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Distinct mechanisms of relaxation to bioactive components from chamomile species in porcine isolated blood vessels

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ABSTRACT

German chamomile (*Matricaria recutita* L.), a widely-used herbal medicine, has been reported to have a wide range of biological effects, including smooth muscle relaxation. The aim of this study was to compare the effects of representative compounds from chamomile (apigenin, luteolin, (−)-α-bisabolol, farnesene, umbelliferone; 3–30 μM) on vascular tone using porcine coronary and splenic arteries mounted for isometric tension recording in isolated tissue baths and precontracted with the thromboxane-mimetic U46619.

Apigenin, luteolin, and (−)-α-bisabolol produced slow, concentration-dependent relaxations in both the coronary and splenic arteries that were not blocked by inhibition of nitric oxide synthase or potassium channels. Removal of extracellular calcium inhibited the relaxations to all three compounds, and these compounds also inhibited calcium re-addition-evoked contractions, indicating that the relaxation response may be mediated through inhibition of calcium influx. Apigenin and luteolin, but not (−)-α-bisabolol, enhanced the relaxation to the nitric oxide donor sodium nitroprusside, indicating that apigenin and luteolin may act to regulate cyclic GMP levels. Umbelliferone produced a rapid, transient relaxation in the splenic artery, but not the coronary artery, that was inhibited by L-NAME and removal of the endothelium, suggesting an influence on nitric oxide production. Farnesene, at concentrations up to 30 μM, was without effect in either blood vessel. In conclusion, hydroxylated compounds (apigenin, luteolin and (−)-α-bisabolol) found in chamomile all caused a slow relaxation of isolated blood vessels through an effect on calcium influx. Umbelliferone, on the other hand, produced a rapid, transient relaxation dependent upon release of nitric oxide from the endothelium.

Introduction

German chamomile (*Matricaria recutita* L.) is a widely-used herbal medicine in the form of an essential oil, and a nutrient in the form of chamomile tea. A wide range of biological effects of *M. recutita* extracts have been reported, including smooth muscle relaxation (McKay and Blumberg, 2006), which may be due to inhibition of phosphodiesterase activity (Maschi et al., 2008). A number of potentially bioactive components have been identified in both chamomile tea and the essential oil. Methanolic extracts of *M. recutita* flowers and tea contain high amounts of the flavonoids apigenin and luteolin (Fig. 1), as well as the coumarin umbelliferone (7-hydroxycoumarin) (Novakova et al., 2010). (−)-α-Bisabolol (Fig. 1) is a sesquiterpene alcohol isolated from chamomile flowers and is found in the essential oil (Orav et al., 2010). It has been reported to have a range of biological activities such as anti-microbial, anti-oxidant and anti-inflammatory effects (McKay and Blumberg, 2006), although no vascular effects have been reported to date. Farnesene is also found in the essential oil and, like (−)-α-bisabolol, is a sesquiterpene, although, unlike (−)-α-bisabolol, it is a simple hydrocarbon and forms a linear molecular structure (Fig. 1).

Chamomile extracts have been reported to inhibit phosphodiesterase activity, and, therefore, would be expected to reduce vascular smooth muscle tone. Some of the compounds present in chamomile have been reported to have effects on vascular tone. For example, apigenin and luteolin have both been reported to induce vasodilatation (Xu et al., 2007). However, the effects on vascular smooth muscle tone of other potentially bioactive compounds in chamomile, such as umbelliferone, (−)-α-bisabolol, and farnesene are unknown.

The aim of this study was, therefore, to compare the *in vitro* vascular effects of potentially bioactive compounds with diverse structures found within chamomile, namely apigenin, luteolin, (−)-α-bisabolol, farnesene, and umbelliferone. Comparisons were made between responses in porcine coronary and splenic artery in order to determine whether effects were generalised phenomena across different arteries.

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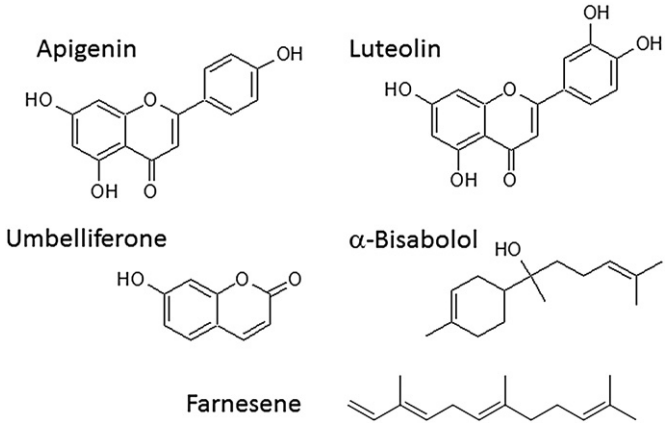

Fig. 1. Chemical structures of the compounds investigated in this study.

Methods

Isolated tissue bath experiments. Porcine hearts and spleens were obtained from a local abattoir, transported to the laboratory in ice-cold Krebs–Henseleit buffer (composition, in mM: NaCl, 128; KCl, 4.8; $MgSO_4$, 1.1; $NaHCO_3$, 25; KH_2PO_4, 1.2; D-glucose, 12; $CaCl_2$, 1.25) pre-gassed with 95% O_2, 5% CO_2. Coronary arteries and splenic arteries were isolated from the appropriate tissue, placed in Krebs–Henseleit buffer, and stored overnight at 4 °C. This method allows the retention of endothelial integrity (Lot and Wilson, 1994). The following day arteries were cleaned of fat and connective tissue, cut into 5 mm ring segments, and suspended in an isolated tissue bath for isometric tension recording as described previously (Roberts et al., 1999).

Effect of chamomile-derived compounds on vascular tone. Tissues were pre-contracted with the thromboxane agonist U46619 (concentration range 4–40 nM) to around 60–70% of the maximum response to 60 mM KCl. Once a stable tension had developed, tissues were exposed to a single concentration of one of the compounds (3—30 μM) or vehicle (0.1% v/v DMSO). Changes in the tone of the arteries were then recorded for 90 min. Where appropriate, inhibitors of nitric oxide synthase (300 μM L-NAME) or potassium channels (10 mM tetraethylammonium, TEA) were incubated with tissues for 30 min prior to pre-contraction with U46619.

In some tissues (see Fig. 8), the endothelium was removed by gently rubbing the inside of the lumen with a fine pair of forceps. Successful removal of the endothelium was confirmed by a lack of relaxation to 100 nM substance P.

Effect of removal of extracellular calcium on vasodilatation. In order to determine whether vasodilatation evoked by the compounds involved extracellular calcium, tissues were incubated in Krebs–Henseleit buffer containing 2 mM EGTA in place of calcium chloride for 5 min prior to pre-contraction with U46619. As removal of extracellular calcium inhibits the U46619-induced contraction, the concentration of U46619 applied to the calcium-free tissues was increased accordingly (140–400 nM compared to 2–45 nM in control tissues), in order to achieve usable levels of tone (around 20–35% of the KCl response in the coronary artery and around 15–25% of the KCl response in the splenic artery). In this paired experiment, tissues examined in calcium-containing Krebs–Henseleit buffer were exposed to lower concentrations of U46619 in order to match the overall level of tone in the calcium-free tissues. After the contraction to U46619 had reached a plateau, single concentrations of chamomile-derived compounds were added as indicated, recording tone for the next 90 min.

Effect of chamomile-derived compounds on contractile responses to calcium. Arterial segments were exposed to 60 mM KCl in Krebs–Henseleit from which calcium was omitted. Increasing concentrations of calcium (1 μM to 3 mM) were then reintroduced to stimulate a contraction in the absence or presence of the indicated concentrations of chamomile-derived compounds.

Effect of chamomile-derived compounds on cyclic nucleotide-mediated relaxations. In order to determine whether the compounds could be acting as phosphodiesterase inhibitors, tissues were exposed to chamomile-derived compounds at the indicated concentrations for 90 min and then contracted with U46619 to 60–70% of the KCl response. U46619 concentrations were adjusted accordingly to take into account the inhibition of the U46619-induced contraction by the compounds. After the contraction to U46619 had reached a plateau, concentration-response curves to the nitric oxide donor sodium nitroprusside (SNP) or the adenylyl cyclase activator forskolin (1 nM to 3 μM) were carried out.

Statistical analysis. Relaxation responses were expressed as a percentage relaxation from the U46619-induced pre-contraction and are presented as means ± S.E.M. Numbers of experiments referred to in the figure legends indicate the number of tissues from separate animals. The effects of chamomile-derived compounds on time courses or concentration-dependent relaxations were analysed by two-way ANOVA followed by Bonferroni post-hoc tests. Effects of interventions on chamomile-derived compound-evoked relaxations were assessed in adjacent segments from the same artery using repeated measures ANOVA. Concentration-relaxation curves were fitted to the four parameter logistic equation (Prism, GraphPad, USA). Parameters from curve fitting were analysed for statistical differences using Student's unpaired *t*-test.

Materials. (−)-α-Bisabolol, tetraethylammonium (TEA), apigenin, luteolin, farnesene (mixed isomers), umbelliferone, sodium nitroprusside (SNP), forskolin were all from Sigma (Poole, Dorset, UK). (5Z, 9α, 11α, 13E, 15 (S))-15-hydroxy-9 (11) methanoepoxyprosta-5,13-dien-1 oic acid (U46619) was obtained from Axxora, Bingham, Notts, UK.

Results

Effect of chamomile-derived compounds on vascular tone

Luteolin, apigenin, and (−)-α-bisabolol produced time and concentration-dependent relaxations in both the porcine coronary artery and the splenic artery (Figs. 2 & 3). Apigenin (Figs. 2A and 3A) and luteolin (Figs. 2C and 3C) produced complete relaxations in both the coronary and splenic arteries at 10 and 30 μM, whereas 3 μM had no significant effect in coronary arteries. Although (−)-α-bisabolol at 3 and 10 μM was without effect, 30 μM (−)-α-bisabolol produced a slow relaxation in both tissues, which was significant from 45 min onward, with a slightly greater effect in the splenic artery compared to coronary artery (Figs. 2B & 3B).

Umbelliferone was without significant effect on the U46619-induced tone in the coronary artery at concentrations up to 30 μM (Fig. 2D). In the splenic artery, however, it produced a small, transient relaxation at 30 μM (Fig. 3D). Fig. 4 shows a typical trace of the umbelliferone-evoked time course of relaxation in the porcine splenic artery.

In comparison, farnesene at concentrations up to 30 μM had no effect on the U46619-induced tone in either the coronary or splenic artery (data not shown).