Pharmacological characterization of 5-hydroxytryptamine-induced excitation of afferent cervical vagus nerve in anaesthetized rats

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1 An excitatory response to 5-hydroxytryptamine (5-HT) was measured from the afferent vagus nerve of anaesthetized rats. Measurements were determined by an extracellular recording from the whole nerve.

2 Intravenous bolus injection of 5-HT ($1.56\text{--}100\text{ }\mu\text{g kg}^{-1}$) evoked a dose-dependent excitation of afferent vagus nerve activity. This response was blocked not only by a selective 5-HT₃ receptor antagonist, GR38032F ($10\text{ and }100\text{ }\mu\text{g kg}^{-1}$), but also by a 5-HT₂ receptor antagonist, ketanserin ($10\text{ and }100\text{ }\mu\text{g kg}^{-1}$).

3 Both a 5-HT₃ receptor agonist, 2-methyl-5-HT ($3.12\text{--}100\text{ }\mu\text{g kg}^{-1}$), and a 5-HT₂ receptor agonist, α -methyl-5-HT ($3.12\text{--}50\text{ }\mu\text{g kg}^{-1}$), produced a dose-dependent excitation of afferent vagus nerve activity. These excitatory effects were antagonized by GR38032F ($10\text{ }\mu\text{g kg}^{-1}$) and ketanserin ($10\text{ }\mu\text{g kg}^{-1}$), respectively.

4 A 5-HT₁ like receptor agonist, 5-carboxamidotryptamine ($50\text{ }\mu\text{g kg}^{-1}$), and a putative 5-HT₄ receptor agonist, 5-methoxytryptamine ($100\text{ }\mu\text{g kg}^{-1}$), failed to produce excitatory effects on the afferent vagus nerve.

5 These results suggest that the 5-HT-induced excitatory response of the afferent vagus nerve might be mediated not only via 5-HT₃ receptors but also via 5-HT₂ receptors in anaesthetized rats. It is unlikely, however, that either 5-HT₁-like or putative 5-HT₄ receptors are involved in the excitatory response of the afferent vagus nerve to 5-HT.

Keywords: 5-Hydroxytryptamine; sensory neurone; vagus nerve activity; 5-HT receptors

Introduction

The excitatory action of 5-hydroxytryptamine (5-HT) is seen in a variety of neurones in the mammalian peripheral nervous system (Fozard, 1984). In particular, 5-HT has a potent depolarizing action on the vagal afferent nerve (Round & Wallis, 1986; Ireland & Tyers, 1987). Most previous reports have concerned *in vitro* experiments, i.e., isolated vagus nerve, nodose ganglion or superior cervical ganglion without nerve endings. The depolarization of these neurones and ganglia by 5-HT *in vitro* is blocked by 5-HT₃ receptor antagonists but not by the cholinergic antagonists, atropine, hexamethonium or the 5-HT₂ receptor antagonists, methysergide or ketanserin (Neto, 1978; Higashi & Nishi, 1982; Ireland *et al.*, 1982; Wallis *et al.*, 1982; Bradly *et al.*, 1986; Round & Wallis, 1986; Ireland & Tyers, 1987). However, many peripheral sensory nerve endings are potently excited by 5-HT and this elicits prominent reflex effects on the cardiopulmonary system (Wallis, 1989). Kirby & McQueen (1984) reported that intracarotid injection of 5-HT in cats produces a triphasic chemoexcitation of the carotid sinus nerve. Both the initial transient chemoexcitation and the subsequent depression are virtually abolished by a 5-HT₃ receptor antagonist, MDL 72222. On the other hand, the delayed excitation is substantially reduced by a 5-HT₂ receptor antagonist, ketanserin. These findings suggest that *in vivo*

responses cannot be extrapolated from *in vitro* responses because 5-HT₂ receptors as well as 5-HT₃ receptors may be involved in the excitatory response to 5-HT *in vivo*. To date, there is no literature concerning the pharmacological characteristics of the effects of 5-HT on afferent vagus nerve activity *in vivo*. In this study, therefore, direct extracellular recordings of the afferent cervical vagus nerve of anaesthetized rats were made in order to elucidate the pharmacological characteristics of the receptors mediating this 5-HT-induced excitatory effect *in vivo*.

Methods

General

Male Wistar rats (320–400 g b.w. and 8–12 weeks old) were used (Shizuoka Laboratory Animal Center, Hamamatsu, Japan). Rats were anaesthetized i.p. with 500 mg kg^{-1} of urethane and 50 mg kg^{-1} of α -chloralose. After immobilization with gallamine triethiodide (10 mg kg^{-1} , i.v.), respiration was maintained through a tracheal cannula connected to a rodent respirator (Harvard apparatus, model 683). End tidal O₂ and CO₂ concentrations were measured with an expired gas monitor (Nippondenki San-ei, Tokyo, Japan, IH26). Ventilation was adjusted to maintain an end tidal O₂ and CO₂ at approximately 15% to 16% and 4.5% to 5.0%, respectively. Drugs were administered i.v. through a catheter inserted into the femoral vein. Rectal temperature was maintained between 37 and 38°C with a heating pad.

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Nerve recording

The right cervical vagus nerve was exposed and sectioned at its junction with the superior laryngeal nerve under an operating microscope. Afferent cervical vagus nerve activity was recorded from the peripheral cut end of the nerve with bipolar platinum-iridium wire electrodes. This portion of the nerve was immersed in a pool of warm paraffin oil. The nerve activity was amplified, passed through a filter (time constant of 0.001 s, and high cut filter of 1000 Hz) and displayed with a thermal array recorder (Nihon Kohden, WS-682G). The discharge rate of this nerve was counted every 1 or 2 s by a real-time data analyzer (Nihon Kohden, Tokyo, Japan, ATAC-450).

Drugs and statistics

The following drugs were used: 5-hydroxytryptamine (5-HT) creatinine sulphate (Sigma Chemical Co., St Louis, MO, U.S.A.); 2-methyl-5-hydroxytryptamine maleate (2-methyl-5-HT, Nippon Glaxo Ltd., Tokyo, Japan); α -methyl-5-hydroxytryptamine maleate (α -methyl-5-HT, Sandoz Ltd., Basel, Switzerland); 5-carboxamidotryptamine maleate (5-CT, Glaxo); 5-methoxytryptamine hydrochloride (Sigma); GR38032F (1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1H-imidazol-1-yl) methyl]-4H-carbazol-4-one, hydrochloride, dihydrate, Glaxo); methoxamine hydrochloride (Nihon Shinyaku, Tokyo, Japan). The agonists were dissolved in the Locke solution; antagonists were dissolved in normal saline. The composition of the Locke solution was (in mM): NaCl 157, KCl 1.88, CaCl₂ 1.08, NaHCO₃ 7.98 and glucose 5.55

(pH = 7.3). The volume injected was kept constant at 500 μ l kg^{-1} . Dose-response curves for 5-HT, 2-methyl-5-HT and α -methyl-5-HT were constructed noncumulatively with a dose-cycle greater than 10 min to avoid tachyphylaxis (Ginzel & Kattegoda, 1954). The antagonists were administered intravenously 5 min before the injection of the agonists throughout the study. Lateral displacements of agonist dose-response curves were measured at the control half-maximum response level (ED_{50}). Data were expressed as the mean $\pm$ s.e.mean. Analysis of variance followed by the Student's *t* test was used for statistical comparison.

Results

Effects of GR38032F on the 5-hydroxytryptamine-induced increase in cervical vagus nerve activity

Intravenous bolus injection of 5-HT (3.12 – $100 \mu\text{g kg}^{-1}$) produced a dose-dependent increase in afferent cervical vagus nerve activity (Figure 1). The ED_{50} was $28.0 \pm 2.1 \mu\text{g kg}^{-1}$ ($n = 36$). Vehicle (saline) did not affect the 5-HT dose-response curve and the ED_{50} value was $28.9 \pm 4.8 \mu\text{g kg}^{-1}$ ($n = 6$, Figure 2a). The maximum response induced by 5-HT was estimated to be $146.4 \pm 8.2\%$ of the pre-injection level. A selective 5-HT₃ receptor antagonist, GR38032F (10 and $100 \mu\text{g kg}^{-1}$), caused a rightward shift in the 5-HT dose-response curve. The ED_{50} s were 50.4 ± 7.8 ($P < 0.05$ vs saline, $n = 11$) and $63.5 \pm 13.4 \mu\text{g kg}^{-1}$ ($P < 0.05$ vs saline, $n = 6$), respectively (Figure 2a). However, GR38032F itself did not alter the discharge of the nerves significantly.

Figure 1 Effect of intravenous injection of 5-hydroxytryptamine (5-HT) on cervical vagus nerve activity in an anesthetized rat. Top two panels, control experiment; lower two panels, pretreatment with GR38032F $10 \mu\text{g kg}^{-1}$, 5 min before the next injection regime of 5-HT. A minimum of 10 min was allowed between the injection of each dose of 5-HT to avoid tachyphylaxis and allow for blood pressure recovery. Traces are the actual records of cervical vagus nerve activity (CVNA), pulse counts per 2 s, blood pressure (BP, mmHg) and heart rate (HR, b.p.m.). All records are from the same animal.

Figure 2 (a) Effect of GR38032F on the 5-hydroxytryptamine (5-HT)-induced increase in cervical vagus nerve activity. Symbols indicate control response (●); saline (○); GR38032F 10 µg kg⁻¹ (Δ) and 100 µg kg⁻¹ (▲). (b) Effect of ketanserin on the 5-HT-induced increase in cervical vagus nerve activity. Symbols indicate control response (●); saline (○); ketanserin 10 µg kg⁻¹ (▲) and 100 µg kg⁻¹ (Δ).

Effects of ketanserin on the 5-hydroxytryptamine-induced increase in cervical vagus nerve activity

A 5-HT₂ receptor antagonist, ketanserin (10 and 100 µg kg⁻¹), caused a rightward shift in the 5-HT dose-response curve. The ED₅₀s were 88.2 ± 26.4 µg kg⁻¹ ($P < 0.05$ vs saline, $n = 6$) and 163.9 ± 32.6 µg kg⁻¹ ($P < 0.001$ vs saline, $n = 4$), respectively (Figure 2b and 3). However, ketanserin itself did not alter the discharge of the nerves significantly.

Effects of 5-hydroxytryptamine agonists on cervical vagus nerve activity

A 5-HT₃ receptor agonist, 2-methyl-5-HT (3.12–100 µg kg⁻¹), also produced a dose-dependent but not marked increase in cervical vagus nerve activity. The ED₅₀ was 18.3 ± 2.4 µg kg⁻¹ ($n = 5$, Figure 4a and 5a). Although the maximum response induced by 2-methyl-5-HT reached only 107.4 ± 2.3% of the pre-injection level, GR38032F (10 µg kg⁻¹) caused a significant rightward shift in the 2-methyl-5-HT dose-response curve. The ED₅₀ was 50.7 ± 11.3 µg kg⁻¹ ($P < 0.05$, Figure 5a).

A 5-HT₂ receptor agonist, α-methyl-5-HT (3.12–50 µg kg⁻¹), produced a marked and dose-dependent increase in cervical vagus nerve activity (Figure 4b). The ED₅₀ was 13.6 ± 2.9 µg kg⁻¹ ($n = 5$, Figure 5b). The maximum response to α-methyl-5-HT reached 162.8 ± 5.4% as compared with the pre-injection level. Ketanserin (10 µg kg⁻¹) caused a significant rightward shift in the α-methyl-5-HT dose-

response curve. The ED₅₀ was 41.2 ± 9.0 µg kg⁻¹ ($P < 0.05$ vs saline, Figure 5b).

Both a 5-HT₁ receptor agonist, 5-carboxamidotryptamine (50 µg kg⁻¹), and a putative 5-HT₄ receptor agonist, 5-methoxytryptamine (100 µg kg⁻¹), failed to increase cervical vagus nerve activity (Figure 6a and b).

In addition to being a potent 5-HT₂ receptor antagonist, ketanserin also has α₁-adrenoceptor blocking effects (Fozard, 1982; McCall & Schuette, 1984). In order to exclude the involvement of the α₁-adrenoceptor mechanism, we have examined the effect of the α₁-agonist, methoxamine, on cervical vagus nerve activity. Methoxamine (100 µg kg⁻¹) failed to produce an increase in cervical vagus nerve activity in spite of its effective pressor response (Figure 6c).

Discussion

In the present study, intravenous bolus injection of 5-HT produced a dose-dependent increase in mass discharges of cervical vagus nerve activity in anaesthetized rats. Treatment with a potent and selective 5-HT₃ receptor antagonist, GR38032F (Butler *et al.*, 1988), and a 5-HT₂ receptor antagonist, ketanserin (Van Nueten *et al.*, 1981), caused a significant rightward shift in the 5-HT dose-response curve. A selective 5-HT₃ receptor agonist, 2-methyl-5-HT (Hoyer & Neijt, 1988), and a selective 5-HT₂ receptor agonist, α-methyl-5-HT (Richardson *et al.*, 1985), also elicited dose-dependent increases in cervical vagus nerve activity. GR38032F and ketanserin caused rightward displacement of the 2-methyl-5-HT and α-methyl-5-HT dose-response curves, respectively. These results suggest that not only 5-HT₃ receptors but also 5-HT₂ receptors might play an important role in the excitatory effect of 5-HT on the afferent vagus nerve *in vivo*. In terms of 5-HT₃ receptors, our results were in agreement with previous *in vitro* reports (Round & Wallis, 1986; Ireland & Tyers, 1987). Thus, the 5-HT-induced excitation of the afferent vagus nerves in anaesthetized rats has been shown to involve the 5-HT₃ receptor as well as the rat isolated vagus nerve.

It is also of interest to note that 5-HT₂ receptors were involved in the 5-HT-induced excitatory effect on cervical vagus nerve activity. In the present study, α-methyl-5-HT was found to produce activation closely resembling that induced by 5-HT. Thus, α-methyl-5-HT was similar to 5-HT in terms of the duration and pattern of evoked discharges of cervical vagus nerve activity (Figures 1 and 4b). The maximum response to α-methyl-5-HT reached 162.8 ± 5.4% and to 5-HT was 146.4 ± 8.2% as compared with their respective pre-injection levels. In contrast, 2-methyl-5-HT had a weak agonist action on cervical vagus nerve activity since the maximum response to this agent was only 107.4 ± 2.3% of the pre-injection level (Figure 4a). Dissimilarity of the agonisms produced by 2-methyl-5-HT and α-methyl-5-HT suggests that 5-HT-induced activation of afferent cervical vagus nerve activity might be mediated mainly by 5-HT₂ receptors rather than the 5-HT₃ receptors in anaesthetized rats. On the other hand, Butler *et al.* (1988) reported that 5-HT and 2-methyl-5-HT produced almost the same fall in heart rate, which is the Bezold-Jarisch reflex. A possible explanation for the difference in efficacy is that we measured the activities of the peripheral cut end of the cervical nerve without the nodose ganglion which is rich in 5-HT₃ receptors (Hoyer *et al.*, 1989), whereas Butler *et al.* used the intact afferent vagus nerve system. In other words, not only the vagus nerve trunk but also the nodose ganglion may be responsible for the initiation of the reflex. This speculation may be supported by a recent observation that 5-HT₃ receptor-mediated apnoea was abolished by vagotomy above, but not below, the nodose ganglia (Yoshioka *et al.*, 1992).

The location of the responsible 5-HT₂ receptor is still unknown; however, 5-HT₂ receptors generally exist in vas-

Figure 3 Effect of ketanserin on the 5-hydroxytryptamine (5-HT)-induced increase in cervical vagus nerve activity in an anaesthetized rat. Top two panels, control experiment; lower two panels, pretreatment with ketanserin 100 μ g kg⁻¹, 5 min before the next injection regime of 5-HT. A minimum of 10 min was allowed between the injection of each dose of 5-HT to avoid tachyphylaxis and allow for blood pressure recovery. Traces are the actual records of cervical vagus nerve activity (CVNA), pulse counts per 2 s, blood pressure (BP, mmHg) and heart rate (HR, b.p.m.). All records are from the same animal.

Figure 4 Effects of intravenous administration of 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) (top 2 panels) and α -methyl-5-HT (lower 2 panels) on cervical vagus nerve activity. Traces are the actual records of cervical vagus nerve activity (CVNA), pulse counts per 2 s, blood pressure (BP, mmHg) and heart rate (HR, b.p.m.).

Figure 5 (a) Effect of GR38032F $10 \mu\text{g kg}^{-1}$ on the 2-methyl-5-hydroxytryptamine (2-methyl-5-HT)-induced increase in cervical vagus nerve activity. Symbols indicate control response (○) and presence of GR38032F (●). (b) Effect of ketanserin $10 \mu\text{g kg}^{-1}$ on the α -methyl-5-HT-induced increase in cervical vagus nerve activity. Symbols indicate control response (○) and ketanserin (●).

cular and nonvascular smooth muscles such as the rat caudal artery, jugular vein, uterine and tracheal tissue etc. (Cohen *et al.*, 1985). The 5-HT₂ receptor is primarily responsible for 5-HT-induced contraction of smooth muscle. Anatomically, the cervical afferent vagus nerve is, in part, a visceral afferent sensory nerve (Hebel & Stromberg, 1986) and spontaneous activity of this nerve might also originate from the sensory nerve endings in the thoracic and abdominal viscera. However, after bilaterally cutting the vagus nerve below the diaphragm, no significant alteration of ongoing cervical vagus nerve activity was observed (data not shown). Furthermore, this surgical denervation did not significantly alter the 5-HT-induced excitation of cervical vagus nerve activity; the dose-ratio for the agonist before and after the surgical denervation was 1.17 ± 0.22 ($n = 4$). Taking these facts into account, the cardiopulmonary afferent nerve might play a much more important role than the abdominal afferents *in vivo*. A definitive conclusion on the receptor site(s) mediating the excitatory effect via 5-HT₂ receptors requires further investigation.

Excitation of cervical vagus nerve activity by 5-HT was not mimicked by 5-CT, a compound known to be a 5-HT₁-like receptor agonist (Saxena & Verdouw, 1985), or by 5-methoxytryptamine, a putative 5-HT₄ receptor agonist (Hoyer & Neijt, 1988), in the present study. In this context, neither a 5-CT-sensitive 5-HT₁-like receptor nor a putative 5-HT₄ receptor is likely to be involved in 5-HT-induced excitation of cervical vagus nerve activity *in vivo*.

Figure 6 Effects of intravenous administration of (a) 5-carboxamidotryptamine (5-CT), (b) 5-methoxytryptamine (5-MeOT) and (c) methoxamine on cervical vagus nerve activity. Traces are the actual records of cervical vagus nerve activity (CVNA), pulse counts per 1 or 2 s, blood pressure (BP, mmHg) and heart rate (HR, b.p.m.). Each record is from a different animal.

Ketanserin inhibited both 5-HT- and α -methyl-5-HT-induced excitatory effects on cervical vagus nerve activity, however, it also has an α_1 -adrenoceptor blocking property (Fozard, 1982; McCall & Schuette, 1984). In order to exclude the possibility of involvement of the α_1 -adrenoceptor-mediated mechanism, we have examined the effect of α_1 -agonist, methoxamine, on cervical vagus nerve activity. A high dose of methoxamine failed to produce an increase in nerve activity in spite of its effective pressor response via the α_1 -adrenoceptor. This result may exclude the possibility that the α_1 -blocking property of ketanserin inhibited the 5-HT induced excitatory effect on cervical vagus nerve activity.

It has been previously mentioned that 5-HT and α -methyl-5-HT have almost the same efficacy but 2-methyl-5-HT has less efficacy than 5-HT or α -methyl-5-HT, according to the percentage changes of pre-injection values. Although this was an *in vivo* experiment in which the ED₅₀ values of agonists were calculated from the dose-response curve, it is interesting

to note that the nature of the antagonism produced by GR38032F against 5-HT and 2-methyl-5-HT was different. With GR38032F ($10 \mu\text{g kg}^{-1}$), the dose-ratio (the means of the quotients: ED_{50} in the absence of antagonist/ ED_{50} in the presence of antagonist) against 5-HT was 1.9 ± 0.3 ($n = 11$) and that against 2-methyl-5-HT was 2.7 ± 0.5 ($n = 5$). Similarly, with ketanserin ($10 \mu\text{g kg}^{-1}$), the ratio against 5-HT was 3.1 ± 0.8 ($n = 6$) and that against α -methyl-5-HT was 3.6 ± 0.5 ($n = 5$). A possible explanation for the differences in the dose-ratios between 5-HT and the agonists is that 5-HT

is a substrate for uptake and the agonists are not. However, the ED_{50} against 5-HT is always smaller than that against selective agonists. These results might support the possibility that more than one receptor is responsible for the 5-HT-induced excitatory effect on cervical vagus nerve activity *in vivo*.

The most noteworthy conclusion of this investigation is that the 5-HT-induced excitatory effect on the afferent vagus nerve *in vivo* may be mediated not only by the 5-HT₃ receptor mechanism but also by the 5-HT₂-receptor mechanism.

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