

# Effect of Indomethacin, Renzapride, Methysergide, Ketanserin, Granisetron and Citalopram on Serotonin-Induced Fluid Accumulation in Pig Jejunum

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## Summary

The purpose of this study was to elucidate the intestinal serotonin (5-HT) receptor subtypes involved in fluid transport in the pig jejunum *in vivo*. The fluid accumulating effect of intraluminally administered 5-HT, renzapride, methysergide, ketanserin, granisetron, citalopram and intravenous indomethacin, was tested in tied-off loops *in vivo*. 5-HT caused a dose-dependent fluid accumulation, which was reduced by indomethacin by about 30 %. Renzapride, methysergide, ketanserin, granisetron and citalopram all caused fluid accumulation. Taking into account these fluid accumulating effects, renzapride, methysergide, ketanserin and granisetron reduced the fluid accumulating effect of 5-HT, giving a maximal reduction of 70, 46, 76, and 80 %, respectively. These data suggest the existence of intestinal 5-HT receptor subtypes involved in fluid transport in the pig jejunum. The antagonistic effects of indomethacin, ketanserin and granisetron, suggest the involvement of prostaglandins, as well as the 5-HT<sub>2</sub> and the 5-HT<sub>3</sub> receptor subtypes in the fluid accumulating response of 5-HT.

## Key words

5-hydroxytryptamine – Receptors – Jejunum – Pig – Secretion – Physiology – Pharmacology

## Introduction

In the pig small intestine, serotonin (5-hydroxytryptamine, 5-HT) is located in both the myenteric and submucosal plexus in addition to the enterochromaffine (EC) cells (Timmermans *et al.* 1990). The pig was chosen as the experimental animal, since it has been reported to be the best model for 5-HT-related processes in the human gastrointestinal (GI) tract (Schworer *et al.* 1992).

The release of 5-HT from EC cells, locally and into the lumen, is a complex calcium-dependent process and is a result of exocytosis predominantly across the basolateral membrane (Schworer *et al.* 1992). Release of 5-HT can be evoked by enterotoxins such as the cholera toxin, mechanical factors, and by chemical stimuli (Schworer *et al.* 1992). Furthermore, 5-HT is one of the endogenous secretagogues responsible for diarrhoea in patients with cholera and with the carcinoid syndrome (Ahlman 1985).

5-HT exerts its effects by activating either G protein-linked receptors (5-HT<sub>1</sub>, 5-HT<sub>2</sub> and 5-HT<sub>4</sub>) or ligand-gated ion channels (5-HT<sub>3</sub> subtype) (Derkach *et al.* 1989). The importance of the different intestinal 5-HT receptor subtypes in intestinal fluid transport is not yet clarified, since there are discrepancies among the reported effects of 5-HT agonists and antagonists on intestinal fluid transport. These differences are assumed to be due to the well-documented segmental and species differences in 5-HT receptor subtypes (Kilpatrick *et al.* 1991, Hartig *et al.* 1992, Hansen 1992, Sjöqvist *et al.* 1992).

To obtain operational data on the intestinal 5-HT receptor subtypes involved in intestinal fluid transport, we tested the effects of intraluminally administered 5-HT and selective 5-HT antagonists.

## Methods

Danish Landrace/Yorkshire crossbred 8-week-old fully weaned pigs (13–15 kg) on a normal diet were used. Animals were fasted for 12 hrs before surgery but had free access to sterile drinking water containing glucose (55 g/l). Twenty minutes before the onset of general anaesthesia, the pigs were sedated by an intramuscular injection (5 mg/kg) of azaperone (Sedaperone, Janssen, Denmark). After an intravenous injection (10 mg/kg) of pentobarbital (Mebumal, University Pharmacy, Denmark), the pigs were intubated, and anaesthesia was maintained by halothane inhalation (2% in oxygen) *via* a closed circuit throughout the experiment.

Using the method described by Lange *et al.* (1987), a midline abdominal incision was made. About twenty jejunal loops were prepared by ligaturing between the mesenteric arcades. The jejunal loops were located 0.6 m distal to the ligament of Treitz. Loops were  $\approx$ 12 cm in length, and the space between loops was 2 cm. Intact blood supply was ensured. Each loop was injected with 3 ml ( $\approx$ 3040 mg) of a buffered solution containing (mM): 10 Na<sup>+</sup>, 7 Cl<sup>-</sup>, 3 HCO<sub>3</sub><sup>-</sup>, 80 glucose; pH 7.4 alone, 95 $\pm$ 1 mOsm (R controls), and containing, in random order, doses of 5-HT (5-hydroxytryptamine creatinine sulfate complex, Sigma Chemicals, St. Louis, MO) alone, 5-HT together with antagonist (see later) or citalopram, antagonist or citalopram alone, or was left unfilled (U controls). Since the loops were only incubated for 15–60 min, a hypotonic control solution was used to ensure total absorption in R loops and to avoid hypertonic solutions after addition of 5-HT with antagonists or citalopram. Using hypotonic solutions also ensured a fast absorption of the water soluble drugs by solvent drag ("bulk flow") (Naftalin and Tripathi 1983, Pappenheimer and Reiss 1987), of the lumenally administered compounds, to the serosal side of the epithelium, where these are expected to exert their effects (Cooke *et al.* 1983). After filling, the loops were returned to the peritoneal cavity, and the abdomen was closed using forceps. The loops occupied about 20% of the small intestine.

Starvation ensured that the intestine was empty, and adding glucose to the drinking water reduced possible starvation reactions and enhanced the effects of secretagogues (Argenzio 1980, White *et al.* 1986). Adding glucose to the R solution also ensured complete absorption of the control solution (Argenzio 1980).

After 45 min the abdomen was reopened, unless otherwise stated. All loops were removed within 2 min and weighed with and without the fluid content. The loops were emptied of fluid by gently squeezing following a transverse incision. Fluid accumulation was calculated by subtraction and is expressed as mg/12 cm loop per 45 min, which gives a similar accuracy as

volumetrically measured values per weight (Lange *et al.* 1987, McEwan *et al.* 1990).

Finally, the lengths of the loops were measured to calculate loop weight/length ratios, which would uncover possible differences in intramural fluid accumulation (McEwan *et al.* 1990). There were no significant differences between 5-HT controls, R controls, U controls or loops treated with agonists and antagonists. All weight/length ratios averaged  $\approx$  0.7.

In experiments reported in the Result sections 1 and 4, the production of prostaglandins was inhibited by giving freshly prepared indomethacin (5 mg/kg, Sigma Chemicals, St. Louis, MO) intravenously 5 min before the abdominal incision.

### Control experiments:

Experiments with R and U control loops, located adjacent to the loops injected with agonists and antagonists, demonstrated that loops did not influence each other with regard to fluid accumulation. Graded doses of NaCl (i.e. absorbable solute) and d-mannitol (i.e. non-absorbable solute) were added to the R control solution to study the putative osmotic effect on fluid accumulation. The R solution with 134 $\pm$ 1, 177 $\pm$ 1, and 207 $\pm$ 1 mOsm NaCl gave the following fluid accumulations: 250 $\pm$ 72, n=4; 288 $\pm$ 106, n=4; and 363 $\pm$ 57 mg, n=3, respectively, N=3. These data are not significantly different from those in R loops. Furthermore, osmotic control experiments with R solution added the non-absorbable d-mannitol, gave the expected osmotic-dependent fluid accumulation in the hypertonic 300 $\pm$ 1 mOsm solution: The R solutions with 200, 250, and 300 mOsm d-mannitol gave a fluid accumulation of 440 $\pm$ 50, n=3; 355 $\pm$ 55, n=4; and 1920 $\pm$ 134 mg, n=6, respectively. In conclusion, the fluid accumulations observed in this paper caused by intraluminal 5-HT agonists or antagonists, are not influenced by the variations in osmolality of the instilled drug solutions.

Animals were killed by an intravenous overdose of pentobarbital.

The study is divided into four major parts;

- 1) 5-HT time-response, 5-HT +/- indomethacin,
- 2) 5-HT +/- antagonists,
- 3) 5-HT +/- citalopram, and
- 4) 5-HT + indomethacin +/- antagonists.

### Drugs

The following compounds were kindly donated from the following sources: ketanserin (R 49945), Janssen, Beerse, Belgium; methysergide, Sandoz, Basel, Switzerland; renzapride (BRL 24924) and granisetron (BRL 43694), SmithKline Beecham, Betsworth, England; citalopram, Lundbeck Pharma, Copenhagen, Denmark. The remaining compounds used were purchased from Sigma Chemicals, St. Louis, MO.

Methysergide is a 5-HT<sub>1</sub>-like antagonist with low affinity for the 5-HT<sub>2</sub> receptor (Bradley *et al.* 1986); renzapride, a substituted benzamide, acts as an agonist at the 5-HT<sub>4</sub> receptor site and as an antagonist to the 5-HT<sub>1P</sub> and 5-HT<sub>3</sub> receptors (Gershon *et al.* 1990); ketanserin is a 5-HT<sub>2</sub> receptor antagonist with low affinity for the  $\alpha_1$  adrenergic and H<sub>1</sub> receptors (Leysen *et al.* 1981); granisetron is a 5-HT<sub>3</sub> receptor antagonist (Sanger and Nelson 1989); citalopram is a specific 5-HT reuptake-inhibitor (Hyttel *et al.* 1984); and indomethacin is an inhibitor of the cyclooxygenase enzyme (Rask-Madsen *et al.* 1990).

### Statistics

The results are expressed as mean fluid accumulation per loop  $\pm$  S.E.M. with *n* equal to the number of loops, and *N* equal to the number of pigs, which was 3 unless otherwise stated. The data were compared to 5-HT controls (26 mM) unless stated otherwise. Student's paired and unpaired t-tests were used for *n* values to calculate significant differences.  $P < 0.05$  value was considered as significant. When data agreed with simple Michaelis-Menten kinetics, the apparent  $E_{MAX}$  and  $EC_{50}$  were calculated using non-linear curve fitting. An estimated  $E_{MAX}$  and  $EC_{50}$  was used when the data did not show simple Michaelis-Menten kinetics and were obtained directly from the figures.

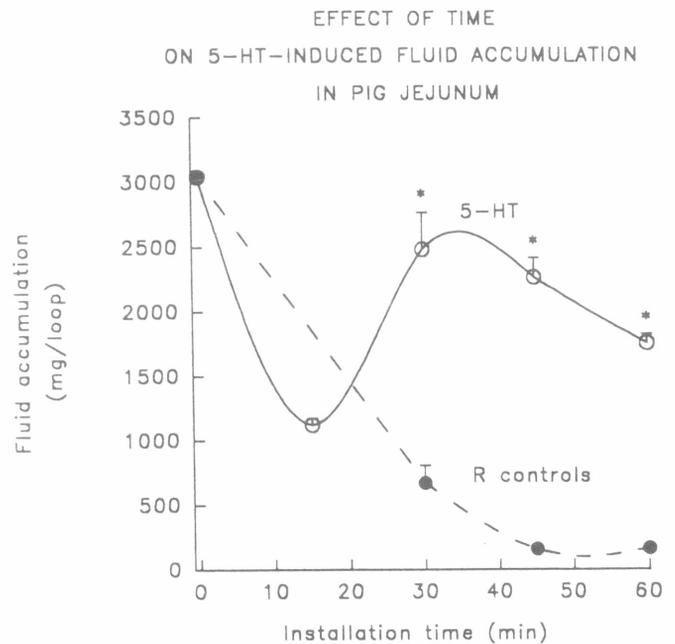
## Results

**Exp. 1:** 5-HT time response and 5-HT +/- indomethacin (Figs 1 and 2).

The time-dependent effects of 26 mM 5-HT on fluid accumulation were measured to determine the time of installation, which would result in maximal fluid accumulation. A maximal effect was observed after 30 min. R controls were completely absorbed after 45 min, but not after 30 min. Thus the 45 min installation time was used in the following experiments. 5-HT induced a dose-dependent fluid accumulation. The apparent  $E_{MAX}$  was  $5962 \pm 348$  mg and the  $EC_{50}$  was  $47 \pm 7$  mM. Indomethacin significantly reduced 5-HT-induced fluid response by about 30 %, giving an apparent  $E_{MAX}$  of  $4252 \pm 611$  mg and  $EC_{50}$  of  $44 \pm 16$  mM of 5-HT.

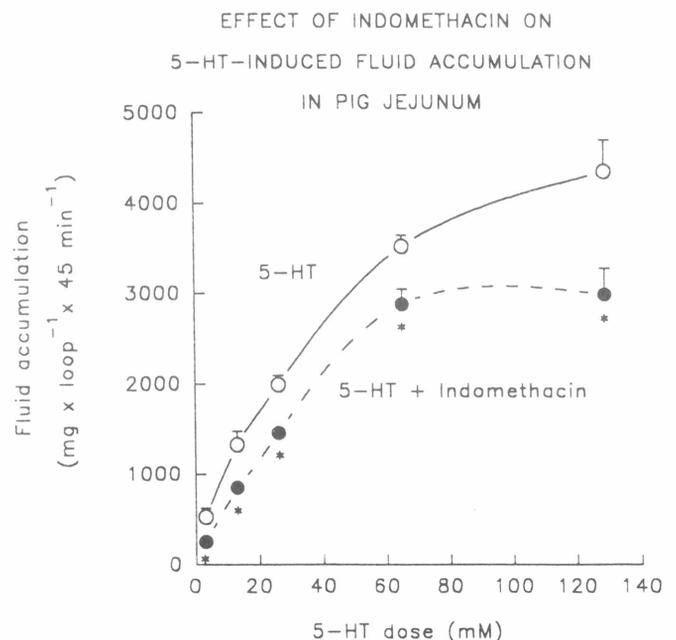
**Exp. 2:** A. Renzapride +/- 5-HT (Fig. 3 and Table 1).

Larger doses than 3 mM of renzapride caused a dose-dependent fluid accumulation. The response-curve of renzapride suggests several sigmoid-shaped curves connected to each other; in the range 0 to 15 mM, 15 to 40 mM, and 40 to 70 mM, respectively.



**Fig. 1**

Time-dependent fluid accumulation for 15, 30, 45 and 60 min, induced by 26 mM serotonin (5-HT, *n*=3, 8, 5 and 3 for the increasing time, *N*=4). Data represent mean fluid accumulation  $\pm$  S.E.M. \*  $p \leq 0.05$  compared to R controls (*n*=4, 4, 45, and 3 for the increasing time, *N*=47).



**Fig. 2**

Indomethacin reduces the dose-dependent 5-HT-induced fluid accumulation. Data represent mean fluid accumulation  $\pm$  S.E.M. in non-treated pigs (*n*=23, 19, 28, 18, and 16 for increasing 5-HT doses, *N*=20) and indomethacin-treated (5-HT + indomethacin, *n*=22, 26, 41, 24, and 10 for increasing 5-HT doses, *N*=12), \*  $p \leq 0.05$ .

**Table 1**

The fractional effect of graded doses of intraluminal antagonist on fluid accumulation induced by intraluminal 5-HT (26 mM, % of control) in pig jejunum *in vivo*.

Antagonist Dose (mg/ml)	1	5	10	15	20	25
Renzapride	76±6 %*	48±11 %*	30±13 %*	-26±10 %*	-21±4 %*	-24±11 %*
Methysergide	107±16 %	94±14 %	75±10 %*	63±16 %*	84±17 %*	-
Ketanserin	75±4 %*	60±6 %*	24±6 %*	29±7 %*	36±9 %*	-
Granisetron	-	75±5 %*	66±6 %*	49±7 %*	20±7 %*	34±5 %*
Indomethacin + Ketanserin	90±5 %	73±5 %*	34±6 %*	-	40±10 %*	-
Indomethacin + Granisetron	-	89±6 %	92±4 %	-	-	38±8 %*

Taking into account the fluid accumulating effect of the antagonist alone, the fractional effect of graded doses of antagonist (renzapride, methysergide, ketanserin, and granisetron) on fluid accumulation (%) induced by 26 mM 5-HT, are presented. Data represent an operational calculation of the mean fluid accumulation ± S.E.M. induced by the combination of antagonist and 5-HT minus the effect of antagonist alone, and compared to 5-HT controls, 100±5 % (1894±103 mg), and 100±3 % (1464±46 mg) for the indomethacin-treated pigs. \*  $p \leq 0.05$  compared to 5-HT controls.

Renzapride, combined with 5-HT (26 mM), did not change the 5-HT-induced secretion except at the 70 mM dose, which significantly increased the effect of 5-HT. Taking into account the fluid accumulating effect of renzapride alone, the fluid accumulating effect of 5-HT was reduced maximally by about 70 % using renzapride in the 40 mM dose. Using higher doses than 40 mM caused negative values in the Table 1, since the fluid accumulating effect of renzapride alone exceeded the effect of combining renzapride and 5-HT.

#### B. Methysergide +/- 5-HT (Fig. 4 and Table 1).

Methysergide alone caused significant fluid accumulation in a dose-dependent manner with an apparent  $E_{MAX}$  of 2348±524 mg and  $EC_{50}$  of 11±8 mM. Methysergide increased the fluid accumulating effect of 5-HT (26 mM) in a dose-independent way. The increase was 30 % on the average. Taking into account the fluid accumulating effect of methysergide alone, the fluid accumulating effect of 5-HT was reduced maximally by about 46 % using methysergide in the 44 mM dose.

#### C. Ketanserin +/- 5-HT (Fig. 5 and Table 1).

Ketanserin alone induced significant fluid accumulation dose-dependently (apparent  $E_{MAX}$  of 3413±778 mg and  $EC_{50}$  of 31±13 mM). Ketanserin did

not change the 5-HT (26 mM) effect in low doses (2–18 mM), but increased the 5-HT effect in larger doses than 18 mM. Taking into account the fluid accumulating effect of ketanserin alone, the fluid accumulating effect of 5-HT was reduced maximally by about 76 % using ketanserin in the 18 mM dose.

#### D. Granisetron +/- 5-HT (Fig. 6 and Table 1).

Granisetron alone evoked significant fluid accumulation. The dose-dependent effect of granisetron gave an estimated  $E_{MAX}$  of 1500 mg using about 60 mM, and  $EC_{50}$  of about 40 mM.

The 5-HT-induced fluid accumulation was unchanged by granisetron except for the highest granisetron dose used (68 mM), which caused a significant increase. Again, the effect of 5-HT plus granisetron showed an additive pattern. Taking into account the fluid accumulating effect of granisetron alone, the fluid accumulating effect of 5-HT was reduced maximally by about 80 % using granisetron in the 54 mM dose.

#### Exp. 3: Citalopram +/- 5-HT (Fig. 7 and Table 1).

Citalopram alone was a very strong and potent stimulator of fluid accumulation. An apparent  $E_{MAX}$  was 16480±3629 mg and the  $EC_{50}$  was 52±19 mM.

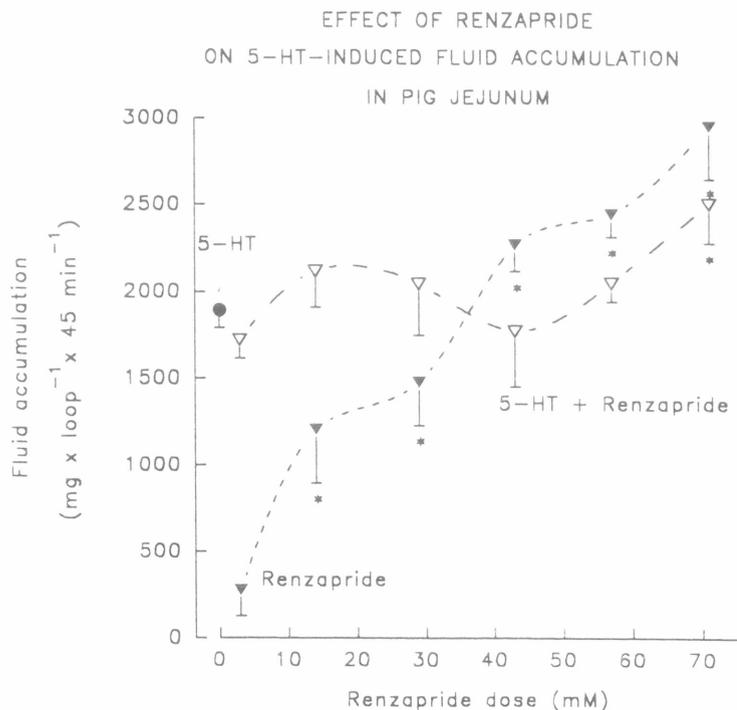


Fig. 3

Dose-dependent effects of renzapride alone (renzapride,  $n=3$  for all doses,  $N=3$ ) and of renzapride plus 26 mM 5-HT on fluid accumulation (5-HT + renzapride,  $n=6$  for all doses,  $N=3$ ) are presented. Data represent mean fluid accumulation  $\pm$  S.E.M. \*  $p \leq 0.05$  for renzapride alone compared to R controls (not shown,  $158 \pm 23$  mg,  $n=45$ ,  $N=45$ ), and 5-HT + renzapride compared to 5-HT controls (5-HT, 26 mM,  $1894 \pm 103$  mg,  $n=42$ ,  $N=42$ ), respectively.

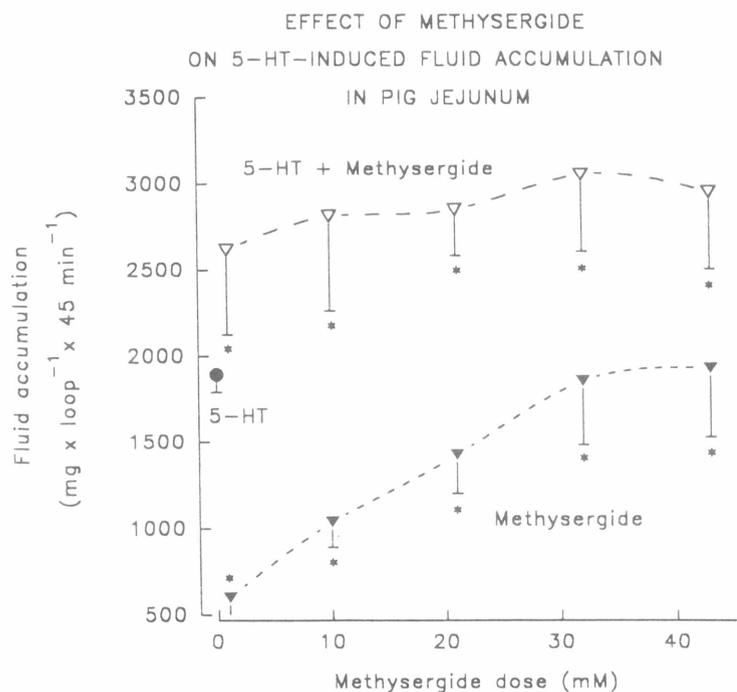


Fig. 4

Dose-dependent effects of methysergide alone (methysergide,  $n=3$  for all doses,  $N=3$ ) and of methysergide plus 26 mM 5-HT on fluid accumulation (5-HT + methysergide,  $n=6$  for all doses,  $N=3$ ) are described. Data represent mean fluid accumulation  $\pm$  S.E.M. \*  $p \leq 0.05$  methysergide alone compared to R controls (not shown,  $158 \pm 23$  mg,  $n=45$ ,  $N=45$ ), and 5-HT + methysergide compared to 5-HT controls (5-HT, 26 mM,  $1894 \pm 103$  mg,  $n=42$ ,  $N=42$ ), respectively.

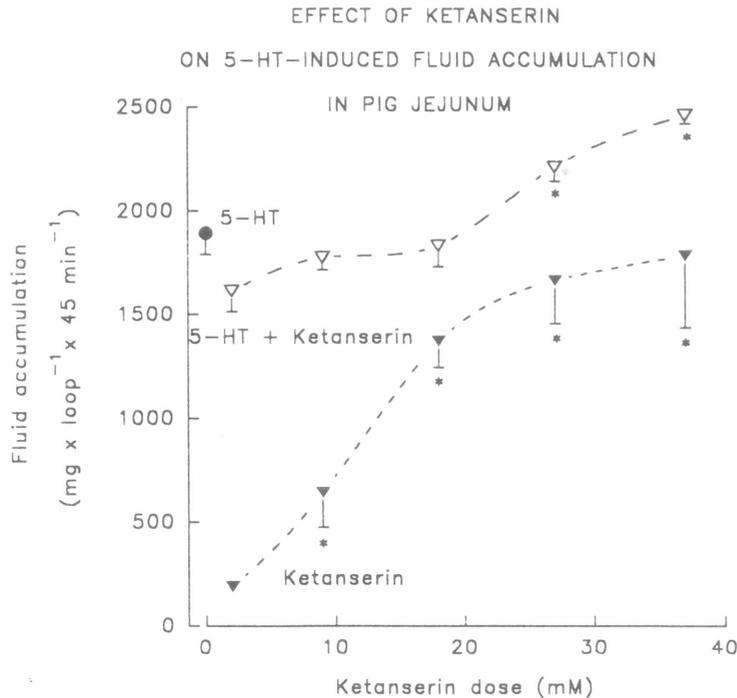
Citalopram caused a dose-dependent increase of 5-HT-induced fluid accumulation, not significantly different from citalopram alone, except with the 2 and 25 mM citalopram doses.

**Exp. 4:** Ketanserin or granisetron +/- 5-HT + indomethacin (Figs 8 and 9, Table 1).

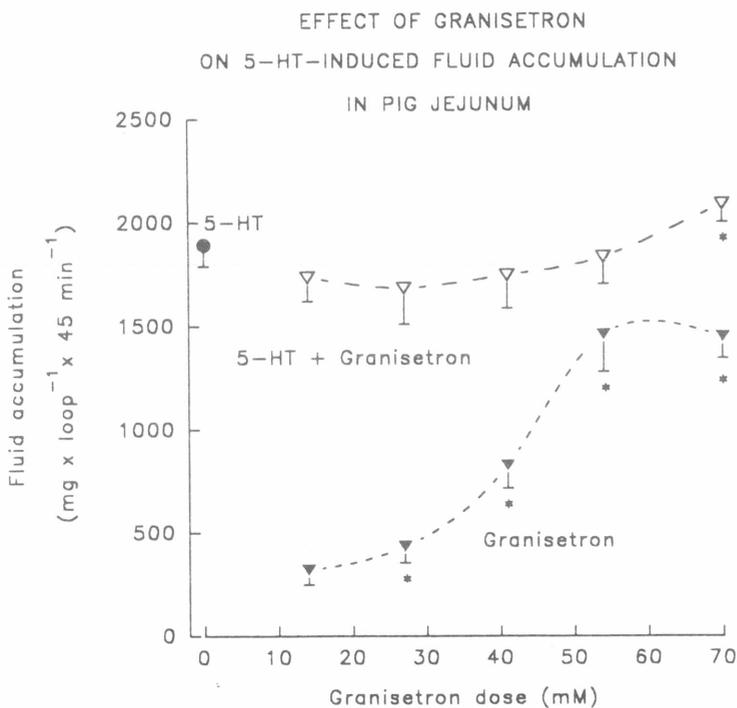
The effects of ketanserin alone, granisetron alone, ketanserin plus 5-HT, and granisetron plus 5-HT

demonstrated in indomethacin-treated pigs the same dose-dependent responses on fluid accumulation as in indomethacin non-treated pigs (part 2C and 2D).

As in the indomethacin non-treated pigs, ketanserin and granisetron reduced the 5-HT effects, maximally by about 66% and 62%, respectively, when the fluid accumulating effect of ketanserin and granisetron were taken into account.

**Fig. 5**

Dose-dependent effects of ketanserin alone (ketanserin,  $n=3$  for all doses,  $N=3$ ) and of ketanserin plus 26 mM 5-HT on fluid accumulation (5-HT + ketanserin,  $n=7$  for all doses,  $N=3$ ) are reported. Data represent mean fluid accumulation  $\pm$  S.E.M. \*  $p \leq 0.05$  ketanserin alone compared to R controls (not shown,  $158 \pm 23$  mg,  $n=45$ ,  $N=45$ ), and 5-HT + ketanserin compared to 5-HT controls (5-HT, 26 mM,  $1894 \pm 103$  mg,  $n=42$ ,  $N=42$ ), respectively.

**Fig. 6**

Dose-dependent effects of granisetron alone (granisetron,  $n=6$  for all doses,  $N=3$ ) and of granisetron plus 26 mM 5-HT on fluid accumulation (5-HT + granisetron,  $n=7$  for all doses,  $N=3$ ) are presented. Data represent mean fluid accumulation  $\pm$  S.E.M. \*  $p \leq 0.05$  granisetron alone compared to R controls (not shown,  $158 \pm 23$  mg,  $n=45$ ,  $N=45$ ), and 5-HT + granisetron compared to 5-HT controls (5-HT, 26 mM,  $1894 \pm 103$  mg,  $n=42$ ,  $N=42$ ), respectively.

## Discussion

The aim of this study was to determine the involvement of the intestinal 5-HT subtypes. Thus the drugs were administered intraluminally. In support of this route of administration, it is known that 5-HT is released intramurally and intraluminally by enterotoxins (Nilsson *et al.* 1983), it passes easily transmurally (Cooke *et al.* 1983) to the serosal side of

the intestinal epithelium, where it is expected to exert its effects (Cooke *et al.* 1983). Since the intramural metabolism of 5-HT is fast (Cooke *et al.* 1983), and high doses of 5-HT are needed for inducing a short-circuit current (i.e. chloride secretion), when administered to the luminal surface of tissue sheets mounted in the Ussing chamber (data not shown), compounds were administered in relatively high doses.

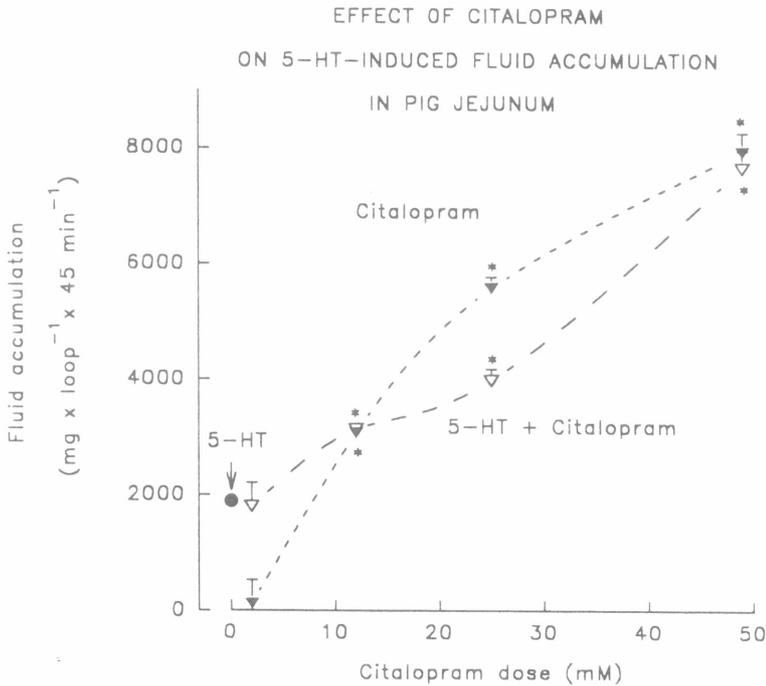


Fig. 7

Dose-dependent effects of citalopram alone (citalopram,  $n=4$  for all doses,  $N=3$ ) and of citalopram plus 26 mM 5-HT on fluid accumulation (5-HT + citalopram,  $n=6$  for all doses,  $N=3$ ) are reported. Data represent mean fluid accumulation  $\pm$  S.E.M. \*  $p \leq 0.05$  citalopram alone compared to R controls (not shown,  $158 \pm 23$  mg,  $n=45$ ,  $N=45$ ), and 5-HT + citalopram compared to 5-HT controls (5-HT, 26 mM,  $1894 \pm 103$  mg,  $n=42$ ,  $N=42$ ), respectively.

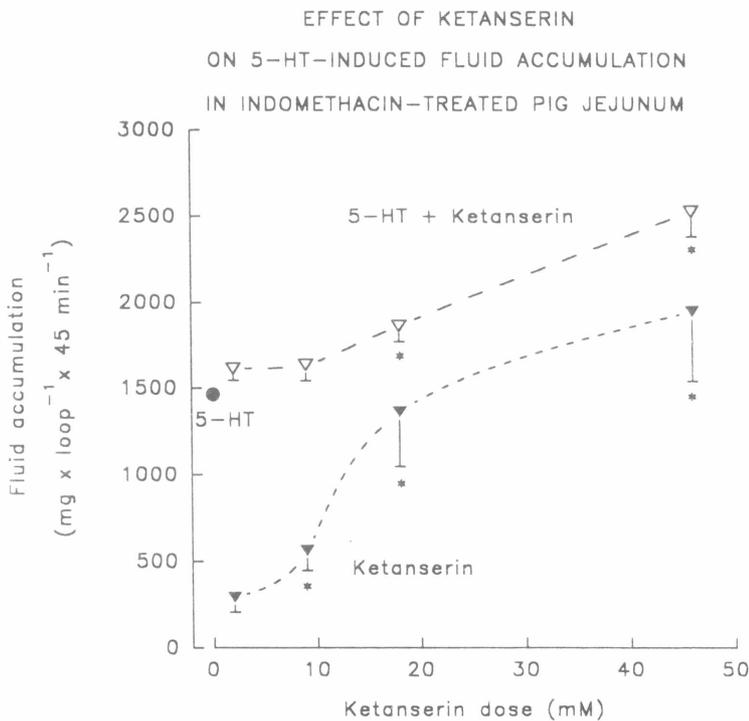


Fig. 8

Dose-dependent effects of ketanserin alone (ketanserin,  $n=3$  for all doses,  $N=3$ ) and of ketanserin plus 26 mM 5-HT on fluid accumulation (5-HT + ketanserin,  $n=7$  for all doses,  $N=3$ ) in indomethacin-treated pigs, are depicted. Data represent mean fluid accumulation  $\pm$  S.E.M. \*  $p \leq 0.05$  ketanserin alone compared to R controls (not shown,  $202 \pm 35$  mg,  $n=12$ ,  $N=12$ ), and 5-HT + ketanserin compared to 5-HT controls (5-HT, 26 mM,  $1464 \pm 46$  mg,  $n=42$ ,  $N=15$ ), respectively.

Furthermore, using the intraluminal route of administration, a minimal systemic effect on the CNS and the cardiovascular system was expected, since experiments were shortlasting and the levels of 5-HT in venous blood are unchanged following intraluminal administration of 5-HT (Hansen and Jaffe 1993a). Finally, the fluid accumulating effect of 5-HT (26 mM) in control experiments did not differ from the effects of

26 mM 5-HT in pigs treated with 5-HT antagonists administered intraluminally in other loops.

In this study, 5-HT was demonstrated to be a potent stimulator of fluid accumulation in the pig jejunum. It has also been demonstrated to be an intestinal secretagogue both *in vivo* and *in vitro* in several other species (Donowitz *et al.* 1977, Cassuto *et al.* 1982, Cooke and Carey 1985, Moriarty *et al.* 1987, Hansen and Bindsvlev 1989, Hansen and Jaffe 1993a).

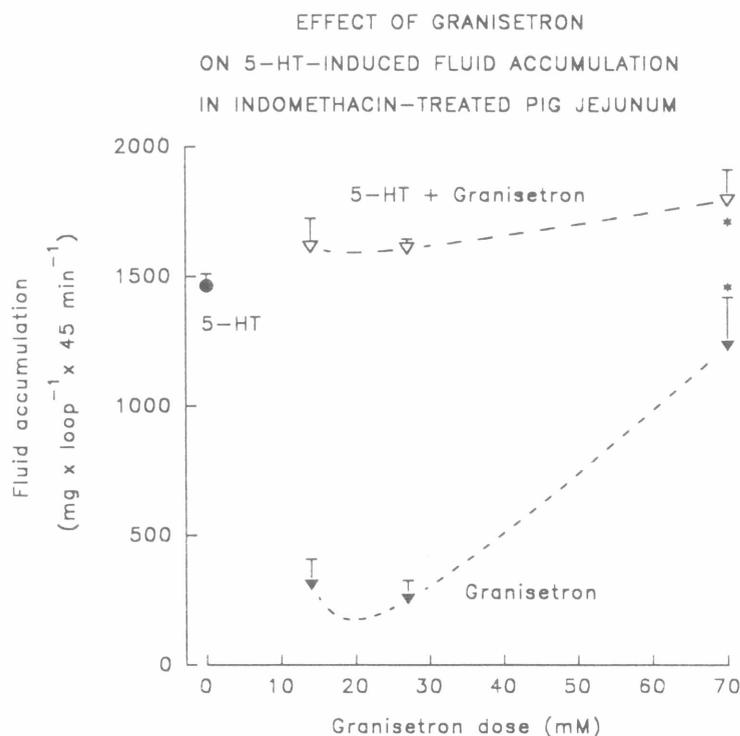


Fig. 9

Dose-dependent effects of granisetron alone (granisetron,  $n=6$  for all doses,  $N=3$ ) and of granisetron plus 26 mM 5-HT on fluid accumulation (5-HT + granisetron,  $n=7$  for all doses,  $N=3$ ) in indomethacin-treated pigs are presented. Data represent mean fluid accumulation  $\pm$  S.E.M. \*  $p \leq 0.05$  granisetron alone compared to R controls (not shown,  $202 \pm 35$  mg,  $n=12$ ,  $N=12$ ), and 5-HT + granisetron compared to 5-HT controls (5-HT, 26 mM,  $1464 \pm 46$  mg,  $n=42$ ,  $N=15$ ), respectively.

Indomethacin reduced the fluid accumulating effect of 5-HT. These results were most likely due to decreased production (and release) of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), since PGE<sub>2</sub> has previously been demonstrated to be an important modulator of intestinal secretion. It is synthesized by non-endocrine cells in response to several neurotransmitters in addition to 5-HT, as well as to hormones, bacterial enterotoxins, luminal antigens, mechanical trauma and hypoxia (Rask-Madsen *et al.* 1990, Hansen and Jaffe 1993b).

The main aim of this study was to elucidate, which of the intestinal 5-HT receptors are involved in 5-HT-induced fluid accumulation. The 5-HT<sub>2</sub> subtype, appears to be present on enterocytes, smooth muscle cells, neurones, and others whereas 5-HT<sub>1A</sub>, 5-HT<sub>1P</sub>, 5-HT<sub>3</sub>, and 5-HT<sub>4</sub> subtypes are solely neuronal (Gershon *et al.* 1990, Frieling *et al.* 1991).

We expected the involvement of 5-HT<sub>2</sub>, 5-HT<sub>3</sub> and 5-HT<sub>4</sub> subtypes, since they have all been demonstrated to be of importance for the secretory response of 5-HT in the small intestine of the rat (Beubler and Horina 1990, Hardcastle and Hardcastle 1991, Hansen and Jaffe 1993a).

The fluid accumulating response of 5-HT was reduced by all tested 5-HT antagonists, when the "intrinsic" fluid accumulating effects of antagonists were taken into account. Using this operational calculation, renzapride, methysergide, ketanserin, and granisetron reduced the fluid accumulating effects of 5-HT maximally by 70, 46, 76, and 80 %, respectively.

As interpreted from studies in the rat (Beubler and Horina 1990, Hansen and Jaffe 1993a), the antagonistic effects of ketanserin and granisetron were

most likely due to a blockade of 5-HT<sub>2</sub> and 5-HT<sub>3</sub> receptors, although ketanserin also has some affinity for  $\alpha_1$ -adrenergic and H<sub>1</sub>-histaminergic receptors (Leysen *et al.* 1981). The mechanisms behind the antagonistic effects of renzapride and methysergide are more speculative. Renzapride has antagonistic properties for both 5-HT<sub>1P</sub> and the 5-HT<sub>3</sub> receptors, while methysergide blocks 5-HT<sub>1</sub> receptors non-selectively in low doses and 5-HT<sub>2</sub> receptors in high doses (Bradley *et al.* 1986, Gershon *et al.* 1990). Taking into account the antagonistic effects of selective 5-HT<sub>2</sub> antagonist ketanserin and the selective 5-HT<sub>3</sub> antagonist granisetron in this study, the antagonistic effects of renzapride and methysergide were therefore most likely due to 5-HT<sub>3</sub> and 5-HT<sub>2</sub> antagonism, respectively.

Listed according to their potency, citalopram > renzapride > methysergide > ketanserin > granisetron *per se* evoked fluid accumulations.

Citalopram, which inhibits the 5-HT transporter in the CNS, most likely non-selectively, activated all the different 5-HT subtypes by increasing the amounts of 5-HT, by inhibiting the 5-HT transporter and reuptake mechanism (Hyttel *et al.* 1984, Bockaert *et al.* 1992, Frieling *et al.* 1991, Gershon *et al.* 1990). Thus the 5-HT transporter in the small intestine does not seem to differ from that in the CNS.

The pathways by which renzapride, methysergide, ketanserin, and granisetron elicited fluid accumulation are uncertain, since they could act at several receptors. Osmotic control experiments using similar high doses of cisapride (data not shown), NaCl, and the non-absorbable mannitol excluded a simple

osmotic mechanism. An intrinsic agonistic activity is possible because Hoyer and Boddeke (1993) have demonstrated that many 5-HT agonists display large differences in activity and potency, depending on the preparation used. This is explained by variations in receptor reserve and by alternate G protein and second messenger coupling.

The present experiments allow the following interpretations concerning the possible mechanisms of action. Renzapride could cause fluid accumulation by antagonizing the 5-HT<sub>1P</sub> receptors, which are responsible for both presynaptic inhibition and postsynaptic excitation (slow EPSP) (Gershon *et al.* 1991), and by stimulating the secretory 5-HT<sub>4</sub> subtype (Bockaert *et al.* 1992, Hardcastle and Hardcastle 1991). The dose-response curve of renzapride included several sigmoid-shaped curves connected to each other, which supports the theory about activation of different receptor subtypes as is known for other 5-HT antagonists, such as ICS 205 930 (Bockaert *et al.* 1992).

Methysergide might cause fluid accumulation by blocking the 5-HT<sub>1A</sub> receptor, which is responsible for inhibitory actions, such as presynaptic inhibition of the release of acetylcholine at nicotinic synapses yielding postsynaptic hyperpolarization (Bradley *et al.* 1986, Gershon *et al.* 1991).

Ketanserin could cause fluid accumulation by blocking  $\alpha_1$ -adrenergic and H<sub>1</sub>-histaminergic receptors (Leysen *et al.* 1981). Ketanserin could also cause an "intrinsic" action at the 5-HT<sub>2</sub> receptors, which have been demonstrated to induce fluid secretion, vasoconstriction, smooth muscle contraction, platelet aggregation, neuronal depolarization and phosphoinositol turnover in the gastrointestinal tract (Sanders-Bush 1988).

Since granisetron is a selective 5-HT<sub>3</sub> receptor antagonist, the fluid accumulation caused by

granisetron most likely was due to "intrinsic" interaction at the myenteric prokinetic, and the submucosal secretory 5-HT<sub>3</sub> receptors, which evoke granisetron-sensitive fast postsynaptic excitatory responses (Sanger and Nelson 1989, Gershon *et al.* 1990, Frieling *et al.* 1991). Another possible mechanism could be an increased release of 5-HT from the EC cells, since activation of the 5-HT<sub>3</sub> receptors on the EC cells have been demonstrated to trigger a positive feedback mechanism leading to an increase of 5-HT release (Gebauer *et al.* 1993).

Treating the animals with indomethacin reduced similarly the secretory response of ketanserin, granisetron and 5-HT indicating an equal importance of presumably PGE<sub>2</sub> in 5-HT, ketanserin and granisetron-evoked fluid accumulation.

In conclusion, this study reveals antagonistic effects of indomethacin, renzapride, methysergide, ketanserin and granisetron on the 5-HT-induced fluid accumulation (i.e. chloride secretion). Results suggest that intestinal 5-HT<sub>2</sub> and 5-HT<sub>3</sub> subtypes, and prostaglandins are involved in the 5-HT secretory cascade. Furthermore, the antagonistic effects were partly concealed by "intrinsic" fluid accumulation (i.e. agonistic effect) caused by the antagonists.

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