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Mechanisms underlying the relaxation caused by the sesquiterpene polygodial in vessels from rabbit and guinea-pig

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Abstract

The sesquiterpene polygodial produces graded relaxation in rings of rabbit pulmonary artery or thoracic aorta and guinea-pig pulmonary artery with endothelium. In rings with rubbed endothelium its vasorelaxant action was largely reduced. The N^{ω}-nitro-L-arginine (L-NOARG), N^{G}-nitro-L-arginine methyl ester (L-NAME), 6-anilino-5,8-quinolinedione (LY 83583) and 1*H*-[1,2,4]Oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), inhibited the endothelium-dependent vasorelaxant action of polygodial. In contrast, N^{ω}-nitro-D-arginine (D-NOARG), indomethacin, N^{2}-[(4*R*)-4-hydroxy-1-(1methyl-1*H*-indol-3yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide (FK 888), (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide (SR 48968), (8*R*,9*S*,11*S*)-(−)-9-hydroxy-9-*n*-hexyloxy-carbonyl-8-methyl-2,3,9,20-tetrahydro-8,11-epoxy-1*H*,8*H*,11*H*-2,7*b*,11*a*-triaqzadibenzo[*a*,*g*]cycloocta[*c*,*d*,*e*]-trinden-1-one (KT 5720), calcitocin gene-related peptide receptor antagonist (CGRP-(8-37), apamin, charybdotoxin and 4-aminopyridine had no effect on polygodial action. However, glibenclamide inhibited partially, but significantly, its relaxant responses. These results demonstrate that the vasorelaxation of polygodial is partly dependent on the release of nitric oxide (NO)or an NO-derived substance from the vascular endothelium through an activation of a guanylyl cyclase-dependent mechanism. Finally, results demonstrate that the polygodial vasorelaxant action is not related with the opening of potassium (K^{+}) channels, release of prostacyclin, substance P, or with the activation of adenylyl cyclase-dependent mechanisms.

Keywords: Polygodial; Relaxation; Aorta, rabbit; Pulmonary artery, rabbit; Pulmonary artery, guinea-pig; Nitric oxide (NO)

1. Introduction

Polygodial is the main sesquiterpene isolated from methanolic extract of the plant *Drymis winteri* (Winteraceae) (El Sayah et al., 1998; Mendes et al., 1998), a native Brazilian medicinal plant used in folk medicine for the treatment of different inflammatory diseases, such as asthma, allergy and bronchitis. This plant is also used as an antispamodic and antipyretic (Morton, 1981), and is sometimes used for the treatment of cancer (Riccieri, 1981).

We have previously reported that the aqueous/ethanolic extract of *D. winteri*, and also its main constituent the sesquiterpene polygodial, produces dose-related and long lasting antinociceptive, antioedematogenic and anti-allergic properties (Tratsk et al., 1997; Mendes et al., 1998). In addition, the extract and the polygodial also interfere with the contractile response induced by several chemical mediators when assessed in the guinea-pig trachea (El Sayah et al., 1997, 1998). The current study was designed to address some of the mechanisms by which the sesquiterpene polygodial induces vasorelaxant action in the thoracic aorta from rabbit and pulmonary arteries from guinea-pig or rabbit in vitro. We found that polygodial induces vasorelaxant action mediated by release of nitric oxide (NO) or by an NO-related substance from vascular endothelium through the activation of a guanylyl cyclase-dependent mechanism.

2. Material and methods

2.1. Tissue preparation

White rabbits (2–3 kg) and guinea-pigs (400–450 g) of both sexes were used. The animals were anaesthetized with

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sodium pentobarbital (rabbits) or with ether (guinea-pigs) were killed by a blow on the head, and were exsanguinated. Thoracic aorta from rabbits and pulmonary arteries from guinea-pigs and rabbits were carefully excised and cleaned from connective tissue and adherent fat, as described previously (Campos and Calixto, 1994; Calixto and Cabrini, 1997). Briefly, the arteries were cut into rings approximately 2–3 mm wide and were suspended in a 5-ml organ bath chamber containing Krebs Henseleit solution at 37°C, pH 7.2, gassed with 95% O_2 and 5% CO_2. The Krebs solution had the following composition (mM): NaCl 113; KCl 4.7; $CaCl_2$ 2.5; $NaHCO_3$ 25; $MgSO_4$ 1.2; KH_2PO_4 0.9 and glucose 11. Care was taken not to damage the endothelium. All procedures were approved by our institutional ethics and are in accordance to NIH Animal Care Guidelines.

In some experiments, the vascular endothelium was denuded intentionally by gently rubbing the internal surface of the artery with a wooden stick. Endothelium integrity was functionally assessed by evaluating the ability of acetylcholine (1 μM) to produce relaxation of preparations pre-contracted with phenylephrine (1 μM). Preparations were considered to contain a viable endothelium when acetylcholine (1 μM) evoked relaxations exceeding 60%, and were considered to be endothelium-denuded when acetylcholine failed to cause relaxation (Furchgott and Zawadski, 1980). Preparations were submitted to a basal tension of 1 g for pulmonary arteries of guinea-pig or rabbit and 2 g for thoracic aorta of rabbit, and were allowed to equilibrate for at least 60 min before drug addition, during which the bath solution was renewed every 20 min. Isometric tension changes were recorded by means of an F-60 force-transducer (Narco Biosystems, USA, or Letica 6006, Spain). The submaximal contractile responses induced by phenylephrine were taken as the 100% values and all subsequent responses were calculated as a percentage of this value.

2.2. Experimental procedure

Following the equilibration period, the preparations were contracted by addition of phenylephrine (1 μM), and once the tonic responses became stable (usually after 5 min), the relaxant cumulative concentration–response curves for polygodial (0.0427–42.74 μM) were obtained in intact or in rubbed-endothelium preparations. To explore further the mechanisms by which polygodial produces relaxation in intact-endothelium preparations, cumulative concentration–response curves for polygodial were obtained in the absence or in the presence of one of the following drugs: soluble guanylyl cyclase inhibitors 1*H*-[1,2,4]Oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ, 1 μM) and NO biological activity inhibitor 6-anilino-5,8-quinolinedione (LY 83583 10 μM), N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and N^ω-nitro-L-arginine (L-NOARG, 100 μM) NO synthase inhibitors, N^ω-nitro-D-arginine (D-NOARG, 100 μM) inactive enantiomer of L-NOARG, tetraethylammonium (1 and 5 mM) an unspecific K^+ channel blocker, glibenclamide (3 and 30 μM,) ATP-sensitive K^+ channel blocker, apamin (1 μM) blocker of Ca^{2+}-activated K^+ channels, 4-aminopyridine (1 and 3 mM) voltage-dependent K^+ channel blocker and charybdotoxin (100 nM) blocker of Ca^{2+}-activated K^+ channels, indomethacin (1 and 10 μM) cyclooxygenase inhibitor, protein kinase A inhibitor (8*R*,9*S*,11*S*)-(−)-9-hydroxy-9-*n*-hexyloxy-carbonyl-8-methyl-2,3,9,20-tetrahydro-8,11-epoxy-1*H*,8*H*,11*H*-2,7*b*,11*a*-triaqzadibenzo[*a*,*g*]cycloocta[*c*,*d*,*e*]-trinden-1-one (KT5720, 1 μM), CGRP-(8-37) (100 nM) calcitocin gene-related peptide receptor antagonist, N^2-[(4*R*)-4-hydroxy-1-(1methyl-1*H*-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide (FK 888, 100 nM) or (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide (SR 48968, 100 nM) tachykinin NK_1 and NK_2 receptors antagonist, respectively. In another set of experiments, we sought to determine whether polygodial could interfere with the endothelium-dependent and independent relaxation produced by acetylcholine (0.001–30 μM) or sodium nitroprusside (0.001–30 μM). With this purpose, a cumulative concentration–response curve for acetylcholine or sodium nitroprusside was obtained 60 min before obtaining the cumulative concentration–response curve for polygodial in rabbit pulmonary artery with intact endothelium. Thereafter, preparations were washed out and after 60 min they were pre-contracted with phenylephrine (1 μM) and again exposed to cumulative concentration up to acetylcholine or sodium nitroprusside . All drugs were left in contact with the tissue for 20 min before contraction with phenylephrine (1 μM). Once the sustained tension was established, polygodial was added cumulatively to the bath. To avoid desensitisation of the preparation, only one complete cumulative concentration–response curve for polygodial was obtained in each preparation. Separate control and test tissues were studied simultaneously in adjacent baths. Only one concentration of each antagonist was tested per preparation.

2.3. Drugs

The drugs used were: acetylcholine iodide, L-phenylephrine hydrochloride, sodium nitroprusside , L-NOARG, L-NAME, D-NOARG, indomethacin, tetraethylammonium, apamin, 4-aminopyridine, charybdotoxin, CGRP-(8-37) and phosphate buffered solution (PBS), all from Sigma (St. Louis, MO, USA). Glibenclamide from Hoescht (Frankfurt, Germany), ODQ from Tocris Cookson (Bailwin, MO, USA), LY 83583 from Research Biochemicals International (Natick, MA, USA), FK 888 from Fujisawa Phamaceutical (Osaka, Japan), SR 48968 from Sanofi Recherche (Montpellier, France), KT 5720 from Calbiochem–Novabiochem (La Jolla, CA). All drugs were

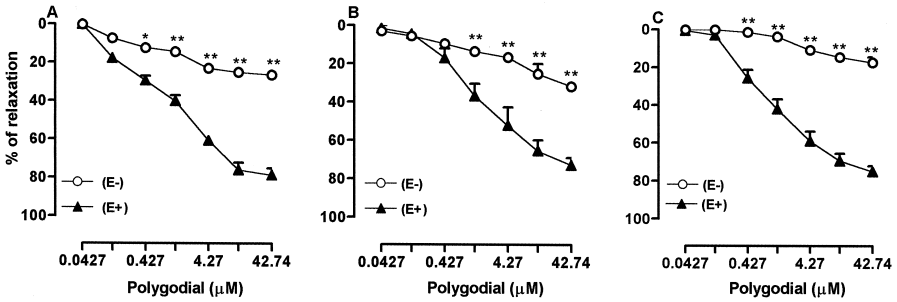

Fig. 1. Mean vasorelaxant concentration–response curves for the polygodial (0.0427–42.74 μM) in rings of pulmonary arteries from guinea-pig (A) or rabbit (B) and thoracic aorta from rabbit (C) with (E +) and without (E −) endothelium, pre-contracted with phenilephrine (1 μM). Each point represents the mean of six experiments and the vertical lines indicate the S.E.M. Differ significantly from control value $^{*}P < 0.05$, $^{**}P < 0.01$.

stored as 1–100 mM stock solutions for up to a week at −20°C and were diluted to the desired concentrations in distilled and deionized water just before use. Stock solutions of all drugs were dissolved in PBS, except glibenclamide which was dissolved in a solution of NaOH plus glucose (5%), KT 5720 in dimethyl sulfoxide (DMSO) and polygodial and indomethacin which were dissolved in absolute ethanol. The final concentration of ethanol and DMSO did not exceed 0.05% and had no effect per se on the basal tonus of the preparations or on phenylephrine or polygodial-mediated responses.

2.4. Statistical analysis

Data are presented as mean ± S.E.M., except for the EC_{50} values (i.e., the concentration of agonists causing half maximal relaxant responses) which are reported as geometric means accompanied by their respective 95%

Table 1
Effect of different drugs on the vasorelaxation induced by polygodial in rings of rabbit and guinea-pig with endothelium
Each group represents the mean ± S.E.M. of four to six experiments.

Drugs	Concentration	Absence		Presence	
		EC_{50} (μM)[a]	E_{max} (%)	EC_{50} (μM)[a]	E_{max} (%)
Rabbit thoracic aorta					
Polygodial		0.9 (0.4–1.8)	75.0 ± 2.9	–	–
Tetraethylammonium	5 mM	0.4 (0.05–3.5)	69.6 ± 5.4	0.9 (0.2–4.3)	62.8 ± 4.4
Apamin	1 μM	1.4 (0.4–5.2)	67.2 ± 5.5	3.1 (0.7–12.0)	65.3 ± 4.9
Glibenclamide	30 μM	2.9 (2.6–3.4)	70.8 ± 3.2	3.7 (1.9–7.4)	64.3 ± 5.7
4-Aminopyridine	1 mM	1.3 (0.2–11.2)	67.6 ± 1.4	2.5 (0.09–6.6)	66.9 ± 1.8
Rabbit pulmonary artery					
Polygodial		0.9 (0.7–1.4)	72.8 ± 3.8	–	–
Tetraethylammonium	5 mM	–	67.0 ± 0.3	–	30 ± 5.0[b]
Apamin	1 μM	2.5 (1.0–6.4)	61.7 ± 0.8	2.7 (1.7–4.3)	59.2 ± 4.6
Charybdotoxin	100 nM	2.7 (1.0–7.0)	75.1 ± 3.7	1.9 (1.1–3.2)	84.1 ± 6.1
Glibenclamide	30 μM	1.47 (1.0–2.4)	75.4 ± 2.9	3.9 (1.7–8.7)	56.7 ± 2.0[b]
4-Aminopyridine	3 mM	1.2 (0.2–9.8)	70.0 ± 9.2	1.8 (0.6–5.3)	79.1 ± 3.1
Indomethacin	1 μM	1.8 (0.9–3.7)	73.5 ± 8.6	2.7 (1.6–4.3)	64.1 ± 6.4
Indomethacin	10 μM	1.6 (0.3–8.1)	76.3 ± 6.2	2.9 (0.1–14.3)	56.4 ± 4.3
FK 888	100 nM	3.2 (0.8–13.3)	73.0 ± 4.2	2.6 (0.4–18.5)	72.6 ± 5.5
SR 48968	100 nM	2.9 (0.3–2.7)	69.8 ± 1.4	3.7 (1.2–11.7)	74.8 ± 9.0
CGRP-(8-37)	100 nM	2.1 (0.6–7.5)	60.8 ± 4.2	1.1 (0.1–8.7)	57.5 ± 7.1
KT 5720	1 μM	0.9 (0.2–3.8)	71.1 ± 2.2	0.9 (0.3–3.3)	66.3 ± 7.9
Guinea-pig pulmonary artery					
Polygodial		1.1 (0.3–3.5)	78.6 ± 3.5	–	–
Tetraethylammonium	1 mM	2.7 (1.5–5.0)	89.3 ± 5.8	2.1 (1.8–2.4)	86.6 ± 1.3
Glibenclamide	3 μM	2.4 (1.0–5.9)	89.1 ± 5.8	3.2 (0.8–12.5)	74.0 ± 4.1
Apamin	1 μM	1.7 (1.0–2.9)	79.7 ± 5.2	1.9 (1.5–25.0)	72.9 ± 2.0

[a] Geometric means accompanied by 95% confidence limits.
[b] Differ significantly from control $P < 0.05$.

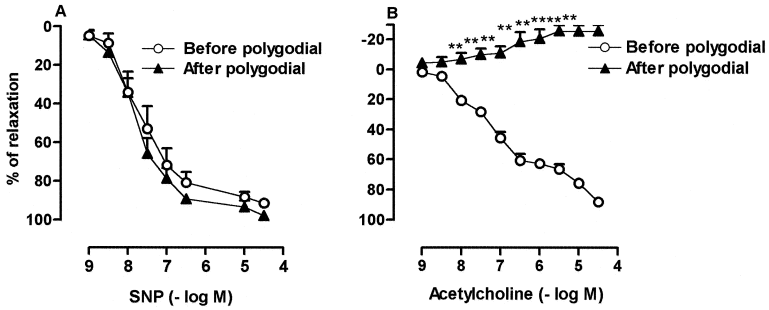

Fig. 2. Mean vasorelaxant concentration–response curves for sodium nitruprusside (0.001–30 μM) (A) and acetylcholine (0.001–30 μM) (B) carried out 1 h before and after a complete concentration–response curve for polygodial (0.0427–42.74 μM) in rings of rabbit pulmonary artery with intact endothelium, pre-contracted with phenilephrine (1 μM). Each point represents the mean of five experiments and the vertical lines indicate the S.E.M. Differ significantly from control value $^{**}P < 0.01$.

confidence limits. The EC_{50} values were determined from individual experiments for the complete agonist concentration–response curves using least-square regression analysis. Statistical significance of the data was assessed by Student's *t*-test for paired or unpaired samples. Differences between groups were considered to be significant at $P < 0.05$.

3. Results

3.1. Influence of endothelium on polygodial vasorelaxant action

The cumulative addition of the polygodial (0.0427–42.74 μM) in preparations pre-contracted with phenylephrine (1 μM) produced concentration-dependent vasorelaxant response in rings of rabbit or guinea-pig pulmonary arteries and rabbit thoracic aorta, with intact endothelium (Fig. 1). The potency and the maximal relaxation caused by polygodial did not significantly differ among the studied tissues. The mean EC_{50} values (and 95% confidence limits) and E_{max} for these responses are shown in Table 1. The vasorelaxant action of polygodial shows a marked tachyphilaxis independent of the vessels investigated or the time elapsed between curves (results not shown). When the vascular endothelium was intentionally removed, the maximal vasorelaxant response induced by polygodial in the three preparations was markedly reduced, $26 \pm 2\%$, $32 \pm 2\%$ and $17 \pm 3\%$ in pulmonary artery of guinea-pigs or rabbits and thoracic aorta of rabbit, respectively (Table 1). As can be seen in Fig. 2, the vasorelaxation caused by acetylcholine, but not that caused by sodium nitroprusside, was completely impaired following polygodial exposure.

3.2. Effect of NO synthase inhibitors and guanylyl cyclase inhibitors on polygodial-mediated vasorelaxant action

Results in Fig. 3 show that pre-incubation of rings of rabbit pulmonary artery with L-NAME (100 μM) or L-NOARG (100 μM) significantly inhibited the endothelium-dependent relaxation caused by polygodial in preparations pre-contracted by phenylephrine (E_{max} of $65 \pm 8\%$, $84 \pm 9\%$ for vehicle and $27 \pm 9\%$; $44 \pm 0.4\%$, in the

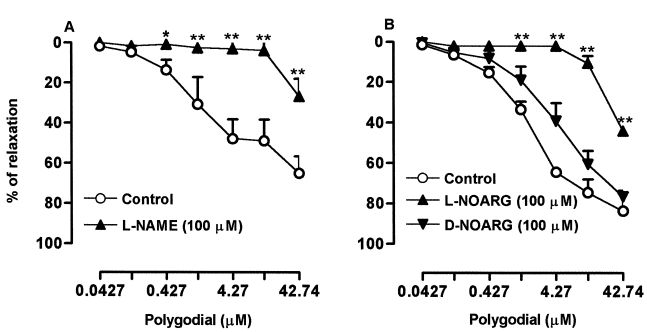

Fig. 3. Mean vasorelaxant concentration–response curves for the polygodial (0.0427–42.74 μM) in rings of rabbit pulmonary artery with intact endothelium and pre-contracted with phenilephrine (1 μM) obtained in the absence (○) or presence (▲) of nitric oxide synthase inhibitors L-NAME (100 μM) (A), L-NOARG (100 μM) and its inactive enantiomer D-NOARG (100 μM) (B). Each point represents the mean of five experiments and the vertical lines indicate the S.E.M. Differ significantly from control value $^{*}P < 0.05$, $^{**}P < 0.01$.

presence of L-NAME and L-NOARG, respectively). The inactive enantiomer D-NOARG (100 μM) (E_{max} of 84 ± 9% (vehicle) and 77 ± 3% for D-NOARG) had no significant effect on the vasorelaxant action of polygodial (Fig. 3). Very similar results were observed in vessels from guinea-pig pulmonary artery and rabbit thoracic aorta (results not shown). Both the NO biological activity inhibitor, LY 83583 (10 μM) and the highly-selective soluble guanylyl cyclase inhibitor ODQ (1 μM), markedly inhibited the endothelium-dependent relaxation caused by polygodial in rings of rabbit aorta pre contracted with phenylephrine (E_{max} of 72 ± 5%, 79 ± 1% for vehicle and 23 ± 1%; 14 ± 3% in the presence of LY 83583 and ODQ, respectively) (Fig. 4). Similar results were observed in rings of guinea-pig and rabbit pulmonary artery (results not shown).

3.3. Effect of K^+ channel blockers and other antagonists on polygodial-mediated vasorelaxant action

The results summarised in Table 1 show that the K^+ channel blockers tetraethylammonium (1 and 5 mM), 4-aminopyridine (1 and 3 mM), apamin (1 μM) or charybdotoxin (100 nM) all failed to significantly affect the vasorelaxant action caused by polygodial (0.0427–42.74 μM) in rings of rabbit thoracic aorta and pulmonary artery and guinea-pig pulmonary artery. In contrast, tetraethylammonium (5 mM) and glibenclamide (30 μM) prevented partially but significantly the vasorelaxant action induced by polygodial in rabbit pulmonary artery. In a similar manner, indomethacin (1 and 10 μM), FK 888 (100 nM), SR 48968 (100 nM), K7 5720 (1 μM) or CGRP-(8-37) (100 nM) had no significant effect on polygodial-mediated relaxation in rabbit pulmonary artery (Table 1).

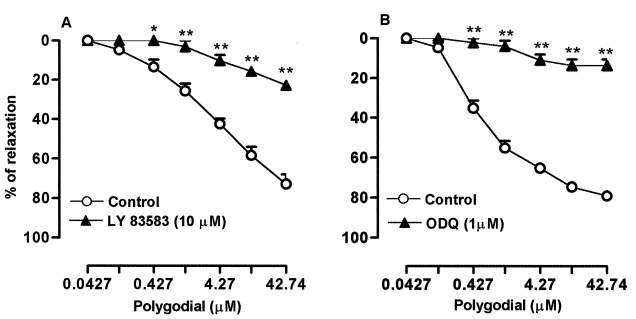

Fig. 4. Mean vasorelaxant concentration–response curves for the polygodial in rings of rabbit thoracic aorta with intact endothelium, pre-contracted with phenilephrine (1 μM), obtained in the absence (○) or presence (▲) of guanylyl cyclase inhibitors LY 83583 (10 μM) (A) or ODQ (1 μM) (B). Each point represents the mean of five experiments and the vertical lines indicate the S.E.M. Differ significantly from control value $^{*}P < 0.05$, $^{**}P < 0.01$.

4. Discussion

In the present study, we have reported for the first time that polygodial, the main sesquiterpene isolated from the bark of *D. winteri*, a medicinal plant used in the folk medicine in Brazil and in many other countries for the management of inflammatory diseases, such as asthma, allergy and bronchitis (Morton, 1981), produces concentration-dependent vasorelaxation in the rabbit and guinea-pig pulmonary arteries and in the rabbit thoracic aorta in vitro. The polygodial-induced vasorelaxant response seems to involve endothelium-dependent and independent mechanisms. These notions are supported by the fact that in our experiments, intentional removal of vascular endothelium from all studied vessels largely reduced (about 80%) the vasorelaxant response produced by polygodial. We have also found that the relaxation observed is due to the action of polygodial and not to the solvent.

Further evidence supporting these views derives from the fact that treatment of the preparations with NO synthase inhibitors such as L-NOARG or L-NAME greatly inhibited the polygodial vasorelaxation. In contrast, the inactive enantiomer D-NOARG failed to interfere with the polygodial vasorelaxation, further suggesting the involvement of NO or NO-related substance in its vasorelaxant response.

Our results also show that the release of prostacyclin from the vascular endothelium seems not to have any major role in the polygodial vasorelaxant response, because indomethacin failed to affect its response. Similarly, the activation of other pathways such as tachykinin NK_1 and NK_2 receptors and CGRP receptor also seems to have no major role in the vasorelaxant action of polygodial, as the selective antagonists of these receptors had no effect on it.

There is now consistent evidence that NO or NO-related substances produce vasorelaxant response in vascular and non-vascular smooth muscles through cGMP-dependent and independent mechanisms (Moncada et al., 1991; Robertson et al., 1993; Bolotina et al., 1994). Thus, we sought to determine in the present study the possible requirement of cGMP pathway in the vasorelaxant action of polygodial. Both the NO biological activity inhibitor , LY 83583 (Mulsch et al., 1988; Luo and Vincent, 1995) and the selective inhibitor of guanylyl cyclase enzyme, ODQ (Garthwaite et al., 1995), at concentrations where they are found active in blocking the soluble guanylyl cyclase enzyme (Brunner et al., 1996), almost completely inhibited the endothelium-dependent vasorelaxant action of polygodial in all vessels studied. Such findings further confirm the involvement of the NO-cGMP pathway in polygodial-mediated vasorelaxant responses.

Because the reported cross-talk between guanylyl cyclase and adenylyl cyclase (Lincoln and Cornwell, 1991) we also examined whether the relaxation response caused by polygodial could be related with activation of cAMP

mechanism. This appear to be not the case, because the selective protein kinase A inhibitor KT 5720, had not apparent effect on polygodial vasorelaxant response.

There is a great body of evidence supporting the view that NO produces relaxation responses in many tissues through direct or indirect activation of K^+ channels, leading to cell hyperpolarisation (Robertson et al., 1993; Bolotina et al., 1994; Koh et al., 1995). Previous studies have also reported that NO modulates the activity of multiple K^+ channel in physiological (Koh et al., 1995) and pathological (Hall et al., 1996) states. Thus, we next examined, by the use of several known selective K^+ channel blockers, whether the polygodial-mediated endothelium-dependent relaxation in vessels from rabbit and guinea-pig could be associated with the opening of K^+ channels.

Our results show that neither Ca^{2+}-activated nor voltage-mediated K^+ channels are likely to be involved in the vasorelaxant action of polygodial in the studied preparations. This derives from the findings demonstrating that the selective neurotoxins, charybdotoxin and apamin, reported to selectively inhibit the high and low conductance Ca^{2+}-activated K^+ channels, respectively (Romey et al., 1984; Cook and Haylett, 1985; Brayden, 1996), did not interfere with the polygodial-mediated vasorelaxant response. Furthermore, the non-selective K^+ channel blocker, tetraethylammonium (Huang et al., 1993) also failed to affect the vasorelaxant action caused by polygodial in guinea-pig artery pulmonary and rabbit thoracic aorta, further suggesting the lack of involvement of K^+ channels in its vasorelaxant action. However in rings of rabbit pulmonary artery, tetraethylammonium and glibenclamide, a selective antagonist of ATP-sensitive K^+ channels, partially but significantly prevented the vasorelaxant action induced by polygodial , suggesting that the activation of ATP-sensitive channels may account, at least in part, for polygodial vasorelaxation.

In summary, our results extend our previous reported action of polygodial (El Sayah et al., 1997, 1998; Mendes et al., 1998) indicating that this sesquiterpene produced a concentration and endothelium-dependent and -independent vasorelaxation in rabbit and guinea-pig pulmonary arteries also in rabbit thoracic aorta in vitro, pre-contracted by phenylephrine. Its vasorelaxant actions are largely mediated by release of NO or NO-related substance from vascular endothelium through the activation of a guanylyl cyclase-dependent mechanism. However, the opening of K^+ channels, prostacyclin, substance P or CGRP release, and activation of adenylyl cyclase, appear to play a minor role, or have no participation in the vasorelaxant action of polygodial in these vessels.

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