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Effects of flavone on the contractile activity of the circular smooth muscle of the rabbit middle colon *in vitro*

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ABSTRACT

The circular smooth muscles of the middle colon of the rabbit generate giant contractions of high amplitude and low frequency. Flavone, at various concentrations, reduces the giant contractions and the tonic contraction induced by 10 μM carbachol and 80 mM KCl. The contractions induced by dequalinium and tetraethylammonium are reduced by flavone (30 μM). At 100 μM, flavone decreases the contraction induced by 100 μM methylene blue and 1 mM orthovanadate. These results suggest that flavone inhibit the giant contractions by (1) inhibition of voltage-dependent Ca^{2+} channels, (2) activation of guanyl cyclase, (3) opening of K^+ channels and (4) inhibition of tyrosines kinases.

1. Introduction

The spontaneous mechanical activity of the proximal, middle and distal colon of the rabbit shows *in vitro* two types of contractions: phasic contractions with low amplitude and high frequency, giant contractions (GCs) with high amplitude and low frequency (Benabdallah et al., 2008). The circular smooth muscle in the colon of different species generate *in vivo* several types of spontaneous contractions which take part in mixture and propulsion: rhythmic phasic contractions, giant migrating contractions (GMCs), and tone (Sarna, 2006). The rhythmic phasic contractions are regulated by slow waves superimposed with spikes. Their maximum frequency is the same as that of slow waves (Gonzalez and Sarna, 2001b). In comparison with the phasic contractions, GMCs are ultrapropulsives contractions of high amplitude and long duration (Gonzalez and Sarna, 2001b; Sarna, 2006). They are not regulated by slow waves, as their duration is much longer than that of a single slow wave cycle (Sarna, 1987). The equivalent of these *in vitro* contractions are the giant contractions (GCs) which are distinguished from the phasic contractions by their high amplitude and low frequency (Gonzalez and Sarna, 2001a).

Flavonoids are members of a class of natural compounds which recently constitute the subject of several studies with scientific and therapeutic interest. These polyphenolic compounds recognized by the presence of a C_6–C_3–C_6 basic ring skeleton are widely distributed throughout the plant kingdom (vegetables, fruits, flowers, wine and tea) (Benavente-Garcia et al., 1997) and potentially interact with physiological processes. Flavonoids are known to exhibit various biological activities such as anti-inflammatory (Vongtau et al., 2000), anti-tumor (Ikemoto et al., 2000), antiproliferative (Zhang et al., 2002), antiviral, anticancer (Di Pietro et al., 2002), antioxidant (Calderone et al., 2004), antiulcer (Lewis and Shaw, 2001),

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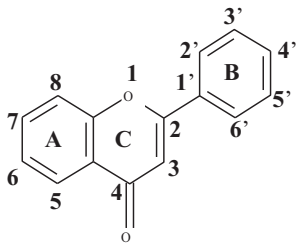

Fig. 1. Structure of flavone.

antiallergic, and anti-hypertensive (Almeida et al., 2006) activities. The effect of different flavonoids on smooth muscle contraction of several tissues also have been described (Hammad and Abdalla, 1997; Ajay et al., 2003; Uydes-Dogan et al., 2005). Quercetin and kaempferol inhibit uterine smooth muscle contraction induced by KCl (60 mM) (Revuelta et al., 1997). Some flavonols, flavones, flavanones, isoflavones and flavanes dose-dependently inhibited the contractions induced by phenylephrine and high K^+ in isolated rat thoracic aorta (Ajay et al., 2003). Several mechanisms, including protein kinases inhibition (Herrera et al., 1996), intracellular inhibition of cAMP and cGMP phosphodiesterase (Herrera et al., 1996; Revuelta et al., 1997) and inhibition of calcium ion influx (Ajay et al., 2003) could be involved in their inhibitory effect.

A report by Benabdallah et al., (2008) indicates that circular muscle strips prepared from the rabbit colon generate regular GCs as well as phasic contractions. This *in vitro* finding and the fact that flavonoids inhibit smooth muscle contractions provide an opportunity to investigate the effects of these compounds on GCs in a muscle bath environment. The specific aim of the present paper was to study the effects of flavones as basic structure of flavonoids (Fig. 1) on the isolated circular muscle from rabbit colon and to examine his possible underlying mechanisms. For that, the interference of this flavonoid with: 1) intracellular Ca^{2+} mobilization, 2) potassium channels, 3) nitrergic pathway, and 4) phosphorylation of proteins were studied.

2. Materials and methods

2.1. Recording of spontaneous contractile activity

The care and handling of the rabbits and the research protocol were in accordance with the institutional guidelines for the use of experimental animals. Adult rabbits of both sex and 1060 ± 339 g body weight were killed by bleeding from the carotid arteries. After a midline laparotomy the whole colon was removed and placed in a Petri dish containing HEPES buffered physiological solution (composition in mM: NaCl 126, KCl 6, $MgCl_2$ 1.2, $CaCl_2$ 2, EDTA 0.01, HEPES 10.5 and glucose 14, pH 7.4). The colon was divided into three different parts: proximal, middle and distal colon. Full thickness circular muscle strips of approximately 2 mm width and 10 mm length were prepared by cutting the tissue parallel to the circular axis. The strips were mounted vertically in organ bath chambers containing 25 ml of the physiological solution warmed at 37 °C and continuously oxygenated. The thread anchoring the upper end of the strips was connected to the lever of a force–displacement transducer (FSG-01/3, Experimetria, Budapest, Hungary) connected to an amplifier (EXP-D, Experimetria, Budapest, Hungary). Muscle activities were stored in a PC and simultaneously visualized (WinDaqLite software, DATAQ Instruments, OH, USA) using an external analog/digital conversion card with a sampling rate of 2 Hz (DI 700-PGL USB, DATAQ Instruments, OH, USA). Before the start of the experiments, the resting tension was adjusted to 1 g and the tissue was allowed to equilibrate for 45 min during which time the solution was renewed every 15 min. To verify the viability and the responsiveness of the smooth muscle, the strips were challenged with 1 μM carbachol for 3 min, a period sufficient for the development of maximal tension. After washing the carbachol, a second period of stabilization (45 min) with periodical washing was started.

2.2. Experimental protocols

After the second equilibration period, strips of colon were incubated with different treatment used in this study.

2.2.1. Effects of flavone on the spontaneous mechanical activity

Flavone in solution in the dimethyl sulphoxide (DMSO) or the vehicle (DMSO) were added to medium cumulatively 1–100 μM) at 15 min intervals.

2.2.2. Effects of flavone on the contraction induced by carbachol and KCl

Strips from the middle colon were precontracted with either 10 μM carbachol or 80 mM KCl for 15 min and 30 min respectively and the relaxant responses to flavone (1–100 μM) were recorded by adding cumulative doses of flavonoid solutions to the tissue bath at 15 min intervals between sussessive concentrations.

2.2.3. Effects of flavone after stimulation of the spontaneous mechanical activity with TEA, dequalinium, methylene blue, and sodium orthovanadate

In order to investigate the mechanisms underlying the relaxant action of flavone, the strips from the middle colon were preincubated for 15 min with 5 mM tetraethylammonium (TEA), a non selective blocker of K^+ channels (Dong et al., 2005), 10 μM dequalinium, an apamin-sensitive K^+ channel blocker (Castle, 1999), 100 μM methylene blue, a guanylate cyclase inhibitor (Börjesson et al., 1999), or 1 mM sodium vanadate, a tyrosine phosphatase inhibitor (Alcon et al., 2000). After this period, the vehicle or the flavone was added to the organ bath separately (30 μM for 30 min) or cumulatively (1 μM for 5 min and 30 μM for 30 min or 30 and 100 μM for 15 min).

2.3. Drugs

The following drugs were used: carbamylcholine chloride (carbachol), tetraethylammonium chloride, dequalinium chloride, methylene blue trihydrate, sodium orthovanadate, and flavone: 2-phenyl-4- H-1-benzopyran-4-one (Sigma, St. Louis, USA). All drugs were prepared as stock solutions (carbachol 100 mM, TEA 500 mM, methylene blue 100 mM, sodium orthovanadate 1 M, and flavone 50 and 100 mM) in distilled water except for dequalinium, and flavone which were prepared in pure DMSO. All dilutions were made with the corresponding solvent of each drug and added in the bathing medium in volumes $< 0.5\%$. Preliminary experiments have shown that the vehicles (distilled water and DMSO) were without any observable effect on the mechanical activities of the preparation.

2.4. Statistical analyses

Several parameters of the GCs were measured during the last three minutes of the incubation period: resting tone (the average minimal tension developed between GCs), maximal tension (g), amplitude (g), frequency (contractions.min^{-1}, cpm) and duration (s). The results are expressed as mean ± S.E.M. with n indicating the number of animals. Differences between treatments were