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Effect of the flavonoid galangin on urinary bladder rat contractility in-vitro

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Abstract

Galangin is a flavanol with several biological activities. We have evaluated the effect of galangin on the contractile response elicited by electrical field stimulation (EFS) in the rat isolated urinary bladder. Galangin ($10^{-8}-10^{-4}$ M) produced a concentration-dependent inhibition of the EFS contractile response without modifying the contractions produced by exogenous acetylcholine (10^{-6} M). Blockade of adrenergic and cholinergic nerves with a combination of atropine (10^{-6} M), phentolamine (10^{-6} M) and propranolol (10^{-6} M) or blockade of tachykinin NK_1 and NK_2 receptors with SR140333 (10^{-7} M) and SR48968 (10^{-6} M) did not modify the inhibitory effect of galangin. However, verapamil (10^{-7} M) significantly reduced the inhibitory effect of galangin. It is concluded that the galangin inhibits EFS-induced contractions of the rat urinary bladder by acting on L-type calcium channels on presynaptic nerves.

Introduction

Galangin, a member of the flavonol class of flavonoids, is present in high concentration both in *Alpinia officinarum*, a plant used as a spice and as a herbal medicine for a variety of ailments in Asia, and in propolis which is a natural composite balsam produced by honeybees (Park et al 1995). Galangin has been demonstrated to possess anti-mutagenic activity by binding to the oestrogen receptor (Wall et al 1988; So et al 1997) and anti-oxidative and radical scavenging activity due to its redox properties (Cholbi et al 1991; Imamura et al 2000). An anti-clastogenic effect against induction of chromosome aberrations by a radiomimetic agent, bleomycin (Heo et al 1994, 1996), and an anti-inflammatory activity by inhibiting the action of cyclo-oxygenase 2 (Gabor & Razga 1991; Kang et al 2000) has also been observed. Metabolic enzyme modulating activity (Shih et al 2000), antiviral activity (Meyer et al 1997) and cytochrome P450 hydroxylase inhibitory activity in human liver microsomes (Buening et al 1981; Ciolino & Yeh 1999) have been also reported. Additionally, in-vivo, galangin reduced the induction of micronuclei by MNU, MNNG and ethyl methane sulfonate in polychromatic erythrocytes in the bone marrow of mice (Heo et al 1992; Sohn et al 1998). Moreover, galangin has been recently proposed as a candidate for cancer chemoprevention (Heo et al 2001).

Previous investigators have shown that flavonoids possess inhibitory effects on both intestinal and bronchial smooth muscle (Di Carlo et al 1999); also, flavonoids, in addition to their diuretic action (Jouad et al 2001), display a protective effect against bladder cancer risk (Garcia et al 1999) and acute renal failure (Avramovic et al 1999).

In this study we have investigated the effect of galangin on the contractions induced by electrical stimulation in the rat bladder.

Materials and Methods

Drugs

Galangin, acetylcholine hydrochloride, atropine sulfate, phentolamine hydrochloride, propranolol hydrochloride, tetrodotoxin and verapamil hydrochloride were purchased from Sigma Chemical Co. Ltd, UK; SR140333 and SR48968 were a gift form SANOFI-

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Reserche (Montpellier, France). Galangin was dissolved in dimethyl sulfoxide (DMSO), SR140333 in DMSO/water (50%, v/v). The other drugs were dissolved in distilled water. DMSO (less than 0.01%) did not modify EFS- or acetylcholine-induced contractions.

Animals

Male Wistar rats (200–220 g) purchased from Charles River UK Ltd were maintained under controlled conditions of temperature (21±1°C) and humidity (30–35%) until used. The rats had free access to water and food. All animal experiments complied with British Home Office Regulations and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC).

Bladder preparations

Rats were killed by asphyxiation with CO_2. The urinary bladder was removed and placed in Krebs solution (mM: NaCl 119, KCl 4.75, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 11). Longitudinal strips of approximately 2×10 mm were cut from the bladder body and placed in 20-mL organ baths containing Krebs solution equilibrated with 95% O_2–5% CO_2 at 37°C. The tissues were connected to an isometric transducer (load 1 g) connected to Servogor 124 recording apparatus (Recorderlab, Surrey, UK). All experiments commenced after a minimal 60-min equilibration period and the strips were subjected to an electrical field stimulation (EFS) of 0.25 Hz for 1 s, 500 mA, 0.25 ms pulse duration, delivered via electrodes placed around the tissue using Harvard (USA) Dual Impendance Research Stimulators.

Stable and reproducible contractions for a time-period of 4 h were obtained with stimulation every 2 s. After stable control contractions evoked by EFS had been recorded, the responses were observed in the presence of increasing cumulative concentrations of galangin ($10^{-8}-10^{-4}$ M). The contact time for each concentration of galangin varied between 30 and 45 min (until the inhibitory effect reached a plateau).

The effect of galangin was also evaluated after the administration in the bath (contact time 30 min) of atropine (10^{-6} M), propranolol (10^{-6} M) and phentolamine (10^{-6} M) (to block the adrenergic and cholinergic nerves), of SR140333 (10^{-7} M) plus SR48968 (10^{-6} M) (to block tachykinin NK_1 and NK_2 receptors) or after verapamil (10^{-7} M) (to block L-type calcium channels). In preliminary experiments the effect of tetrodotoxin (3×10^{-7} M, contact time 10 min) on EFS-induced contractions was evaluated. These concentrations were selected on the basis of previous work (Elliott et al 1996; Nocerino et al 2002).

The effect of galangin was also evaluated (contact time 45 min) on the contractions produced by exogenous acetylcholine (10^{-6} M). Acetylcholine was left in contact with the tissue for 30 s and then washed out.

Statistical analysis

Results are expressed as means±s.d. Non-linear regression analyses for all concentration response curves were performed. Data were analysed by one-way analysis of variance followed by Dunnett's test. A value of $P<0.05$ was considered significant. The concentration of galangin that produced 50% inhibition of EFS-induced contractions (IC50) was used to characterise its potency. IC50 values (geometric means±95% confidence limits) were calculated using the computer programme of Tallarita & Murray (1996).

Results

Electrical stimulation (0.25 Hz for 1 s, 500 mA, 0.25 ms pulse duration) of the bladder strips gave contractile responses which were abolished by tetrodotoxin (3×10^{-7} M, n = 7, $P<0.001$) but left unchanged by a combination of atropine (10^{-6} M), propranolol (10^{-6} M) and phentolamine (10^{-6} M) (5±2% inhibition, n = 7, $P>0.2$), SR144333 (10^{-7} M) plus SR48968 (10^{-6} M) (4±3% inhibition, n = 7, $P>0.2$) or verapamil (10^{-7} M) alone (5±4% inhibition, n = 8, $P>0.2$). Galangin (10^{-8}–10^{-4} M) decreased the amplitude of the EFS-evoked contractions in a concentration-dependent manner (Figure 1). The IC50 value (95% confidence limits) was 1.5×10^{-6} (9.3×10^{-7} to 2.4×10^{-6}) M. Statistical significance ($P<0.01$) was achieved starting from the 10^{-5} M concentration.

Acetylcholine (10^{-6} M) produced contractile responses of the bladder strips which were abolished by atropine (10^{-6} M, 100% inhibition, n = 6, $P<0.001$) but unaffected by

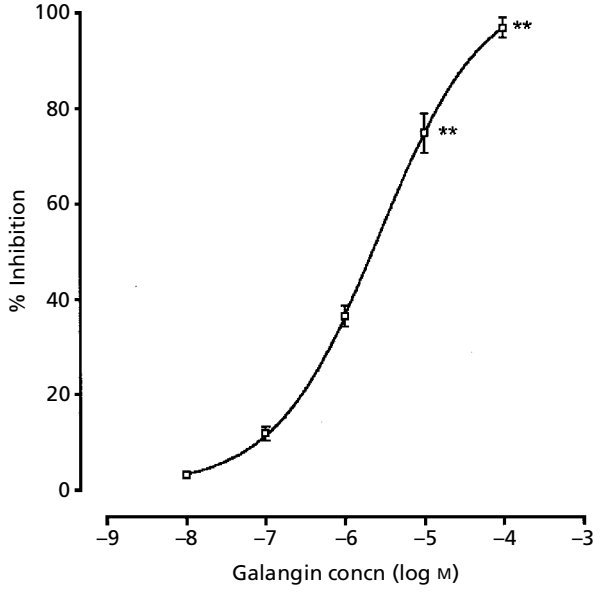

Figure 1 Effect of galangin on the contractile response produced by electrical field stimulation (EFS). Each point represents the mean of 6 or 7 experiments; bars show s.d. $^{**}P<0.01$ vs control.

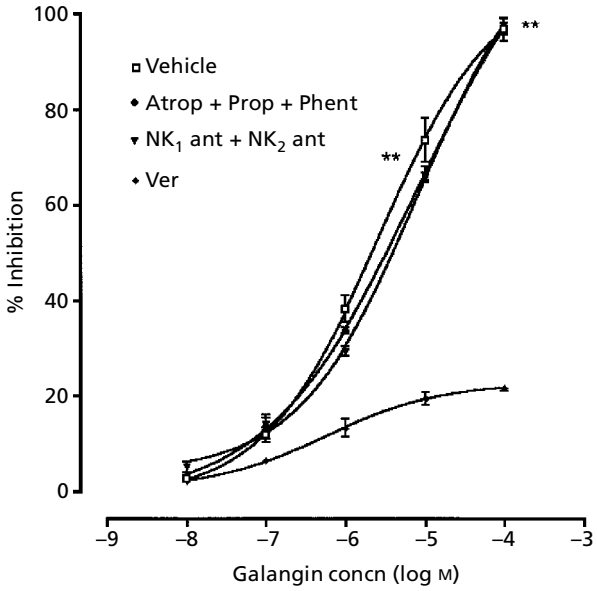

Figure 2 Effect of various drugs on the inhibitory effect of galangin (vehicle) on EFS-induced contractions in the rat urinary bladder. Each point represents the mean of 7 or 8 experiments; bars show s.d. $^{**}P < 0.001$ vs corresponding control. Atrop = atropine 10^{-6} M; Prop = propranolol 10^{-6} M; Phent = phentolamine 10^{-6} M; NK_1 ant = NK_1 receptor antagonist SR140333 10^{-7} M; NK_2 ant = NK_2 receptor antagonist SR48968 10^{-6} M; Ver = verapamil 10^{-7} M.

tetrodotoxin (3×10^{-7} M, 2 ± 3% inhibition, n = 7, $P > 0.2$). However, galangin did not modify the contractions evoked by acetylcholine 10^{-6} M (% inhibition: galangin 10^{-8} M, 2.1 ± 1.8; galangin 10^{-7} M, 4.8 ± 5.2; galangin 10^{-6} M 6.8 ± 0.4; galangin 10^{-5} M 14.0 ± 6.2; galangin 10^{-2} M 14.7 ± 5.4; n = 6, $P > 0.2$).

The inhibitory effect of galangin in the EFS-induced contractions was unaffected by a combination of atropine (10^{-6} M), propranolol (10^{-6} M) and phentolamine (10^{-6} M) or by SR144333 (10^{-7} M) plus SR48968 (10^{-6} M) (Figure 2). However galangin was without significant effect when tested after verapamil (10^{-7} M) (Figure 2).

Vehicle (DMSO 2 μL/20 mL) did not modify EFS-induced contractions (2 ± 3% inhibition, n = 6, $P > 0.2$).

Discussion

Flavonoids are a group of about 4000 naturally occurring compounds that are ubiquitous in vascular plants. Flavonoids are generally non-toxic and show a variety of beneficial biological activity and have been investigated as potential drugs against various diseases (Di Carlo et al 1999). In view of their many reported biological actions (Wall et al 1988; Cholbi et al 1991; Gabor & Razga 1991; Heo et al 1994, 1996; So et al 1997; Imamura et al 2000; Kang et al 2000; Shih et al 2000), we studied the effects of galangin on rat bladder motility.

We have shown that galangin, in a concentration-dependent manner, reduced the contractions evoked by electrical stimulation of the rat bladder. These contractions were almost completely abolished by tetrodotoxin, a specific Na^+-channel blocker. This suggests that the main effect of the electrical stimuli that we applied to the bladder was to provoke contractions indirectly by stimulating the release of contractile transmitters from prejunctional nerve terminals. However, it is unlikely that electrical stimulation induced a significant release of acetylcholine or tachykinins as these contractions were unaffected by either atropine or tachykinin NK_1 and NK_2 antagonists. Thus, it is likely that contractions induced by electrical stimulation are mediated by the release of endogenous ATP, as previously documented in the mouse (Santicioli et al 1986), rat (Tong et al 1997; Khattab et al 2002) and guinea-pig (Westfall et al 2000) bladder.

The ability of galangin to inhibit electrically evoked bladder contractions may well depend on an ability to suppress the release of these transmitters (presynaptic action) rather than on some ability to interact directly with smooth muscle. Acetylcholine is well known to contract the urinary bladder of rodents through a direct action on bladder smooth muscle in-vitro (Burnstok et al 1972). Our experiments with rat bladder showed that galangin did not attenuate contractions induced by acetylcholine. These data suggest a presynaptic site of action for galangin.

It is well known that Ca^{2+} is important for the contractile activity of smooth muscle, including the bladder. It has been suggested that blockade of L-type Ca^{2+} channels reduces the neural release of contractile mediators (Somogyi et al 1997). As verapamil abolished the inhibitory effect of galangin, it is likely that the inhibitory effect of galangin could involve L-type Ca^{2+} channels.

Our findings enable us to exclude a number of factors as potential contributory mechanisms involved in the inhibition produced by galangin. In particular, we can exclude an inhibition of the release of acetylcholine and noradrenaline (norepinephrine) from neural or non-neural sources, as atropine, propranolol and phentolamine did not modify the inhibitory effect of galangin. We can also exclude an inhibition of the release of tachykinins, as tachykinin NK_1 and NK_2 receptor antagonists did not modify the effect of galangin. Previous investigators have shown that both tachykinin NK_1 and NK_2 receptors are involved in the contractile response of the rat isolated urinary bladder to tachykinins (Hall et al 1992). The effect of tackykinin NK_3 antagonists was not studied as it is well known that the rat urinary bladder is devoid of tachykinin NK_3 receptors (Torrens et al 1995).

Conclusion

In this study, we have demonstrated that galangin exerts an inhibitory effect on rat urinary bladder contractility by acting on L-type calcium channels located on presynaptic nerve terminals. If these data are confirmed in-vivo, there might be a possible role for galangin in treating urinary incontinence.

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