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Feverfew Extracts and Parthenolide Irreversibly Inhibit Vascular Responses of the Rabbit Aorta

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Abstract—Samples prepared from chloroform extracts of fresh leaves of feverfew (*Tanacetum parthenium*) strongly inhibited responses of rabbit aortic rings to phenylephrine, 5-hydroxytryptamine, thromboxane mimetic U46619 (9,11-dideoxy-11α,9α-epoxy-methano-$PGF_{2\alpha}$), and angiotensin II, but the inhibition to contractions induced by potassium depolarization was much less. The inhibition was concentration- and time-dependent, non-competitive, and irreversible, and also occurred in endothelium-denuded preparations. The feverfew extracts also caused a progressive loss of tone of pre-contracted aortic rings and appeared to impair the ability of acetylcholine to induce endothelium-dependent relaxations of the tissue. These effects were mimicked by a purified preparation of an α-methylenebutyrolactone, parthenolide, obtained from the extract. Our results demonstrate a nonspecific and potentially toxic response to feverfew on the vasculature.

Extracts or leaves from the feverfew plant (*Tanacetum parthenium*) have long been used as a folk remedy for the treatment of various diseases (Berry 1984), including migraine (Johnson 1984), but the mechanism of action is not known. Two recent clinical studies in the United Kingdom have supported claims for prophylactic benefit in patients with migraine (Johnson et al 1985; Murphy et al 1988). Despite intense interest in the role of changes in vascular reactivity in the pathogenesis of migraine (Fozard & Gray 1989; Glover & Sandler 1989; Saxena & Ferrari 1989; Sandler & Collins 1990), we are not aware of any studies of feverfew extracts on blood vessel function in-vitro. We have therefore studied the effects of crude chloroform extracts of fresh feverfew on contractile and relaxant responses of isolated rings prepared from rabbit aorta.

Materials and Methods

Rings of 2 mm thickness were carefully cut from the thoracic segment of the aorta taken from freshly killed male New Zealand White rabbits, 2–3 kg, killed by anaesthetic overdose. Rings were attached via Harvard heart clips to cotton threads and mounted in 3 mL tissue baths, and tension was monitored using Harvard FT03 isometric transducers coupled to Lectromed or Grass pen recorders. After applying 2 g tension, preparations were allowed to equilibrate at 37°C for 90 min in gassed (95% O_2–5% CO_2) Krebs solution (composition mM): NaCl 117, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·2, $NaHCO_3$ 24·8, KH_2PO_4 1·2, glucose 11·1), and their viability established by eliciting contractions with 10^{-6} M phenylephrine. Preparations were also made from aortic segments stored in oxygenated Krebs solution for 16–24 h at 4°C; these behaved in exactly the same manner as those taken immediately post-mortem.

Crude feverfew extracts were prepared by stirring freshly gathered leaves in 20 vol chloroform, using plants grown at Castle Donington, Leicestershire (Begley et al 1989). The solvent was removed and samples of the resulting viscous dark green oil were resuspended in methanol for addition to the baths. Addition of an equivalent amount of methanol (up to 0·25% v/v) did not affect any of the parameters measured. Parthenolide was isolated from an aqueous extract of fresh leaves by back-extraction with chloroform. The dried chloroform extracts were evaporated and the residue subjected to chromatography on a silica gel column and eluted with chloroform. TLC and NMR analysis showed that the sample was pure. The chemical composition of the crude extracts was determined by HPLC analysis using a 10 μ Partisil column and ethyl acetate/hexane 1:1 as solvent. The crude extracts appeared to be chemically and pharmacologically stable for at least 6 months if stored at −20°C. Supplies of all the agonists used were from Sigma Chemical Company; other chemicals were from Aldrich or BDH.

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Results

Cumulative addition of phenylephrine (3×10^{-8}–3×10^{-4} M), 5-hydroxytryptamine (5-HT, 3×10^{-7}–10^{-4} M), angiotensin II (10^{-11}–10^{-8} M) or thromboxane mimetic U46619 (9,11-dideoxy-9α,11α-methano-epoxy-$PGF_{2\alpha}$) (10^{-8}–10^{-6} M) produced characteristic concentration-dependent increases in tension of the aortic rings. Addition of feverfew extract for 10 min or longer led to a progressive time-dependent and irreversible reduction in the responses to the spasmogenic agonists, in both endothelium-intact and endothelium-denuded aortic ring preparations.

Fig. 1 shows typical results in which cumulative concentration-response curves for phenylephrine were obtained before, 17 min after adding 200 μg mL^{-1} feverfew extract, and 27 min after prolonged washing of the tissue to remove added drugs. Responses to phenylephrine were completely abolished by the feverfew treatment, even after its attempted removal. In contrast, addition of 50 mM potassium chloride elicited a contraction in the feverfew-treated tissue, implying that functional integrity of the contractile mechanism of the

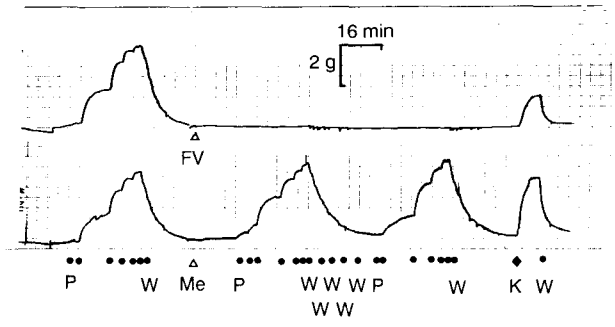

FIG. 1. Cumulative concentration-response curves to phenylephrine (P) were obtained by successive addition of doses at the times indicated by ● to give final concentrations of 1×10^{-7}–3×10^{-5} M. After completion of each set of responses, the tissues were extensively washed (at W) to remove drugs and to restore the baseline. At △, 6 μL of methanol (control, bottom trace) or 6 μL of a chloroform extract of feverfew resuspended in methanol (final bath concentration 200 μg mL^{-1}, top trace) were added and left in contact for 17 min before phenylephrine was added. After 45 min the feverfew was washed out (W) and the tissues left to re-equilibrate with repeated washings for 27 min before adding cumulative doses of phenylephrine for the third time. In the final part, 50 mM potassium chloride (K) was added to the control and test preparations at ◆. A representative example of four separate experiments is shown.

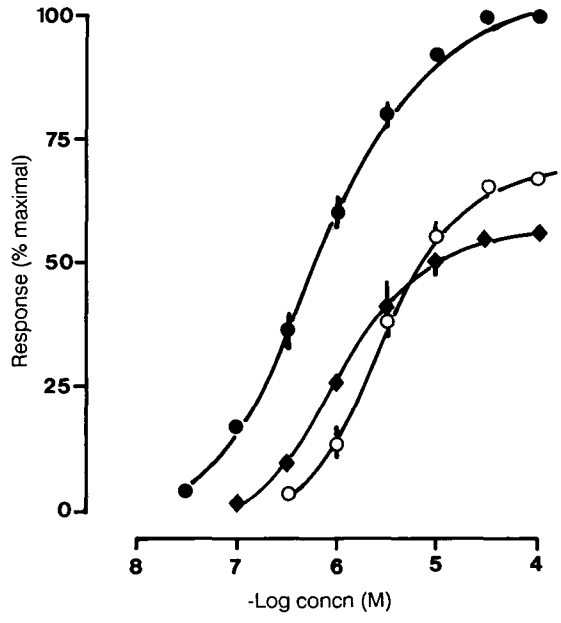

FIG. 2. Irreversible inhibition of cumulative responses to phenylephrine by 50 μg mL^{-1} feverfew extract. The feverfew was maintained in contact with the tissues for 10 min before beginning the second set of phenylephrine tests (protocol similar to that shown in Fig. 1). The third set of tests with phenylephrine was performed 20 min after extensively washing the tissues. Results show mean ± s.e.m. from 6–12 tests. Responses before adding feverfew, ●; responses in presence of feverfew, ◆; responses after washing out feverfew, ○.

muscle was retained. In ten further tests it was found that the response to 50 mM potassium chloride was reduced to 63·5 ± 10·1% of its previous magnitude after treatment with feverfew under conditions that yielded 80–100% inhibition to spasmogenic agonists. None of these inhibitory effects were observed in tissues treated with the methanol vehicle used to dissolve the feverfew extract (Fig. 1).

Fig. 2 summarizes the results of further experiments using phenylephrine. Cumulative doses of this agonist were tested before, during and after exposure of the aortic rings to a lower concentration (50 μg mL^{-1}) of feverfew extract. The data show that feverfew produced a reduction in the maximal response to phenylephrine and that the inhibitory effect persisted after washing. Similar results (depression of the maximal response, suggestive of noncompetitive antagonism, and irreversible blockade) were obtained using other agonists such as 5-HT and U46619 (results not shown).

In further experiments, responses to near-maximal concentrations of phenylephrine, 5-HT, angiotensin II and U46619 were inhibited 36, 76, 100 and 47%, respectively, after 10 min incubation with 200 μg mL^{-1} feverfew extract, and 100% after 30 min incubation, emphasizing the time-dependent nature of the inhibition. To check the dose-dependence of the effect, feverfew was added at 50, 75, 100 and 200 μg mL^{-1} to aortic rings for 30 min and the responses to 10^{-6} M phenylephrine determined; responses were inhibited by 4, 8, 37 and 93% of control (mean values from 2 tests).

Addition of feverfew to aortic rings precontracted with any of the four spasmogens caused a slow progressive loss of tone. This was not observed after addition of the methanol vehicle. This response occurred in both endothelium-intact and endothelium-denuded preparations, and is shown in Fig. 3 for phenylephrine-precontracted preparations. In 6 experiments, 48·5 ± 3·3% of the tone induced by 10^{-6} M phenylephrine was lost 15 min after adding feverfew, compared with 6·7 ± 3·2% lost after adding methanol (corresponding to losses in tension of 126 ± 9 mg min^{-1} compared with 18 ± 9, mg min^{-1} respectively, $P < 0.01$ by Student's unpaired t-test). Following the attempted washout of the feverfew, phenylephrine failed to cause an increase in tension, as expected (Fig. 3), although the methanol-treated controls were fully responsive.

Addition of acetylcholine to endothelium-intact preparations induced rapid relaxations due to the generation of EDRF (nitric oxide), but did not cause this effect in endothelium-denuded rings, as shown in Fig. 3. In preparations that had been contracted with phenylephrine and then treated for several minutes with feverfew (which induced slow loss of tone, as described in the previous paragraph), prompt EDRF-like relaxations were no longer observed in response to acetylcholine, implying that feverfew impairs this mechanism of vascular relaxation. This action of feverfew required several minutes incubation with the aortic ring, by which time it had begun to relax (as in Fig. 3); thus it was not possible to quantify this effect of feverfew accurately because the baseline from which the changes caused by acetylcholine were to be measured had altered appreciably. Furthermore, a feverfew-treated tissue could not be contracted again in order to reassess acetylcholine-induced relaxations. However, we observed that sodium nitroprusside (2·5 μM) induced prompt relaxation in both endothelium-intact and endothelium-denuded preparations, even in the presence of feverfew, implying that feverfew does not abolish the inherent ability of the smooth muscle to relax.

Purified parthenolide, the major sesquiterpene α-methylene butyrolacetone isolated from feverfew (Begley et al 1989), was also tested. Fig. 4 shows that parthenolide

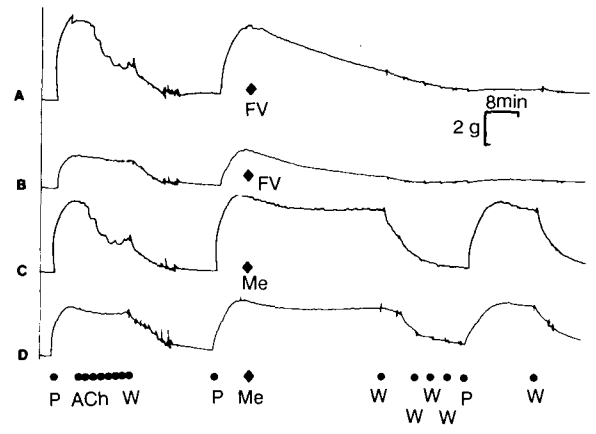

FIG. 3. Effects of acetylcholine and feverfew on phenylephrine-contracted rabbit aortic rings: comparison between endothelium-intact and endothelium-denuded preparations. Two of the four preparations (B and D) were removed from the baths and the endothelium gently rubbed with moist tissue, and then remounted. Increases in developed tension were obtained by adding phenylephrine at P (10^{-6} M). At peak tension, cumulative doses of acetylcholine (ACh) were added to all baths at the times indicated by the dots (range 10^{-8}–10^{-5} M) to elicit relaxations in the endothelium-intact rings. The endothelium-denuded preparations did not respond in this fashion. Addition of feverfew extract (100 μg mL^{-1}) to the upper two preparations (A and B) at the point marked (FV) caused a gradual loss of tension in both endothelium-intact and endothelium-denuded preparations. Addition of methanol (Me) to preparations C and D had no such effect. After repeated washing, phenylephrine was again added to all preparations, but only elicited responses from the methanol-treated controls. Washouts indicated at dot W. Representative of 6 similar experiments.

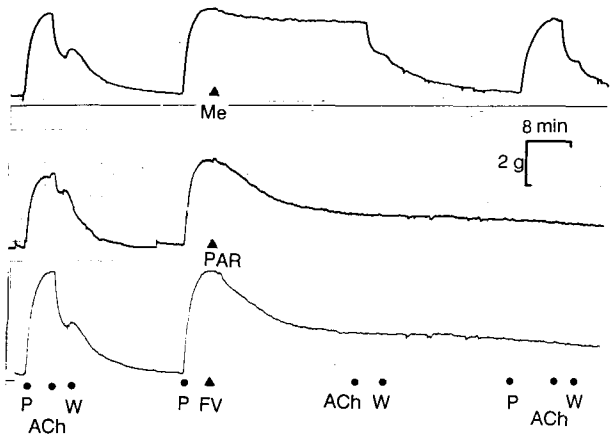

FIG. 4. Inhibitory effects of feverfew are mimicked closely by parthenolide. The actions of purified parthenolide (250 μg mL^{-1} final concentration), feverfew extract (500 μg mL^{-1}) and methanol control were compared on endothelium-intact preparations. Additions of phenylephrine (10^{-6} M) were made at P; acetylcholine (10^{-4} M) at ACh and methanol (top preparation), parthenolide (middle preparation) or feverfew (bottom preparation) at Me, PAR and FV, respectively. W indicates bath washout. The feverfew and parthenolide both caused loss of sustained tension, loss of response to acetylcholine, and loss of response to phenylephrine. One of three similar experiments.

treatment closely mimicked the effects of the crude feverfew extract: it induced a slow loss of tone in the phenylephrine-precontracted endothelium-intact preparation, and prevented any subsequent response to phenylephrine. In contrast, addition of the methanol vehicle did not affect maintained tone of the aortic ring, or its subsequent response to a further dose of phenylephrine. Fig. 4 also shows that acetylcholine does not induce relaxation of the phenylephrine-contracted aortic ring after treatment with either feverfew extract or purified parthenolide, as described above. Finally, we treated feverfew extracts with excess cysteine in order to inactivate the α-methylene butyrolactone function in any parthenolide they might contain (absence of α-methylenelactones was verified by HPLC assay). In four experiments, addition of cysteine-treated feverfew extracts at 300 μg mL^{-1} failed to cause a loss of tone in precontracted aortic rings, and incubation at this concentration for 15 min or more failed to inhibit subsequent responses to phenylephrine, suggesting that the inhibitory principle had indeed been removed.

Discussion

These experiments show that resuspended chloroform extracts of fresh leaves of the feverfew plant (*Tanacetum parthenium*) have three inhibitory effects on the physiological function of isolated rings of rabbit aorta, causing a non-specific inhibition of the increases in tension elicited by all spasmogenic agonists so far tested, and loss of tone of precontracted rings. The feverfew extracts also appear to interfere with acetylcholine-induced endothelium-dependent relaxation, although this could not be examined in detail.

The inhibition by feverfew of the responses to the spasmogens has several characteristic features which distinguish it from the inhibitory effects of therapeutically useful specific competitive antagonists such as phentolamine and ketanserin. In this preparation these two compounds caused surmountable and specific antagonism in which the same maximal responses were obtained at higher doses of phenylephrine and 5-HT, respectively (data not shown). Thus feverfew causes inhibition which is nonspecific, time-dependent, irreversible, and noncompetitive (i.e. maximal responses to the spasmogens cannot be re-established by increasing their dose). The inhibitory effect is exerted on the smooth muscle, as it does not require the presence of a functional endothelium. The underlying mechanism needs further analysis at the cellular and molecular level, but the implication is that feverfew contains a substance which interacts irreversibly, possibly covalently, with a component of the smooth muscle that is essential for linking receptor stimulation to contractile function. A similar argument can be used to explain the loss of tone caused by feverfew in precontracted preparations.

That the active constituent responsible for these effects is parthenolide, is supported by the very close similarity between its effects and those of the crude extracts, and the finding that the extracts contained approximately 7·5% by weight of parthenolide, as measured by HPLC. It is possible that the activated α-methylene butyrolactone function present in the parthenolide molecule enables it to react covalently with essential sulphydryl or amino groups in tissue proteins by a Michael-type addition reaction (Kupchan et al 1970), as suggested for inhibition of platelet function (Groenewegen et al 1986; Heptinstall et al 1987). We have found that addition of cysteine or glutathione to aqueous solutions of partheno-

lide at 37°C, pH 7·4, causes complete disappearance of signals characteristic of butyrolactone groups, as determined by NMR (unpublished experiments), and that feverfew extracts treated in this manner lose their inhibitory activity on the smooth muscle.

The present results show that feverfew extracts and the parthenolide derived from them, interfere markedly with both contractile and relaxant mechanisms in blood vessels. The finding that the effects are irreversible implies a potentially toxic consequence of feverfew on vascular function, if they translate to the clinical use of the drug and to all blood vessels (to date we have observed similar effects on isolated aortic rings of rat and guinea-pig, but have not investigated intact perfused vascular preparations). It is not known whether these inhibitory properties of feverfew are relevant to its claimed anti-migraine action or side effects such as tendency to mouth ulceration observed in patients who chew fresh leaves (Berry 1984; Johnson 1984).

Acknowledgement

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