A substance P antagonist inhibits vagally induced increase in vascular permeability and bronchial smooth muscle contraction in the guinea pig

(vascular permeability/respiratory tract/bronchoconstriction)

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ABSTRACT Electrical stimulation of the cervical vagus nerve in anesthetized guinea pigs induced a rapid increase in respiratory insufflation pressure, suggesting increased airway resistance. After intravenous administration of a substance P (SP) antagonist, [D-Arg¹,D-Pro²,D-Trp⁷⁹,Leu¹¹]SP, the insufflation pressure response to vagal stimulation was reduced by 78% while the cardiovascular effects were unchanged. Histamine receptor-blocking agents were used to inhibit the effects of histamine release induced by the SP-antagonist. [D-Arg¹,D-Pro²,D-Trp⁷⁹,Leu¹¹]SP also reduced the increase in insufflation pressure caused by intravenous SP or capsaicin. The long-lasting noncholinergic contraction of the main and hilus bronchi induced by field stimulation in vitro, as well as the contractile effects of SP and capsaicin, were also blocked by the SP antagonist. The cholinergic contractions and the noncholinergic tracheal relaxation on field stimulation in vitro were, however, not blocked by the antagonist. Vagal stimulation in vitro also increased vascular permeability in the respiratory tract and esophagus, causing a subepithelial edema as indicated by Evans blue extravasation. Previous treatment with [D-Arg¹,D-Pro²,D-Trp⁷⁹,Leu¹¹]SP inhibited the permeability increase induced by both vagus nerve stimulation and exogenous SP. SP release from vagal sensory nerves was indirectly shown by reduction in the bronchial levels of SP after nerve stimulation in vivo. The data suggest that most of the vascular or capsaicin-induced increase in bronchial smooth muscle tone is caused by SP release from sensory neurons. In addition, activation of vagal SP-containing sensory nerves induces local edema. Tracheobronchial afferent SP-containing C fibers may thus exert local control of smooth muscle tone and vascular permeability in normal and pathophysiologi- cal conditions.

Activation of sensory neurons leads to local vasodilation (1) and increased vascular permeability in the skin (2). Both effects are abolished in animals that have been treated with capsaicin (3, 4), the pungent agent of hot peppers, which causes selective degeneration of chemosensitive primary afferent neurons when given neonatally (3). Administration of capsaicin in vitro releases substance P (SP) from unmyelinated sensory nerves (5) and it also causes long-term depletion of SP in these neurons (6). SP is a strong candidate to mediate neurogenic inflammatory responses and antidiromic vasodilatation, because it induces increases in vascular permeability (4) and vasodilatation (7). Antidiromic trigeminal nerve stimulation has been shown to cause SP release from nerve terminals in the dental pulp (8, 9) and eye (10). Furthermore, it has been shown that the antidiromic vasodilation in the pulp (11) and the inflammation in the eye on IR irradiation (12) can be inhibited by SP antagonists. Recent studies suggest that capsaicin-sensitive sensory nerves of vagal origin can induce neurogenic inflammation and bronchoconstriction in the respiratory tract (13, 14).

In this paper, we report that the increase in vascular permeability in the respiratory tract and esophagus and the noncholinergic bronchoconstriction induced by vagus stimulation can be blocked by [D-Arg¹,D-Pro²,D-Trp⁷⁹,Leu¹¹]SP (15), which is a specific SP antagonist (16).

MATERIALS AND METHODS

Experiments were performed on adult guinea pigs (250–350 g) of both sexes. Respiratory insufflation pressure was recorded in anesthetized guinea pigs (sodium pentobarbital at 40 mg/kg and suxamethon at 1 mg/kg) as an indication of changes in tracheobronchial resistance to air (17). The respiratory rate was 40/min and the respiration volume was 3 ml per stroke. Heart beat rate and systemic arterial blood pressure were continuously recorded via a catheter in a carotid artery connected to a pressure transducer and a Grass polygraph. Both cervical vagal nerves were cut and the distal ends were stimulated at 10 V, 5 ms, 10 Hz for 30 s. Atropine (0.5 mg/kg), SP, capsaicin, or the SP antagonist ([D-Arg¹,D-Pro²,D-Trp⁷⁹,Leu¹¹]SP) were administered intravenously (i.v.) via a jugular vein catheter. Because of the histamine-releasing properties of the SP antagonist (18), the animals were first treated with meperidine at 1 mg/kg and ephedrine at 1 mg/kg. These histamine antagonists did not influence the insufflation pressure or permeability response to SP, capsaicin, or vagus nerve stimulation. The first dose of [D-Arg¹,D-Pro²,D-Trp⁷⁹,Leu¹¹]SP (1 μmol/kg) was administered i.v. for 15 min. Then, there was a waiting period of 30 min, which was followed by a dose of 0.3 μmol/kg given i.v. 1 min before vagus nerve stimulation or SP or capsaicin injections. For in vitro studies, guinea pigs were sacrificed by stunning them. Pieces of trachea and stem and hilus bronchi were removed by dissection and placed in a 5-ml organ bath containing Krebs’ solution/glucose bubbled with 97% O₂/3% CO₂. The organ bath was maintained at 37°C and the isolated tissues were allowed to equilibrate for about 1 hr at a constant load of 250 mg (stem and hilus bronchi) or 500 mg (trachea). Electrical field stimulation (50 V, 1 ms, 10 Hz for 30 s) was given by means of platinum electrodes. The mechanical activity of the tissues was recorded isometrically. Capsaicin (Sigma) was first dissolved in ethanol and then diluted in saline to give a stock solution of 10 mg/ml. Further dilutions were made in saline. [D-Arg¹,D-Pro²,D-Trp⁷⁹,Leu¹¹]SP (50 μM) was added 10 min before electrical field stimulation or addition of SP and capsaicin. Meperidine (10 μM) was present in the organ bath throughout the experiments to minimize the contractile action of the SP antagonist.

Vascular permeability was studied in vitro using the Evans

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Abbreviations: SP, substance P; i.v., intravenous(hy).
blue extravasation technique. After electrical stimulation of the cervical vagal nerves for 5 min or 5 min after i.v. injection of SP and capsaicin, the animals were perfused with saline via the aorta. The Evans blue content in the tissue was extracted with formamide and quantified fluorimetrically (19). To study the microscopic localization of the extravasated Evans blue, tissue pieces were immersion fixed in formalin, sectioned in a cryostat, and examined in a fluorescence microscope. Evans blue exhibits a bright red fluorescence when excited at an absorption maximum of ~600 nm (19).

For determination of tissue concentrations of SP-like immunoreactivity (referred to below as SP), tissues were extracted by homogenization and boiling for 10 min in 10 vol of 1.0 M acetic acid. Then, the extracts were centrifuged and lyophilized, and SP was determined by using a highly specific radioimmunoassay (20, 21). The SP immunoreactivity was characterized by gel chromatography on a Sephadex G-25 column.

RESULTS

I.v. injection of SP (2 nmol/kg) or capsaicin (10 nmol/kg) induced an increase in respiratory insufflation pressure, as well as vascular changes as evidenced by a decrease in blood pressure (Fig. 1). A marked increase in insufflation pressure was also induced by vagus nerve stimulation (Fig. 1). After i.v. administration of [D-Arg1,D-Pro2,D-Trp7,9,Leu11]SP, the changes in insufflation-pressure caused by SP, capsaicin, or vagus nerve stimulation were significantly reduced (Figs. 1 and 2). The decrease in blood pressure and changes in heart rate after SP, capsaicin, or vagal stimulation were not reduced by the SP antagonist (Fig. 1).

The concentrations of SP in the trachea and stem bronchi from control guinea pigs were 3.17 ± 0.51 and 7.35 ± 0.54 pmol/g (wet weight) of tissue (mean ± SEM; n = 4 or 5), respectively. After 5 min of bilateral vagal stimulation, the corresponding SP levels were 2.23 ± 0.32 and 3.24 ± 0.59 pmol/g (wet weight) of tissue (n = 5). The reduction in SP level of the stem bronchi was 56% (P < 0.01; Student's t test), suggesting a nervous SP release during vagal stimulation. The total amount of SP extracted from the stem bronchi was also reduced after vagal stimulation (P < 0.05), thus excluding the possibility that the effect on the SP level was due to solely an increase in tissue weight caused by local edema. Gel permeation chromatography of an extract of guinea pig trachea showed that the immunoreactive material detected by the assay to a major extent (>90%) eluted at the position of synthetic bovine SP (data not shown).

Electrical field stimulation of isolated tissues from the respiratory tract showed different responses in the various regions (Fig. 3). Thus, in the trachea, an atropine-sensitive contraction was followed by a long-lasting noncholinergic relaxation. Addition of [D-Arg1,D-Pro2,D-Trp7,9,Leu11]SP did not significantly modify these responses in the trachea (Fig. 3). The main bronchus responded by an initial atropine sensitive contraction that was followed by a long-lasting atropine-resistant increase in bronchial tone. The SP antagonist did not reduce the atropine-sensitive portion of the contraction in the main bronchus but almost completely blocked the atropine-resistant contraction (Fig. 3). After 1 hr of repeated washing, the contractile response gradually returned to control value. The hilus bronchus usually responded to field stimulation with a long-lasting atropine-resistant contraction (Fig. 3). This contraction was almost totally blocked by [D-Arg1,D-Pro2,D-Trp7,9,Leu11]SP (Fig. 3). In two out of six animals, a small relaxation of the hilus bronchus was observed on field stimulation in the presence of the SP antagonist. These differences in the response to field stimulation of the various parts of the respiratory tract were seen in most preparations although, in some, a small cholinergic component was also observed in the hilus bronchi. SP (0.1 μM) or capsaicin (0.1 μM) also induced long-lasting contractions in all three regions of the bronchial tree. In the hilus bronchi, the

![Graph](image-url)

**Fig. 1.** Effects of i.v. injection of SP (2 nmol/kg) and capsaicin (10 nmol/kg) and of bilateral cervical vagus nerve stimulation (10 Hz, 5 ms, 10 V) on respiratory insufflation pressure (kPa), systemic arterial blood pressure (mm Hg), and heart rate (beats per min) in an anesthetized guinea pig. After the decrease of the responses, the animal was allowed to rest for 10 min. Time scale is 1 min between large bars. [D-Arg1,D-Pro2,D-Trp7,9,Leu11]SP (total dose, 10 μmol/kg) was given after control responses. Then, an additional dose (3 μmol/kg) was administered 1 min before injection of SP or capsaicin or nerve stimulation. The animal had been treated previously with mesyramine/cimetidine (each, 1 mg/kg).
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DISCUSSION

The present data show that a SP antagonist, ([D-Arg¹,D-Pro²,D-Trp⁷⁹,Leu¹¹]SP, markedly reduced the vagally or capsaicin-induced increase in bronchial smooth muscle tone, as well as the increased extravasation of Evans blue in the respiratory tract and esophagus after vagus nerve stimulation. This suggests that antidiromic or local activation of sensory neurons of vagal origin induces a local release of SP, causing bronchoconstriction and local edema in the respiratory tract. The usefulness of the SP antagonist was enhanced by the finding that virtually all of its stimulatory effects were blocked by histamine antagonists. The SP-immunoreactive nerves in the trachea, stem bronchi (17), and esophagus are capsaicin sensitive and originate mainly from vagal sensory ganglia (23). The occurrence of a noncholinergic excitatory component in the regulation of bronchial smooth muscle tone has recently been demonstrated both in vitro (22) and in vivo (14). This component, which occurs mainly in the distal bronchial tree (22), is characterized by a long-lasting contraction. Recent observations indicate that this type of contraction is absent in animals previously treated with capsaicin (14).

In vivo experiments with capsaicin-treated animals (14) and the present data from experiments using the SP antagonist suggest that SP release from sensory nerves causes most of the vagally induced increase in insufflation pressure. Sensory SP nerves also exist in the tracheal smooth muscle, although no certain reduction of the contractile response in the trachea to field stimulation was observed in capsaicin-treated animals (14) or after incubation with the SP antagonist. Furthermore, capsaicin also contracts the trachea. The present data suggest that this contraction is due to SP release from sensory nerves. This is in agreement with the effects of capsaicin on the central branch of

![Graph of response of guinea pig trachea (upper trace), main bronchus (middle trace), and hilus bronchus (lower trace) in vitro to electrical field stimulation (50 V, 1 ms, 10 Hz) for 30 s. After the decrease of the response to nerve stimulation, the preparations were allowed to rest for at least 15 min. Mepyramine (10 µM) was present throughout the experiment. Atropine (1 µM) was present in B, C, and D and [D-Arg¹,D-Pro²,D-Trp⁷⁹,Leu¹¹]SP (50 µM) was present in C. The response in D was obtained in the presence of atropine and mepyramine as above 1 hr after washout of the SP antagonist. Time scale is 1 min between large bars.](image-url)
FIG. 4. Fluorescence micrographs of guinea pig trachea (A–C) and esophagus (D–F). (A and D) Control animals receiving Evans blue at 20 mg/kg. Yellow autofluorescence in the trachea (A and C) and esophagus (D and F) represent connective tissue components; the innermost layer of the squamous epithelium of the esophagus also has a yellow autofluorescence. (B and E) Extravasation of Evans blue as indicated by bright red fluorescence (mainly in the subepithelial layers) after 5 min of cervical vagus nerve stimulation. (C and F) Absence of Evans blue extravasation in the trachea and esophagus on vagal nerve stimulation in an animal previously treated with the SP antagonist (total dose, 10 μmol/kg).

sensory nerves (5). The simultaneous presence of the strong dilatory component in the trachea may, however, mask the effects of SP release from local sensory nerves. Earlier data from capsaicin-treated animals indicate an interaction between acetylcholine and SP neurons in vivo (14). This result is supported by the finding that the vagally induced insufflation-pressure response was reduced by 78% after administration of the SP antagonist although the atropine-resistant component has been found to be about 40% (14).

The selectivity of the SP antagonist was substantiated by several observations. The vagally induced decrease in blood pressure or bradycardia was unaffected. Furthermore, the vascular effects of SP and capsaicin were virtually unchanged, confirming previous findings that the decrease in blood pressure induced by SP is much more resistant to SP antagonists than is that of smooth muscle contractions (24). This may be explained by the existence of different types of SP receptors (16, 24). The specificity of the SP antagonist was also supported by the observation that the atropine-sensitive contractions and atropine-resistant relaxations in vitro were not blocked by [D-Arg²,D-Pro⁵,D-Trp⁹,Leu¹⁴]SP. Vagally induced neurogenic inflammation has been described in the rat respiratory tract (13, 25). Also, this reaction was absent in capsaicin-treated animals, which, together with the present data from experiments using the SP antagonist, indicates that SP is mediating this response. Many capsaicin-sensitive SP nerves are also present where the Evans
blue leakage was most pronounced—i.e., close to the lining respiratory epithelium (17). In contrast to observations from the skin (4), the effect of SP on permeability in the lung and esophagus did not seem to be indirect via histamine release.

Capsaicin-sensitive SP nerves are generally believed to belong to the C-fiber group and have characteristics of chemo-sensitive nociceptive neurons (3, 6). Vagal afferent C fibers are present in the respiratory tract (26). These C fibers appear to have little influence on the normal control of breathing (26) but exhibit functional properties of visceral nociceptor nerves, which are activated by chemical stimuli (such as capsaicin), local tissue damage, or anaphylactic reactions (26). The strong inhibition of the vagally induced bronchoconstriction and edema formation by [D-Arg1, D-Pro2, D-Trp7,9, Leu11]SP indicates that the SP nerves may have a major role in the local regulation of airway resistance and interstitial fluid transfer in various pathological conditions.

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