Assessment of Mechanisms Involved in Antinociception Caused by Sesquiterpene Polygodial

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ABSTRACT

Polygodial, a sesquiterpene isolated from the bark of Drymis winteri given systemically, intraplatrally, or by spinal or supraspinal sites, produced antinociception when assessed in both phases of the formalin test and against capsaicin-induced pain. Polygodial, even at high doses, had no antinociceptive or anithyperalgesic effect when assessed in hot-plate assay or in glutamate-induced hyperalgesia, nor did it significantly interfere with the motor coordination of animals when tested in the rotarod test. The polygodial antinociception assessed in the formalin test was not affected by i.p. treatment of animals with cyprodepin, yohimbine, phaclofen, bicusculine, or nitric oxide precursor or by intrathecal administration of potassium channel blockers such as apamin, charybdotoxin, glibenclamide, or tetraethylammonium. In contrast, polygodial antinociception was significantly attenuated by i.p. treatment of animals with naloxone, naltindrole, 2-(3,4-dichlorophenyl)-η-methyl-η-[(15)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinilyl)acetamide, p-chlorophenylalanine, prazosin, or by i.c.v. treatment with pertussis toxin. In addition, polygodial antinociception was not cross-tolerant to morphine, nor was its effect affected by the adrenalectomy of animals. Together, these results show that polygodial produces pronounced systemic, spinal, and supraspinal antinociception in mice, mainly preventing the neuropenic pain produced by formalin and capsaicin. The mechanism by which polygodial produces antinociception seems likely to involve an interaction with the opioid system, mainly κ and δ subtypes, depend on the activation of G i/o protein sensitive to pertussis toxin, α 1-adrenoceptors, and the serotoninergic system. Collectively, these results suggest that polygodial itself or its derivatives may have potential therapeutic value for the development of new analgesic drugs.

Previous studies from our group have shown that the extract obtained from the bark of Drymis winteri, known popularly as “Casca de Anta,” antagonizes in a graded manner contractions induced by several neurotransmitters known to participate in pain transmission and inflammatory states when assessed in rat uterus and guinea pig ileum and trachea in vitro (El Sayah et al., 1997). We also have shown that the major constituent isolated from this extract, the sesquiterpene polygodial, at micromolar concentrations produces similar inhibition of neurotransmitter-induced contractions in the guinea pig trachea (El Sayah et al., 1998). In addition, the extract of D. winteri produced dose-related and long-lasting antinociception when assessed against acetic acid, kaolin, zymozan, formalin, and capsaicin in mice, as well as against bradykinin and Substance P, but not carrageenan or prostaglandin E 2-induced hyperalgesia in rats (Mendes et al., 1998). We also have demonstrated that the sesquiterpene polygodial given i.p. prevented, in a dose-related manner, the pain responses elicited by i.p. injections of mice with acetic acid, kaolin, or zymozan. At the ID 50 level, polygodial was ~14- to 27-fold more potent than the extract obtained from D. winteri (Mendes et al., 1998).

In this study, we therefore describe the peripheral, topical, spinal and supraspinal antinociceptive properties produced by the naturally-occurring sesquiterpene polygodial in different chemical and thermal behavioral models of nociception. A second objective of this study was to evaluate, by use of selective antagonists of receptors or drugs that interfere with second messengers and ion channels, the possible mechanisms that may be involved in the antinociceptive action of polygodial.

ABBREVIATIONS: i.t., intrathecal; DIPPA, 2-(3,4-dichlorophenyl)-η-methyl-η-[(15)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinilyl)acetamide; TEA, tetraethylammonium; PCPA, D,L-p-chlorophenylalanine methyl ester hydrochloride; l-NOARG, Nω1-nitro-l-arginine; GABA, y-aminobutyric acid; SHO, sham-operated.
Materials and Methods

Animals
Male Swiss mice (25–35 g), housed at 22 ± 2°C under a 12-h light/dark cycle and with access to water and Purina chow ad libitum, were used throughout the experiments. Animals were acclimatized to the laboratory for at least 1 h before testing and were used once throughout the experiments. The experiments reported were carried out in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983).

Pharmacological Analysis

Formalin Test. The procedure used was essentially similar to that described previously (Corrêa and Calixto, 1993; Santos and Calixto, 1997a). Animals were injected with 20 μl of 2.5% formalin solution (0.92% formaldehyde), made up in phosphate-buffered solution, intraplantarly in the right hindpaw of the mouse, with a microsyringe with a 26-gauge needle. Animals were pretreated with polygodial (12.8–420.7 μmol/kg) by i.p. or p.o. routes, 0.5 and 1 h before formalin injection or intraplantarly (10–300 nmol/site, injected in association with formalin). Other groups of animals were treated with polygodial (10–300 nmol/site) or with vehicle (5 μl/site) by i.c.v. or intrathecal (i.t.) routes as described previously (Hylden and Wilcox, 1980; Vaz et al., 1996; Santos et al., 1999), 10 min before formalin injection. After intraplantar injection of formalin, the animals were immediately placed in a glass cylinder 20 cm in diameter, and the time spent licking the injected paw was timed with a chronometer and considered as indicative of pain. To investigate whether the antinociceptive activity of polygodial was associated with anti-emetogenic activity, at the end of all experiments the animals were sacrificed by cervical dislocation, and the paws were cut at the knee joint and weighed on an analytical balance (Corrêa and Calixto, 1993; Santos and Calixto, 1997a; Santos et al., 1999).

Capsaicin-Induced Pain. To evaluate the possible analgesic effect of the polygodial on neurogenic pain, we also investigated whether polygodial antagonized capsaicin-induced pain in the mouse paw. The procedure used was similar to that described previously (Corrêa et al., 1996; Santos and Calixto, 1997a,b; Santos et al., 1999). Animals were observed individually for 5 min after capsaicin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of pain. Animals were treated either with i.p. or p.o. injection of vehicle (10 ml/kg) or polygodial (2.1–128.1 μmol/kg), 0.5 and 1 h before capsaicin injection. Other groups of animals were treated with polygodial (10–300 nmol/site) or with vehicle (5 μl/site) by i.c.v. or i.t. routes, 10 min before capsaicin injection, or intraplantarly (10–300 nmol/site, injected in association with capsaicin).

Glutamate-Induced Hyperalgesia. To test the hypothesis of whether the excitatory amino acids were involved in the polygodial antinociception, we assessed the effect of polygodial (2.1–42.7 μmol/kg) given by i.p. route on the hyperalgesic response caused by spinally administered glutamate (100 nmol/site i.t.) in mice in the hot-plate test, as reported previously (Beirith et al., 1998; Ferreira et al., 1999). The hyperalgesic response was measured on the hot-plate apparatus (model-DS 37; Ugo Basile, Varese, Italy) maintained at 50 ± 1°C as described in the hot-plate test. The maximal hyperalgesic response caused by i.t. injection of glutamate was observed for 1 h after the injection. A cutoff of 30 s was used for the hot-plate test. The maximal percentage of effect of glutamate-induced hyperalgesia was calculated as follows: % maximal percentage of effect = postdrug – predrug/30 – predrug × 100.

Hot-Plate Test. The hot-plate test was used to measure the response latencies according to the method described previously (Beirith et al., 1998; Santos et al., 1999). In these experiments, the hot-plate was maintained at 56 ± 1°C. Animals were placed into a glass cylinder and the time (s) between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. The reaction time was recorded for animals pretreated with the polygodial (42.7 μmol/kg i.p.) or with morphine (26.6 μmol/kg s.c.), which was used as a positive control. Animals that remained on the apparatus for an average of 6 s were selected 24 h previously on the basis of their reactivity in the model. A latency period (cutoff) of 30 s was defined as complete antinociception. Animals were treated with polygodial or with morphine 0.5 h before. Control animals received the vehicle used to dilute these drugs.

Rota-Rod Test. To exclude the possible nonspecific muscle-relaxant or sedative effects of polygodial, the mice were tested in the rota-rod test, which was used to measure motor performance according to the method described previously (Vaz et al., 1996; Santos et al., 1999). The apparatus (model-DS 37; Ugo Basile) consisted of a bar with a diameter of 2.5 cm, subdivided into six compartments by disks, 25 cm in diameter (Duham and Miya, 1957). The bar rotated at a constant speed of 22 rpm. The animals were selected 24 h previously by eliminating those mice that did not remain on the bar for two consecutive periods of 60 s. Animals were treated with polygodial (42.7 μmol/kg i.p.) or with vehicle (10 ml/kg i.p.), and were retested. The time they remained on the rotating bar (maximum of 60 s) was recorded.

Analysis of Possible Mechanism of Action of Polygodial. To investigate the participation of the opioid system in the antinociceptive effect of polygodial, animals were pretreated with naloxone (13.7 μmol/kg i.p.), a nonselective opioid receptor antagonist; cyprodime (2.3 μmol/kg i.p.), a selective μ-opioid receptor antagonist; naltrindole (2.2 μmol/kg i.p.), a selective δ-opioid receptor antagonist; or 2-(3,4-dichlorophenyl)-n-methyl-n-((1S)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl)acetamide (DIPPA) (0.2 μmol/kg i.p.), a selective κ-opioid receptor antagonist, as reported previously (Chang et al., 1994a,b; Craft et al., 1995; Frey and Schicht, 1996; Ossipov et al., 1996; Schwartz et al., 1997; Santos et al., 1999), 15 min before administration of polygodial (42.7 μmol/kg i.p.) or vehicle (10 ml/kg i.p.) injection. The other groups of animals received polygodial, naloxone, cyprodime, naltrindole, DIPPA, or vehicle 0.5 h before the formalin injection. To assess the possible participation of GluR protein (sensitive to pertussis toxin) on the antinociceptive action of polygodial, animals were pretreated with pertussis toxin (1.0 μg/site i.c.v.) 7 days before the administration of polygodial (42.7 μmol/kg i.p.) or morphine (13.3 μmol/kg s.c.; used as positive control). Other groups of animals were pretreated with saline (5 μl/site i.c.v.), and 7 days after they received polygodial (42.7 μmol/kg i.p.), morphine (13.3 μmol/kg s.c.), or vehicle injection, 0.5 h before the formalin injection (Santos et al., 1999). The possible cross-tolerance between morphine and polygodial also was investigated. Animals were pretreated with morphine (13.3 μmol/kg s.c.) or with vehicle (10 ml/kg s.c.) by repetitive administration over a 7-day period before testing for formalin-induced pain. The animals received one s.c.-administered injection per day at 9:00 AM for the first 6 days and a single injection at 8:00 AM on day 7 with morphine or vehicle. Animals tolerant to morphine (i.e., treated with morphine for 7 days) or nontolerant (i.e., treated with vehicle for 7 days) received polygodial (42.7 μmol/kg i.p.) or morphine (13.3 μmol/kg s.c.) and the antinociceptive effects were evaluated 0.5 h later in the formalin test (Santos et al., 1999).

Next we investigated the possible role played by various potassium channel blockers in the antinociceptive effect caused by polygodial. For this purpose, mice were pretreated with apamin [50 ng/site i.t.; a blocker of small (or low)-conductance calcium-gated potassium channels], charybdotoxin [250 pg/site i.t.; a blocker of large (or fast)-conductance calcium-gated potassium channels], tetraethylammonium (TEA) [1 μg/site i.t.; a blocker of voltage-gated potassium channels], or glibenclamide (100 μg/site i.t.; a blocker of ATP-gated potassium channels), and after 15 min they received polygodial (42.7 μmol/kg i.p.) or vehicle (10 ml/kg i.p.). The used doses of the potassium channel blockers were the same as reported previously (Welch and Dunlow, 1993; Welch et al., 1995). The nociceptive responses caused by formalin were recorded 0.5 h after ad-
administration of polygodial or vehicle. Other groups of animals received vehicle (5 μL/site) by i.t. route 15 min before the administration of polygodial or vehicle and 0.5 h after received the formalin injection. To assess the possible contribution of serotonin to the antinociceptive effect of polygodial, animals were pretreated with D,L-p-chlorophenylalanine methyl ester hydrochloride (PCPA) (399.8 μmol/kg i.p., an inhibitor of serotonin synthesis) once a day for four consecutive days. The selected dose of PCPA was based in previous studies (Pini et al., 1996; Vaz et al., 1996; Santos et al., 1999). One-half hour after the last injection of PCPA, animals received polygodial (42.7 μmol/kg i.p.), morphine (13.3 μmol/kg s.c.), or vehicle (10 ml/kg i.p.). Other groups of mice were treated with saline (10 ml/kg, s.c.), and 4 days after received polygodial, morphine, or vehicle injection, 0.5 h before formalin injection.

In a separate series of experiments, we also investigated the role played by the nitric oxide–l-arginine pathway in the antinociception caused by polygodial. To this end, animals were pretreated with l-arginine (3444 μmol/kg i.p.) and after 15 min they received polygodial (42.7 μmol/kg i.p.), l-α-nitro-l-arginine (l-NOARG) (342.0 μmol/kg i.p.), or vehicle (10 ml/kg i.p.) as reported previously (Santos et al., 1995, 1999; Vaz et al., 1996). The algesic responses caused by formalin injection were recorded 0.5 h after administration of polygodial, l-NOARG, or vehicle. Other groups of animals received only polygodial, l-NOARG, l-arginine, or vehicle 0.5 h before formalin injection. To assess the possible contribution of the γ-aminobutyric acid (GABA) system, animals were pretreated with phaclofen (a GABA A antagonist; 40 μmol/kg i.p.) or bicuculline (a GABA A antagonist; 1.9 μmol/kg i.p.) and after 15 min they received a polygodial (42.7 μmol/kg i.p.), baclofen (a GABA A agonist; 4.8 μmol/kg i.p.), muscimol (a GABA A agonist; 8.7 μmol/kg i.p.), or vehicle injection (Santos et al., 1999). Other groups of animals received only a polygodial, phaclofen, baclofen, bicuculline, muscimol, or saline injection 0.5 h before formalin injection. To examine the possible contribution of α 1 - and α 2 -adrenoceptors in the antinociceptive effect caused by polygodial, animals were pretreated with prazosin (0.4 μmol/kg i.p.) or with yohimbine (0.4 μmol/kg i.p.) and after 15 min the animals received polygodial (42.7 μmol/kg i.p.), phenylephrine (49.1 μmol/kg i.p.), clonidine (3.7 μmol/kg i.p.), or saline (10 ml/kg i.p.) injection. Other groups of animals were pretreated with vehicle (10 ml/kg i.p.) and after 15 min received polygodial, phenylephrine, clonidine, prazosin, yohimbine, or vehicle injection (Santos et al., 1995; Vaz et al., 1996). The algesic responses caused by formalin injection were recorded 0.5 h after administration of drugs or vehicle injection. Finally, to investigate the possible contribution of endogenous glucocorticoids in the antinociceptive effect caused by polygodial, animals were anesthetized with 2,2,2-tribromoethanol (0.25 g/kg i.p.) and both adrenal glands were removed through dorsal incision, as described previously by Vaz et al. (1996). After surgery, animals were returned to their cages, with free access to water and food, but water was replaced by saline (0.9% NaCl solution) to maintain physiological sodium plasma concentration. Another group of animals was sham-operated (SHO) and allowed free access to water and food. After 1 week, the animals received polygodial (42.7 μmol/kg i.p.) or vehicle (10 ml/kg i.p.) injection, 0.5 h before formalin injection. The SHO animals were used as control.

Drugs. The following substances were used: formalin, morphine hydrochloride (E. Merck, Darmstadt, Germany), PCPA, pertussis toxin, 2,2,2-tribromoethanol, l-arginine, l-NOARG, yohimbine, clonidine, l-phenylephrine, prazosin, bicuculline, capsaicin, apamin, TEA chloride, charybdotoxin (Sigma Chemical Co., St. Louis, MO), naloxone hydrochloride, glibenclamide, baclofen, phaclofen, cypridine hydrobromide, naltindrole hydrochloride, DIPPA (Research Biochemicals Inc., Natick, MA), muscimol (Tocris, Ballwin, MO), and Tween 80 (LabSynth, São Paulo, Brazil). The polygodial was isolated from the bark of D. winteri at the Chemistry Department of the Federal University of Santa Catarina, Brazil, as described previously (El Sayah et al., 1998; Ceccineli Filho et al., 1998). Its degree of purity was >96%. Drugs were prepared just before use in 0.9% NaCl solution, with the exception of capsaicin and polygodial, which were dissolved in absolute ethanol and Tween 80, respectively. The final concentration of Tween and ethanol did not exceed 5% and did not cause any effect per se.

Statistical Analysis. Results are presented as means ± S.E., except the ID 50 values (i.e., the dose or the concentration of drugs reducing the pain responses by 50% relative to control value), which are reported as geometric means accompanied by their respective 95% CL. The ID 50 values were determined by linear regression from individual experiments with linear regression GraphPad software (GraphPad Software, San Diego, CA). The statistical significance between groups was performed by ANOVA followed by Newman-Keuls multiple comparison test. P values <.05 were considered as indicative of significance.

Results

Formalin-Induced Licking. Figure 1, A and B shows that polygodial given by p.o. or i.p. routes produced significant inhibition of the early (0–5 min) and the late phase (15–30 min) of the formalin-induced licking. The calculated mean ID 50 values shown in Table 1 reveal that polygodial given orally was ∼2- to 8-fold less potent than when it was given by i.p. route. Independent of the route of administration used, polygodial did not affect the edema response associated with the second phase of the formalin test (data not shown).

The i.c.v., i.t., or intraplantar injection of the polygodial also inhibited both phases of formalin-induced licking (Fig. 2, A and B). At the ID 50 level, polygodial was 3- to 6-fold more active when given by i.c.v. route (Table 1).

Capsaicin-Induced Pain. Figure 3, A and B, and data summarized in Table 1 show that polygodial, given systemically (i.p. or p.o.) or by i.c.v., i.t., or intraplantar routes, caused significant inhibition of the capsaicin-induced licking.

Glutamate-Induced Hyperalgesia. The i.p. administration of polygodial (up to 42.7 μmol/kg) did not cause any significant inhibition of the glutamate-induced hyperalgesia (data not shown).

Hot-Plate Test. Polygodial (42.7 μmol/kg i.p.) at similar doses to those at which it was active in other models of pain, did not significantly increase the latency response in the hot-plate test (control response of 6.6 ± 0.6 s versus polygodial)}
dial-treated group response of 8.7 ± 0.9 s). Under similar conditions, morphine (26.6 μmol/kg s.c.) caused a significant and marked (control response of 6.6 ± 0.6 s versus morphine-treated group response of 24.0 ± 1.5 s (P < .01) increase in the latency on the hot-plate assay (N = 10).

**Rota-Rod Test.** The i.p. injection of polygodial (up to 42.7 μmol/kg) did not significantly affect the motor response of animals. Control response in the rota-rod test was 59.1 ± 0.7 versus 58.5 ± 1.0 s in the presence of tested compound (N = 7). In addition, polygodial at all doses used failed to produce any detectable effect (data not shown).

**Analysis of Mechanism of Action of Polygodial.** Figure 4, A and B and the data summarized in Table 2 show that the pretreatment of animals with naloxone, naltirindole, or DIPPA before injection of polygodial largely reverted the antinociception caused by polygodial against both phases of the formalin test. In contrast, pretreatment of animals with cyprodime did not significantly change the antinociceptive effect caused by polygodial when assessed against both phases of the formalin test. The i.c.v. administration of pertussis toxin, an inactivator of Gi/o protein, produced significant inhibition of morphine-induced antinociception when assessed against both phases of formalin-induced pain. Un-
under the same conditions, pertussis toxin treatment significantly antagonized the antinociceptive action of the polygodial against the second (but not the first) phase of the formalin test (Table 2 and Fig. 5, A and B).

Figure 6, A and B show that the pretreatment of animals with morphine (once a day for seven consecutive days) produced significant tolerance to the antinociceptive effects caused by morphine, but did not change the antinociception caused by polygodial compared with the animals pretreated with saline. Figure 7, A and B also shows that the pretreatment of animals with PCPA (once a day for 4 days) prevented significantly the antinociception caused by polygodial or morphine, when assessed against the second phase of formalin-induced pain (Table 2).

Table 2 shows that the i.t. administration of various potassium channel blockers, including amphin (50 ng/site), charybdotoxin (250 μg/site), TEA (1 μg/site), or glibenclamide (100 μg/site), given 15 min beforehand, did not significantly modify the antinociception caused by polygodial against both phases of formalin-induced nociception (data not shown). The treatment of animals with L-arginine (3444 μmol/kg i.p.), given 15 min prior, significantly reversed the antinociception caused by L-NOARG, but did not significantly change the action caused by polygodial when analyzed against both phases of the formalin test (data not shown and Table 2).

Previous treatment of the animals with phaclofen (40 μmol/kg i.p.) or bicuculline (1.9 μmol/kg i.p.), 15 min before, significantly reversed the antinociception caused by baclofen or muscimol, but had no effect on the antinociceptive action caused by polygodial when analyzed against both phases of the formalin test (data not shown and Table 2). Treatment of the animals with prazosin (0.4 μmol/kg i.p.) or with yohimbine (0.4 μmol/kg i.p.), 10 min before, significantly reversed the antinociception caused by phenylephrine and clonidine, respectively (Fig. 8, A and B). The same treatment with prazosin significantly antagonized the antinociceptive action caused by polygodial against both phases of the formalin test. In contrast, treatment with yohimbine did not significantly modify the antinociceptive effect caused by polygodial against both phases of formalin-induced nociception (Table 2 and Fig. 8, A and B). In addition, bilateral adrenalectomy of the animals, performed 1 week before experiments, did not significantly affect the antinociceptive effect caused by polygodial in this same model (data not shown).

Discussion

Polygodial is the major naturally occurring sesquiterpene present in the extract obtained from the bark of D. winteri and related species (Apel and Dohr, 1958; Torres et al., 1992; Brown, 1994; Cechinel Filho et al., 1998; El Sayah et al., 1998). Confirming and extending our previous findings reported for polygodial and the extract obtained from the bark of D. winteri (Mendes et al., 1998), the data presented in this study show that systemic (i.p. and p.o.), i.c.v. and i.t., or intraplantar injections of polygodial, at doses that it did not produce motor dysfunction or any detectable side effect, produced marked and dose-related antinociception when assessed in chemical, but not in thermal assays of nociception. Compared with reference analgesic drugs, polygodial was 4- to 30-fold more potent than aspirin and dipyreone, respectively, but it was 2- to 69-fold less active than morphine, depending on the route of administration and also on the behavioral model of pain used (Vaz et al., 1996; Beirith et al., 1998).

To investigate the possible mechanisms by which polygodial produces antinociception, we have assessed in the present study the effect of several in vivo procedures and also, by the use of selective antagonists of receptors, the effect(s) of ion channel and inactivation of G_i protein on its antinociceptive action. Our data demonstrate that the activation of the opioid naloxone-sensitive pathway is certainly involved in the antinociception produced by polygodial because naloxone significantly reversed both morphine and polygodial antinociception. By using more selective opioid antagonists, it was possible to demonstrate that polygodial antinociceptive action involves the k- and δ- (but not μ-) opioid receptors. This evidence derives from the fact that naltrindole and DIPPA (but not cyprodime) significantly inhibited polygodial antinociceptive when assessed in the formalin test (Chang et al., 1994a,b; Craft et al., 1995; Frey and Schicht, 1996; Ossipov et al., 1996; Schwartz et al., 1997; Santos et al., 1999). In spite of this evidence suggesting the involvement of opioidlike peptides in the antinociception produced by polygodial, this sesquiterpene was completely devoid of antinociception when assessed in the acute thermal model of pain, the hot-plate test, and it did not present any evidence of cross-tolerance with morphine when it was administered to animals that had received an s.c. injection of this opioid once a day for 7 consecutive days. Further behavioral and biochemical studies are now in progress to address these issues. However, results of the present study support, at least in part, the role played by the serotonergic system in the antinociceptive effect of polygodial because the pretreatment of animals with PCPA, at a dose known to inhibit serotonin synthesis (Pini et al., 1996), significantly reversed both polygodial and morphine antinociceptions in the formalin model of pain. Furthermore, results of the present study provide consistent evidence supporting the involvement of α_1- (but not α_2-) adrenoceptors in the antinociception caused by polygodial, evident by the fact that prazosin, at similar doses known to prevent phenylephrine-induced antinociception (Uhlón et al., 1990; Tasker et al., 1992; present study),

![Fig. 3. Effect of i.p. (C; A), oral (●; A), i.c.v. (△; B) i.t. (●; B), or intraplantar (△; B) administration of polygodial on capsaicin-induced licking in mice. The total time spent licking the hindpaw (0–5 min) was measured after intraplantar injection of capsaicin. Each point represents the mean for 8 to 10 animals and the vertical bars indicate S.E.M. The point (0) indicates the control values (animals injected with the vehicle) and the asterisks denote significance levels compared with control groups. ANOVA, *P < .05, **P < .01.](image-url)
consistently attenuated polygodial-induced antinociception in the formalin test. In marked contrast, the nitric oxide pathway, GABA_A and GABA_B receptors, and activation of small- or large-conductance calcium-gated potassium channels, ATP-gated potassium channels, or voltage-gated potassium channels seem unlikely to be involved in the antinociceptive action of polygodial, evident by the fact that selective antagonists of these receptors or ion channel, in conditions where they produce significant inhibition of the antinociception caused by the selective agonists and/or enzyme precursors (Welch and Dunlow, 1993; Raffa and Martínez, 1995; Shewade and Ramaswamy, 1995; Welch et al., 1995; Shafizadeh et al., 1997; Santos et al., 1999), had no significant effect on the polygodial antinociception. Finally, polygodial antinociceptive action was not modulated by endogenous glucocorticoids hormones because previous bilateral adrenalectomy of animals, carried out 1 week before testing, did not significantly modify its analgesic action compared with SHO animals.

An interesting finding of the present study was that, like morphine, polygodial antinociception was significantly attenuated after i.c.v. treatment of animals with pertussis toxin (1 μg/site; 7 days before experiments) at a dose that has been shown previously to suppress the antinociceptive effect caused by morphine through ADP ribosilation (Hernandez et al., 1995; Santos et al., 1999; present study). These results, therefore, are consistent with the hypotheses that polygodial antinociception, similar to that of morphine, is probably cou-
pled to G_i/o pertussis toxin-sensitive mechanisms. An additional mechanism that also could contribute to the mechanism involved in the antinociception of polygodial, particularly against the neurogenic pain, is its possible interaction with tachykinin receptors and/or actions. We have recently demonstrated that the extract, and also the sesquiterpene polygodial, antagonized through a selective manner contraction elicited by neurokinin2 (but not neurokinin 1) tachykinin agonists in the guinea pig trachea “in vitro” (El Sayah et al., 1997, 1998). In addition, it also was reported that the extract of D. winteri dose-dependently reversed Substance P- and bradykinin-induced hyperalgesia in the rat paw (Mendes et al., 1998).

Szallasi et al. (1998) have shown that several naturally occurring unsaturated dialdehyde sesquiterpenes and related bioactive terpenoids are capable of inhibiting the specific binding of [3H]resiniferatoxin by rat spinal cord membranes. Most of these compounds are pungent on the human tongue (Szallasi et al., 1996, 1999). The pungent and non-pungent terpenoids were referred as vanilloid receptor antagonists. Whether or not polygodial produces its antinociceptive action through inhibition of the [3H]resiniferatoxin binding site remains to be determined.

In conclusion, results from the present study extend our previous findings (El Sayah et al., 1997, 1998; Mendes et al., 1998) by demonstrating that the major constituent isolated from the bark of the Brazilian medicinal plant D. winteri, the sesquiterpene polygodial, produced systemic, local, spinal, and...
supraspinal antinociception when assessed in chemical (formal- 
lin and capsaicin-induced pain) but not in thermal (hot-plate 
test) models of nociception in the mouse. The precise site by 
which polygodial induces antinociception is currently under 
investigation, but an interaction with an opiate-like system, i.e., 
through κ- and δ-receptors, the α1-adrenergic receptor; the 
serotonergic system, and an interaction with a G protein 
sensitive to treatment with pertussis toxin, has an important 
modulatory role in its antinociceptive action. Thus, polygodial 
or its derivatives might be of interest in the development of new 
analgesic drugs for the management of neurogenic pain.

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