Levodropropizine reduces capsaicin- and substance P-induced plasma extravasation in the rat trachea

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We investigated the effect of the non-opioid, peripherally acting antitussive agent levodropropizine to reduce neurogenic plasma extravasation in the rat trachea. Levodropropizine (10, 50 and 200 mg/kg) reduced in a dose-dependent manner the extravasation of Evans blue dye evoked by capsaicin. Levodropropizine inhibited also substance P-evoked extravasation, whereas it did not affect the extravasation evoked by platelet activating factor. Levodropropizine (10 and 100 μM) did not affect the contraction produced by [Sar9,Met(O2)ll]substance P, a selective agonist for tachykinin NK1 receptors, in the rat urinary bladder in vitro. These data indicate that levodropropizine inhibits capsaicin-induced plasma extravasation: (a) acting at a postjunctional level; (b) exhibiting neuropeptide selectivity and; (c) via a mechanism independent of tachykinin NK1 receptor blockade. Irrespective of the mechanism, this novel antiinflammatory action of levodropropizine underlines its potential role in inflammatory airway diseases such as bronchial asthma.

Levodropropizine, Capsaicin; Substance P; Sensory nerves; Neurogenic inflammation; Plasma extravasation

1. Introduction

Stimulation of capsaicin-sensitive sensory nerves in rodent airways produces a variety of reflexly mediated responses or responses due to release of peptides such as substance P and neurokinin A from the peripheral endings of these nerves. These responses include increase in blood flow (Piedimonte et al., 1992b), neutrophil adhesion (Umeno et al., 1989), bronchocstriction (Szolcsanyi and Bartho, 1982; Lundberg et al., 1983), mucus secretion (Kuo et a., 1990) and cough (Forsberg and Karlsson, 1986). Capsaicin also produces plasma extravasation (Lundberg et al., 1983; Saria et al., 1983) in the rat trachea by releasing tachykinins, which in turn activate tachykinin NK1 receptors (Abelli et al., 1991; Eglezos et a., 1991; Piedimonte et al., 1992a).

Levodropropizine is a new phenylpiperazine antitussive derivative which does not interact with opioid, GABA, muscarinic or β-adrenoceptors. A slight interaction with histamine H1 and 5-HT receptors has been observed with a calculated Kd of about 10 μM (Mellilo et al., 1988). Levodropropizine prevents cough in various experimental models evoked by irritants such as citric acid, NH4OH, H2SO4 and by electrical stimulation of the vagus nerve (Malandrino et al., 1988). Recently, levodropropizine was shown to inhibit the cough induced by capsaicin and a peripheral site of action has been indicated (Lavezzi et al., 1992). Because codeine, but not levodropropizine, prevented cough when these drugs were given intracerebroventricularly, whereas they were equipotent when they were given by aerosol, it was hypothesized that levodropropizine may reduce cough induced by capsaicin by interfering with capsaicin activation of peripheral endings of sensory nerves (Lavezzi et a., 1992). Levodropropizine might act also on the mechanism brought about by substance P, because substance P is released by capsaicin and may be involved in the cough reflex (Kohrogi et al., 1988).

To investigate the action of levodropropizine on the response produced by sensory nerve activation, we studied the effect of levodropropizine on the increase in vascular permeability evoked by capsaicin in the rat trachea. Then, the effect of levodropropizine on capsaicin-evoked increase in vascular permeability was compared with the effect exerted by this drug on the
responses produced by substance P and by platelet activating factor (PAF). Because levodropropizine inhibited the vascular extravasation evoked by capsaicin and by substance P, but not by PAF, to discriminate whether this inhibitory effect was due to antagonism on tachykinin NK₁ receptors, we also studied the effect of levodropropizine on the smooth muscle contraction evoked by the selective NK₁ receptors agonist [Sar⁹,Met(O₂)₁₀]substance P (Regoli et al., 1988) on the rat urinary bladder in vitro.

2. Materials and methods

2.1. Description of experiments

We used pathogen-free F344 rats (10–12 weeks of age) from Simonsen Laboratories, Inc., Gilroy, CA, weighing 200–300 g. On the day of the experiment, the rats were anesthetized with sodium pentobarbital (65 mg/kg i.p.; Anthony Products Co., Arcadia, CA). We used Evans blue dye (3% solution in 0.9% saline; Polysciences, Inc., Warrington, PA) to measure vascular extravasation. Immediately after the injection of the dye (30 mg/kg, i.v. over 5 s), capsaicin (325 nmol/kg, over 2 min), substance P (5 nmol/kg) or PAF (20 nmol/kg) was given by i.v. injection. Capsaicin (Sigma, St. Louis, MO) was dissolved in a vehicle having a final concentration of 0.75% ethanol, 0.375% Tween 80 and 0.85% saline in aqueous solution. PAF (Bachem Inc., Torrance, CA) was prepared as a stock solution (1 mg/ml) in 100% ethanol, stored at −80°C and then diluted to the final concentration in 0.25% bovine serum albumin in 0.9% saline. Substance P (Peninsula, Gilroy, CA) was prepared as a stock solution in 0.1 N acetic acid and then diluted to the final concentration in 0.9% saline.

Five minutes after injection of the tracer, the chest was opened, a cannula was inserted into the ascending aorta through the left ventricle, and the circulation was perfused for 2 min with phosphate buffer (pH 5; Sigma Chemical Co., St Louis, MO) at a pressure of 120 mm Hg. The trachea was dissected and opened along the ventral midline, blotted, weighed, and then incubated in 3 ml of formamide (Fisher Scientific, Santa Clara, CA) at 50°C for 18 h to extract the extravasated Evans blue dye.

First, we studied the time course of the effect of levodropropizine on capsaicin-evoked increase in vascular extravasation. Levodropropizine (Dompe' Farmaceutici S.p.A., Milan, Italy) was injected i.p., 15, 30 or 60 min before the stimulus. Next, we investigated the effect of different doses of levodropropizine (5–200 mg/kg = 21–847 μmol/kg, i.p., administered 30 min before the stimulus) on the response produced by capsaicin or substance P. The effect of the two doses of levodropropizine (10 and 200 mg/kg, i.p., 30 min before the stimulus) was also studied on the response produced by PAF. Levodropropizine (200 mg) was administered in a final volume of 10 ml/kg of 0.9% saline containing 5% dimethyl sulfoxide. To obtain lower doses, levodropropizine was diluted in 0.9% saline. Control animals were pretreated with the vehicle of 200 mg/kg levodropropizine.

2.2. Measurement of plasma extravasation

The extravasation of Evans blue-labeled macromolecules from the microcirculation in different tissues was quantified by measuring the optical density of the formamide extracts at a wavelength of 620 nm with a spectrophotometer (Model UV160M, Shimatzu Scientific Instruments, Inc., Columbia, MD). The amount of Evans blue dye extravasated in the tissues, expressed in nanograms per milligram of wet weight, was interpolated from a standard curve of Evans blue concentrations (0.1–5 μg/ml).

2.3. Measurement of blood pressure and heart rate

Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.; Anthony Products Co., Arcadia, CA). A catheter (ID 0.8 mm, length 2.5 cm; Angiocath, Deseret Medical, Utah) was inserted into the left femoral artery and advanced to the abdominal aorta and then connected to a pressure transducer (model 1270A, Hewlett-Packard) for measurement of arterial pressure. The amplified signal from the transducer (module M2102B, Electronics for Medicine) was displayed continuously on a video monitor (model OM, Electronics for Medicine) and recorded by an oscillographic recorder (model DASH-8, Astro-Med). Heart rate was derived from the pressure pulse signal by a cardiotachometer coupler.

2.4. In vitro study

Rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.), and exanguinated and the urinary bladder was removed immediately and placed in ice-cold modified Krebs solution with the following composition (mM): NaCl 118; KCl 5.9; CaCl₂ 2.5; MgSO₄ 1.2; NaH₂PO₄ 1.2; NaHCO₃ 25; and glucose 10. Bladders were divided into two longitudinal halves and mounted in 5 ml organ baths containing modified Krebs solution maintained at 37°C and bubbled continuously with 95% O₂ and 5% CO₂. The free end of the strips was connected with a fine thread to an isometric transducer (Model 1030, UFI, Morro Bay, CA). The strips were placed under a resting tension of one gram, and then allowed to equilibrate for at least 60 min. Changes in the tension were recorded on a Beckman R 611
polygraph (Beckman, Fullerton, CA). Concentration-response curves were constructed with [Sar\(^9\),Met(O\(_2\))\(^{11}\)] substance P, a selective agonist for tachykinin NK\(_1\) receptors (kindly provided by Dr. D. Regoli, Department of Pharmacology, University of Sherbrooke, Canada). We investigated the effect of levodropropizine (10 or 100 \(\mu\)M, administered 5 min before the stimulus) on the response produced by [Sar\(^9\),Met(O\(_2\))\(^{11}\)] substance P. Values are expressed as percent of the maximal response to 3 \(\mu\)M [Sar\(^9\),Met(O\(_2\))\(^{11}\)] substance P.

2.5. Statistical analysis

All data reported in this study are expressed as the mean \(\pm\) standard error of the mean (S.E.M.). Mean values of spectrophotometric measurements of Evans blue dye extravasation were analyzed by one-way analysis of variance (ANOVA). Comparisons between means in each condition were performed by the Dunnett's multiple range test. Differences having a P value \(< 0.05\) were considered significant.

3. Results

3.1. Time course of the effect of levodropropizine

Administration of capsaicin vehicle did not significantly increase the vascular extravasation of Evans blue dye in the rat trachea (fig. 1). Capsaicin (325 nmol/kg) evoked a marked increase in vascular extravasation (fig. 1). Levodropropizine (200 mg/kg), by itself did not affect the baseline extravasation of the Evans blue dye (10.2 \(\pm\) 1.8 ng/mg, \(n = 5\)). Pretreatment with levodropropizine 15 min before capsaicin significantly reduced the vascular extravasation (fig. 1). A more pronounced reduction was obtained when levodropropizine was given 30 and 60 min before capsaicin injection. Vehicle indicates the effect of the vehicle of capsaicin. Columns are mean \(\pm\) S.E.M. of at least six experiments. * \(P < 0.05\); ** \(P < 0.01\).

3.2. Dose dependence of the effect of levodropropizine

A small dose of levodropropizine (5 mg/kg) did not affect the response to capsaicin (325 nmol/kg). However, 10 and 50 mg/kg of the drug significantly reduced the response to capsaicin, and an almost complete inhibition of the vascular extravasation was observed after pretreatment with 200 mg/kg of levodropropizine (fig. 2). Substance P (5 nmol/kg) produced a marked increase in vascular extravasation not statistically different from that evoked by capsaicin. A small dose of levodropropizine (5 mg/kg) did not affect the response to substance P (fig. 3). Similarly to the response to capsaicin, levodropropizine (10–50 mg/kg) inhibited the effect of substance P and the highest dose of levodropropizine (200 mg/kg) virtually abolished the substance P-evoked vascular extravasation. The vascular extravasation induced by 20 nmol of PAF was comparable to the responses evoked by capsaicin and substance P (fig. 3). The two high doses of
Fig 3 Effect of pretreatment with different doses of levodropropizine (Lp.) on the vascular extravasation of Evans blue dye evoked by substance P (5 nmol/kg, i.v.) in the rat trachea. Levodropropizine or its vehicle (0) was administered 30 min before substance P injection. Vehicle indicates the effect of the vehicle of substance P. Columns are means ± S E M of at least five experiments * P < 0.05, ** P < 0.01.

levodropropizine (10 and 200 mg/kg) failed to affect the response to PAF (fig. 4).

3.3. Cardiovascular parameters

One minute before injection of capsaicin, mean arterial pressure (108 ± 3 mmHg, n = 5) and heart rate (415 ± 28 beats/min) in the animals treated with levodropropizine (200 mg/kg) was not significantly different from blood pressure (110 ± 4 mmHg) and heart rate (418 ± 18 beats/min) observed in animals pretreated with vehicle of levodropropizine (n = 5). Capsaicin (325 nmol/kg) produced a biphasic response in blood pressure. The initial hypotension (-14 ± 2% from baseline mean arterial pressure; n = 5 rats) was followed by a hypertensive response (36 ± 6%). Similar responses were observed in rats pretreated with levodropropizine (200 mg/kg) (initial hypotension, +16 ± 3%; hypertension, 38 ± 7%, n = 5). Capsaicin induced a long-lasting tachycardia (20 ± 3% from baseline heart rate) that was not significantly different in rats pretreated with levodropropizine (20 ± 4%). Substance P (5 nmol/kg) decreased blood pressure (-15 ± 2%, n = 5) and increased heart rate (15 ± 1%). Similar responses were observed in rats pretreated with levodropropizine (200 mg/kg) (blood pressure, -17 ± 2%; heart rate, 16 ± 2%).

3.4. In vitro studies

[Sar⁹,Met(O₂)¹¹] substance P evoked a rapidly developing contraction in strips of the rat urinary bladder. The response to [Sar⁹,Met(O₂)¹¹] substance P was concentration (1 nM to 3 μM)-dependent: the threshold concentration was 10 nM, and the maximal effect was attained with 1–3 μM [Sar⁹,Met(O₂)¹¹] substance P. EC₅₀ was 95 ± 10 nM. The concentration-response curve to [Sar⁹,Met(O₂)¹¹] substance P was not affected by either 10 or 100 μM levodropropizine (fig. 5).
4. Discussion

In a previous study levodropropizine was found to inhibit the cough response evoked by capsaicin aerosol in guinea pigs (Lavezzo et al., 1992). In addition, it was observed that after aerosol administration, levodropropizine and codeine were equipotent in inhibiting capsaicin-induced cough. However, after intracerebroventricular administration, codeine, but not levodropropizine produced an inhibitory effect (Lavezzo et al., 1992). Furthermore, in capsaicin-pretreated animals codeine, but not levodropropizine, reduced the cough evoked by stimulation of the vagus nerve (Lavezzo et al., 1992). These observations suggested that levodropropizine exerted its antitussive effect at the peripheral level, and possibly involved capsaicin-sensitive sensory nerves. However, these data do not give information about the mechanism by which levodropropizine could affect the function of sensory nerves.

To study this issue we have investigated the effect of levodropropizine on capsaicin-evoked increase in vascular extravasation. An increase in vascular extravasation is a major feature of the local response produced by activation of sensory nerves in rat airways (Lundberg et al., 1983; Saria et al., 1983). Although the series of events that lead from sensory nerve stimulation to the opening of the gaps between endothelial cells of post-capillary venules has not been clarified completely, both tachykinin release from sensory nerve endings and NK₁ receptor activation are necessary steps (Lundberg et al., 1983; Saria et al., 1988; Abelli et al., 1991; Eglezos et al., 1991; Piedimonte et al., 1992a). Because the preferred ligand for NK₁ receptors, among the natural tachykinins, is substance P, this peptide plays a prominent role in neurogenic plasma extravasation.

In the present study we observed that levodropropizine dose dependently reduced the Evans blue dye extravasation evoked by capsaicin. The finding that levodropropizine, at the highest concentration used (200 mg/kg), did not affect the changes in blood pressure and heart rate evoked by substance P or by capsaicin indicates that the inhibitory action of levodropropizine was not due to gross changes in cardiovascular parameters. Inhibition of capsaicin-evoked increase in vascular extravasation by levodropropizine could be due to prejunctional or postjunctional factors. The observation that levodropropizine inhibited with a similar potency capsaicin- and substance P-evoked vascular extravasation points on a possible site of action of levodropropizine at the postjunctional level. There are examples of agents, such as β₂-adrenoceptor agonists, that can inhibit vascular extravasation produced by different agents irrespective of the fact that they produce this effect via a neurally dependent or neurally independent mechanism (O'Donnell and Persson, 1978; McDonald, 1992; Sulakvelidze and McDonald, 1992). Therefore, the hypothesis may be advanced that levodropropizine reduces plasma extravasation in a manner independent from the stimulus. However, the finding that levodropropizine did not affect the response to PAF indicates that it does not affect vascular permeability non-specifically.

The ability of levodropropizine to limit the increase in vascular extravasation evoked both by capsaicin and by substance P could be easily explained if this drug exhibited antagonistic property for NK₁ receptors. To test this possibility we studied the effect of levodropropizine on a rat in vitro preparation that contains NK₁ receptors. In vitro experiments on the rat urinary bladder show that levodropropizine did not affect the contractile response to [Sar⁹, Met⁶] substance P. Both NK₁ and NK₂ receptors mediate the response to tachykinins in the rat urinary bladder (Regoli et al., 1988). Because we used a selective NK₁ receptor agonist (Regoli et al., 1988), we can assume that the contraction observed was due to NK₁ receptor activation. Hence, the absence of inhibition by levodropropizine of [Sar⁹, Met⁶] substance P-evoked contraction clearly indicate that levodropropizine does not exhibit any appreciable antagonistic property on NK₁ receptors in the rat.

In conclusion, the antitussive agent, levodropropizine, shows a marked inhibitory effect on plasma extravasation mediated by sensory nerve activation. The inhibitory activity might due to different mechanisms, including a functional antagonism on tachykinin-evoked vascular extravasation at the level of the opening of the gaps between endothelial cells. Irrespective of the mechanism, this novel antiinflammatory effect of levodropropizine underlines the potential therapeutic role of this drug in inflammatory airway diseases such as bronchial asthma.

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