5-Hydroxytryptamine<sub>3</sub> Receptor Antagonism Modulates a Noxious Visceral Pseudoaffective Reflex

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Summary—5-Hydroxytryptamine (5-HT) receptor agonists and antagonists were dosed intravenously (i.v.) and studied for their effects on the depressor cardiovascular pseudoaffective reflex evoked by acute noxious colo-rectal distension in the anaesthetized rat. Methiothepin (100 μg kg<sup>-1</sup>) caused an initial, unsustained blockade of evoked depressor responses whilst ketanserin (100 μg kg<sup>-1</sup>) was without effect. By comparison, ondansetron dose dependently inhibited evoked depressor responses and was maximally active at 100 μg kg<sup>-1</sup>, causing a 57.5 ± 0.9% reduction. An ID<sub>50</sub> value of 36.7 μg kg<sup>-1</sup> was estimated by regression analysis. In contrast, granisetron caused complete blockade of the depressor response with an ID<sub>50</sub> of 0.4 μg kg<sup>-1</sup>. Bell-shaped dose-effect curves were demonstrated for both granisetron and ondansetron. Intrathecal dosing with granisetron (100 ng) into the thoracolumbar region of the spinal cord prevented the depressor response to colo-rectal distension, suggesting a spinal site of action. The pseudoaffective depressor responses were not facilitated by pre-dosing with the 5-HT<sub>1</sub> receptor agonists, 8-OH DPAT, α-methyltryptamine or 1-phenylbiguanide. However, 8-OH DPAT (100 μg kg<sup>-1</sup>) facilitated pressor responses. It is suggested that 5-HT<sub>3</sub>-like receptors may have a role in modulating depressor responses to visceral pain and that in this action different 5-HT<sub>3</sub> receptor antagonists are not necessarily equi-effective.

Keywords—5-HT<sub>3</sub>, receptor, 5-HT<sub>3</sub>, receptor antagonists, pseudoaffective reflex, colo-rectal distension.

Pseudoaffective responses, which are brain stem or spinal reflex induced emotional or affective responses to tissue damaging stimuli, can be evoked experimentally by noxious distension of visceral organs [see Ness and Gebhart (1990), for review]. These reflexes have most commonly been observed as changes in blood pressure, heart rate and visceromotor effects such as hunching. For example, marked decreases in blood pressure correlate with the magnitude of distension of the proximal jejunum in anaesthetized rats (Lembeck and Skofitsch, 1982). Ness and Gebhart (1988) reported that two cardiovascular pseudoaffective responses, namely pressor and depressor effects, were demonstrable with acute noxious colo-rectal distension in anaesthetized rats. However, the depressor response to colo-rectal distension, which is observed under certain anaesthetics, was not investigated pharmacologically by these workers.

Moss and Sanger (1990) observed depressor responses to acute distension of the duodenum in anaesthetized rats. The 5-hydroxytryptamine, (5-HT)<sub>3</sub> receptor antagonists granisetron and ICS 205-930 reduced the response to distension, whilst ondansetron had no effect. Thus, there appeared to be a possible role for certain 5-HT<sub>3</sub> receptor antagonists in visceral nociception. However, the failure of ondansetron to behave in a similar manner indicates that this may be due to other actions for either compound. In the present study the effects of 5-HT receptor antagonists on depressor responses to noxious colo-rectal distension in anaesthetized rats were investigated. Furthermore, pharmacological distinctions between granisetron and ondansetron were examined and a site of action for granisetron suggested.

METHODS

Animals

Male Wistar rats (200–300 g; Charles River) were fasted individually overnight, prior to induction of non-recovery anaesthesia using intravenous (i.v.) propofol (25–50 mg kg<sup>-1</sup> h<sup>-1</sup>). When compared with urethane, pentobarbital, α-chloralose and alphaxalone/alphadolone ('Saffan', Pitman-Moore Ltd, Cheshire, U.K.), propofol (Rapinovet, Pitman-Moore Ltd, Cheshire, U.K.) was determined to allow the most frequent occurrence of depressor pseudoaffective responses to colo-rectal distension. That the pseudoaffective response was dependent on the nature of the anaesthetic used is in

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agreement with Ness and Gebhart (1988). Animal care and protocol review were in accordance with the UK Animals (Scientific Procedures) Act 1986.

**Experimental protocol**

Under propofol (25–50 mg kg\(^{-1}\) h\(^{-1}\)) anaesthesia a thin-walled balloon 6–7 cm in length was inserted anally, with an exteriorized cannula taped in place, to prevent balloon expulsion, and connected to a pressure transducer and a 10 ml syringe. A tracheostomy was performed and the right carotid artery and the left jugular vein cannulated to facilitate blood pressure recording and drug administration respectively. After a 30 min equilibration period acute colorectal distensions were initiated with an intraluminal pressure of 80 mmHg for 20 sec [see Ness and Gebhart (1988)], repeated at 10 min intervals. Rats were rejected from studies if no response could be obtained even after pharmacological manipulation, or if the response was consistently too small. Those animals exhibiting pressor responses were used for other studies, not reported here. After 3 consistent depressor responses, where the pseudoadverse reflexes measured >10 mmHg, a single concentration of the antagonist was dosed i.v. and the distensions repeated.

In one group of rats intrathecal catheters were placed using the method of Yaksh and Rudy (1976). Briefly, the catheter (Portex 0.61 mm o.d. polyethylene tubing, 8.5 cm long) was inserted under non-recovery propofol anaesthesia through the atlanto-occipital membrane into the subarachnoid space and passed down to the lumbar enlargement. Precise positioning of the catheter was determined with post-mortem examination.

**Compounds**

Methiothepin maleate (10–100 pg kg\(^{-1}\)), 1-phenylbiguanide (10 pg kg\(^{-1}\)-1 mg kg\(^{-1}\)), \(\alpha\)-methyl-5-hydroxytryptamine maleate (\(\alpha\)-Me-5-HT; 10 pg kg\(^{-1}\)) and ketanserin tartrate (100 pg kg\(^{-1}\)) were from Hoffman LaRoche, Sigma, Aldridge and Research Biochemicals Inc. respectively. 8-Hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH DPAT; 1 pg kg\(^{-1}\)-100 pg kg\(^{-1}\)), granisetron (0.3 pg kg\(^{-1}\)-1 mg kg\(^{-1}\)) and ondansetron (1 pg kg\(^{-1}\)-1 mg kg\(^{-1}\)) were synthesized by SB Pharmaceuticals. All drugs were administered i.v. with 0.9% w/v saline vehicle. Granisetron was dosed intrathecally (0.1 \(\mu\)g and 10 \(\mu\)g), in a volume of 10 \(\mu\)l, with a 10 \(\mu\)l wash of saline vehicle.

**Analysis and statistics**

All values are expressed as the mean ± SEM unless otherwise stated. Pseudoadverse reflexes resulting from colorectal distension were expressed as a percentage of the mean consistent responses prior to drug administration. ID\(_{50}\) values for inhibition of the depressor response were calculated after regression analysis of the maximum response at each effective dose.

**RESULTS**

Acute noxious colorectal distension usually caused pseudoadverse vascular reflexes but did not alter blood pressure basal tone. The depressor responses were unaffected by i.v. administration of the vehicle (n = 7).

**Antagonists**

Methiothepin (100 pg kg\(^{-1}\)) caused a fall in resting blood pressure, which may have had the effect of inhibiting subsequent pseudoadverse reflexes. Thus, depressor responses were inhibited by 56.8 ± 14.1%; n = 5, at 5 min post-dose, thereafter the blockade by methiothepin recovered in some animals but not all (Fig. 1). This recovery was also associated with the return of blood pressure to pre-dose levels. By comparison, ketanserin (100 pg kg\(^{-1}\)) caused a slight fall in resting blood pressure; but had no effect on the pseudoadverse reflex (Fig. 1). Ondansetron dose dependently inhibited depressor pseudoadverse reflexes without affecting resting blood pressure. At 1 pg kg\(^{-1}\) ondansetron was without effect but demonstrated good activity at 100 pg kg\(^{-1}\), causing a 57.5 ± 0.9% reduction (Fig. 2). These data were used
The depressor response was also reduced dose dependently by granisetron (Fig. 3). The lowest effective dose was 0.3 \( \mu g \) kg\(^{-1}\) and the pseudoaffective response evoked by colo-rectal distension was completely prevented at 10 \( \mu g \) kg\(^{-1}\) granisetron. Over this range of doses in ID\(_{50}\) value of 0.4 \( \mu g \) kg\(^{-1}\) (95% confidence limits 0.3–0.5 \( \mu g \) kg\(^{-1}\)) was estimated by regression analysis. The dose dependent action of granisetron was altered at higher doses when the effects of the drug were variable. Thus, at 100 \( \mu g \) kg\(^{-1}\) complete blockade of the response was only demonstrable in 3 of 6 rats tested, whilst in 2 there was slight inhibition (22.0 ± 8.0%) and in 1 rat granisetron was inactive. At the higher dose of 1 mg kg\(^{-1}\) granisetron also appeared to be inactive \((n = 3)\). As observed with ondansetron, the onset of blockade was rapid yet the time to reach maximal effect was slow; for example, at 10 \( \mu g \) kg\(^{-1}\) granisetron required a mean of 46 ± 8 min, \( n = 6 \); before complete inhibition of the depressor response was achieved.

Intrathecal administration of granisetron at the thoracolumbar (T13–L2) level of the spinal cord prevented the depressor response to colo-rectal distension. Thus, whilst saline vehicle was without effect, granisetron at 100 \( \mu g \) required a mean of 45.0 ± 3.7 min \( (n = 4) \) to completely inhibit the depressor response to colo-rectal distension. However, in experiments where 10 \( \mu g \) granisetron were applied intrathecally no effect was observed \((n = 3, \text{data not shown})\).

Agonists

In rats in which colo-rectal distension did not evoke a pseudoaffective reflex after 3 consecutive distensions, 5-HT receptor agonists were administered, in an attempt to enhance the sensitivity of the reflex. As such, 8-OH DPAT (1–100 \( \mu g \) kg\(^{-1}\)) caused a fall in resting blood pressure but at a dose of 100 \( \mu g \) kg\(^{-1}\) facilitated pressor responses to colo-rectal distension (Fig. 4). Neither
pressor nor depressor responses were facilitated by the addition of the 5-HT_{3} receptor agonist, phenylbiguanide (10–1000 μg kg^{-1}, n = 5), doses which evoked the von Bezold–Jarisch reflex. Neither were they facilitated by the non-selective 5-HT_{2} receptor agonist, α-Me-5-HT at doses up to 100 μg kg^{-1}, n = 3.

**DISCUSSION**

Ness and Gebhart (1988) demonstrated that pseudo-affective reflexes could be evoked by colo-rectal balloon inflation in anaesthetized animals, whilst Lembeck and Skofitsch (1982) had previously shown that the pressor and depressor vascular pseudoaffective reflexes evoked by jejunal distension are mediated via different pathways. The pressor response was determined to be a spinal pathway whilst the depressor response was lost after spinalization thereby indicating the involvement of a central reflex centre. Under propofol anaesthesia either pressor or depressor responses have been observed, or no effect was apparent from noxious colo-rectal distension and consequently, this preparation would prove unsuitable to use for a specific pseudoaffective reflex only.

Moss and Sanger (1990) have proposed a role for 5-HT_{3}-like receptors in mediating the depressor response whilst Dauzebrink and Gebhart (1991) demonstrated in the conscious rat, where the depressor response is not observed, that 5-HT_{1}, 5-HT_{2}, and 5-HT_{3} receptors all modulate the pressor pseudoaffective response at the spinal level. Consequently they proposed a role for each of these receptors in visceral antinociception.

The data presented here for methiothepin and ketanserin, at doses consistent with their ability to antagonize the receptors supra-maximally (Connor et al., 1986), suggests that the 5-HT_{1} and 5-HT_{2} receptors may not contribute substantially to the pseudoaffective depressor response, although it is possible that these antagonists did not reach the spinal cord, a potential site of action. The high dose of methiothepin (100 μg kg^{-1}) has also been shown to cause a decrease in the diastolic blood pressure of cats (Connor et al., 1986) and it is suggested that this effect may contribute to a non-specific inhibition of the distension evoked depressor response. However, it would prove interesting to determine the effects of antihypertensive agents and compare them with the observed effects of methiothepin.

A role for 5-HT_{3} receptors in visceral pain modulation may be inferred from their presence on primary afferent terminals in the dorsal horn (Hamon et al., 1989). However, there are some notable differences between the efficacy of granisetron and ondansetron as first suggested by Moss and Sanger (1990). Thus in the present experiments, granisetron was more effective and approx. 100 times more potent than the submaximally-effective action of ondansetron, contrasting with an expected 5–10-fold difference in potency seen in various other models of 5-HT_{3} receptor mediated activity [e.g. inhibition of the von Bezold–Jarisch reflex in rats (Sanger, 1990)]. The time course of 5-HT_{3} receptor antagonist activity was comparatively slow, thus whilst the time to onset was rapid, the time to maximum effect was not achieved until approx. 50 min after dosing. This contrasts with the ability of granisetron to abolish vomiting evoked by cytotoxic treatment within seconds of administration (Bermudez et al., 1988) and the rapid blockade of the von Bezold–Jarisch reflex in rats, occurring within 5 min of i.v. administration (Sanger and Nelson, 1989). Furthermore, bell-shaped dose–response curves were also observed with these compounds, a phenomenon previously shown in experiments designed to measure the potential anxiolytic properties of the compounds (Kilpatrick and Tyers, 1992). The explanation for this type of dose response curve is currently unknown. Nevertheless, the differences in efficacy and time course of action for granisetron and ondansetron do not conform exactly to their definition as 5-HT_{3} receptor antagonists. Consequently, their activity must be described as being due to antagonism at a 5-HT_{3}-like receptor, with subtle differences in the pharmacology and/or the neuronal connections made by these receptors and by those receptors which can be readily classified as 5-HT_{3}.

Our observations are at least partly supported by clinical observations that have been reported for these two 5-HT_{3} receptor antagonists. Thus, in contrast to the failure of ondansetron to affect the pain and sensory disorders associated with the irritable bowel syndrome (Steadman et al., 1990; Maxton et al., 1991), pilot studies with granisetron have suggested a potential analgesic activity in both irritable bowel syndrome (Prior and Read, 1990) and in migraine patients (Couturier et al., 1991; Rowat et al., 1991). This correlation supports the validity of measuring depressor responses in anaesthetized animals even though pressor responses are observed in conscious rats (Ness and Gebhart, 1988). Similarly in 5-HTP-treated conscious rats subjected to colo-rectal distension, the analgesic and lack of analgesic properties of 5 HT_{3} receptor antagonists (Banner and Sanger, 1994) is remarkably similar to the results presented here, further supporting measurements of depressor responses as an index of visceral nociception. Thus, although the depressor response does not in itself represent pain it may be a meaningful reflex to study to determine the potential analgesic properties of compounds such as the 5-HT_{3}-like receptor antagonists used here. A further consideration is that the activity of these compounds may not be limited to inhibition of pseudoaffective reflexes and nociception but they could also suppress the complete gradation of sensations that are perceived as afferent activity increases.

It is also apparent that there are differences between different regions of the gut with respect to nociceptive processing. It is possible that 5-HT_{3}-like receptors do not play such a fundamental role in modulating nociceptive activity evoked from the duodenum, where granisetron only partially inhibited the depressor response and ondansetron had no activity (Moss and Sanger, 1990). By
comparison in the colo-rectal region both antagonists were more effective inhibitors of the same pseudoaffec-
tive reflex.

5-HT may sensitize primary afferents existing periplu-
erally within the gut, thereby modulating transmission of nociceptive levels of information to the spinal cord. However, Cramer and Rademaker (1989) found that the 5-HT3 receptor antagonist ICS 205-930 did not affect rat mesenteric nerve activity when dosed intraperitoneally, but did cause a reduction in depressor pseudoaffective responses to noxious distension of the proximal ileum. These experiments would suggest that ICS 205-930 acted within the brain or spinal cord. Here, a similar action intrathecally to the i.v. action of granisetron has been demonstrated, including apparent bell-shaped dose response curves, suggesting that the 5-HT3-like receptors mediating the depressor response are probably located at a site where colo-rectal afferents are known to enter the spinal cord (Nadellaft and Booth, 1984; Ness and Gebhart, 1990). Furthermore, the observation that phenylbiguanide did not facilitate depressor responses may be indicative of a brain or spinal cord site of action for these 5-HT3-like receptors, as phenylbiguanide does not easily cross the blood brain barrier. However, it also remains to be determined if it has a high affinity for this 5-HT3-like receptor. As it has been observed that certain 5-HT3 receptor antagonists inhibit the depressor response to noxious stimulation, a reflex which involves a central reflex centre (Lembeck and Skofitsch, 1982), it may be suggested that rather than a blockade of descending noxious inhibitory pathways involving 5-HT, antagonism of these receptors could inhibit rostral transmission, possibly via the spinoparabrachial and spinothalamic tracts from lamina I cells (Menetrey and de Pommery, 1991).

In conclusion 5-HT3-like receptors, present in the spinal cord, may modulate depressor responses to nox-
ious visceral stimuli evoked in different regions of the gut. The precise roles for 5 HT in visceral nociceptive processing requires further investigation.

REFERENCES

Bannor S. E. and Sanger G. J. (1995) Differences between 5-HT3 receptor antagonists in modulation of visceral hyper-
The anti-emetic potential of the 5-hydroxytryptamine, recep-
Connor H. E., Fenik W., Humphrey P. A. and Perren M. J. 
Couturier E. G., Hering R., Foster C. A., Steiner T. J. 
and Clifford R. F. (1991) First clinical study of the 
selective 5-HT3 antagonist, granisetron (BRL 43694), in the
Cramer W. C. M. and Rademaker B. (1989) 5-HT3 receptors 
and visceral nociception: involvement and localization. Dig. 
Dis. Sci. 34: 972.
spinal 5-HT1, 5-HT3, and 5-HT4 receptor subtypes modulate responses to noxious colo-rectal distension in the rat. Brain 
Res. 538: 64–75.
Hamon M., Gallisost M. C., Menard F., Gozlan H., Bourgoin 
S. and Verge D. (1989) 5-HT, receptor binding sites are on 
capsaicin-sensitive fibres in the rat spinal cord. Eur. J. 
Pharmac. 164: 315–322.
pretreatment with capsaicin and morphine. Naunyn- 
trial of ondansetron, a selective 5-HT, antagonist in irritable 
bowel syndrome (IBS). Gastroenterology 100: A468.
ascending pathways that reach central areas involved in 
visceroreception and visceronociception in the rat. Eur. J. 
Neurosci. 3: 249–259.
Moss H. E. and Sanger G. J. (1990) The effects of granisetron, 
ICS 205-930 and ondansetron on the visceral pain reflex 
induced by duodenal distension. Br. J. Pharmac. 100: 
497–501.
Nadellaft I. and Booth A. M. (1984) The location and 
morphology of preganglionic neurons and the distribution of visceral afferents from the rat pelvic nerve: a horseradish 
a noxious visceral stimulus: physiologic and pharmacologic 
characterization of pseudoaffective reflexes in the rat. Brain 
and post-prandial motility by granisetron, a 5-HT3 receptor 
antagonist, in patients with irritable bowel syndrome (IBS). 
Gut 31: A1174.
A double-blind comparison of granisetron and placebo for 
treatment of acute migraine in the emergency department. 
Cephalalgia 11: 207–213.
Pharmac. 68: 314–324.
Steadman C. J., Talley N. J., Phillips S. F. and Zimmerle A. 
R. (1992) Selective 5-hydroxytryptamine type 3 receptor 
antagonism with ondansetron as treatment for diarrhoea-
predominant irritable bowel syndrome: a pilot study. Mayo 
Yaksh T. L. and Rudy T. A. (1976) Chronic catheterization of 
the spinal subarachnoid space. Physiol. Behav. 17: 1031–1036.