5-Hydroxytryptamine-induced bronchoconstriction in the guinea-pig: effect of 5-HT$_2$ receptor activation on acetylcholine release

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1 The bronchoconstrictor responses to 5-hydroxytryptamine (5-HT) were studied in the guinea-pig to establish whether they are partly attributable to parasympathetic activation within the airways. 5-HT dose-response curves were constructed in anaesthetized and ventilated guinea-pigs pretreated with saline, or by bilateral cervical vagotomy or vagotomy plus atropine 3 mg kg$^{-1}$ i.v. Vagotomy had no effect on 5-HT-induced bronchoconstriction but vagotomy plus atropine significantly reduced it.

2 To determine whether parasympathetic activation within the airways resulted from pre- or postganglionic stimulation, 5-HT dose-response curves were constructed for two groups of vagotomized guinea-pigs treated with hexamethonium 2 mg kg$^{-1}$, or hexamethonium 2 mg kg$^{-1}$ plus atropine 3 mg kg$^{-1}$. Guinea-pigs treated with hexamethonium plus atropine experienced significantly less 5-HT-induced bronchoconstriction than those treated with hexamethonium alone.

3 To characterize the subtype of 5-HT receptors involved in the activation of the parasympathetic system by 5-HT, dose-response curves to 5-HT were constructed for four groups of vagotomized guinea-pigs treated with saline, 1 mg kg$^{-1}$ of the 5-HT$_2$ antagonist ICS 205-930, or either 0.01 or 0.1 mg kg$^{-1}$ of the 5-HT$_2$ antagonist ketanserin. ICS 205-930 enhanced 5-HT-induced bronchoconstriction but 0.01 mg kg$^{-1}$ ketanserin inhibited it significantly and 0.1 mg kg$^{-1}$ ketanserin abolished it. To confirm the involvement of 5-HT$_2$ receptors in these responses, we studied the effects in vagotomized guinea-pigs of atropine on the bronchoconstriction induced by the 5-HT$_2$ agonist, a-methyl-5-HT, infused at rates of 40 and 80 ng kg$^{-1}$ s$^{-1}$. At both rates, atropine significantly reduced the bronchoconstrictor responses to a-methyl-5-HT.

4 The above results indicate that 5-HT-induced bronchoconstriction is indeed partly mediated by parasympathetic activation within the airways. This activation is mediated by stimulation of 5-HT$_2$ receptors which are probably located on the postganglionic parasympathetic nerve endings.

Keywords: 5-HT-induced bronchoconstriction; respiratory system; ketanserin; ICS 205-930; a-methyl-5-HT; parasympathetic system

Introduction

5-Hydroxytryptamine (5-HT) is a bronchoconstrictor agent which has been widely used to study bronchial reactivity in several species. 5-HT-induced bronchoconstriction is attributed to the direct action of 5-HT on airway smooth muscle and to a central vagal reflex resulting from the stimulation of irritant receptors in the airways. Parasympathetic nerve activation within the airways may also contribute to the bronchoconstrictor effects of 5-HT, independently of central reflex pathways. 5-HT has been shown to facilitate ganglionic transmission (Wallis & Woodward, 1975; Round & Wallis, 1986) and has been demonstrated in isolated tracheal smooth muscle preparations to induce the release of acetylcholine by interacting with presynaptic neuronal receptors (Aas, 1983).

In vitro results suggest that in the guinea-pig, 5-HT-induced bronchoconstriction could indeed result from parasympathetic system activation in the airways. Thus, in an innervated preparation of guinea-pig trachea, McCaig (1986) reported that atropine partially blocked the increase in intraluminal pressure induced by 5-HT. In guinea-pig tracheal strips, Baumgartner et al. (1990) showed that atropine significantly reduced the contractile response to 5-HT. In guinea-pig isolated perfused lungs, Bhattacharya (1955) demonstrated that atropine prevented 5-HT-induced bronchoconstriction. However, the existence of local parasympathetic activation within the airways in the bronchoconstriction induced by 5-HT in vivo has not been established.

The aims of our study were therefore: (1) to determine whether the bronchoconstrictor response to 5-HT in the guinea-pig is in part attributable to parasympathetic activation within the airways and (2) to characterize the subtype of 5-HT receptors involved in the interaction between the parasympathetic system and 5-HT, using selective 5-HT receptor agonists and antagonists.

Methods

Animals and general procedures

All experiments were performed on male Hartley strain guinea-pigs weighing 250-300 g (Charles River, France), housed in a temperature-controlled room (21°C) with food and water freely available. Guinea-pigs were anaesthetized i.p. with 40 mg kg$^{-1}$ pentobarbitone sodium. A repeat dose of 10 mg kg$^{-1}$ was given i.p. every 45 min throughout the experiments. These doses were selected so that the animals were surgically anaesthetized during the experiments. The trachea was cannulated, and polyethylene catheters were placed in the external jugular veins for drug administration and in the left common carotid artery for monitoring of blood pressure and heart rate via an indwelling cannula connected to a pressure transducer (Gould). The animals were mechanically ventilated...
and were paralysed by i.v. injection of 0.1 mg kg\(^{-1}\) vecuronium bromide repeated every 30 min throughout the experiments. Respiratory frequency was set at 60 cycles min\(^{-1}\) and tidal volume at 6 ml kg\(^{-1}\). Body temperature was kept constant at 38°C by placing the animals on a thermostatically controlled heated blanket (Animal Blanket Unit, Harvard) to avoid non-specific changes in bronchial reactivity (Macquin-Mavier et al., 1989). Before the experiments began, the guinea-pigs were allowed 20 min to recover from the preparation procedure.

**Measurement of respiratory mechanics**

Airway responses to 5-HT were assessed by measuring respiratory system conductance and compliance. Both parameters were monitored continuously and determined on line from the total respiratory pressure, inspiratory flow and tidal volume signals, as described previously (Lorino et al., 1988). Tracheal pressure was measured with a Validyne MP45 + 56 cmH\(_{2}\)O differential pressure transducer. Inspiratory flow was measured with a heated Fleisch +000 pneumotachograph connected to a Validyne MP45 + 2.5 cmH\(_{2}\)O differential pressure transducer. Airflow was integrated for measurement of tidal volume. Pressure and flow signals were digitized at a sample rate of 16 Hz and fed into an Apple IIE microcomputer. Respiratory conductance and compliance were calculated for each breath and their averages displayed every 15 s. A large inflation was performed manually every 5 min by occlusion of the expiratory valve for three respiratory cycles to prevent alveolar atelectasis.

**Experimental protocol**

The following procedure was used for all series of experiments: after the 20 min stabilization period, the guinea-pigs were injected i.v. with a bolus of 1 mg kg\(^{-1}\) propranolol to minimize potential changes in bronchoconstrictor responses resulting from changes in levels of circulating catecholamines. Ten minutes later, they were given three sequential 5-HT challenges with increasing doses of 5-HT (40, 60 and 80 ng kg\(^{-1}\) s\(^{-1}\) respectively) at 15 min intervals. During each challenge, 5-HT was infused i.v. for 5 min until a plateau response was reached. Ten minutes elapsed between each challenge to allow respiratory parameters to return to within 5% of baseline values.

Three studies were performed. In the first, we explored the bronchoconstrictor responses to i.v. administered 5-HT, to see if they included a central vagal reflex and/or a significant peripheral cholinergic component. We reasoned that, if the local effects of 5-HT on cholinergic nerves in the airways was important in the mediation of 5-HT-induced bronchoconstriction, then in vagotomized guinea-pigs, treatment with atropine should reduce the airway responses to 5-HT. Accordingly, three groups of eight guinea-pigs each were pretreated i.v. with saline, bilateral cervical vagotomy or vagotomy plus 3 mg kg\(^{-1}\) atropine respectively, 10 min before 5-HT was injected and the dose-response curve recorded.

In the second study, we attempted to establish whether the peripheral cholinergic component of the airway responses to 5-HT may represent a stimulatory action of 5-HT on the efferent parasympathetic ganglia and/or at a site distal to the ganglia. For this purpose, we evaluated the airway responses to 5-HT in two groups of seven vagotomized guinea-pigs each, treated i.v. with either 2 mg kg\(^{-1}\) of the ganglion blocking agent hexamethonium or 2 mg kg\(^{-1}\) hexamethonium plus 3 mg kg\(^{-1}\) atropine 10 min before 5-HT injection and dose-response curve recording.

In the third study, we aimed to characterize the subtype of 5-HT receptors involved in the interaction between 5-HT and the peripheral cholinergic system. We evaluated the airway responses to 5-HT in four groups of vagotomized guinea-pigs treated i.v. with saline (n = 6), the 5-HT\(_{3}\) antagonist ICS 205-930 (1 mg kg\(^{-1}\)) (n = 6) or one of two doses of the 5-HT\(_{2}\) antagonist ketanserin (0.01 or 0.1 mg kg\(^{-1}\)) (n = 5 and 3 respectively) 10 min before 5-HT injection and dose-response curve recording. Since we found that ketanserin abolished 5-HT-induced bronchoconstriction, we studied two additional groups of six vagotomized guinea-pigs each, to confirm the involvement of 5-HT\(_{2}\) receptors in the peripheral cholinergic activation by 5-HT. These guinea-pigs were treated i.v. with saline or 3 mg kg\(^{-1}\) atropine before two consecutive challenges with the 5-HT\(_{3}\) agonist, a-methyl-5-hydroxytryptamine maleate (a-methyl-5-HT), administered as 5 min infusions at rates of 40 and 80 ng kg\(^{-1}\) s\(^{-1}\) respectively.

**Drugs**

5-Hydroxytryptamine hydrochloride, hexamethonium bromide and atropine sulphate were purchased from Sigma and dissolved in physiological saline. Ketanserin was a gift from Janssen and ICS 205-930 (3-tropanyl-indole-3-carboxylate), from Sandoz Ltd. a-Methyl-5-hydroxytryptamine was purchased from Research Biochemicals Incorporated. Ketanserin, ICS 205-930, and a-methyl-5-hydroxytryptamine were dissolved in distilled water. All drugs were injected in a final volume of 1 ml kg\(^{-1}\). All doses are expressed in terms of their corresponding base.

**Analysis of data**

Data are expressed as means ± s.e. Dose-response curves for 5-HT were analysed by repeated-measures analysis of variance (BMDP statistical software). If there was an interaction between the dose of 5-HT and the treatment, the analysis of variance was followed by the Mann-Whitney U test. P values of less than 0.05 were considered statistically significant.

**Results**

**Effects of vagotomy and of vagotomy plus atropine on 5-hydroxytryptamine-induced bronchoconstriction**

5-HT (40-80 ng kg\(^{-1}\) s\(^{-1}\)) caused dose-related bronchoconstriction. As shown in Figure 1, bilateral cervical vagotomy had no significant effect on 5-HT-induced bronchoconstriction. In contrast, this bronchoconstriction was significantly reduced when the vagotomized guinea-pigs were pretreated with atropine, whatever the degree of bronchoconstriction. Baseline respiratory conductance and compliance were not significantly different in guinea-pigs which underwent bilateral cervical vagotomy or vagotomy plus atropine compared to control levels (Table I).

![Figure 1](attachment:image.png)  
**Figure 1** Effects of bilateral cervical vagotomy (stippled columns) or bilateral cervical vagotomy plus atropine (solid columns) on the decreases in respiratory system conductance and compliance induced by 5-hydroxytryptamine (5-HT) infused at rates of 40, 60 and 80 ng kg\(^{-1}\) s\(^{-1}\); controls, open columns. Each group included 8 guinea-pigs. Vagotomy did not affect the bronchoconstrictor response to 5-HT, whereas vagotomy plus atropine significantly reduced it, whatever the degree of bronchoconstriction. (**P < 0.01 versus controls).
Effects of atropine on 5-hydroxytryptamine-induced bronchoconstriction in vagotomized guinea-pigs treated with hexamethonium

In vagotomized guinea-pigs pretreated with 2 mg kg\(^{-1}\) hexamethonium, bronchoconstrictor responses to 5-HT were significantly enhanced when 5-HT was infused at rates of 40 and 60 ng kg\(^{-1}\) s\(^{-1}\) (P < 0.05). As shown in Figure 2, in vagotomized guinea-pigs pretreated with hexamethonium, atropine significantly reduced the bronchoconstrictor responses to 5-HT, whatever the degree of bronchoconstriction.

Effects of ICS 205-930 and ketanserin on 5-hydroxytryptamine-induced bronchoconstriction in vagotomized guinea-pigs

Treatment with 1 mg kg\(^{-1}\) ICS 205-930 i.v. slightly but significantly enhanced the decrease in respiratory conductance induced by 5-HT; it also tended to enhance the decrease in respiratory compliance induced by 5-HT, although not significantly (P = 0.056). Treatment with 0.01 mg kg\(^{-1}\) ketanserin i.v. significantly reduced 5-HT-induced bronchoconstriction, and treatment with 0.1 mg kg\(^{-1}\) i.v. ketanserin completely abolished it (Figure 3).

Effects of atropine on the bronchoconstrictor induced by \(\alpha\)-methyl-5-HT in vagotomized guinea-pigs

\(\alpha\)-Methyl-5-HT (40–80 ng kg\(^{-1}\) s\(^{-1}\)) induced dose-dependent bronchoconstriction, which was significantly reduced by atropine (Figure 4).

Discussion

In this study, we attempted to determine whether parasympathetic activation within the airways is involved in the bronchoconstrictor responses to 5-HT in the guinea-pig. We conducted all our experiments in guinea-pigs pretreated with propranolol, and some of them in vagotomized guinea-pigs, to eliminate any possible modulation of airway contraction by activation of the sympathetic nervous system and/or the non-adrenergic inhibitory system (Clerici et al., 1989).

We found that atropine plus bilateral cervical vagotomy attenuated 5-HT-induced bronchoconstriction, whereas bilateral vagotomy alone had no effect. While vagotomy blocks only the central reflexes, atropine antagonizes the actions of acetylcholine resulting from either centrally or locally induced activation of the cholinergic system. While it is theoretically possible that atropine inhibits 5-HT-induced bronchoconstriction by a direct action at 5-HT receptors, there is no evidence that it exhibits such non-specific activity. Furthermore, Advenier et al. (1984) have previously shown that in guinea-pig trachea in vitro, atropine is of the order of 10,000 times more potent against acetylcholine than against 5-HT. Our results strongly suggest therefore that, in addition to exerting direct action on airway smooth muscle, 5-HT caused bronchoconstriction which was partly mediated by cholinergic activation within the airways, and independently of central reflex pathways. In contrast to the results of previous studies in the dog and cat (Hahn et al., 1978; Parratt et al., 1982) demonstrating marked reduction of the bronchoconstrictor responses to 5-HT after bilateral vagotomy, we did not find a vagally mediated reflex component in the bronchoconstriction.
that 5-HT induced in the guinea pig. However, our findings do not rule out the possibility that reflex-mediated parasympathetic activation is involved in the bronchoconstrictor responses to 5-HT, since in the guinea-pig, vagotomy was earlier found, not only to suppress parasympathetic pathways but also to block reflex activation of the non adrenergic inhibitory system which modulates 5-HT-induced bronchoconstriction (Clerici et al., 1989). In the dog and rabbit, parasympathetic activation within the airways was recently demonstrated to play a significant role in the airway responses to other bronchoconstrictor agents, e.g. histamine, platelet activating factor (PAF) and substance P (Shore et al., 1985; Leff et al., 1987; Grunstein et al., 1984). As regards PAF and substance P, parasympathetic activation within the airways appears to be the sole contribution of the parasympathetic system to their bronchoconstrictor effects. Our results demonstrate that this mechanism also contributes to 5-HT-induced bronchoconstriction in the guinea-pig.

Because parasympathetic ganglia lie within the airway wall (Skogh, 1988), the parasympathetic nerve structures which might be activated within the airways by 5-HT after vagotomy include not only postganglionic pathways but also the parasympathetic ganglia. 5-HT has been shown to activate pre- and postganglionic sympathetic nerve terminals in guinea-pig tracheal strips (Baumgartner et al., 1990), and may also stimulate pre synaptic receptors on cholinergic nerve terminals, as demonstrated in the isolated bronchial smooth muscle of the rat (Aas, 1983). To determine the site of the peripheral cholinergic action of 5-HT, we compared the bronchoconstrictor responses to 5-HT by vagotomized guinea-pigs after ganglionic blockade with hexamethonium and after ganglionic plus muscarinic blockade with hexamethonium plus atropine. The observation that hexamethonium alone did not inhibit bronchoconstrictor responses to 5-HT, but that additional atropine treatment did cause a significant reduction suggests that the predominant site of the cholinergic action of 5-HT is located distal to the airway parasympathetic ganglia, i.e. on the parasympathetic nerve terminals. The additional observation that hexamethonium alone appeared actually to enhance 5-HT-induced responses was unexpected, and of uncertain significance.

To characterize the 5-HT receptor subtype involved in the interaction between 5-HT and the parasympathetic system, we studied the effects of the selective 5-HT2 antagonist, ICS 205-930 (Richardson et al., 1985) and of the selective 5-HT1 antagonist, ketanserin on the bronchoconstrictor responses to 5-HT in vagotomized guinea-pigs. The release of acetylcholine by 5-HT may result from the excitation of the 5-HT1 receptors on parasympathetic nerve terminals, as reported for rat bronchi and rabbit heart (Wallis, 1989). However, in our experiments, ICS 205-930 did not reduce but rather enhanced 5-HT-induced bronchoconstriction. In contrast, ketanserin inhibited it. Similar inhibition by ketanserin of the bronchoconstrictor responses to the 5-HT was previously reported in guinea-pig isolated perfused lung by Selig et al. (1989) and in cats by Ball et al. (1983). It is well established that the 5-HT receptors mediating tracheal smooth muscle contraction are of the 5-HT1 subtype (Cohen et al., 1985; Lemoine & Kauman, 1986). Since there is no evidence that ketanserin interacts with muscarinic receptors (Awouters et al., 1982), the most likely explanation for our results is that ketanserin prevented acetylcholine release. If true, this would suggest that the 5-HT receptors located on the parasympathetic nerve terminals are of the 5-HT1 subtype. To confirm such involvement of 5-HT1 receptors in the interaction between 5-HT and the parasympathetic system within the airways, we induced bronchoconstriction by infusing vagotomized guinea-pigs with the selective 5-HT1 agonist, a-methyl-5-HT (Richardson et al., 1985), and found that atropine significantly reduced a-methyl-5-HT-induced bronchoconstriction.

In conclusion, our data suggest that the bronchoconstriction induced by infusion of 5-HT is elicited by two mechanisms: direct constriction of bronchial smooth muscle and indirect constriction of this muscle by postganglionic stimulation of the parasympathetic nerves. Both mechanisms are mediated by the stimulation of 5-HT1 receptors.

References


25


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