal smooth muscle; antispasmodic effect**

INTRODUCTION

Originating from Mexico, *Opuntia ficus indica* is a member of the *Cactaceae* family that is also found in Latin America, South Africa, and in the Mediterranean area. Folk medicine has traditionally used it for centuries. The green part of the plant (cladode) has a number of positive health effects (*1*, *2*), to which studies in recent years have provided experimental support. Various preparations from cladodes have been shown to be helpful for treatment of ulcers (*3*, *4*), have been used as antiuric and diuretic agents (*5*), have hypoglycemic and hypolipidemic effects in humans and experimental animals, (*6*−*10*) and may improve the platelet functions (*11*). In addition, tablets of lyophilized cladodes are marketed and recommended by herbalists as a coadjuvant against obesity and diabetes. In contrast to the stems, healthy properties of cactus pear fruits have been assessed only recently. Nutritional studies have shown that diets including cactus pear fruits are protective against oxidative-stress in humans (*12*). Antiulcer and hepathoprotective effects of the fruit juice have been shown in rats (*3*, *4*), and antitumor activities of fruit extracts have been demonstrated in cell cultures (*13*).

Nutritional components as well as the characteristic pigments of *Opuntia ficus indica* fruit pulp have long been determined. The betalains such as the purple-red betanin and the orange-yellow indicaxanthin occur in quite high amounts, whereas polyphenol compounds such as flavonoids are represented only in minute quantities. Bioactivities and effects of betalains are still scarcely known, although various in vitro activities and in vivo effects of the cactus pear fruits have been attributed to these pigments (*12*, *14*−*21*).

Many plant extracts and phytochemicals affect intestinal functions, with reported antispasmodic effects, delay of the intestinal transit, suppression of gut motility, stimulation of water adsorption, and decrease of electrolyte secretion (*22*). Because effects of cactus pear fruit on the intestinal motor function are not known, the purpose of the present study was to investigate the influence of a hydrophilic extract of fresh fruit pulp from the yellow cultivar of the Sicilian cactus pear (cactus fruit extract; CFE) on both spontaneous and evoked contractions of ileal smooth muscle and to eventually research component(s) responsible for the observed effects. Mouse ileum has been chosen since previous studies from our lab (*23*, *24*) have shown that its spontaneous mechanical activity is stable for long periods. On the other hand, ileum represents a significant portion of the small intestine and undergoes motor changes under various pathological conditions.

MATERIAL AND METHODS

Drugs. Ascorbic acid, atropine sulfate, carbachol (CCh), N_ω-nitro-L-arginine methyl ester (L-NAME), KCl, tetraethylammonium chloride (TEA), and tetrodotoxin (TTX) were purchased from Sigma Chemical

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Corp. (St. Louis, MO, USA). All solvents were of the highest purity or HPLC grade.

Preparation of Cactus Pear Fruit Extracts (CFE). Cactus pear fruits (*Opuntia ficus indica*, L. Mill), yellow cultivar (Sulfarina), were obtained from cactus plants grown in Sicily and processed within 72 h from the collection. Fruits were manually peeled, finely chopped, and briefly homogenized with a tissue blender. The pulp was then strained through a gauze (0.6-mm mesh size) to eliminate the seeds and mesocarp fibres. Samples of 100 g were extracted with 100 mL of methanol over 2 h at 10 °C with stirring. After centrifugation at 70000*g* for 30 min at 4 °C, the extracts were subjected to rotary evaporation to remove the organic solvents, subjected to cryo-dessiccation, stored at −80 °C, and used within two months. Immediately before use, the dried extracts were resuspended in suitable volumes of 5 mM saline phosphate buffer, pH 7.4 (PBS). The solubilized CFE is expressed as mg of fresh fruit pulp/mL.

Purification of Indicaxanthin. Indicaxanthin was isolated from CFE and resuspended in PBS by liquid chromatography on Sephadex G-25 (Sigma Chemical Corp.; St Louis, MO, USA) (*25*). Fractions containing the pigment were subjected to cryo-dessiccation. The dessiccated material was resuspended in 1% acetic acid in water and submitted to semipreparative HPLC using a Varian Pursuit C18 column (250 × 10 mm i.d.; 5 μm; Varian, Palo Alto, Ca, USA), eluted with a 20 min linear gradient elution from solvent A (1% acetic acid in water) to 20% solvent B (1% acetic acid in acetonitrile) with a flow-rate of 3 mL/min (*26*). Spectrophotometric revelation was at 482 nm. The elution volumes relevant to indicaxanthin were collected. Samples after cryo-dessiccation were stored at −80 °C. Immediately before use, the samples were resuspended in PBS and the concentration of indicaxanthin was evaluated spectrophotometrically at 482 nm, with a DU-640 Beckman spectrophotometer by using a molar absorbance of 42 800 (*27*).

Analysis of Ascorbic Acid. The ascorbic acid in the CFE resuspended in PBS was quantified by reversed-phase HPLC, with spectrophotometric revelation at 266 nm, as reported (*25*). Quantization was by reference to curves constructed with 5−100 ng of pure compound.

Animals. Adult male mice (C57BL/10SnJ) (weighing 25 ± 1.5 g) obtained from Harlan Laboratories (San Pietro di Natisone-Udine, Italy) were used for the study. Animals were maintained under controlled conditions of temperature (22 ± 2 °C) and humidity (55 ± 5%) until used. The animals had free access to water and food. All animal procedures were in conformity with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC). Mice were killed by cervical dislocation. The abdomen was immediately opened and the ileum was removed and placed in Krebs solution of (mM): NaCl 119; KCl 4.5; $MgSO_4$ 2.5; $NaHCO_3$ 25; KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.1.

Experimental Procedure. Ileal segments (20 mm in length) were suspended in a four channel organ bath containing 6 mL of oxygenated (95% O_2 and 5% CO_2) Krebs solution maintained at 37 °C. The distal end of each segment was tied to organ holders and the proximal end was secured with a silk thread to an isometric force transducer (FORT 25, Ugo Basile, Biological Research Apparatus, Comerio VA, Italy) to record contractions from the longitudinal axis. Mechanical activity was digitized on a A/D converter, visualized, recorded, and analyzed using the PowerLab/400 system (Ugo Basile, Italy). Longitudinal preparations were subjected to an initial tension of 200 mg and were allowed to equilibrate for at least 30 min. Rhythmic spontaneous contractions developed in all preparations. CFE (10−320 mg fresh pulp equivalents/mL of organ bath) was then tested cumulatively into the bath to obtain dose-dependent response curves. The contact time for each amount of extract was 5 min. Preliminary experiments had showed a maximal effect of the extract within this time period, and noncumulative responses were similar to those obtained in a cumulative manner. When required, the effect of CFE was evaluated in the presence of TTX (1 μM), L-NAME (300 μM), atropine (1 μM), or tetraethylammonium (TEA) (20 mM), added to the organ bath 30 min before CFE. The concentrations of the inhibitors used were determined from previous experiments, in which they have been shown to be effective (*23*, *28*). All drugs were dissolved in distilled water and stock solutions were prepared. The working solutions were prepared fresh on the day of the experiments by diluting the stock solutions in Krebs.

In other experiments, the effect of a 5 min contact of CFE (80−320 mg of fresh pulp equivalents/mL of organ bath) was evaluated on the contractions evoked by either exogenous carbachol (CCh, 10 μM) or high extracellular K^+ (KCl, 60 mM). Control experiments showed that these concentrations of CCh and KCl resulted in constant contractile responses. CCh or KCl were left in contact with the tissue for 2 min and then washed out.

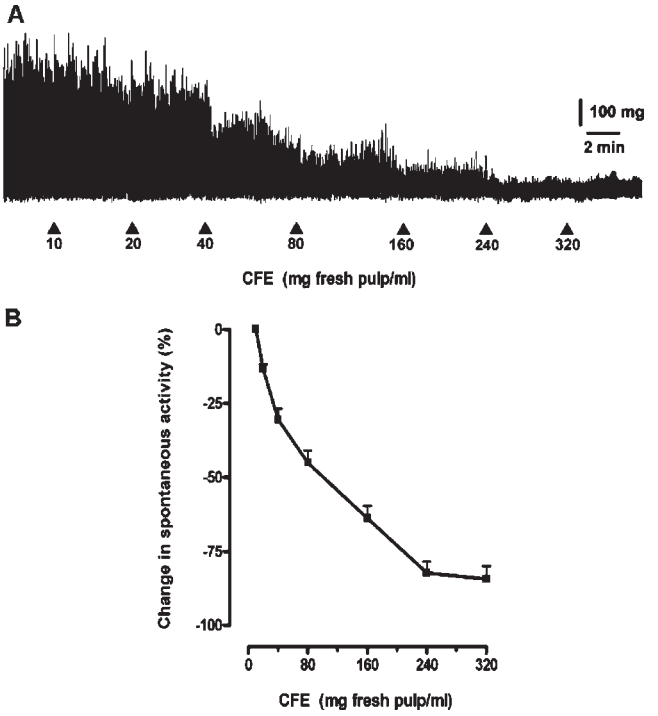

Figure 1. (A) Typical recording showing the inhibitory effects induced by increasing amount of CFE on the spontaneous mechanical activity of mouse ileal longitudinal muscle. (B) Dose−response curve for the inhibitory effects induced by CFE on the spontaneous contractions of mouse ileal longitudinal muscle. The inhibitory response is expressed as percent change of the phasic resting activity (−100% corresponds to the abolition of spontaneous activity). Each value is the mean ± SEM of 10 separate experiments. SEM is shown only if it exceeds the dimensions of the symbol.

When required, ascorbic acid (18−600 μM) or indicaxanthin (3−100 μM) were tested on the ileal spontaneous mechanical activity by adding, in a cumulative manner, increasing concentrations of the compounds alone or in combination. The contact time for each amount was 5 min. Lastly, the contraction induced by CCh was evaluated in the presence of indicaxanthin (25−50−100 μM) or in the presence of a combination of indicaxanthin (100 μM) and ascorbic acid (600 μM).

Statistical Analysis. For data analysis, the mean amplitude of the spontaneous contractions was determined for 10 min before drug administration. The inhibitory effects of the compounds were estimated as the decrease in the amplitude of the spontaneous contraction and reported as percent change from the resting phasic spontaneous activity (i.e., 100% corresponds to the abolition of spontaneous phasic activity). Effects on the contractions evoked by CCh and KCl were expressed as a percentage of the phasic component of the response obtained in control conditions. All data are mean values ± SEM of *n* experimental animals. Statistical analysis was performed by means of 2-way ANOVA followed by Bonferroni post hoc test, using Prism 4.0, GraphPad Software (San Diego, CA, USA). A probability value of less than 0.05 was regarded as significant.

RESULTS

CFE Effects. Isolated segments of mouse ileum exhibited spontaneous phasic contractions with an amplitude of about 300 mg and a frequency of about 30 cpm. CFE caused a dose-dependent inhibitory effect, characterized by a decrease of the mean amplitude of spontaneous contractions (**Figure 1**A). Occasionally, a small decrease in the resting tone was also observed at

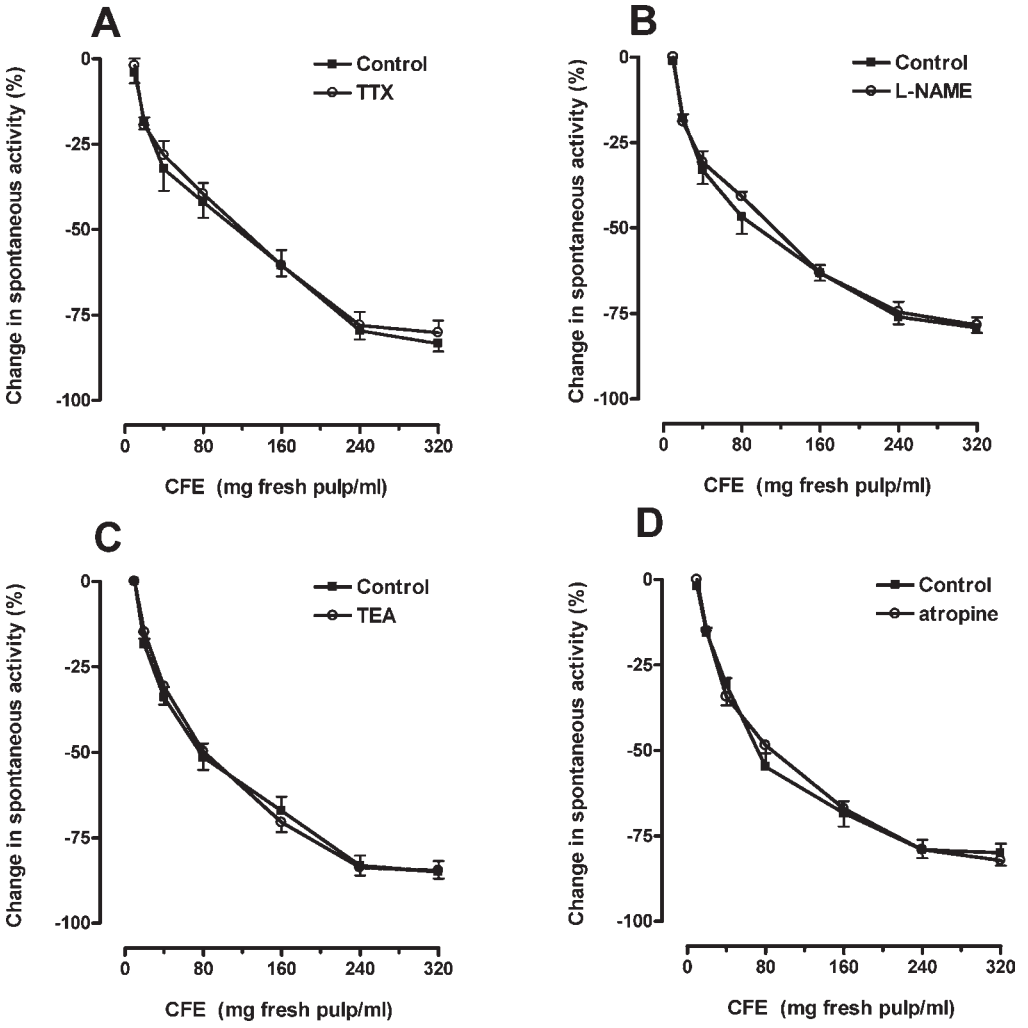

Figure 2. Dose−response curves for the inhibitory effects induced by CFE on the spontaneous contractions of mouse ileal longitudinal muscle in the absence (control) or in the presence of (A) TTX (1 μM), (B) L-NAME (300 μM), (C) TEA (20 mM), or (D) atropine (1 μM). The inhibitory response is expressed as percent change of the resting activity (−100% corresponds to the abolition of spontaneous activity). Each value is the mean ± SEM of five separate experiments. SEM is shown only if it exceeds the dimensions of the symbol.

the highest dose tested. The effects of CFE were reversible, returning to normal spontaneous contractions within 5 min of washing the tissue with fresh Krebs solution. The CFE inhibitory effects started at 20 mg of fresh pulp equivalents/mL of organ bath and the maximal response, consisting in an inhibition by about 80% of the spontaneous contractions, was reached at the dose of 240 mg/mL; a CFE amount as high as 320 mg/mL did not cause further inhibition (**Figure 1**B).

The inhibitory effects of CFE on spontaneous phasic contractions were unaffected by TTX (1 μM) (**Figure 2**A), a blocker of neuronal voltage-dependent Na^+ channels, L-NAME (300 μM) (**Figure 2**B), a blocker of the synthesis of nitric oxide (NO), TEA (20 mM), a nonselective blocker of potassium channels (**Figure 2**C), or by atropine (1 μM), a muscarinic receptor antagonist (**Figure 2**D), which per se decreased the spontaneous activity.

CCh, a muscarinic receptor agonist, and high extracellular potassium concentrations were used to evaluate whether CFE also inhibited evoked contractions. CCh (10 μM) and KCl (60 mM) induced reproducible and constant contractile responses, characterized by a fast initial peak, the phasic component, followed by a decline to a lower maintained tension level, the tonic component. The contractions induced by 10 μM CCh (phasic component: 0.91 ± 0.1 mg; tonic component: 0.73 ± 0.1 mg, n = 10) or by

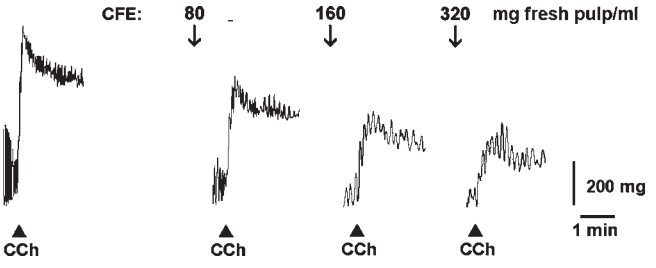

Figure 3. Typical recording showing contractions evoked by 10 μM CCh in the presence of increasing amounts of CFE. The arrows indicate application of CFE.

60 mM KCl (phasic component: 0.86 ± 0.18 mg; tonic component: 0.76 ± 0.1 mg, n = 10) were not significantly different. CFE remarkably inhibited both components of the CCh-induced contraction in a dose-dependent manner, being more effective on the phasic component than on the tonic one (**Figures 3** and **4**A). The preparation recovered 100% of its contractile ability after 10 min wash out. On the contrary, the KCl-evoked contractions were not inhibited by CFE up to 320 mg/mL (**Figure 4**B).

Effects of Ascorbic Acid and Indicaxanthin. Indicaxanthin is the characteristic and most abundant phytochemical of CFE, that

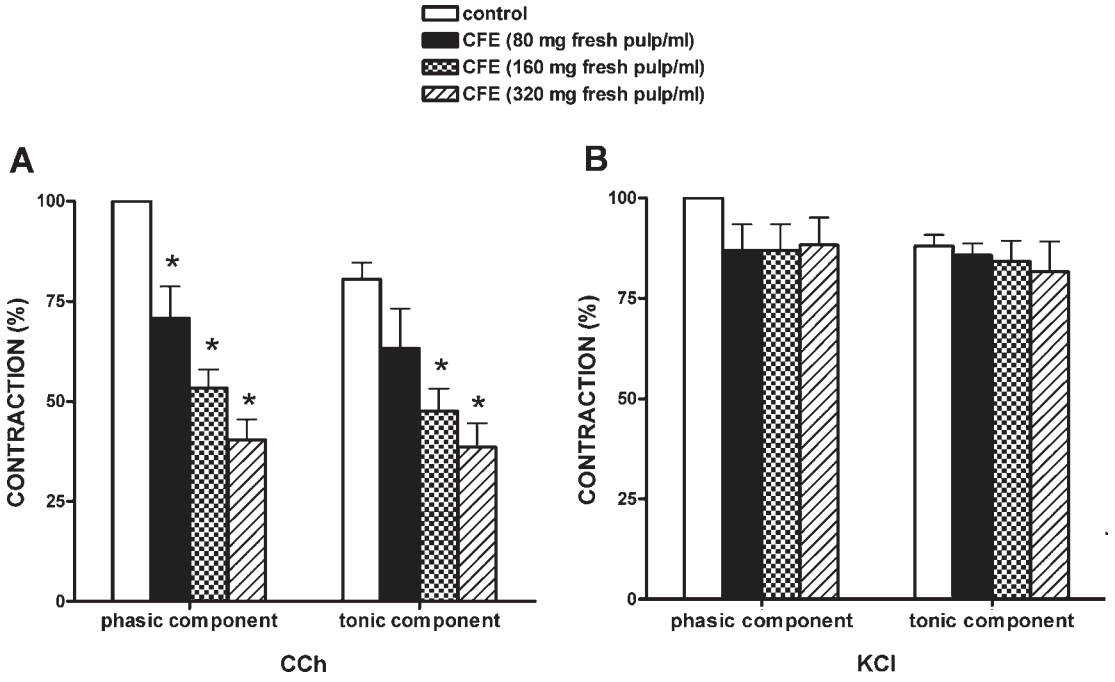

Figure 4. Effect of CFE on (A) CCh (10 μM) or (B) KCl (60 mM)-evoked phasic and tonic contractile components in mouse ileal longitudinal muscle. Data are expressed as a percentage of the phasic responses obtained in the absence of CFE (control). Each value is the mean ± SEM of six separate experiments. *$P < 0.01$ when compared to control (ANOVA followed by Bonferroni *t*-test).

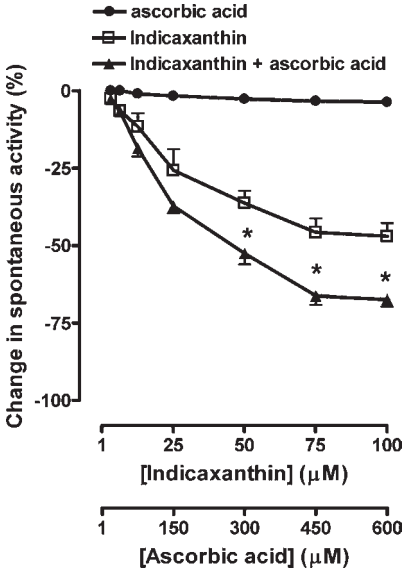

Figure 5. Concentration−response curves for the inhibitory effects induced by ascorbic acid ($n = 3$), indicaxanthin alone ($n = 5$), or in combination with ascorbic acid ($n = 5$) on the spontaneous contractions of mouse ileal longitudinal muscle. The inhibitory response is expressed as percent change of the resting activity (−100% corresponds to the abolition of spontaneous activity). Each value is the mean ± SEM which is shown only if it exceeds the dimensions of the symbol. *$P < 0.05$ when compared to indicaxanthin alone (ANOVA followed by Bonferroni *t*-test).

also contained relevant amounts of ascorbic acid. The effect of these components, alone or combined, was then investigated taking into account their amount in our CFE preparations (indicaxanthin, 9.6 mg/100 g fresh pulp; ascorbate, 33 mg/100 fresh pulp). Ascorbic acid (18−600 μM) did not affect the mechanical spontaneous activity of ileal longitudinal muscle (**Figure 5**) nor the CCh-evoked contractions. On the contrary, indicaxanthin (3−100 μM) caused a reversible and concentration-dependent decrease of the mean amplitude of spontaneous contractions (**Figure 5**). Under the conditions applied, the maximal inhibitory effect (E_{max}) was observed with 75 μM indicaxanthin with a percent reduction of contraction amplitude of about 50%. Then, though the spontaneous ileal contractility appeared substantially inhibited, the effect of indicaxanthin alone (−47.0 ± 4.2; $n = 5$) was significantly lower than the maximal response induced by CFE with a comparable indicaxanthin amount (see **Figure 1**; −84.3 ± 4.3; $n = 10$) ($P < 0.01$). The combination of indicaxanthin (3−100 μM) and ascorbic acid (18−600 μM) inhibited the spontaneous contractions with an effect remarkably higher than indicaxanthin alone ($E_{max} = -67.3 \pm 2.3$; $n = 5$) (**Figure 5**). E_{max} of CFE was not reached, but the values were not significantly different ($P > 0.05$). Indicaxanthin (25−50−100 μM) inhibited both components of the CCh-induced contraction in a dose-dependent manner, being more effective on the phasic component than on the tonic one, and it failed to affect KCl-evoked contractions (**Figure 6**). Once more, the combined indicaxanthin (100 μM) and ascorbic acid (600 μM) were more effective in inhibiting CCh-evoked contractions (phasic component: 50% ± 5.0; tonic component: 44 ± 4.0; $n = 4$) than indicaxanthin alone (phasic component: 69% ± 1.5; tonic component: 55.4 ± 1.5; $n = 4$) (**Figure 6**) and the percent inhibition caused by their combination was quite comparable with that observed with CFE (phasic component: 40.3% ± 5.1; tonic component: 38.5 ± 6.0; $n = 6$; $P > 0.05$) (see **Figure 4**).

DISCUSSION

Recent studies in vivo and/or in vitro showed that preparations and extracts from either cladodes or fruits of cactus pear possess various biological activities and effects, including anticancer, antiviral, anti-inflammatory, antidiabetic and antihyperlipidemic, as well as antioxidant properties (*4*). The present work for the first time shows that an aqueous extract of fruits from the yellow cultivar of cactus pear (*Opuntia ficus indica*) exerts a direct inhibitory effect on both spontaneous and CCh-evoked contractions of mouse ileal longitudinal muscle, which has

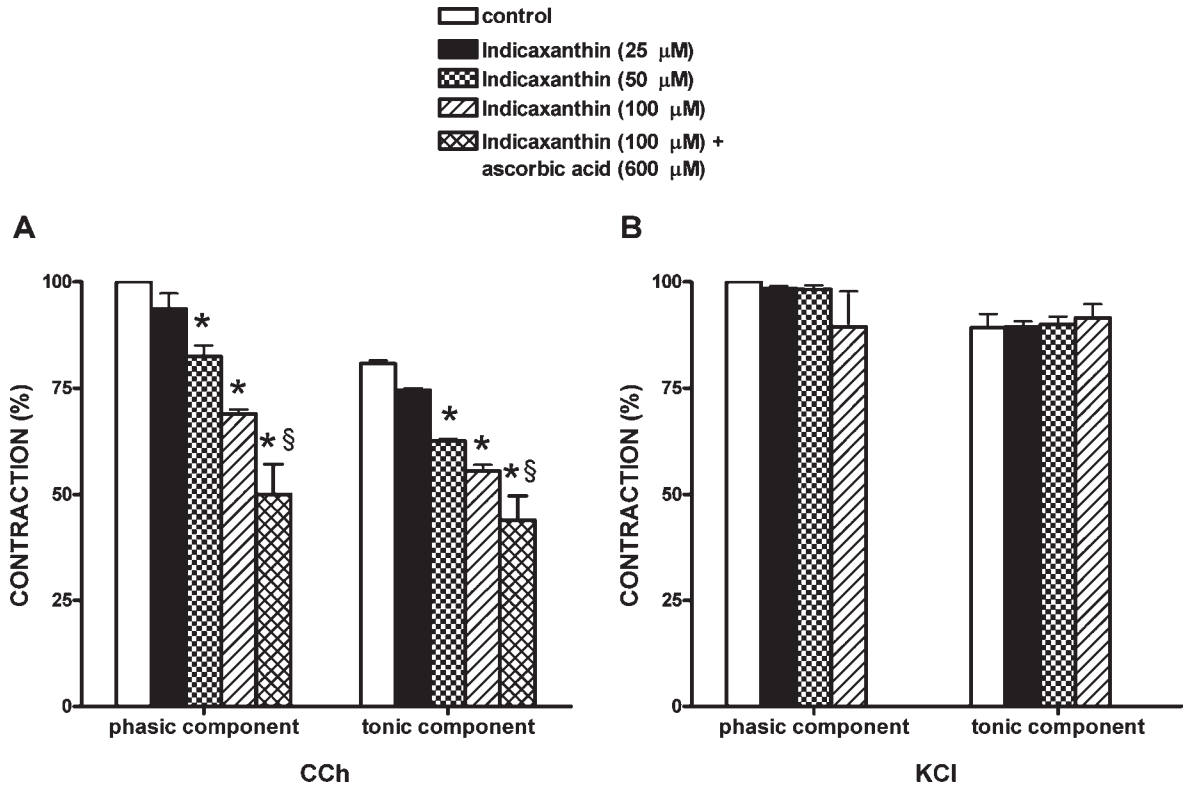

Figure 6. Effect of indicaxanthin on (A) CCh (10 μM) or (B) KCl (60 mM)-evoked phasic and tonic contractile components in mouse ileal longitudinal muscle. Data are expressed as a percentage of the phasic responses obtained in the absence of indicaxanthin (control). Each value is mean ± SEM of four separate experiments. SEM is shown only if it exceeds the dimensions of the symbol. *$P < 0.01$ when compared to control; §$P < 0.01$ when compared to 100 μM indicaxanthin (ANOVA followed by Bonferroni *t*-test).

appeared mainly but not simply due to the activity of indicaxanthin.

The myorelaxant effect of CFE is possibly to be ascribed to a direct action on the smooth muscle cell rather than indirect activity on neurotransmitter release. Indeed, the observation that TTX, a blocker of neuronal voltage-dependent Na^+ channels, did not reduce the inhibitory effects of the extract suggests that the effect does not depend on neural action potentials, but may be due to an action on smooth muscle cells. On the other hand, these results cannot rule out a prejunctional action through production and release of some inhibitory neurotransmitters from nerve terminals. Because NO is the main inhibitory neurotransmitter mediating inhibitory effect in the mouse ileum longitudinal muscle (*24*, *29*) and can also originate from non-neural cells of the intestinal tract (*30*, *31*), the involvement of NO in the mechanism underlying the inhibitory responses of CFE was investigated by blocking NO synthesis. L-NAME did not affect the inhibitory effects of the extract, suggesting that NO was not involved in the CFE action.

The spasmolytic effects of various medicinal plants have appeared to be mediated by K^+ channel opening mechanisms (*32*−*34*). These channels control the contraction of the gastrointestinal smooth muscles by setting resting potential and influencing slow waves and action potential configuration (*35*). Activation of K^+ channels causes membrane hyperpolarization of smooth muscle cells and therefore inhibition of Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels. In our experimental preparation, a potential role of the K^+ channels was ruled out since TEA, an inhibitor of the K^+ channel, did not antagonize the myorelaxant action of the CFE.

In the attempt to clarify the mechanism responsible for the observed effects, we used two different agents, KCl and CCh, which induce contractile responses through distinct pathways, namely, sarcolemma Ca^{2+} influx and phospholipase C (PLC), inositol triphosphate (IP_3), and diacylglicerol (DAG) pathway, respectively. In particular, high K^+ (> 30 mM) causes smooth muscle contractions through membrane depolarization leading to an increased calcium influx through voltage-dependent L-type Ca^{2+} channels (*36*), and any substance causing inhibition of high K^+-induced contraction is considered as an inhibitor of Ca^{2+} influx (*37*). On the other hand, muscarinic receptor activation by CCh induces contraction due to IP_3-dependent Ca^{2+} release from the internal store (phasic component), followed by Ca^{2+} entry via voltage-dependent Ca^{2+} channels (tonic component), after depletion of the store. Therefore, each contractile phase requires IP_3 (*38*). The observation that CFE selectively reduced the CCh-induced contractile effects without affecting the KCl-induced contraction suggests that the inhibitory effect is not mediated by blockade of voltage-dependent L-type Ca^{2+} channels, but possibly some component/s of CFE can interfere directly or indirectly with muscarinic receptor activation and/or the PLC-IP_3 pathway, and consequently with Ca^{2+} release from intracellular stores. The potential involvement of the muscarinic receptors in the inhibitory effect of CFE on the spontaneous contractions was investigated by testing the extract in the presence of atropine, a muscarinic receptor antagonist. Atropine per se caused a reduction of the amplitude of spontaneous contractions suggesting that muscarinic receptors are tonically activated, as reported in previous investigations (*23*, *39*). However, the observation that the CFE inhibitory effects were not significantly reduced in the presence of atropine suggests that some component of the extract interferes with the contractile machinery independently of the activation of muscarinic receptors. Further studies are required to clarify the level at which the

PLC/IP_3-dependent signaling and the pathway leading to calcium release are blocked.

The fruits of *Opuntia ficus indica* are a very good source of water-soluble nitrogenous chromo alkaloids called betalains, the nutraceutical properties and bioactivity of which are presently the subject of many reports (*40*). Indicaxanthin is the most abundant pigment in the fruit extract of the yellow cultivar of cactus pear, whereas only traces of polyphenols occur (*41*). On the other hand, as in most fruits and vegetables, ascorbic acid represents a major component of the water-soluble fraction of cactus pear fruit (*25*). In accordance, we stated to investigate whether and to what extent these CFE components were responsible for the observed effects. Effects of ascorbic acid on the smooth muscle of different regions from rodent gastrointestinal tract have been reported (*42*−*44*); however, in our system this component has appeared totally ineffective to modify the spontaneous mechanical activity, suggesting that, at least per se, it was not involved in the CFE inhibitory effects. Indicaxanthin, instead, induced inhibitory effects on both spontaneous and CCh-evoked contractions of mouse ileal longitudinal muscle, being able to reduce in a concentration-dependent manner and reversibly the contractility of the intestinal segment, though with a lower effectiveness than CFE. Interestingly, the combination of indicaxantin with ascorbic acid induced an inhibitory effect remarkably higher than that of indicaxantin alone and comparable with that observed with the whole CFE. Therefore, while showing cooperative interactions between ascorbic acid and indicaxanthin, our findings suggest that this pigment is to be considered the main component providing CFE with the spasmolytic activity. Vitamin C is known to preserve the indicaxanthin integrity when present with the betalain under a variety of conditions of pH and temperature (*45*), and then an increase of the pigment stability can potentiate its effects. At this stage, molecular mechanisms underlying the indicaxanthin activity in the present system are far from being envisaged. Redox properties of this molecule (*25*) and possibly other chemico-physical interactions with cell components (*46*) may be involved. In any instance, the reversibility of the observed effects rules out structural changes or covalent binding of the molecule to cell components.

In conclusion, present findings show that a hydrophilic extract from pulp of fruit from yellow cultivar of *Opuntia ficus indica* effectively inhibits the contractility of mouse ileum by interefering with pathways of intracellular Ca^{2+} release in the smooth muscle cells. Purified indicaxanthin may mimic the whole extract effects, and the mechanism of its activity is under investigation. When considering the high bioavailability of indicaxanthin from cactus pear in humans (*47*), these results provide a basis for the potential use of cactus pear extracts or purified indicaxanthin in gastrointestinal disorders, such as abdominal cramps and constipation. Our data provide added value to the nutritional characteristic of the fruits of *Opuntia ficus indica*.

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