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The effects of granisetron, ICS 205-930 and ondansetron on the visceral pain reflex induced by duodenal distension

[1]Helen E. Moss & Gareth J. Sanger

Beecham Pharmaceuticals, Medicinal Research Centre, Coldharbour Road, The Pinnacles, Harlow, Essex. CM19 5AD

1 Distension of the duodenum in anaesthetized rats, by rapid application of intraluminal pressures (10–75 cmH_2O), evoked falls in diastolic blood pressure and intragastric pressure.

2 The distension-induced responses were blocked by pretreatment with morphine (20 $mg\,kg^{-1}$, s.c.), an action reversible by injection of naloxone (5 $mg\,kg^{-1}$, i.v.).

3 Bilateral cervical vagotomy reduced the distension-evoked fall in intragastric pressure but had no effect on the corresponding fall in blood pressure.

4 Granisetron or ICS 205-930 (1–1000 $\mu g\,kg^{-1}$, i.v.) had no effects on duodenal intraluminal pressure, but reduced the responses to distension with a bell-shaped dose-response relationship. Ondansetron (1–1000 $\mu g\,kg^{-1}$, i.v.) did not reduce the reflex responses.

5 These results show that the 5-HT_3 receptor antagonists used exerted different effects on the reflex responses to duodenal distension.

Introduction

Distension of the human biliary system (Gaensler, 1951), ileum (Lipkin & Sleisenger, 1958) or colon (Latimer *et al.*, 1979; Swarbrick *et al.*, 1980) evokes abdominal pain. In anaesthetized animals, the effect of distension of hollow organs can be assessed by measuring a pseudoaffective reflex. Woodworth & Sherrington (1904) described pseudoaffective reflexes as '... mimetic movements simulating expression of certain affective states' and these include movement of the head towards the stimulus, dilatation of the pupils, vocalisation and transient changes in arterial blood pressure.

In anaesthetized ferrets and cats, distension of the biliary system increased blood pressure (Cervero, 1982; 1983) and firing of high threshold afferent fibres which respond only to noxious distension. In anaesthetized rats, distension of the jejunum (Lembeck & Skofitsch, 1982), renal pelvis (Brasch & Zetler, 1982) or ileum (Clark & Smith, 1985) induced a decrease in blood pressure in 60–70% of animals. This response could be blocked by morphine, an action reversed by naloxone. Capsaicin, which selectively destroys unmyelinated afferent fibres (Buck & Burks, 1986), also abolished the response to distension of the rat jejunum (Lembeck & Skofitsch, 1982) and no response could be evoked in rats treated neonatally with capsaicin (Skofitsch & Lembeck, 1980). Thus it is possible that distension-induced changes in blood pressure can be used to assess pain in anaesthetized animals.

In the present study with anaesthetized rats, duodenal distension evoked a fall in blood pressure and intragastric pressure. It has been shown that 5-hydroxytryptamine (5-HT) can be released by increasing the intraluminal pressure of the small intestine (Bülbring & Crema, 1959; Burks & Long, 1966). A potential role of endogenous 5-HT in modulating distension-induced reflexes was therefore examined by use of the selective 5-HT_3 receptor antagonists granisetron (BRL 43694; Sanger & Nelson, 1989), ICS 205-930 (Richardson *et al.*, 1985) and ondansetron (GR 38032F; Butler *et al.*, 1988). Preliminary results have been previously reported to the British Pharmacological Society (Moss & Sanger, 1987).

Methods

Following overnight fasting, male Wistar rats (200–300 g) were anaesthetized by intraperitoneal injection of 25% urethane (6 $ml\,kg^{-1}$). The right jugular vein and a carotid artery were catheterised to facilitate drug injection and blood pressure recording respectively; the trachea was cannulated to maintain a clear airway. The whole stomach was cannulated via the oesophagus and tied distal to the pyloric sphincter. The stomach was filled with 5 ml 0.9% w/v saline to incresae resting gastric tone. A 3–4 cm segment of duodenum was isolated by ligature immediately below the stomach, with major blood vessels and nerves remaining intact. This segment was flushed with saline and connected orally via a 37°C heating coil to a series of reservoirs containing 0.9% w/v saline and set at various heights above the animal. Drainage from the duodenum was achieved aborally. Animals were allowed 30 min recovery after surgery, before the experiment was started.

Pressure-response curves to duodenal distension were constructed using rapid application of pressures of 10, 25, 50 or 75 cmH_2O; each increase in pressure was maintained for 20 s before the pressure was removed and the duodenum drained of fluid. In order to measure the effects of drugs, distension with 75 cmH_2O was applied every 5 min until constant responses were achieved. Drugs were then injected and the distensions repeated at 5 min intervals.

Granisetron, ICS 205-930, ondansetron (1–1000 $\mu g\,kg^{-1}$) or ((3α-tropanyl)-1H-indole-3-carboxylic acid ester) saline (0.9% w/v in an equivalent volume) were given intravenously (i.v.) via the jugular vein and their effects monitored up to 20 min following administration. Morphine (20 $mg\,kg^{-1}$; Evans Medical) was administered subcutaneously (s.c.) and then naloxone (5 $mg\,kg^{-1}$, i.v.; Endo) 25 min later. All drugs were dissolved in saline; 5-HT_3 receptor antagonists were synthesized by Beecham Pharmaceuticals.

Responses to duodenal distension following drug administration were expressed as a percentage of the response immediately prior to dosing (taken as 100% response). Means were compared by a one sample t test and considered significantly different if $P < 0.05$. The effects at 20 min (expressed as a percentage of the maximum inhibition obtained) of granisetron and ICS 205-930 on the fall in blood pressure were also subjected to regression analysis and analysis of variance using methods outlined by Draper & Smith (1981). ID_{50} values were calculated as the dose required to give 50% of the actual maximum blocking effect obtained with the compound.

Results

Duodenal distension for 20 s, by use of rapid application of pressures in the range 10–75 cmH_2O, induced a decrease in diastolic blood pressure. This decrease was rapid in onset, the maximum fall lasting for 10–15 s with a return to normal pressures occurring 1–2 min later. The initial amplitude of the

[1] Author for correspondence.

response was related to the pressure applied to the duodenum (Figure 1). Distension of the duodenum also induced a fall in intragastric pressure which occurred at pressures of 25 cmH_2O and above (Figure 1). Compared with the fall in blood pressure, this fall was less rapid in onset, reaching a maximum after approximately 30 s, recovering over the subsequent 1–2 min. These blood and gastric pressure responses to duodenal distension were present in 80% of rats. Except where stated, those that did not respond were discarded. In all subsequent experiments, a distension pressure of 75 cmH_2O was used.

Following bilateral cervical vagotomy, a small fall was seen in resting blood pressure and intragastric pressure but not in the intraluminal pressure of the duodenum. The distension-evoked fall in intragastric pressure was unaffected in 3 rats, reduced by 60–90% in 7 rats and abolished in 4 rats even after the baseline intragastric pressure had been raised with the further addition of saline to the stomach (1.5 ml). The fall in blood pressure in response to distension was unaffected by vagotomy in all of these rats ($n = 14$).

Morphine (20 mg kg^{-1}, s.c.) had no effects on resting blood, intragastric or intraduodenal pressure, but prevented or greatly reduced the distension-evoked fall in blood and intragastric pressure; these effects were highly significant 15–25 min after dosing ($P < 0.005$; $n = 6$; Figure 2). Administration of naloxone (5 mg kg^{-1}, i.v.) at 25 min reversed the action of morphine (Figure 2).

Granisetron (1–1000 $\mu g\,kg^{-1}$, i.v.) was without effect on resting blood, intragastric or intraduodenal pressure. However, granisetron (1–100 $\mu g\,kg^{-1}$, i.v.) reduced the distension-evoked fall in blood pressure and at a dose of 10 $\mu g\,kg^{-1}$ i.v. reduced the fall in intragastric pressure (Figures 3 and 4). The most consistently effective dose of granisetron was 10 $\mu g\,kg^{-1}$; reductions were seen in all animals tested. Higher doses produced higher (50 $\mu g\,kg^{-1}$) or less consistent (100 $\mu g\,kg^{-1}$) antagonism of the fall in blood pressure, but with no reduction in the fall in intragastric pressure. Granisetron at 1000 $\mu g\,kg^{-1}$ increased or had no effect on the fall in blood and intragastric pressures in 4 of 7 and 3 of 7 animals respectively. Regression analysis of the effect of granisetron (1, 10 and 50 $\mu g\,kg^{-1}$) on the fall in blood pressure revealed a significant correlation (Figure 4; $F = 5.006$, $n = 19$, $P < 0.03$). Taking a maximum effect of granisetron at 50 $\mu g\,kg^{-1}$, then an ID_{50} value was calculated as 1.2 (95% confidence limits: 0.00026–140) $\mu g\,kg^{-1}$.

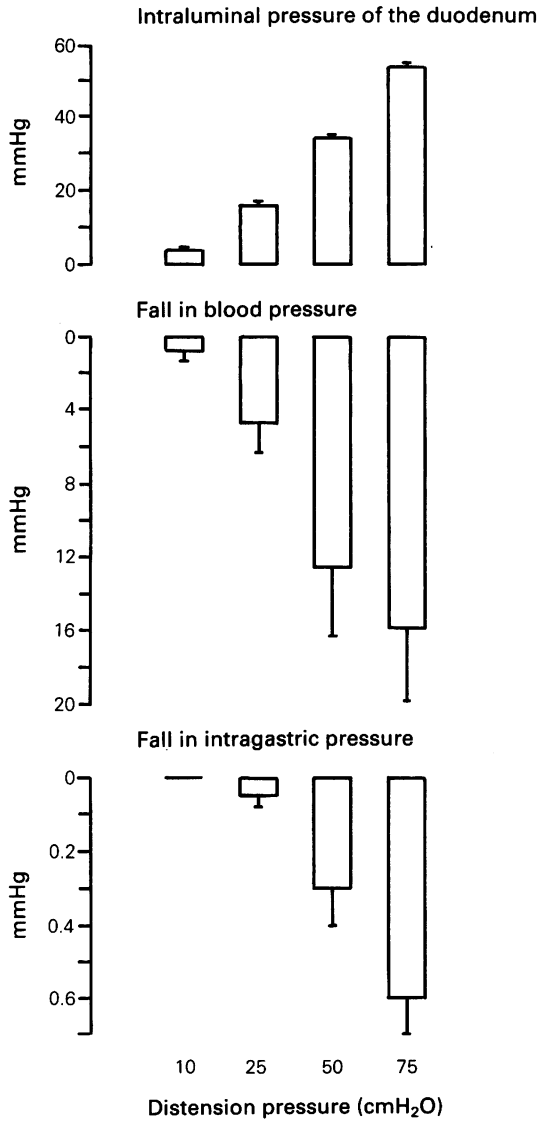

Figure 1 Effect of duodenal distension using pressures of 10, 25, 50 and 75 cmH_2O for 20 s every 5 min on the intraluminal pressure of the duodenum, diastolic blood pressure and intragastric pressure ($n = 6$). In each case, the maximum pressure change evoked by duodenal distension was recorded. Results are expressed as means (columns) with standard errors indicated by vertical bars.

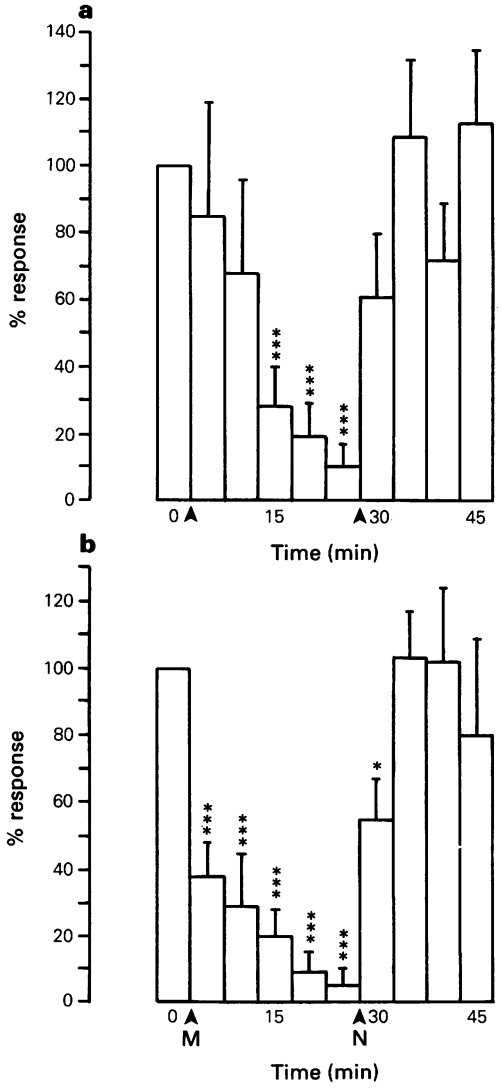

Figure 2 Antagonism by morphine (20 mg kg^{-1}, s.c.) and its reversal by naloxone (5 mg kg^{-1}, i.v.) on the fall in (a) blood pressure and (b) intragastric pressure induced by duodenal distension with 75 cmH_2O for 20 s every 5 min. Morphine (M) and naloxone (N) are given at the arrows. The first column represents the pre-morphine control response taken as 100%. Results are expressed as means (columns) with standard errors shown by vertical bars. * $P < 0.05$; *** $P < 0.005$, with one sample t test ($n = 6$).

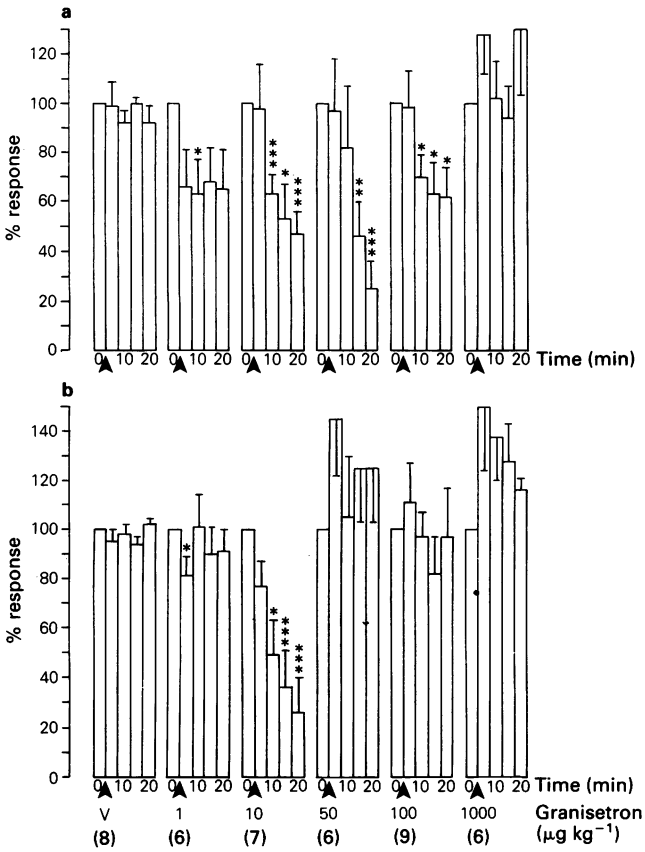

Figure 3 Effects of granisetron (1–1000 $\mu g\,kg^{-1}$, i.v.) or vehicle (V = 0.9% w/v saline) administered after a control distension (arrow) on the fall in (a) blood pressure and (b) intragastric pressure induced by duodenal distension with 75 cmH_2O for 20 s every 5 min. The first column in each group represents the pre-dose control response taken as 100%. Results are expressed as means (columns) with standard errors shown by vertical bars. * $P < 0.05$; *** $P < 0.005$, with one sample t test. Numbers in parentheses indicate the number of rats used.

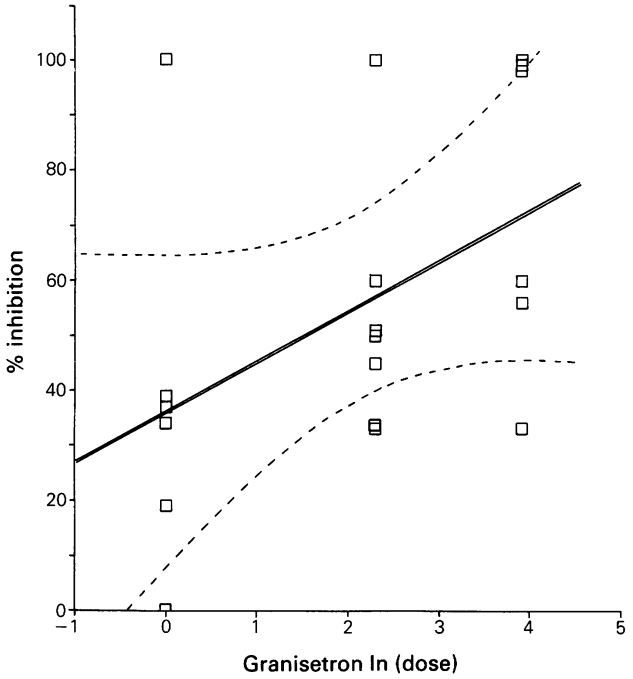

Figure 4 Regression analysis of the effect of granisetron (1, 10 and 50 $\mu g\,kg^{-1}$) on the fall in blood pressure induced by duodenal distension with 75 cmH_2O for 20 s every 5 min. The dose is plotted on a natural log scale and the effect of granisetron as a percentage of the maximum inhibition of the distension-evoked response. Squares represent each data point; $n = 6$ for 1 $\mu g\,kg^{-1}$, $n = 7$ for 10 $\mu g\,kg^{-1}$ and $n = 6$ for 50 $\mu g\,kg^{-1}$. Dotted lines represent 95% confidence limits. Regression line has equation $Y = 9.2 + 36.2$ ($F = 5.006$, $n = 19$, $P < 0.03$).

ICS 205-930 (1–1000 $\mu g\,kg^{-1}$, i.v.) had no effect on resting blood, intragastric or intraduodenal pressure. As with granisetron, ICS 205-930 (10 and 100 $\mu g\,kg^{-1}$, i.v.) reduced the responses to duodenal distension by varying degrees; ICS 205-930 1 $\mu g\,kg^{-1}$ was inactive and 1000 $\mu g\,kg^{-1}$ increased the distension-evoked blood and intragastric pressure responses in, respectively, 5 of 6 and 3 of 6 rats (Figure 5). When the effect of ICS 205-930 (1, 10 and 100 $\mu g\,kg^{-1}$) on the fall in blood pressure was subjected to regression analysis, there was no statistically significant correlation ($F = 2.315$, $n = 15$, $P = 0.15$).

Ondansetron (1–1000 $\mu g\,kg^{-1}$, i.v.) had no effects on resting intragastric or duodenal pressure. However, 100–1000 $\mu g\,kg^{-1}$ ondansetron caused the resting blood pressure to become irregular in 11 out of 22 animals receiving these doses. Furthermore, in 7 of 11 rats dosed with 1000 $\mu g\,kg^{-1}$, ondansetron consistently caused a rapid but transient fall in blood pressure of 16 ± 2 mmHg. Overall, these effects on blood pressure were so marked in these animals ($n = 11$) that it was impossible to test the effect of ondansetron on their response to duodenal distension. In those rats where distension evoked responses could be reliably measured after dosing with ondansetron, there was no statistically significant inhibitory effect of ondansetron on the responses to duodenal distension at any one of the doses tested (1–1000 $\mu g\,kg^{-1}$, i.v.; Figure 6); a lower dose (0.1 $\mu g\,kg^{-1}$; $n = 2$, data not shown) was also inactive. As with the highest dose of granisetron and ICS 205–930 (1000 $\mu g\,kg^{-1}$, i.v.), ondansetron increased the intragastric responses to distension in some animals, but increases also occurred at all other doses tested (3 of 6 rats at 1 $\mu g\,kg^{-1}$, 3 of 9 at 10 $\mu g\,kg^{-1}$, 4 of 7 at 100 $\mu g\,kg^{-1}$ and 2 of 4 at 1000 $\mu g\,kg^{-1}$). Ondansetron also caused small increases in the distension-induced fall in blood pressure at 1 and 100 $\mu g\,kg^{-1}$, i.v. Because of these effects, ondansetron (100 $\mu g\,kg^{-1}$) was tested in 4 rats which failed to respond to duodenal distension; 20 min after dosing, duodenal distension evoked a fall in blood pressure in 2 animals and a fall in intragastric pressure in the other two.

Discussion

In this study, distension of the duodenum has been used as a model for visceral pain in the urethane-anaesthetized rat. Distension evoked falls in blood and intragastric pressure, the magnitude of which was dependent on the degree of pressure used, as previously shown in the jejunum (Lembeck & Skofitsch, 1982). By contrast, the renal pelvis may have an all-or-none response to distension (Brasch & Zetler, 1982).

Following bilateral cervical vagotomy, the distension-evoked fall in blood pressure was unchanged. This was in agreement with previous observations on rat ileum and jejunum (Clark & Smith, 1985; Lembeck & Skofitsch, 1982), suggesting that the neurones involved run in the sympathetic nerves. In contrast, the fall in intragastric pressure produced by duodenal distension was usually greatly reduced by vagotomy; in some animals the response was abolished. These results contrasted with those in the anaesthetized ferret where vagotomy did not affect the gastric relaxation evoked by duodenal distension (Andrews & Lawes, 1984).

The falls in blood and intragastric pressures induced by duodenal distension were blocked by morphine, an action reversed by naloxone. Similar distension pressure within the intestine of conscious rats or rabbits also elicits a morphine-sensitive behavioural response indicative of pain or discomfort (Jensen *et al.*, 1988; Colburn *et al.*, 1989). The fall in blood pressure induced by intestinal distension in anaesthetized rats

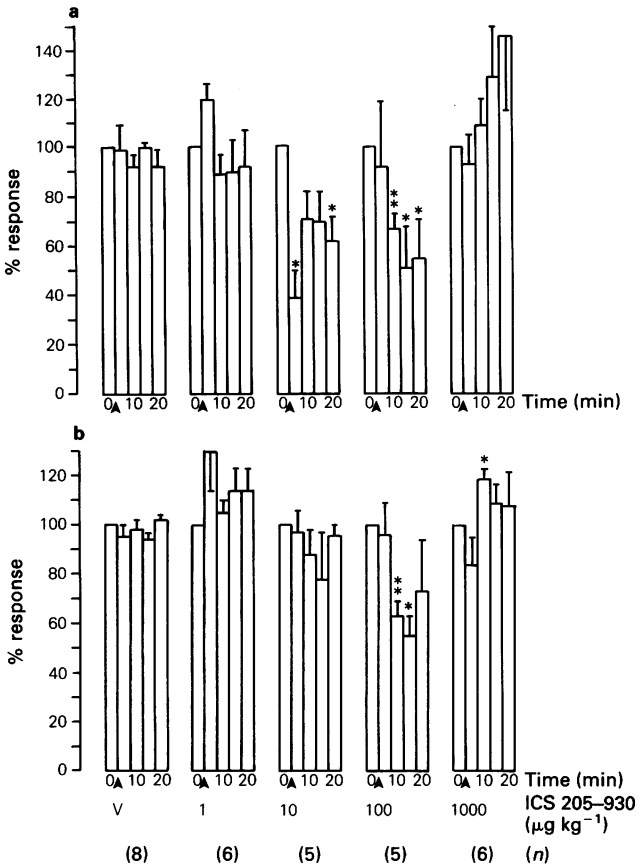

Figure 5 Effect of ICS 205-930 (1–1000 $\mu g\,kg^{-1}$, i.v.) or vehicle (V = 0.9% w/v saline) administered after a control distension (arrow) on the fall in (a) blood pressure and (b) intragastric pressure induced by duodenal distension with 75 cmH_2O for 20 s every 5 min. The first column in each group represents the pre-dose control response taken as 100%. Results are expressed as means (columns) and standard errors (vertical bars). * $P < 0.05$; ** $P < 0.01$, with one sample t test. Numbers in parentheses indicate the number of rats used.

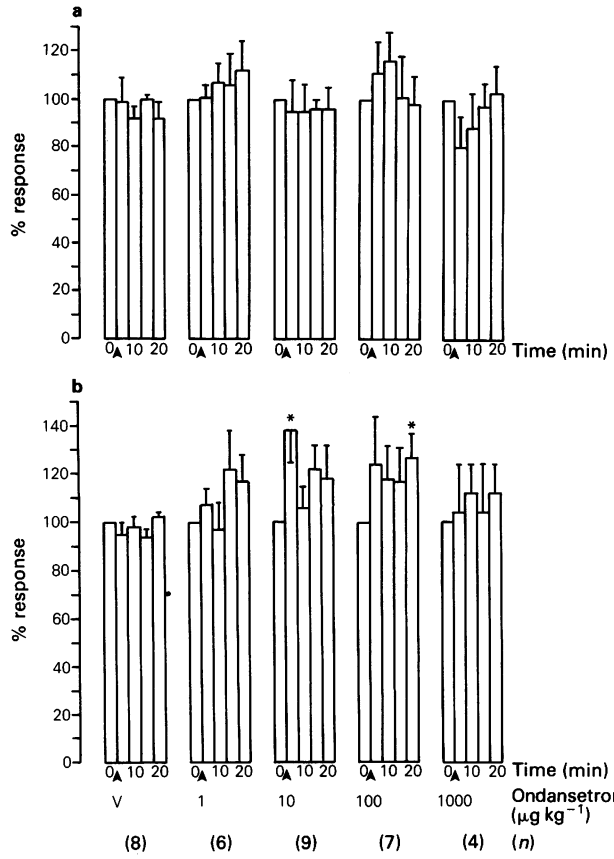

Figure 6 Effect of ondansetron (1–1000 $\mu g\,kg^{-1}$, i.v.) or vehicle (V = 0.9% w/v saline) administered after a control distension (arrow) on the fall in (a) blood pressure and (b) intragastric pressure induced by duodenal distension with 75 cmH_2O for 20 s every 5 min. The first column in each group represents the pre-dose control response taken as 100%. Results are expressed as means (columns) and standard errors (vertical bars). * $P < 0.05$ with one sample t test. Numbers in parentheses indicate the number of rats used.

may, therefore, form part of a pain response (see Introduction).

Granisetron and ICS 205-930 reduced, but did not abolish the responses to duodenal distension. For both compounds, the dose-response curve was bell-shaped. Such curves have previously been reported for ondansetron, granisetron and ICS 205-930 in rat models of anxiety (Costall *et al.*, 1988a) and psychosis (Costall *et al.*, 1988b), and for ondansetron against cytotoxic drug-induced emesis in cancer patients (Kris *et al.*, 1988). The mechanisms which underlie bell-shaped curves are often unknown, but one possibility is that two actions are involved, with the higher doses reducing the activity of the lower doses. With this in mind, interpretation of an ID_{50} value must be treated with caution. Nevertheless, the ID_{50} for granisetron in the present experiments (1.2 $\mu g\,kg^{-1}$, i.v.) is similar to the ID_{50} obtained for granisetron against the 5-HT_3 receptor-mediated Bezold-Jarisch reflex in anaesthetized rats (0.7 $\mu g\,kg^{-1}$, i.v., Sanger & Nelson, 1989). For ICS 205-930, the ability to antagonize the distension-evoked reflex was less consistent and the effective doses (10 and 100 $\mu g\,kg^{-1}$; not 1 $\mu g\,kg^{-1}$, i.v.) are greater than the range of active doses detected in the Bezold-Jarisch reflex test (ID_{50} = 0.4 or 1.4 $\mu g\,kg^{-1}$, i.v., Richardson *et al.*, 1985; Sanger, 1990; $ID_{100} > 10\,\mu g\,kg^{-1}$, i.v., Cohen *et al.*, 1989).

Ondansetron did not reduce the distension-evoked falls in either blood or intragastric pressures. This observation is in marked contrast to the effects of granisetron or ICS 205-930 and conflicts with the potent ability of this compound to antagonize the Bezold-Jarisch reflex in anaesthetized rats (ID_{50} against 2-methyl-5-HT = 0.4 $\mu g\,kg^{-1}$, i.v., Butler *et al.*, 1988; ID_{50} against 5-HT = 3.6 $\mu g\,kg^{-1}$, i.v., Sanger, 1990) and to antagonize the 5-HT_3 receptor in general. Our observations may be explained if a novel 5-HT_3 receptor subtype is involved, if certain compounds have properties which are additional to their ability to antagonize 5-HT_3 receptors and/or if the compounds have different pharmacodynamic profiles. Evidence in favour of a pharmacodynamic difference between the compounds has been suggested by Cohen *et al.* (1989), who found that ondansetron antagonism of the 5-HT-evoked Bezold-Jarisch reflex was of shorter duration than that of ICS 205-930. However, against 2-methyl 5-HT, the duration of action of ondansetron seems to be greater than 2 h (Butler *et al.*, 1988). Alternatively, our experiments show that ondansetron tended to increase the response to duodenal distension at lower doses than granisetron and ICS 205-930. If the affinity of ondansetron for a receptor causing an increase in the distension-induced reflex is higher than its affinity for a receptor which would inhibit the reflex, then no change or an increase in the distension-evoked reflex would be seen. For granisetron and to a lesser extent, ICS 205-930, these affinities could be reversed, resulting in a bell-shaped dose-response curve. For either of these activities, the compounds could, therefore, act at the 5-HT_3 receptor, at an as yet unclassified 5-HT_3 receptor subtype or at a non-5-HT_3 receptor. Further analysis is clearly required, so that these suggestions can be tested.

The site of action for granisetron and ICS 205-930 is not known. It is unlikely to be a direct effect on the smooth muscle of the blood vessels and the stomach. Neither compound affected resting blood pressure or intragastric pressure and granisetron has no effect on the fall in blood pressure

evoked by vagal stimulation (Sanger & Nelson, 1988). If granisetron and ICS 205-930 act by antagonizing a 5-HT receptor, this may be present on the peripheral afferent nerve fibres, being activated by 5-HT released from enterochromaffin cells by distension (Bülbring & Crema, 1959). Alternatively, they may also act within the central nervous system or spinal cord. Cramer & Rademaker (1989) found that in anaesthetized rats, ICS 205-930 (9 μmol kg^{-1}, i.p.) reduced the fall in blood pressure evoked by noxious distension of the proximal ileum, but did not affect the afferent nerve activity arising from the ileum. A site of action outside the gut was, therefore, suggested.

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