



SPECIAL REPORT

Evidence that 5-HT_{1D} receptors mediate inhibition of sympathetic ganglionic transmission in anaesthetized cats*James F.X. Jones, †Graeme R. Martin & ¹Andrew G. Ramage

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In anaesthetized cats, 5-carboxamidotryptamine (5-CT) or 5-hydroxytryptamine (5-HT) (0.3–300 µg kg⁻¹, i.v.) inhibited the postganglionic compound action potential evoked by preganglionic electrical stimulation (0.5 Hz) with a similar potency in the stellate and splanchnic ganglia. In the 5-HT experiments transmission thorough the inferior mesenteric ganglia was also recorded. The maximal inhibitory effect of 5-HT was greater on the stellate and splanchnic ganglia (60 ± 4 and 52 ± 5%) than on the inferior mesenteric (15 ± 2%). The effects of 5-HT were unaffected by pretreatment with antagonists (1 mg kg⁻¹, i.v.) for 5-HT₂ (BW501C67), 5-HT_{1A} (WAY-100635) and 5-HT₃ receptors (ondansetron). However, responses to both 5-HT and 5-CT were attenuated significantly by GR127935 (1 mg kg⁻¹) except the responses to 5-HT at the inferior mesenteric ganglia. These results are consistent with the involvement of 5-HT_{1D} receptors mediating inhibition of sympathetic ganglionic transmission *in vivo*.

Keywords: Sympathetic ganglia of cat; 5-HT_{1D} receptors; 5-hydroxytryptamine, 5-carboxamidotryptamine; GR127935; ondansetron; WAY-100635; BW501C67

Introduction 5-Hydroxytryptamine (5-HT) has been reported to cause hyperpolarization of the superior cervical ganglia either *in vitro* (rat and rabbit; see Wallis & Elite, 1990) and *in vivo* (cat; Haefely, 1974). In the rat this effect appears to be mediated by 5-HT₁-like receptors, since 5-HT effects are mimicked by 5-carboxamidotryptamine (5-CT) with a similar potency (Ireland & Jordan, 1987). The present experiments were carried on anaesthetized cats to determine the effects of intravenous (i.v.) 5-HT on sympathetic ganglionic transmission and the receptor subtype involved. Since 5-CT was originally found to have a similar hyperpolarizing action to 5-HT (Ireland & Jordan, 1987) experiments were initially carried out to determine if 5-CT could inhibit the electrically evoked compound action recorded postganglionically from the stellate and the splanchnic ganglia. Then another series of experiments was carried out with 5-HT in the presence of a 5-HT₂ receptor antagonist, BW501C67, to prevent the activation of 5-HT₂ receptors in causing bronchoconstriction (see Ramage *et al.*, 1993). In these experiments the effect of 5-HT was repeated in the presence of a 5-HT_{1A} receptor antagonist, WAY-100635 (Fletcher *et al.*, 1994) and then repeated in the presence of a 5-HT₃ receptor antagonist, ondansetron, in case the well documented 5-HT₃ receptor-mediated depolarization (see Wallis & Elliott, 1990) was masking the inhibitory action of 5-HT. The effect of 5-HT followed by 5-CT was then examined in the presence of the 5-HT_{1D} receptor antagonist, GR127935 (Skingle *et al.*, 1993). In the 5-HT experiments, in addition to the stellate and splanchnic ganglia, compound action potentials were also recorded from the inferior mesenteric ganglia.

Methods *Preparation* Cats were anaesthetized with a mixture of i.v. α-chloralose (80 mg kg⁻¹) and pentobarbitone sodium (6 mg kg⁻¹) and artificially ventilated after neuromuscular blockade with vecuronium bromide (200 µg kg⁻¹). The level of anaesthesia was assessed by the absence of cardiovascular response to paw-pinch and additional anaesthetic administered (10–15 mg kg⁻¹) as required. Recordings of

blood and tracheal pressure, arterial pH and gases were carried out as previously-described (Ramage *et al.*, 1993). Left stellate, splanchnic and inferior mesenteric ganglia were exposed by a retro-pleural or retro-peritoneal approach. Compound action potentials were recorded from postganglionic fibres (cut distally) using silver hook electrodes. The signal was amplified (Neurolog NL104) and filtered (50–500 Hz; NL125).

Stimulation protocol The preganglionic fibres, cut centrally, were stimulated supramaximally (1 ms, 5–15 V) at 0.5 Hz (see Haefely, 1974). Thirty sweep signal averages (200 ms duration) were obtained from the evoked postganglionic compound action potentials. These signals were rectified and cumulatively integrated (CED, Spike2, Sigavg). At the end of each experiment injection of trimetaphan (100 µg kg⁻¹; i.v.) was used to confirm that the potentials recorded were indeed primarily due to cholinergic transmission.

Protocol and analysis Two groups of 4 cats were used. In group 1 only a cumulative doses-response curve to 5-CT (i.v.; 0.3–300 µg kg⁻¹) was constructed. Intervals between injections were 3, 3, 5, and 5 min. In group 2 a dose-response curve to 5-HT (i.v.; 0.3–300 µg kg⁻¹) was constructed in the presence (i.v.) of BW501C67 (2-anilino-*N*-[2-(3-chlorophenoxy)propyl] acetamidine HCl) (1 mg kg⁻¹) and repeated in the presence of WAY-100635 (*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl) cyclohexanecarboxamide tri-chloride) (1 mg kg⁻¹), followed by ondansetron (1 mg kg⁻¹) and then GR127935 (*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide) (1 mg kg⁻¹). After construction of the last 5-HT dose-response curve in the presence of GR127935, a cumulative dose-response curve was constructed for 5-CT. In these group 2 experiments, the animals were vagotomized bilaterally. 5-HT was always administered 3 min after each antagonist. Changes (%) in the response to all test drugs were taken from the 30 sweep averages prior to their injection. Changes caused by 5-CT were taken 1 min prior to the next dose and 5 min after the last dose while for 5-HT the changes were measured within the first minute after administration. For antagonists, changes were taken 1 min prior to administration of the first dose of 5-HT. Changes in the

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compound potentials caused by each antagonist were compared by Student's paired *t* test. Comparison between dose-response curves and between ganglia were statistically analysed

by a three-way ANOVA and least significant differences. All doses of drugs refer to their salts.

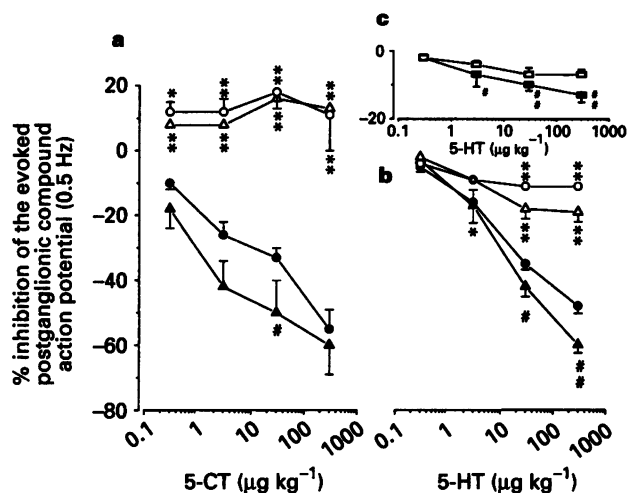


Figure 1 Anaesthetized cats: (a) shows the effect of cumulative doses (i.v.) of 5-carboxamidotryptamine (5-CT) while (b) and (c) show the effects of doses (i.v.) of 5-HT on low frequency (0.5 Hz) transmission through the stellate (▲) and splanchnic (●) ganglia and in (c) the inferior mesenteric ganglia (■). The 5-HT curve was carried out in the presence (1 mg kg⁻¹) of BW501C67, ondansetron and WAY-100635. Open symbols show the effect of 5-CT and 5-HT in the presence of GR127935 (1 mg kg⁻¹ i.v.) in the group 2 experiments. Each point represents the mean value (*n*=4) with s.e.mean. Comparisons were made by three-way analysis of variance and the least significant difference test to compare means: **P* < 0.05; **P* < 0.01 for comparison between non and GR127935 pretreated effects. #*P* < 0.05; ###*P* < 0.01 for comparison, of mesenteric versus splanchnic and stellate versus splanchnic, of the effect of the agonists between ganglia.

Results Both 5-CT and 5-HT caused a dose-dependent inhibition of the evoked compound action potential in the splanchnic and stellate ganglia, in both cases achieving a similar maximum inhibition (Figures 1 and 2). The inhibition by 5-HT was unaffected by WAY-100635 and ondansetron but significantly (*P* < 0.05) attenuated by GR127935 (Figures 1b and 2a). In the 5-HT experiments addition of 5-CT in the presence of all these antagonist also failed to inhibit the evoked compound action potential (Figure 1a). The antagonists used had no significant effect on the baseline evoked compound action potential (Figure 2a).

The inhibitory effect of 5-HT on the inferior mesenteric ganglia was significantly less than on the other ganglia (Figure 2a) although trimetaphan inhibition was not significantly different between the ganglia (stellate, $-81 \pm 5\%$ splanchnic $-61 \pm 7\%$; inferior mesenteric $-72 \pm 2\%$; mean \pm s.e.mean; Figure 2b). Whilst the inhibitory effect of 5-HT on the inferior mesenteric ganglia was attenuated by GR127935, this failed to reach statistical significance.

Discussion The present experiments demonstrate that low frequency transmission in sympathetic ganglia is inhibited by intravenous 5-HT and that this inhibition is more marked in some ganglia (stellate and splanchnic) than others (inferior mesenteric). The reasons for these inter-ganglionic differences remain unknown, but they may reflect differences in 5-HT receptor-effector coupling or drug access. The ability of 5-CT to mimic, with similar potency, the effects of 5-HT on stellate and splanchnic ganglia is consistent with the study by Ireland & Jordan (1987) showing that both drugs likewise hyperpolarize the superior cervical ganglia of the rat at similar concentrations. Their proposal that 5-HT-induced inhibition of ganglionic transmission involves a receptor of the 5-HT₁ class is supported by the present results at least in the cardiac and

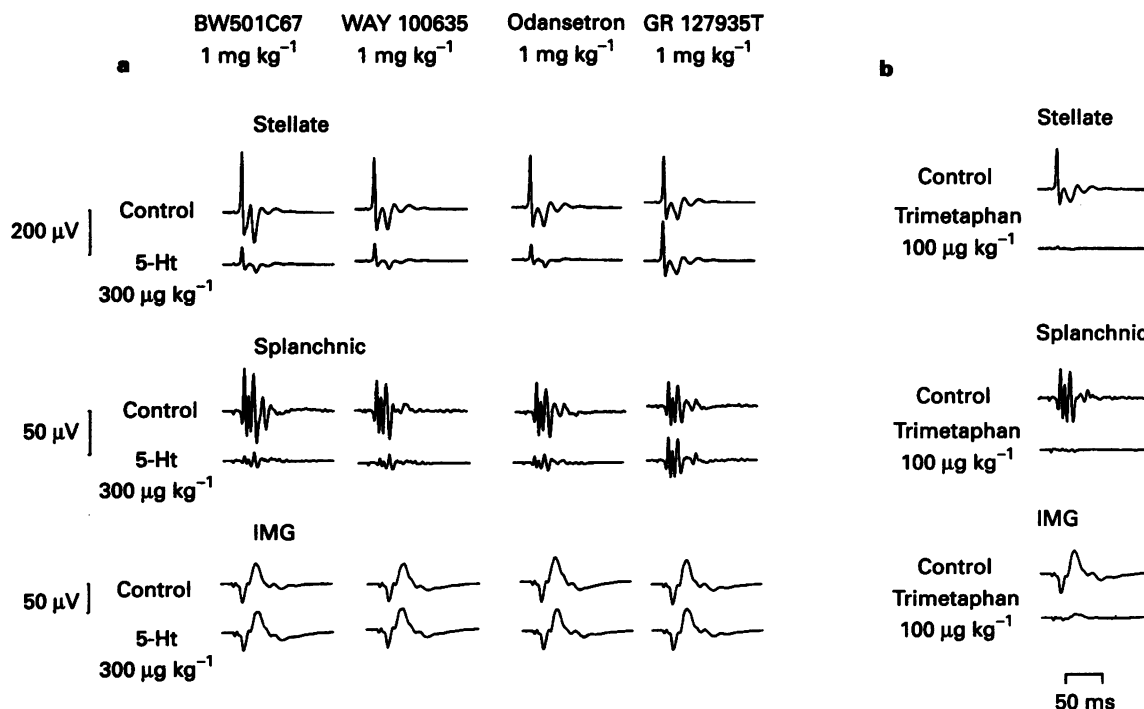


Figure 2 Anaesthetized cat: (a) shows traces of the effect of the largest dose of i.v. 5-HT (300 µg kg⁻¹) on the evoked compound action potentials (0.5 Hz) recorded postganglionically from the stellate, splanchnic and inferior mesenteric ganglia in the presence (i.v.) of 1 mg kg⁻¹ of BW501C67, ondansetron, WAY-100635 and GR127935. (b) Shows traces of the effect of i.v. trimetaphan (100 µg kg⁻¹) on evoked postganglionic action potentials on the three ganglia at the end of the same experiment.

splanchnic ganglia. Hence, the failure of the selective antagonists, BW501C67 and ondanestron, to modify 5-HT effects rules out a contribution of 5-HT₂ and 5-HT₃ receptors, respectively. Furthermore, the selective antagonist WAY-100635 excludes a role for their neuroinhibitory receptors of the 5-HT_{1A} subtype. Only the antagonist, GR127935, effectively blocked inhibition of ganglionic transmission in the cardiac

and splanchnic ganglia by either 5-HT or 5-CT, indicating that the inhibition is mediated by a 5-HT_{1D} receptor. Studies are in progress with selective 5-HT_{1D} agonists to substantiate further a role for this receptor subtype in the modulation of cardiac and splanchnic sympathetic ganglionic transmission and to investigate the nature of the receptors mediating the weak inhibitory effect of 5-HT in the inferior mesenteric ganglia.

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