

RESEARCH PAPER

Alosetron, cilansetron and tegaserod modify mesenteric but not colonic blood flow in rats

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Background and purpose: As the use of the 5-HT₃ receptor antagonist alosetron (GlaxoSmithKline) and the 5-HT₄ receptor agonist tegaserod (Novartis) in patients with irritable bowel syndrome has been associated with cases of ischaemic colitis, the effects of alosetron, cilansetron (Solvay) and tegaserod on the rat splanchnic circulation were evaluated.

Experimental approach: Phenobarbital-anaesthetised rats were instrumented to record blood flow in the superior mesenteric artery and transverse colon and to calculate mesenteric and colonic vascular conductance.

Key results: Intravenous alosetron (0.03–0.3 mg·kg⁻¹) did not alter blood pressure or heart rate but reduced mesenteric blood flow and vascular conductance by 15–20%. This activity profile was also seen after intraduodenal alosetron and shared by the 5-HT₃ receptor antagonist cilansetron. In contrast, blood flow, vascular conductance and intraluminal pressure in the colon were not modified by alosetron and cilansetron. Intravenous or intraduodenal tegaserod (0.3–1.0 mg·kg⁻¹) had no inhibitory effect on mesenteric and colonic blood flow. Peroral treatment of rats with alosetron or tegaserod for 7 days did not modify mesenteric haemodynamics at baseline and after blockade of nitric oxide synthesis. Mild inflammation induced by dextran sulphate sodium failed to provoke a vasoconstrictor effect of cilansetron in the colon.

Conclusions and implications: Alosetron and cilansetron, not tegaserod, caused a small and transient constriction of the rat mesenteric vascular bed, whereas blood flow in the colon remained unaltered. The relevance of these findings to the treatment-associated occurrence of ischaemic colitis in patients with irritable bowel syndrome remains open.

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Abbreviations: CBF, colonic blood flow; CVC, colonic vascular conductance; DSS, dextran sulphate sodium; GI, gastrointestinal; HR, heart rate; IBS, irritable bowel syndrome; i.d., intraduodenal; L-NAME, N-nitro-L-arginine methyl-ester; MAP, mean arterial blood pressure; MBF, mesenteric blood flow; MPO, myeloperoxidase; MVC, mesenteric vascular conductance; NMP, 1-methyl-2-pyrrolidone

Introduction

5-Hydroxytryptamine (5-HT) acting via 5-HT₃ and 5-HT₄ receptors (Alexander *et al.*, 2008) participates in the control of gastrointestinal (GI) motility, epithelial ion and fluid transport as well as sensation (De Ponti, 2004; Gershon and Tack, 2007). As there is evidence that a disturbance of the 5-HT system is a contributory factor in irritable bowel syndrome (IBS), 5-HT₃ receptor antagonists such as alosetron (GlaxoSmithKline) and cilansetron (Solvay) as well as 5-HT₄ receptor agonists such as tegaserod (Novartis) were developed for the

therapy of diarrhoea- and constipation-predominant IBS, respectively (Schoenfeld, 2004; Chey and Cash, 2005; Evans *et al.*, 2007; Andresen *et al.*, 2008; Rahimi *et al.*, 2008). Subsequent clinical observations showed that the most significant adverse effect of the 5-HT₃ receptor antagonist alosetron was constipation and, in very rare cases, ischaemic colitis (Miller *et al.*, 2003; Chang *et al.*, 2006; Andresen *et al.*, 2008; Rahimi *et al.*, 2008). Meta-analyses of the clinical trials indicate that 0.15–0.20% of the patients on alosetron may develop ischaemic colitis compared with none under placebo (Chang *et al.*, 2006; Andresen *et al.*, 2008; Rahimi *et al.*, 2008). Later on, a very few cases of ischaemic colitis were also observed in patients under therapy with cilansetron or tegaserod (Brinker *et al.*, 2004; Chey and Cash, 2005; DiBaise, 2005; Andresen *et al.*, 2008). In this context, it is important to consider that patients with IBS or chronic constipation are at

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greater risk to develop ischaemic colitis than healthy subjects in whom this vascular failure is extremely rare (Higgins *et al.*, 2004; Chang *et al.*, 2008). The mechanisms whereby these drugs may give rise to ischaemic colitis are not known (Camilleri, 2007), but it is worth noting that idiopathic constipation is associated with a reduction of blood flow through the colonic mucosa (Emmanuel and Kamm, 2000). In an experimental study in anaesthetised rats, both acute and short-term alosetron administration failed to significantly alter baseline mesenteric and colonic blood flow (CBF) or to interfere with splanchnic vascular control mechanisms during occlusion and reactive hyperaemia (Grundy *et al.*, 2007). Because cilansetron and tegaserod have not yet been studied in their effects on the splanchnic circulation, it was the overall aim of this study to compare tegaserod, cilansetron and alosetron in their influence on mesenteric and colonic circulation of anaesthetised rats.

By measuring blood flow and vascular conductance in the superior mesenteric artery and within the wall of the transverse colon, which is supplied by the superior mesenteric artery, several factors that might determine the effects of alosetron and tegaserod on the splanchnic circulation were evaluated. The experimental models were validated by their sensitivity to the vasoconstrictor effect of the nitric oxide synthase inhibitor N-nitro-L-arginine methylester (L-NAME, Bachem, Basel, Switzerland) and the vasodilator effect of the α_2 -adrenoceptor agonist clonidine. In study 1, we set out to explore the dose dependency of any acute effects of alosetron, cilansetron and tegaserod, injected *i.v.*, on CBF measured by the hydrogen gas clearance technique, mesenteric blood flow (MBF) measured by the ultrasonic transit time shift technique and intracolonic pressure. Study 2 was designed to compare the time-dependent effects of alosetron and tegaserod, injected *i.v.*, on CBF measured by laser Doppler flowmetry and MBF in fasted and non-fasted rats. Studies 3 and 4 addressed the possibility that the effects of alosetron and tegaserod on splanchnic haemodynamics depended on the route and duration of administration. In study 3, alosetron and tegaserod were administered *i.d.*, and their acute influence on CBF and MBF was evaluated. Study 4 was designed to test whether peroral treatment of rats with alosetron or tegaserod for 1 week modified the splanchnic circulation at baseline and affected the vasoconstrictor effect of L-NAME. Finally, study 5 addressed the question of whether a vasoconstrictor effect of cilansetron in the colon might be provoked under conditions of mild colitis, given that IBS can be associated with low-grade inflammation in the colon (Bercik *et al.*, 2005; Spiller, 2007; De Giorgio and Barbara, 2008). A preliminary account of some of the findings obtained in study 1 has been published in abstract form (Holzer *et al.*, 2003b).

Methods

Animals

All animal care and experimental procedures complied with the 1986 Directive of the European Communities Council and were approved by an ethics committee at the Federal Ministry of Science and Research of the Republic of Austria. The experiments were conducted in female Sprague Dawley

rats (body weight: 180–230 g) (Division of Laboratory Animal Science and Genetics, Department of Biomedical Research, Medical University of Vienna, Himgberg, Austria). Unless stated otherwise, the rats were deprived of food for 20 h before blood flow measurement, while tap water was available *ad libitum*. The animals were anaesthetised with phenobarbital (230 mg·kg⁻¹ injected *i.p.*) between 7.00 and 8.00 AM. After the loss of the righting reflex, they were placed on a thermostated table and, when surgical anaesthesia was achieved, fitted with a tracheal cannula to facilitate spontaneous respiration and to allow for the administration of hydrogen gas. A cannula in a jugular vein was used for continuous infusion of saline (1.5 mL·h⁻¹) for dehydration of the animals to be avoided and for the *i.v.* administration of drugs.

Induction of mild colitis

Dextran sulphate sodium (DSS, MP Biochemicals, Illkirch, France) was added to the drinking water at a concentration of 3% (w/v) for 7 days. The control animals received normal tap water. The DSS-containing drinking water was made fresh every day. For protocol consistency, the drinking bottles containing normal tap water were also renewed daily.

Measurement of blood pressure and heart rate (HR)

Arterial blood pressure was recorded from a cannula in a common carotid artery, and the blood pressure signal was sampled at a rate of 1 kHz and fed into a personal computer, which calculated mean arterial blood pressure (MAP) and HR online (Heinemann *et al.*, 1998).

Measurement of CBF with the hydrogen gas clearance technique

The hydrogen clearance technique has been established to measure microcirculatory blood flow in the digestive tract and other tissues (Leung *et al.*, 1984; Livingston *et al.*, 1989; Holzer *et al.*, 1991). Based on the washout of inhaled hydrogen, which clears essentially with a single passage of blood through the lungs, the hydrogen clearance method has the advantage of providing absolute estimates of microcirculatory blood flow at the position of a platinum electrode, which catalyses the dissociation of molecular hydrogen into protons and electrons (Guth and Leung, 1987). The major disadvantage of the hydrogen clearance technique is that blood flow recording is discontinuous because periods of hydrogen inhalation alternate with periods of hydrogen washout. For this reason, only changes in blood flow that persist for some time can be recorded.

In the present experiments (study 1), the transverse colon was instrumented with a needle-type platinum electrode, while a reference electrode was placed in the peritoneal cavity. As has been reported for the stomach (Holzer *et al.*, 1991), the platinum electrode was inserted from the serosa tangentially to the colon at an angle of about 30 degrees and positioned in the submucosa of the colon. The reproducibility of placing the needle electrode in the submucosal layer of the colonic wall was confirmed by histology. To this end, the needle electrode was stained with blue ink before being placed in the colonic wall. After removal of the electrode, the tissue was

rapidly excised and frozen. Sections (35 μm thick) of the tissue perpendicular to the needle track were taken in a cryostat and examined under a microscope. The needle track was invariably localised to the submucosal layer of the colonic wall, as observed in three rats.

The measurement of CBF via the clearance of inhaled hydrogen gas was discontinuous, as the experimental protocol involved alternating 15 min periods of saturation, and desaturation, of the tissue with hydrogen gas (Holzer *et al.*, 1991; Heinemann *et al.*, 1999). The current representing the actual hydrogen concentration at the site of the electrode was taken up by a polarographic unit, amplified, digitised at a frequency of 1 Hz and recorded on a personal computer with a custom-made software (Heinemann *et al.*, 1999). The washout curve was then fitted to a monoexponential curve, the power of which was used to calculate the average CBF during the 15 min period of desaturation (Livingston *et al.*, 1989). Average values of MAP and HR were determined for the same time periods. In addition, the colonic vascular conductance (CVC) value was calculated as CBF divided by MAP.

Measurement of CBF with laser Doppler flowmetry

The principle of laser Doppler flowmetry is based on the reflectance of monochromatic light by moving particles in the tissue. As the volume of tissue that is sampled is not precisely known, the technique cannot record blood flow in absolute values but in arbitrary perfusion units (PUs). The uncertainty about the tissue volume sampled also makes it difficult to compare recordings taken from different animals with each other because the vascular geometry of the tissue sample (i.e. the relative proportion of arteries, arterioles and capillaries) may differ substantially but cannot be controlled adequately. The major advantages of the technique are that it provides continuous recordings of blood flow and that it is able to detect rapidly occurring and short-lasting changes in this variable following an intervention.

In all studies, except study 1, CBF was measured by laser Doppler flowmetry. In *studies 2 and 4*, a side delivery endoscopic fibre probe (type: P6asd; external diameter: 2.1 mm; Moor Instruments, Axminster, Devon, UK) was inserted into the lumen of the transverse colon through an incision in the colonic wall at least 1 cm proximally to the site of measurement. The probe was held in place by a ligature at the site of incision and connected to a dual-channel laser Doppler monitor (model MBF3D; Moor Instruments), which fed the data into a personal computer using a custom-made software. In *studies 3 and 5*, CBF was recorded with a two-channel, side delivery endoscopic fibre probe (415–263 rat intestine probe; external diameter: 3.5 mm; Perimed AB, Stockholm, Sweden) connected to a four-channel laser Doppler monitor (PeriFlux System 5000 equipped with four PF 5010 LDPM Units; Perimed AB). The data (PU) were recorded on a personal computer with the software PSW 2.1 Perisoft for Windows (Perimed AB). The CVC value was calculated as PU divided by MAP.

Measurement of MBF with the ultrasonic transit time shift technique

Blood flow in the superior mesenteric artery was recorded with the ultrasonic transit time shift technique, which mea-

sures the net volume of MBF with a factory-calibrated sensor (Heinemann *et al.*, 1998). To this end, the superior mesenteric artery at its exit from the aorta was separated from the surrounding tissue over a length of 4 mm. A perivascular ultrasonic flow probe was placed around the artery and connected to a small animal flowmeter (model T206, Transonic, Ithaca, New York, USA), which calculated the volume of MBF and fed the signal at a rate of 1 Hz into a personal computer. The data acquisition programme calculated the mesenteric vascular conductance (MVC; MBF divided by MAP) online (Heinemann *et al.*, 1998). In addition, the software allowed for the calculation of average values of MAP, HR, MBF, MVC, CBF and CVC during specified periods of time.

Measurement of intraluminal pressure in the colon

Motor activity in the colon was estimated by the method of Wager-Page *et al.* (1992). The ascending colon was fitted with an inflow cannula (outer diameter: 2.5 mm) through which saline was slowly (0.02 mL \cdot min $^{-1}$) infused in an aboral direction throughout the experiment. The inflow cannula was connected with a pressure transducer with which the contractile activity of the colon was recorded via changes in the intraluminal pressure. After amplification, the intracolonic pressure signal was digitised at a rate of 1 Hz and fed into a personal computer (Holzer *et al.*, 2003a). Infusion of saline into the colon resulted in an intraluminal pressure of about 400 Pa at equilibrium. Phasic increases in colonic pressure superimposed on the tonic pressure were quantified by averaging the amplitude of the phasic contractions that occurred during the 15 min observation periods and by expressing the average amplitude as Pa \cdot (10 s) $^{-1}$.

Myeloperoxidase in the colon

The tissue level of myeloperoxidase (MPO) was used to quantify inflammation-associated infiltration of neutrophils and monocytes into the tissue (Krawisz *et al.*, 1984). Full-thickness pieces of the descending colon were excised, shock-frozen in liquid nitrogen and stored at -70°C until assay. After weighing, the frozen tissues were placed, at a ratio of 1 mg: 0.02 mL, in MPO lysis buffer. The composition of this buffer was 200 mmol \cdot L $^{-1}$ NaCl, 5 mmol \cdot L $^{-1}$ EDTA, 10 mmol \cdot L $^{-1}$ Tris, 10% glycine, 0.1 mmol \cdot L $^{-1}$ phenylmethylsulphonyl fluoride, 1 $\mu\text{g}\cdot$ mL $^{-1}$ leupeptide and 28 $\mu\text{g}\cdot$ mL $^{-1}$ aprotinin, pH 7.4. The samples were homogenised on ice with an Ultraturrax (IKA, Staufen, Germany) and then subjected to two centrifugations at 6000 \times g and 4 $^{\circ}\text{C}$ for 15 min. The MPO (donor: H $_2$ O $_2$ oxidoreductase, EC 1.11.1.7) content of the supernatants was measured with an enzyme-linked immunosorbent assay kit specific for the rat and mouse protein (Hycult Biotechnology, Uden, the Netherlands). The sensitivity of this assay is 1 ng \cdot mL $^{-1}$ at an intra- and inter-assay variation of around 10%.

Experimental protocols

After the completion of surgery, the variables under study were monitored for up to 120 min when the cardiovascular parameters had become stable. Thereafter, baseline values

were recorded and averaged during a period of 15 min. In study 1, baseline recordings were taken during the period of 25–10 min before i.v. injection of vehicle ($1 \text{ mL}\cdot\text{kg}^{-1}$), alosetron (0.03 , 0.1 or $0.3 \text{ mg}\cdot\text{kg}^{-1}$), cilansetron (0.1 or $0.3 \text{ mg}\cdot\text{kg}^{-1}$), tegaserod (0.3 or $1 \text{ mg}\cdot\text{kg}^{-1}$) or L-NAME ($0.02 \text{ mmol}\cdot\text{kg}^{-1}$). Post-injection recordings were made during the periods of 5–20 min and 35–50 min. Study 2 was performed to evaluate the effects of select drug doses in fasted and non-fasted rats over a prolonged period of time. After the baseline values during the period of 25–10 min pre injection were recorded, vehicle ($1 \text{ mL}\cdot\text{kg}^{-1}$), alosetron ($0.03 \text{ mg}\cdot\text{kg}^{-1}$), tegaserod ($1 \text{ mg}\cdot\text{kg}^{-1}$) or L-NAME ($0.02 \text{ mmol}\cdot\text{kg}^{-1}$) was injected i.v.; post-injection recordings were taken for a period of 140 min.

In study 3, the drugs were administered i.d. via a soft infant feeding tube (outer diameter 1.5 mm; Rüschi, Montevideo, Uruguay), which, during surgery, had been passed down through the oesophagus, stomach and pylorus so that its tip was positioned in the duodenum. After baseline recordings of the parameters under study had been made during the period of 15–0 min pre injection, vehicle ($1 \text{ mL}\cdot\text{kg}^{-1}$), alosetron ($0.3 \text{ mg}\cdot\text{kg}^{-1}$), tegaserod ($30 \text{ mg}\cdot\text{kg}^{-1}$) or clonidine ($0.03 \text{ mg}\cdot\text{kg}^{-1}$) was administered i.d. Post-injection recordings were taken for 90 min.

Study 4 was carried out to investigate whether the splanchnic circulation at baseline and after injection of L-NAME is modified by short-term peroral pretreatment with alosetron or tegaserod for 7 days. Each day, the animals were given vehicle ($10 \text{ mL}\cdot\text{kg}^{-1}$), alosetron ($0.3 \text{ mg}\cdot\text{kg}^{-1}$) or tegaserod ($1 \text{ mg}\cdot\text{kg}^{-1}$) at 7.30–8.00 AM, 1.00–1.30 PM and 6.00–6.30 PM. The solutions were administered intragastrically (IG) through a soft infant feeding tube (outer diameter: 2.2 mm; Portex, Hythe, UK). On the eighth day, they received the last dose at 7.30 AM, 1 h before they were anaesthetised for blood flow measurement. After baseline recordings had been taken during the period of 25–10 min pre injection, L-NAME ($0.02 \text{ mmol}\cdot\text{kg}^{-1}$) was injected i.v. Thereafter, the cardiovascular parameters were recorded for a period of 110 min.

In study 5, a group of rats was treated with DSS (3% added to the drinking water) for 7 days before they were anaesthetised for measurement of CBF by laser Doppler flowmetry. The control animals received normal tap water. On day 7, the animals were inspected to calculate a disease activity index based on fur appearance, locomotion, blood on faeces and diarrhoea. Each condition was rated as 0 if no abnormality was observed, or as 1 if the fur appeared neglected, locomotion was reduced, signs of diarrhoea were present and/or traces of blood on the faeces were observed, with the maximum disease activity index scoring as 4. After baseline recordings had been taken during the period of 15–0 min pre injection, cilansetron ($0.3 \text{ mg}\cdot\text{kg}^{-1}$) was injected i.v., and MAP, HR, CBF and CVC were recorded for 50 min. At the end of the experiments, a segment of the descending colon was excised for determination of the MPO tissue concentration.

In all experiments, only one dose of drug or vehicle was tested in each anaesthetised animal. Rats with a MAP lower than 60 mmHg at the time of vehicle or drug administration were excluded from the study.

Drugs and solutions

For i.v. injection in studies 1 and 2, alosetron (Novartis, Basel, Switzerland) was dissolved at a concentration of $1 \text{ mg}\cdot\text{mL}^{-1}$ in saline (0.9% NaCl) whose pH was adjusted to approximately 4 by adding solid tartaric acid. This stock solution was diluted with vehicle (saline of pH 4 adjusted with tartaric acid) to obtain injection solutions containing 0.03 and $0.1 \text{ mg}\cdot\text{mL}^{-1}$. Cilansetron (Novartis) was dissolved and diluted with saline. Tegaserod (Novartis) was dissolved in 100% 1-methyl-2-pyrrolidone (NMP) at concentrations of 3 and $10 \text{ mg}\cdot\text{mL}^{-1}$ and diluted with saline to yield injection solutions containing 10% NMP. The vehicle control for tegaserod was saline containing 10% NMP.

For i.d. administration in study 3, the vehicle for all drugs was 10% NMP in saline. While alosetron ($0.3 \text{ mg}\cdot\text{mL}^{-1}$) and clonidine ($0.03 \text{ mg}\cdot\text{mL}^{-1}$; Sigma, Vienna, Austria) were directly dissolved in the vehicle, tegaserod used in study 3 was suspended in 100% NMP at a concentration of $300 \text{ mg}\cdot\text{mL}^{-1}$ and diluted with saline to yield a homogeneous injection suspension of $30 \text{ mg}\cdot\text{mL}^{-1}$ tegaserod in 10% NMP. In study 4, the vehicle for all drugs was 1% NMP in saline. In this instance, tegaserod was dissolved in 100% NMP at a concentration of $10 \text{ mg}\cdot\text{mL}^{-1}$ and diluted with saline to yield an injection solution of $0.1 \text{ mg}\cdot\text{mL}^{-1}$ tegaserod containing 1% NMP.

L-NAME hydrochloride was dissolved in saline at a concentration of $0.02 \text{ mmol}\cdot\text{mL}^{-1}$. DSS (molecular weight: 36 000–50 000) was added to the drinking water (tap water) at a concentration of 3% (w/v).

Data presentation and statistics

The cardiovascular parameters (MAP, HR, MBF, MVC, CBF and CVC estimated via hydrogen gas clearance) recorded immediately before i.v. or i.d. administration of vehicle or drugs (baseline values) are given in absolute terms. The parameters measured after i.v. or i.d. administration of vehicle or drugs are presented as a percentage of the baseline values in order to compare drug-induced changes independently of differing baseline values. When CBF and CVC were estimated by laser Doppler flowmetry, only relative changes are given, except in study 5, where absolute values are reported. All data are shown as means \pm SEM. Statistical evaluation of the original data (absolute values) was performed with Student's *t*-test (drug vs. vehicle), one-way analysis of variance (ANOVA; drugs vs. vehicle) or one way ANOVA for repeated measures (post-administration vs. pre-administration) followed by the Bonferroni test, as appropriate. *P* values less than 0.05 were considered significant.

Results

Dose-dependent effects of i.v. injection of alosetron, cilansetron and tegaserod (study 1)

The rationale of study 1 was to examine whether acute i.v. injections of alosetron, cilansetron and tegaserod have any dose-dependent effect on MAP, HR, mesenteric and colonic haemodynamics as well as intracolonic pressure. In addition, the ability of the vascular recording techniques to pick up vasoconstrictor effects was tested with L-NAME. After baseline

Table 1 Baseline values of cardiovascular parameters

Cardiovascular parameter	Study 1: fasted rats	Study 2: fasted rats	Study 2: non-fasted rats	Study 3: fasted rats	Study 4: fasted rats
MAP (mmHg)	94.7 ± 1.5 (90)	84.0 ± 1.4 (85)	86.5 ± 2.8 (22)	88.2 ± 1.9 (38)	81.8 ± 3.6 (21)
HR (beats·min ⁻¹)	342 ± 7.2 (81)	346 ± 7.2 (85)	357 ± 14.6 (18)	362 ± 7.1 (38)	333 ± 12.3 (21)
MBF (mL·min ⁻¹)	14.8 ± 0.54 (90)	12.6 ± 0.31 (85)	17.4 ± 1.0 (22)**	13.0 ± 0.45 (38)	15.7 ± 0.90 (21)
MVC [μ L·min ⁻¹ ·(mmHg) ⁻¹]	159 ± 6.5 (90)	153 ± 4.9 (85)	209 ± 16.2 (22)**	150 ± 6.5 (38)	200 ± 13.6 (21)
CBF ^a [mL·min ⁻¹ ·(100 g) ⁻¹]	92.2 ± 4.4 (88)	N.M.	N.M.	N.M.	N.M.
CVC ^a [mL·min ⁻¹ ·(100 g) ⁻¹ ·(mmHg) ⁻¹]	1.0 ± 0.06 (88)	N.M.	N.M.	N.M.	N.M.

^aCBF and CVC were measured by the hydrogen gas clearance technique. The cardiovascular parameters were recorded immediately before administration of any drug or vehicle. Means ± SEM, n in parenthesis.

***P* < 0.01 versus respective values recorded in fasted rats of study 2 (Student's *t*-test).

CBF, colonic blood flow; CVC, colonic vascular conductance; HR, heart rate; MAP, mean arterial blood pressure; MBF, mesenteric blood flow; MVC, mesenteric vascular conductance; N.M., not measured.

Table 2 Effect of acute i.v. injection of vehicle, alosetron, cilansetron, tegaserod and L-NAME on MAP, HR, CBF, CVC as well as tonic and phasic pressure in the colon of fasted rats

Treatment and recording period	MAP (mmHg)	HR (beats·min ⁻¹)	CBF [mL·min ⁻¹ · (100 g) ⁻¹]	CVC [mL·min ⁻¹ · (100 g) ⁻¹ · (mmHg) ⁻¹]	Tonic pressure in colon (Pa)	Phasic pressure in colon (Pa)
Vehicle (alosetron) 25–10 min pre	93.4 ± 4.75	314 ± 8.65	72.9 ± 9.64	0.76 ± 0.08	416 ± 73.5	29.7 ± 3.30
Vehicle (alosetron) 5–20 min post	91.7 ± 8.11	335 ± 22.1	80.1 ± 13.7	0.89 ± 0.12	434 ± 72.9	32.7 ± 3.25
Vehicle (alosetron) 35–50 min post	90.2 ± 8.03	336 ± 22.9	65.0 ± 9.32	0.73 ± 0.09	429 ± 75.6	30.4 ± 3.61
Alosetron (0.03 mg·kg ⁻¹) 25–10 min pre	97.1 ± 4.02	338 ± 29.4	97.3 ± 7.22	1.00 ± 0.07	328 ± 58.0	26.7 ± 1.73
Alosetron (0.03 mg·kg ⁻¹) 5–20 min post	102 ± 4.19	354 ± 41.8	107 ± 10.7	1.04 ± 0.08	345 ± 63.8	27.3 ± 2.45
Alosetron (0.03 mg·kg ⁻¹) 35–50 min post	101 ± 4.64	369 ± 55.5	104 ± 8.85	1.03 ± 0.07	353 ± 61.0	28.6 ± 1.73
Alosetron (0.1 mg·kg ⁻¹) 25–10 min pre	92.4 ± 5.37	361 ± 40.3	110 ± 13.9	1.19 ± 0.14	463 ± 91.7	23.4 ± 0.37
Alosetron (0.1 mg·kg ⁻¹) 5–20 min post	95.0 ± 4.47	347 ± 39.8	108 ± 16.4	1.13 ± 0.17	455 ± 91.8	25.0 ± 1.33
Alosetron (0.1 mg·kg ⁻¹) 35–50 min post	89.7 ± 6.83	351 ± 32.9	97.7 ± 16.2	1.19 ± 0.31	449 ± 100	23.4 ± 1.73
Alosetron (0.3 mg·kg ⁻¹) 25–10 min pre	95.3 ± 6.18	321 ± 11.6	119 ± 17.0	1.28 ± 0.19	420 ± 87.6	26.0 ± 2.62
Alosetron (0.3 mg·kg ⁻¹) 5–20 min post	99.6 ± 4.53	349 ± 28.5	117 ± 13.8	1.23 ± 0.22	440 ± 88.7	27.7 ± 3.43
Alosetron (0.3 mg·kg ⁻¹) 35–50 min post	98.1 ± 5.36	320 ± 12.4	111 ± 10.2	1.18 ± 0.17	432 ± 88.8	24.9 ± 2.54
Vehicle (cilansetron) 25–10 min pre	87.1 ± 6.17	364 ± 25.0	106 ± 26.6	1.36 ± 0.46	427 ± 41.8	31.9 ± 3.12
Vehicle (cilansetron) 5–20 min post	89.0 ± 6.04	387 ± 42.7	100 ± 26.6	1.30 ± 0.48	448 ± 47.0	29.0 ± 3.54
Vehicle (cilansetron) 35–50 min post	84.7 ± 8.64	350 ± 11.5	80.8 ± 19.8	1.26 ± 0.56	487 ± 54.7	31.4 ± 3.51
Cilansetron (0.1 mg·kg ⁻¹) 25–10 min pre	87.9 ± 4.62	374 ± 35.7	106 ± 14.6	1.23 ± 0.19	426 ± 60.6	43.7 ± 8.66
Cilansetron (0.1 mg·kg ⁻¹) 5–20 min post	93.3 ± 5.27	371 ± 32.8	104 ± 12.8	1.11 ± 0.11	473 ± 64.5	50.1 ± 9.76
Cilansetron (0.1 mg·kg ⁻¹) 35–50 min post	92.0 ± 6.66	382 ± 28.3	91.9 ± 7.24	1.01 ± 0.07	477 ± 46.1	47.1 ± 6.92
Cilansetron (0.3 mg·kg ⁻¹) 25–10 min pre	89.2 ± 4.72	392 ± 24.5	92.7 ± 22.4	1.02 ± 0.23	464 ± 92.8	29.0 ± 4.42
Cilansetron (0.3 mg·kg ⁻¹) 5–20 min post	86.7 ± 2.20	376 ± 14.0	89.2 ± 18.6	1.03 ± 0.22	473 ± 96.1	28.0 ± 3.67
Cilansetron (0.3 mg·kg ⁻¹) 35–50 min post	85.5 ± 3.84	384 ± 18.5	80.1 ± 9.44	0.95 ± 0.13	474 ± 95.5	27.7 ± 3.76
Vehicle (tegaserod) 25–10 min pre	105 ± 4.37	359 ± 17.7	68.4 ± 11.4	0.64 ± 0.10	409 ± 114	21.3 ± 4.68
Vehicle (tegaserod) 5–20 min post	103 ± 5.52	361 ± 19.2	66.8 ± 7.83	0.66 ± 0.07	409 ± 118	21.7 ± 3.84
Vehicle (tegaserod) 35–50 min post	106 ± 6.02	371 ± 20.2	66.0 ± 10.8	0.63 ± 0.09	404 ± 112	20.6 ± 2.92
Tegaserod (0.3 mg·kg ⁻¹) 25–10 min pre	94.1 ± 3.19	317 ± 17.1	66.9 ± 8.97	0.75 ± 0.11	425 ± 75.1	21.6 ± 1.81
Tegaserod (0.3 mg·kg ⁻¹) 5–20 min post	86.6 ± 3.33**	321 ± 18.0	58.4 ± 8.24	0.67 ± 0.10	437 ± 83.4	20.8 ± 1.41
Tegaserod (0.3 mg·kg ⁻¹) 35–50 min post	88.1 ± 3.06*	316 ± 20.4	56.2 ± 9.91	0.64 ± 0.12	468 ± 83.4	24.6 ± 2.54
Tegaserod (1 mg·kg ⁻¹) 25–10 min pre	100 ± 5.41	327 ± 23.9	75.5 ± 12.8	0.68 ± 0.15	443 ± 75.1	24.8 ± 3.26
Tegaserod (1 mg·kg ⁻¹) 5–20 min post	94.1 ± 4.61	293 ± 11.0	63.4 ± 11.5	0.63 ± 0.14	431 ± 73.4	23.4 ± 3.06
Tegaserod (1 mg·kg ⁻¹) 35–50 min post	97.3 ± 4.90	302 ± 11.1	67.9 ± 11.3	0.61 ± 0.14	445 ± 73.4	21.9 ± 2.66
L-NAME (0.02 mmol·kg ⁻¹) 25–10 min pre	89.9 ± 4.80	328 ± 16.3	106 ± 19.4	1.20 ± 0.21	392 ± 36.1	27.7 ± 3.24
L-NAME (0.02 mmol·kg ⁻¹) 5–20 min post	123 ± 4.19**	324 ± 14.1	78.5 ± 7.99*	0.66 ± 0.10**	400 ± 32.6	37.4 ± 5.59
L-NAME (0.02 mmol·kg ⁻¹) 35–50 min post	122 ± 5.51**	333 ± 11.7	69.8 ± 12.6**	0.60 ± 0.14**	399 ± 32.1	30.4 ± 3.07

CBF and CVC were measured by the hydrogen gas clearance technique. Note that the vehicle for L-NAME (saline) was the same as for cilansetron. The experimental parameters were recorded and averaged during the pre-injection and post-injection periods as indicated. Means ± SEM, *n* = 6–9.

P* < 0.05, *P* < 0.01 versus pre-injection (one-way ANOVA for repeated measures followed by the Bonferroni test).

CBF, colonic blood flow; CVC, colonic vascular conductance; HR, heart rate; L-NAME, N-nitro-L-arginine methylester; MAP, mean arterial blood pressure.

recordings of the cardiovascular parameters under study (Table 1) had been taken, vehicle, alosetron, cilansetron, tegaserod or L-NAME was injected i.v. CBF in this study was measured with the hydrogen gas clearance technique. The vehicles for alosetron, cilansetron, tegaserod and L-NAME were devoid of any effect on the cardiovascular parameter and intracolonic pressure recordings (Table 2).

Alosetron (0.03, 0.1 and 0.3 mg·kg⁻¹) failed to alter MAP and HR during the 50 min observation period post injection (Table 2). All three doses of alosetron, though, led to a small and, in some cases, significant decrease of MBF and MVC (Figure 1A,B), whereas CBF and CVC were not significantly altered (Table 2). Like alosetron, cilansetron (0.1 and 0.3 mg·kg⁻¹) did not modify MAP and HR during the 50 min

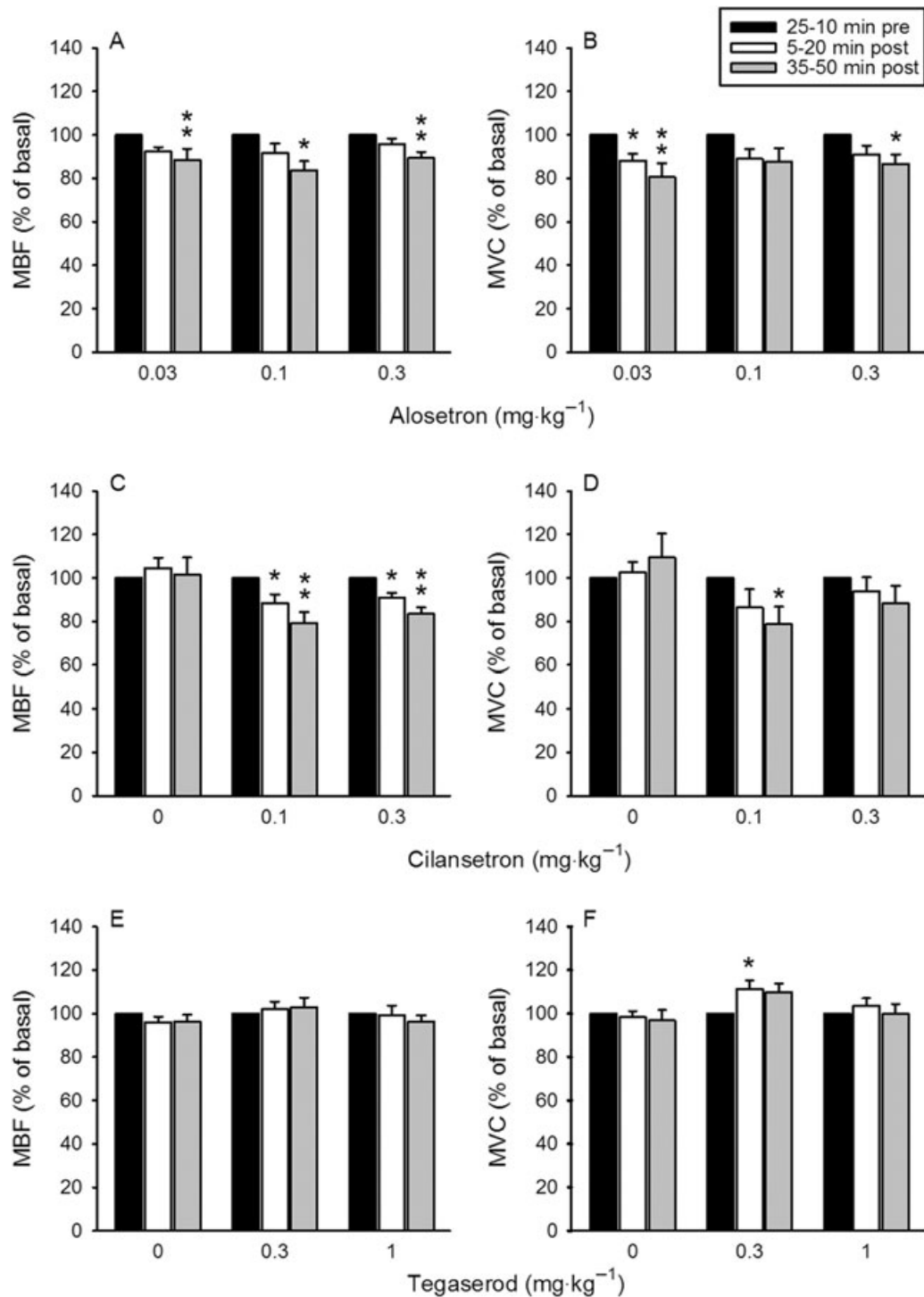


Figure 1 Dose-dependent effects of i.v.-injected (A,B) alosetron, (C,D) cilansetron and (E,F) tegaserod on mesenteric blood flow (MBF, left panels) and mesenteric vascular conductance (MVC, right panels). The doses of the drugs are indicated below the panels: 0 refers to the effects of vehicle. The basal values of the cardiovascular parameters were recorded and averaged during the 25–10 min period pre injection and set as 100%. The values recorded and averaged during the 5–20 min and 35–50 min periods post injection are expressed as a percentage of the baseline recordings. Means + SEM, $n = 6-9$. * $P < 0.05$, ** $P < 0.01$ versus basal (one-way ANOVA for repeated measures followed by the Bonferroni test).

observation period post injection (Table 2). However, both doses of cilansetron caused a slight fall of MBV and MVC, which, in some groups, reached statistical significance (Figure 1C,D). In contrast, both CBF and CVC remained unchanged by either dose of cilansetron (Table 2).

After injection of 0.3 mg·kg⁻¹ tegaserod, MAP fell slightly but to a significant extent, whereas, after injection of 1 mg·kg⁻¹ tegaserod MAP did not change significantly (Table 2). HR stayed unaltered by either dose of tegaserod (Table 2). Tegaserod (0.3 and 1 mg·kg⁻¹) also failed to modify MBF and MVC,

with the exception of a small but significant rise of MVC during the 5–20 min observation period after administration of 0.3 mg·kg⁻¹ tegaserod (Figure 1E,F). CBF and CVC remained unchanged by either dose of tegaserod (Table 2).

For the current *in vivo* preparation to be validated, i.v. administration of the vasoconstrictor drug L-NAME was used. Relative to vehicle, L-NAME (0.02 mmol·kg⁻¹) led to a sustained rise of MAP in the absence of any significant change of HR (Table 2). The hypertension caused by L-NAME was accompanied by a pronounced and sustained decrease of MBF and MVC, with the magnitude of these effects being identical to those recorded in study 2 (Figure 2A,B,C,D). CBF and CVC were also significantly attenuated after injection of L-NAME (Table 2).

At baseline conditions, the tonic intraluminal pressure in the ascending colon was 424 ± 21.5 Pa (*n* = 89), and the phasic increases in colonic pressure superimposed on the tonic pressure amounted to 27.5 ± 1.2 Pa·(10 s)⁻¹ (*n* = 89). Neither the tonic intraluminal pressure nor the phasic pressure increases were altered by L-NAME (0.02 mmol·kg⁻¹), alosetron (0.03, 0.1 and 0.3 mg·kg⁻¹), cilansetron (0.1 and 0.3 mg·kg⁻¹) and tegaserod (0.3 and 1 mg·kg⁻¹) in any consistent manner (Table 2).

Time-dependent effects of i.v. injection of alosetron and tegaserod in fasted and non-fasted rats (study 2)

Study 2 pursued three aims. The first aim was to evaluate whether it takes a prolonged period of time (140 min) to observe changes in colonic haemodynamics after acute i.v. injection of alosetron, tegaserod and, for validation purposes, L-NAME. The second aim was to test whether the drug effects on CBF and CVC recorded with the hydrogen gas clearance technique can be reproduced with laser Doppler flowmetry. The third aim was to examine whether food deprivation before the experiments modifies the effect of acute i.v. injection of alosetron and tegaserod on the splanchnic circulation.

These experiments were carried out with only one dose of each drug, which was chosen on the basis of the results of study 1. The dose of 0.03 mg·kg⁻¹ alosetron was chosen because it was most active in reducing MVC in study 1 (Figure 1). As tegaserod had no consistent effects in study 1, the highest dose (1 mg·kg⁻¹) used in those experiments was selected. The cardiovascular parameters recorded at baseline are summarised in Table 1.

Fasted rats. The extension of the recording period to 140 min showed that, following i.v. injection of the vehicles for L-NAME, alosetron and tegaserod, MAP and HR tended to rise over time, whereas MBF, MVC, CBF and CVC tended to fall, and that these changes were statistically significant in some experimental groups (Figures 2–4, Table 3). The prompt effect of L-NAME (0.02 mmol·kg⁻¹) to increase MAP in the absence of any significant change of HR was still evident 90–140 min post injection (Figure 2A,B). This was also true for the action of L-NAME to reduce MBF and MVC (Figure 2C,D). While CBF measured with the laser Doppler flowmetry was not significantly diminished by L-NAME (Figure 2E), CVC was significantly attenuated throughout the 140 min observation period (Figure 2F).

Alosetron (0.03 mg·kg⁻¹) did not consistently affect MAP and HR during the 140 min observation period post injection nor did it alter CBF and CVC (Table 3). As found in study 1, MBF and MVC were significantly (*P* < 0.05) decreased 5–20 min after injection of alosetron when compared with the values measured after vehicle injection (Figure 3A,B). However, this effect of alosetron was no longer seen 35–140 min post injection (Figure 3A,B). Compared with vehicle, tegaserod (1 mg·kg⁻¹) led to an initial rise of MBF and MVC in this series of experiments (Figure 4A,B), an effect that was gone 95–140 min post injection (Figure 4A,B). CBF and CVC did not differ throughout the 140 min observation period after injection of vehicle or tegaserod (Table 3). MAP was not significantly altered by tegaserod, whereas HR first fell

Table 3 Effect of acute i.v. injection of vehicle, alosetron and tegaserod on MAP, HR, CBF and CVC of fasted rats during a prolonged period of time (140 min)

Treatment and recording period	MAP (mmHg)	HR (beats·min ⁻¹)	CBF (PU)	CVC [PU·(mmHg) ⁻¹]
Vehicle (alosetron) 25–10 min pre	85.5 ± 4.14	340 ± 7.47	164 ± 49.7	1.81 ± 0.45
Vehicle (alosetron) 5–20 min post	87.9 ± 3.90	353 ± 10.2	159 ± 51.5	1.71 ± 0.45
Vehicle (alosetron) 65–80 min post	92.3 ± 3.75*	369 ± 8.07**	139 ± 40.5	1.45 ± 0.36**
Vehicle (alosetron) 125–140 min post	91.0 ± 3.77	376 ± 10.5**	136 ± 32.2	1.46 ± 0.31**
Alosetron (0.03 mg·kg ⁻¹) 25–10 min pre	86.5 ± 4.86	341 ± 32.6	155 ± 30.3	1.75 ± 0.27
Alosetron (0.03 mg·kg ⁻¹) 5–20 min post	89.0 ± 5.71	353 ± 36.6	155 ± 31.0	1.69 ± 0.26
Alosetron (0.03 mg·kg ⁻¹) 65–80 min post	93.6 ± 4.90	376 ± 39.9	146 ± 29.6	1.52 ± 0.25
Alosetron (0.03 mg·kg ⁻¹) 125–140 min post	91.5 ± 5.45	382 ± 37.2*	141 ± 31.8	1.50 ± 0.29
Vehicle (tegaserod) 25–10 min pre	75.8 ± 2.25	344 ± 11.9	258 ± 63.0	3.45 ± 0.90
Vehicle (tegaserod) 5–20 min post	83.2 ± 2.09**	363 ± 14.3	233 ± 50.7	2.89 ± 0.59
Vehicle (tegaserod) 65–80 min post	89.9 ± 2.04**	389 ± 15.5**	205 ± 44.1	2.42 ± 0.54*
Vehicle (tegaserod) 125–140 min post	89.8 ± 2.81**	391 ± 19.8**	196 ± 47.6	2.26 ± 0.57**
Tegaserod (1 mg·kg ⁻¹) 25–10 min pre	89.1 ± 4.53	354 ± 18.1	216 ± 29.6	2.49 ± 0.36
Tegaserod (1 mg·kg ⁻¹) 5–20 min post	86.5 ± 5.22	332 ± 15.9*	218 ± 39.8	2.54 ± 0.45
Tegaserod (1 mg·kg ⁻¹) 65–80 min post	87.0 ± 2.57	360 ± 15.8	203 ± 43.8	2.26 ± 0.47
Tegaserod (1 mg·kg ⁻¹) 125–140 min post	93.1 ± 2.48	382 ± 14.9**	184 ± 39.3	2.02 ± 0.41

CBF and CVC were measured by laser Doppler flowmetry. The experimental parameters were recorded and averaged during the pre-injection and post-injection periods as indicated. Means ± SEM, *n* = 8–12.

P* < 0.05, *P* < 0.01 versus pre-injection (one-way ANOVA for repeated measures followed by the Bonferroni test).

CBF, colonic blood flow; CVC, colonic vascular conductance; HR, heart rate; MAP, mean arterial blood pressure; PU, perfusion unit.

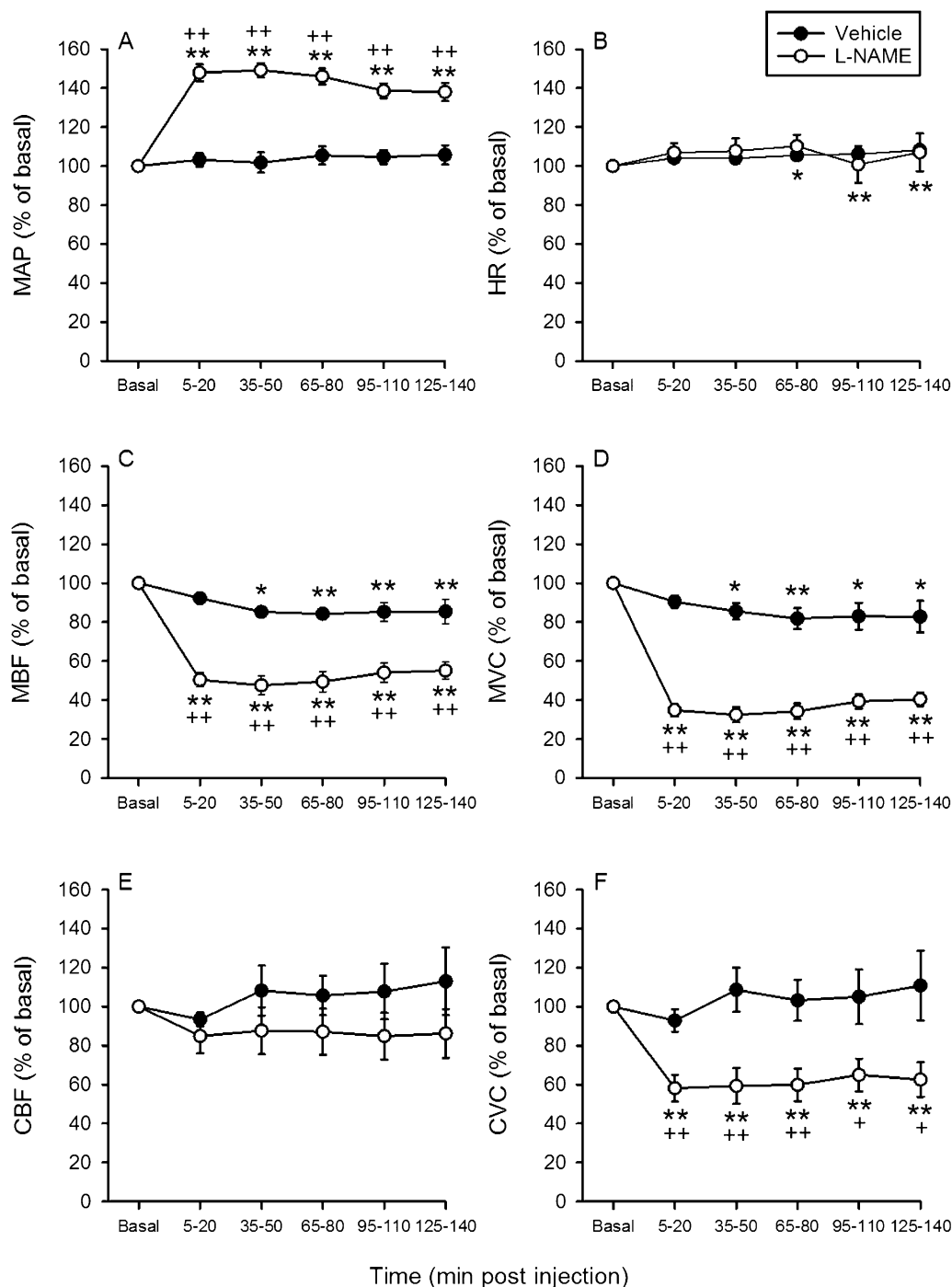


Figure 2 Time-dependent effects of i.v.-injected vehicle and N-nitro-L-arginine methylester ($0.02 \text{ mmol}\cdot\text{kg}^{-1}$) on (A) mean arterial blood pressure (MAP), (B) heart rate (HR), (C) mesenteric blood flow (MBF), (D) mesenteric vascular conductance (MVC), (E) colonic blood flow (CBF) measured by laser Doppler flowmetry and (F) colonic vascular conductance (CVC). The basal values of the cardiovascular parameters were recorded and averaged during the 25–10 min period pre injection and set as 100%. The values recorded and averaged during the 5–20 min, 35–50 min, 95–110 min and 125–140 min periods post injection are expressed as a percentage of the baseline recordings. Means \pm SEM, $n = 9-10$. * $P < 0.05$, ** $P < 0.01$ versus basal (one-way ANOVA for repeated measures followed by the Bonferroni test); + $P < 0.05$, ++ $P < 0.01$ versus vehicle (Student's t -test).

and later increased relative to the pre-injection value (Table 3).

Non-fasted rats. In these experiments, CBF and CVC were not estimated because intraluminal placement of the endoscopic laser Doppler flowmetry probe required that the colon

be emptied by fasting. The baseline values of MBF and MVC measured in non-fasted rats turned out to be significantly larger than those measured in fasted rats, whereas MAP and HR values did not differ to a significant extent (Table 1). After i.v. administration of alosetron ($0.03 \text{ mg}\cdot\text{kg}^{-1}$), MBF and MVC tended to fall but, with the exception of one value, did not

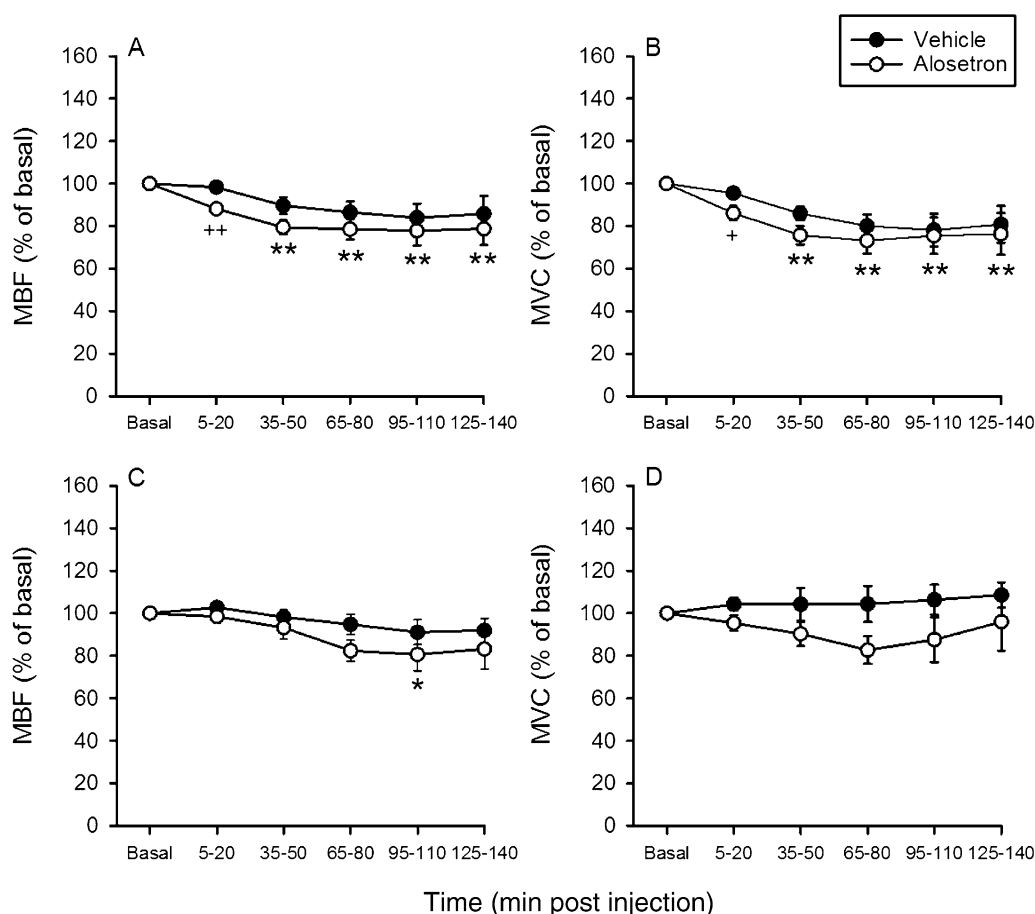


Figure 3 Time-dependent effects of i.v.-injected vehicle and alosetron (0.03 mg·kg⁻¹) on mesenteric blood flow (MBF, left panels) and mesenteric vascular conductance (MVC, right panels) in fasted (A,B) and non-fasted (C,D) rats. The basal values of the cardiovascular parameters were recorded and averaged during the 25–10 min period pre injection and set as 100%. The values recorded and averaged during the 5–20 min, 35–50 min, 95–110 min and 125–140 min periods post injection are expressed as a percentage of the baseline recordings. Means \pm SEM, $n = 7$ –10. * $P < 0.05$, ** $P < 0.01$ versus basal (one-way ANOVA for repeated measures followed by the Bonferroni test); + $P < 0.05$, ++ $P < 0.01$ versus vehicle (Student's *t*-test).

become significantly different from the values measured after vehicle injection (Figure 3C,D). Like the vehicle, tegaserod (1 mg·kg⁻¹) failed to alter MBF and MVC during the 140 min observation period post injection (Figure 4C,D). MAP and HR did not significantly change after injection of alosetron and tegaserod, while, after administration of vehicle, MAP and HR decreased with a delay of some 125 min (Table 4).

Effects of i.d. injection of alosetron and tegaserod (study 3)

The aim of study 3 was to test whether alosetron and tegaserod are able to modify CBF and vascular conductance after acute i.d. administration. In addition, the ability of the vascular recording techniques to pick up vasodilator effects was tested with clonidine, a drug with proven oral bioavailability that has been found to increase gastric mucosal vascular conductance in a sustained manner (Holzer and Painsipp, 2001). In view of its GI bioavailability in humans (50–60%), the highest dose of alosetron used in study 1 (0.3 mg·kg⁻¹) was selected for i.d. injection. The dose of tegaserod chosen for i.d. administration was 30 mg·kg⁻¹ because the oral bioavailability of this drug in humans is only about 10% (Rivkin, 2003; Evans *et al.*, 2007).

Table 4 Effect of acute i.v. injection of vehicle, alosetron and tegaserod on MAP and HR of non-fasted rats during a prolonged period of time (140 min)

Treatment and recording period	MAP (mmHg)	HR (beats·min ⁻¹)
Vehicle (10% NMP) 25–10 min pre	81.6 \pm 5.51	362 \pm 19.1
Vehicle (10% NMP) 5–20 min post	80.1 \pm 4.85	363 \pm 20.6
Vehicle (10% NMP) 65–80 min post	74.0 \pm 5.27	353 \pm 18.9
Vehicle (10% NMP) 125–140 min post	69.6 \pm 5.61**	342 \pm 17.9**
Alosetron (0.03 mg·kg ⁻¹) 25–10 min pre	89.8 \pm 3.79	355 \pm 39.2
Alosetron (0.03 mg·kg ⁻¹) 5–20 min post	92.8 \pm 3.54	356 \pm 40.6
Alosetron (0.03 mg·kg ⁻¹) 65–80 min post	90.3 \pm 4.65	363 \pm 42.1
Alosetron (0.03 mg·kg ⁻¹) 125–140 min post	80.9 \pm 7.33	372 \pm 49.1
Tegaserod (1 mg·kg ⁻¹) 25–10 min pre	87.7 \pm 5.60	354 \pm 13.4
Tegaserod (1 mg·kg ⁻¹) 5–20 min post	83.0 \pm 5.64	333 \pm 11.9
Tegaserod (1 mg·kg ⁻¹) 65–80 min post	82.3 \pm 4.08	347 \pm 15.4
Tegaserod (1 mg·kg ⁻¹) 125–140 min post	74.3 \pm 3.78	352 \pm 14.2

The experimental parameters were recorded and averaged during the pre-injection and post-injection periods as indicated. Means \pm SEM, $n = 7$ –8.

** $P < 0.01$ versus pre-injection (one-way ANOVA for repeated measures followed by the Bonferroni test).

HR, heart rate; MAP, mean arterial blood pressure; NMP, 1-methyl-2-pyrrolidone.

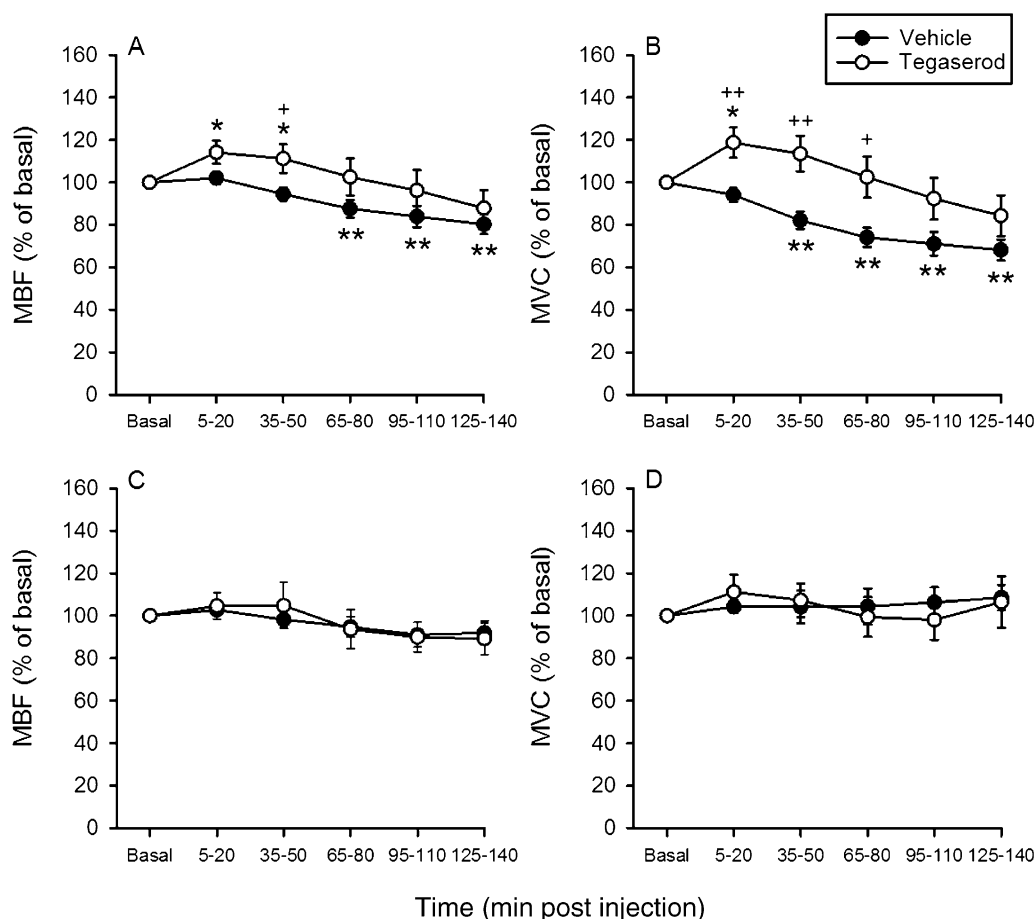


Figure 4 Time-dependent effects of i.v.-injected vehicle and tegaserod ($1 \text{ mg}\cdot\text{kg}^{-1}$) on mesenteric blood flow (MBF, left panels) and mesenteric vascular conductance (MVC, right panels) in fasted (A,B) and non-fasted (C,D) rats. The basal values of the cardiovascular parameters were recorded and averaged during the 25–10 min period pre injection and set as 100%. The values recorded and averaged during the 5–20 min, 35–50 min, 95–110 min and 125–140 min periods post injection are expressed as a percentage of the baseline recordings. Means \pm SEM, $n = 7\text{--}12$. * $P < 0.05$, ** $P < 0.01$ versus basal (one-way ANOVA for repeated measures followed by the Bonferroni test); + $P < 0.05$, ++ $P < 0.01$ versus vehicle (Student's *t*-test).

Once baseline recordings of the cardiovascular parameters (Table 1) had been made, vehicle, alosetron or tegaserod was administered i.d., whereafter post-injection recordings were taken for 90 min. Following i.d. injection of clonidine ($0.03 \text{ mg}\cdot\text{kg}^{-1}$), MAP, HR, MBF and CBF (measured by laser Doppler flowmetry) decreased, whereas MVC, and CVC in particular, increased to a significant extent, with these changes being sustained throughout the observation period (Figure 5).

Throughout the observation period, i.d. injection of vehicle, alosetron ($0.3 \text{ mg}\cdot\text{kg}^{-1}$) or tegaserod ($30 \text{ mg}\cdot\text{kg}^{-1}$) failed to alter MAP, CBF and CVC (Table 5). The HR also remained unchanged, except that there was a late increase of this parameter following vehicle or tegaserod administration (Table 5). Alosetron led to a delayed fall of MBF, which was statistically significant when compared with the pre-injection baseline value (Figure 6A). Relative to vehicle, however, neither alosetron nor tegaserod caused a significant change of MBF and MVC (Figure 6A,B).

Effects of short-term peroral pretreatment with alosetron and tegaserod (study 4)

The rationale of study 4 was to test whether prolonged peroral administration causes alosetron and tegaserod to induce

colonic vasoconstriction and to modify the cardiovascular effects of L-NAME after repeated peroral administration for 1 week. Rats were pretreated for 7 days by thrice-daily IG administrations of vehicle, alosetron ($0.3 \text{ mg}\cdot\text{kg}^{-1}$) or tegaserod ($1 \text{ mg}\cdot\text{kg}^{-1}$), the highest doses tested in study 1. The last dose of each drug was administered 1 h before the rats were anaesthetised. Once baseline recordings of the cardiovascular parameters (Table 1) had been made, L-NAME ($0.02 \text{ mmol}\cdot\text{kg}^{-1}$) was injected i.v., whereafter post-injection recordings were made for 110 min. The baseline parameters for MBF and MVC did not differ significantly between rats that had been pretreated with IG vehicle, alosetron or tegaserod for 7 days (Table 6). As found in study 2 (Figure 2), i.v. injection of L-NAME led to a prompt increase of MAP, which was accompanied by a marked reduction of MBF, MVC and CVC, whereas CBF (measured with laser Doppler flowmetry) was not significantly modified. The magnitude of the hypertensive, mesenteric and colonic vasoconstrictor responses to L-NAME was indistinguishable in rats that had been pretreated with IG vehicle, alosetron or tegaserod for 7 days (Table 6). In contrast, the increase in HR, which, in vehicle-pretreated rats, accompanied the hypertensive response to L-NAME, was absent in rats pretreated with alosetron or tegaserod (Table 6).

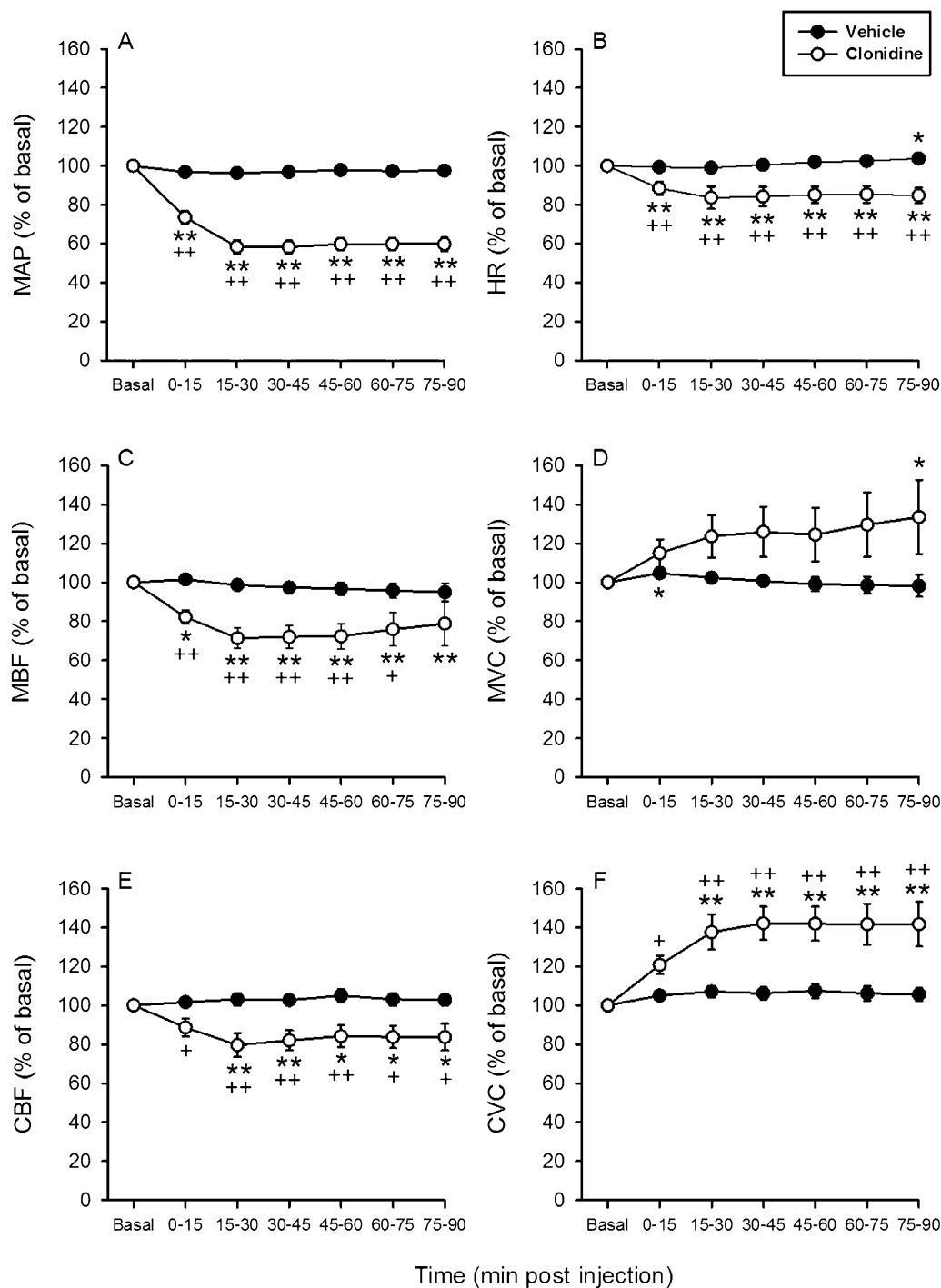


Figure 5 Time-dependent effects of i.d.-injected vehicle and clonidine (0.03 mg·kg⁻¹) on (A) mean arterial blood pressure (MAP), (B) heart rate (HR), (C) mesenteric blood flow (MBF), (D) mesenteric vascular conductance (MVC), (E) colonic blood flow (CBF) measured by laser Doppler flowmetry and (F) colonic vascular conductance (CVC). The basal values of the cardiovascular parameters were recorded and averaged during the 15–0 min period pre injection and set as 100%. The values recorded and averaged during the 0–15 min, 15–30 min, 60–75 min and 75–90 min periods post injection are expressed as a percentage of the baseline recordings. Means \pm SEM, $n = 7-8$. * $P < 0.05$, ** $P < 0.01$ versus basal (one-way ANOVA for repeated measures followed by the Bonferroni test); + $P < 0.05$, ++ $P < 0.01$ versus vehicle (Student's *t*-test).

Effect of cilansetron in rats with mild colitis (study 5)

Study 5 was carried out to test whether mild inflammation causes cilansetron to evoke vasoconstriction in the colon. Treatment of rats with DSS (3% added to the drinking water) for 7 days led to a significant increase in the MPO content of

the colonic wall (Figure 7A), which was associated with a significant rise of the disease activity index (Figure 7B). Pilot experiments had demonstrated that treatment of rats with 1 or 2% DSS for 7 days ($n = 6-9$) failed to significantly enhance the colonic MPO level. Baseline MAP (106.5 ± 5.9 mmHg in

Table 5 Effect of acute i.d. injection of vehicle, alosetron and tegaserod on MAP, HR, CBF and CVC of fasted rats

Treatment and recording period	MAP (mmHg)	HR (beats·min ⁻¹)	CBF (PU)	CVC [PU·(mmHg) ⁻¹]
Vehicle (10% NMP) 15–0 min pre	89.3 ± 5.39	396 ± 22.1	392 ± 48.6	4.52 ± 0.73
Vehicle (10% NMP) 0–15 min post	86.6 ± 5.54	393 ± 23.0	399 ± 49.8	4.75 ± 0.80
Vehicle (10% NMP) 15–30 min post	86.1 ± 5.64	392 ± 22.8	405 ± 52.0	4.84 ± 0.83
Vehicle (10% NMP) 45–60 min post	87.1 ± 4.84	403 ± 22.7	409 ± 51.9	4.87 ± 0.88
Vehicle (10% NMP) 75–90 min post	87.0 ± 5.21	422 ± 24.5*	405 ± 56.3	4.86 ± 0.95
Alosetron (0.3 mg·kg ⁻¹) 15–0 min pre	92.7 ± 3.58	358 ± 16.8	399 ± 51.5	4.34 ± 0.59
Alosetron (0.3 mg·kg ⁻¹) 0–15 min post	90.3 ± 4.95	352 ± 14.2	396 ± 53.3	4.46 ± 0.63
Alosetron (0.3 mg·kg ⁻¹) 15–30 min post	91.7 ± 5.63	348 ± 14.2	395 ± 57.2	4.36 ± 0.61
Alosetron (0.3 mg·kg ⁻¹) 45–60 min post	91.7 ± 5.81	355 ± 14.4	404 ± 53.6	4.42 ± 0.55
Alosetron (0.3 mg·kg ⁻¹) 75–90 min post	91.3 ± 4.96	357 ± 13.1	410 ± 51.0	4.50 ± 0.55
Tegaserod (30 mg·kg ⁻¹) 15–0 min pre	81.9 ± 3.37	372 ± 8.93	392 ± 60.1	4.83 ± 0.74
Tegaserod (30 mg·kg ⁻¹) 0–15 min post	79.1 ± 3.86	372 ± 11.7	391 ± 61.5	5.06 ± 0.86
Tegaserod (30 mg·kg ⁻¹) 15–30 min post	79.3 ± 3.60	371 ± 11.8	370 ± 46.6	4.77 ± 0.66
Tegaserod (30 mg·kg ⁻¹) 45–60 min post	84.9 ± 3.70	383 ± 14.0	377 ± 60.3	4.56 ± 0.81
Tegaserod (30 mg·kg ⁻¹) 75–90 min post	85.1 ± 2.72	386 ± 13.5**	379 ± 56.3	4.49 ± 0.70

The experimental parameters were recorded and averaged during the pre-injection and post-injection periods as indicated. Means ± SEM, *n* = 7–8.

P* < 0.05, *P* < 0.01 versus pre-injection (one-way ANOVA for repeated measures followed by the Bonferroni test).

CBF, colonic blood flow; CVC, colonic vascular conductance; HR, heart rate; MAP, mean arterial blood pressure; NMP, 1-methyl-2-pyrrolidone; PU, perfusion unit.

Table 6 Effect of short-term peroral administration of vehicle, alosetron and tegaserod on MAP, HR, MBF, MVC, CBF and CVC of fasted rats at baseline (before) and after acute i.v. injection of L-NAME

Treatment and recording period	MAP (mmHg)	HR (beats·min ⁻¹)	MBF (μL·min ⁻¹)	MVC [μL·min ⁻¹ (mmHg) ⁻¹]	CBF (PU)	CVC [PU (mmHg) ⁻¹]
25–10 min before L-NAME (vehicle pretreatment)	77.3 ± 4.23	316 ± 11.8	16.4 ± 0.74	215 ± 16.0	196 ± 47.8	2.68 ± 0.71
5–20 min post L-NAME (vehicle pretreatment)	124 ± 3.41**	350 ± 10.0**	7.91 ± 0.81**	64.3 ± 8.26**	169 ± 45.7	1.38 ± 0.38**
95–110 min post L-NAME (vehicle pretreatment)	125 ± 2.46**	365 ± 16.2**	7.59 ± 0.99**	60.2 ± 7.48**	183 ± 43.1	1.44 ± 0.33**
25–10 min before L-NAME (alosetron pretreatment)	77.3 ± 5.90	316 ± 24.2	14.0 ± 1.76	192 ± 30.8	349 ± 73.9	5.05 ± 1.35
5–20 min post L-NAME (alosetron pretreatment)	120 ± 2.50**	340 ± 10.7	8.02 ± 1.63**	67.3 ± 14.4**	303 ± 64.1	2.57 ± 0.58**
95–110 min post L-NAME (alosetron pretreatment)	116 ± 5.95**	328 ± 17.1	8.33 ± 1.41**	73.2 ± 12.9**	428 ± 123	4.01 ± 1.28
25–10 min before L-NAME (tegaserod pretreatment)	83.1 ± 5.68	340 ± 20.8	16.0 ± 1.23	199 ± 21.4	328 ± 94.4	3.94 ± 1.22
5–20 min post L-NAME (tegaserod pretreatment)	126 ± 2.88**	331 ± 10.1	8.37 ± 0.68**	66.5 ± 5.69**	310 ± 93.2	2.39 ± 0.70**
95–110 min post L-NAME (tegaserod pretreatment)	125 ± 2.28**	342 ± 9.44	7.41 ± 0.89**	59.6 ± 7.51**	221 ± 63.4	1.74 ± 0.48**

Rats were pretreated for 7 days by 3 daily IG administrations of vehicle, alosetron (0.3 mg·kg⁻¹) or tegaserod (1 mg·kg⁻¹). The cardiovascular parameters were recorded before and after i.v. injection of L-NAME (0.02 mmol·kg⁻¹) and averaged for the periods indicated. Means ± SEM, *n* = 7.

***P* < 0.01 versus pre-injection (one way ANOVA for repeated measures followed by the Bonferroni test).

CBF, colonic blood flow; CVC, colonic vascular conductance; HR, heart rate; L-NAME, N-nitro-L-arginine methylester; MAP, mean arterial blood pressure; MBF, mesenteric blood flow; MVC, mesenteric vascular conductance; PU, perfusion unit.

the control rats, 100.4 ± 5.4 mmHg in the DSS-treated rats, *n* = 10–11) and baseline HR (334 ± 8.5 beats·min⁻¹ in the control rats, 364 ± 8.7 beats·min⁻¹ in the DSS-treated rats, *n* = 10–11) did not differ significantly between the control animals and rats with mild colitis. Cilansetron (0.3 mg·kg⁻¹), a dose found to reduce MVC in study 1 (Figure 1), failed to alter any of these parameters in both control and DSS-treated animals. The recordings of CBF and CVC are shown in Figure 7C,D.

Discussion

The major results of the current study can be summarised as follows:

- Treatment with the 5-HT₃ receptor antagonist alosetron had the potential of causing a minor constriction of the vascular bed of the superior mesenteric artery of anaesthetised rats, whereas a minor dilatation of this arterial bed was seen after treatment with the partial 5-HT₄ receptor agonist tegaserod.

- The small mesenteric vasoconstriction caused by alosetron was observed after both i.v. and i.d. administration of the drug and appears to be a class effect of 5-HT₃ receptor antagonists, as it was shared by cilansetron.
- The vasoconstrictor response to 5-HT₃ receptor antagonism was transient, and the short-term peroral administration of alosetron was without effect on the haemodynamics in the superior mesenteric artery.
- The minor vasoconstrictor action of 5-HT₃ receptor antagonism on the mesenteric artery was not paralleled by any appreciable change in colonic motor activity.
- Alosetron, cilansetron and tegaserod failed to modify blood flow in the colon.
- Fasting had no influence on the action profile of alosetron and tegaserod in the mesenteric and colonic vascular beds.
- Mild colitis failed to provoke a vasoconstrictor effect of 5-HT₃ receptor antagonism in the colon.

These data add significantly to the results of another study in which alosetron did not alter mesenteric and CBF of

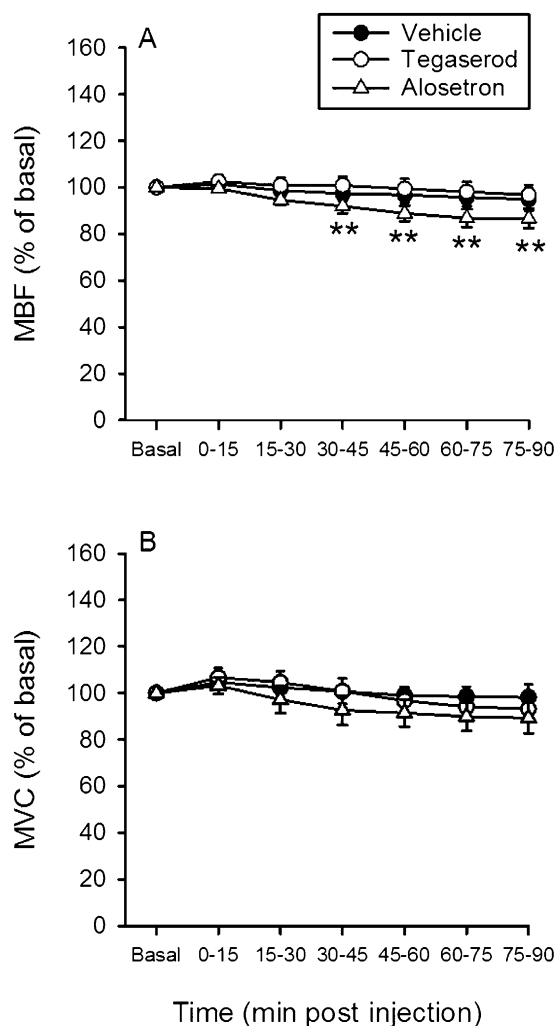


Figure 6 Time-dependent effects of i.d.-injected vehicle, alosetron ($0.3 \text{ mg}\cdot\text{kg}^{-1}$) and tegaserod ($30 \text{ mg}\cdot\text{kg}^{-1}$) on (A) mesenteric blood flow (MBF) and (B) mesenteric vascular conductance (MVC). The basal values of the cardiovascular parameters were recorded and averaged during the 15–0 min period pre injection and set as 100%. The values recorded and averaged during the 0–15 min, 15–30 min, 60–75 min and 75–90 min periods post injection are expressed as a percentage of the baseline recordings. Means \pm SEM, $n = 7$ –8. $**P < 0.01$ versus basal (one-way ANOVA for repeated measures followed by the Bonferroni test).

anaesthetised rats and failed to interfere with splanchnic vascular control mechanisms during occlusion and reactive hyperaemia (Grundy *et al.*, 2007).

Blood flow in the superior mesenteric artery was recorded with the ultrasonic transit time shift technique (Holzer *et al.*, 1994; Heinemann *et al.*, 1999), whereas blood flow in the transverse colon, which is supplied by the superior mesenteric artery, was estimated by hydrogen gas clearance and laser Doppler flowmetry. Both techniques have been validated and used to measure nutrient blood flow in the wall of the GI tract (Johansson, 1988; Livingston *et al.*, 1989; Holzer *et al.*, 1991). By calculating the vascular conductance, it was possible to obtain a measure of dilatation or constriction of the vascular bed under study (Heinemann *et al.*, 1998). The sensitivity of

our experimental model to i.v.-administered vasoactive drugs was proved by the vasoconstrictor response to the nitric oxide synthase inhibitor L-NAME ($0.02 \text{ mmol}\cdot\text{kg}^{-1}$). Intravenous injection of this drug is known to decrease the conductance in various vascular beds including the rat gastric mucosa, which causes sustained hypertension (Gardiner *et al.*, 1990; Holzer *et al.*, 1994). In the current study, L-NAME constricted not only the mesenteric arterial bed but also the colonic vasculature, as revealed by hydrogen gas clearance and laser Doppler flowmetry. The ability of our preparation to respond to i.d.-administered vasoactive drugs was verified by the vasodilator response to clonidine, an α_2 -adrenoceptor agonist with oral bioavailability (Arndts *et al.*, 1983). In our experiments, clonidine ($0.03 \text{ mg}\cdot\text{kg}^{-1}$) enhanced the vascular conductance both in the mesenteric and colonic vascular bed. Our *in vivo* preparation is thus able to reveal constriction and dilatation of the mesenteric and colonic vasculature.

Relative to the pronounced changes in the mesenteric and colonic haemodynamics elicited by L-NAME and clonidine, alosetron, cilansetron and tegaserod caused only minor alterations of the splanchnic circulation. The i.v. doses of alosetron (0.03 , 0.01 and $0.3 \text{ mg}\cdot\text{kg}^{-1}$), cilansetron (0.1 and $0.3 \text{ mg}\cdot\text{kg}^{-1}$) and tegaserod (0.3 and $1 \text{ mg}\cdot\text{kg}^{-1}$) used here are considered to be clinically relevant because the standard single doses used in humans are 1 mg alosetron, 2 mg tegaserod and 6 mg tegaserod (Evans *et al.*, 2007; Andresen *et al.*, 2008). For a patient weighing 70 kg , these doses would amount to $0.014 \text{ mg}\cdot\text{kg}^{-1}$ alosetron, $0.28 \text{ mg}\cdot\text{kg}^{-1}$ cilansetron and $0.086 \text{ mg}\cdot\text{kg}^{-1}$ tegaserod. The dose equivalents of these drugs in rodents have not been reported, but it can be assumed that equiactive drug doses in rats are higher than in humans because of faster metabolism processes in small animals. Hence, we suppose that, at the doses used, both alosetron and cilansetron retain their selectivity for 5-HT_3 receptors (De Ponti, 2004). In contrast, tegaserod is not only a partial 5-HT_4 receptor agonist but also a 5-HT_{1B} and 5-HT_{2B} receptor antagonist (Beattie *et al.*, 2004; Weber *et al.*, 2006).

Our findings with alosetron differ from those made by Grundy *et al.* (2007), who failed to see an effect of i.v. alosetron (0.01 – $0.1 \text{ mg}\cdot\text{kg}^{-1}$) on MBF and CBF. In contrast, we observed that alosetron consistently reduced MBF and MVC by a maximum of 20%. Following i.v. injection, the mesenteric vasoconstrictor effect of alosetron was prompt and transient because it was seen only during a 50 min post-injection period. Because, therapeutically, alosetron is taken *per os*, it was also tested after i.d. administration, in which case the effect of alosetron to decrease MBF was delayed, which can be explained by the time taken until alosetron was absorbed from the small intestine. The high potency of alosetron to cause mesenteric vasoconstriction at i.v. doses as small as $0.03 \text{ mg}\cdot\text{kg}^{-1}$ is consistent with the high potency of alosetron as a 5-HT_3 receptor antagonist (De Ponti, 2004). As the therapeutic dosage of alosetron in humans is also low (De Ponti, 2004), we conclude that the mesenteric vasoconstrictor effect of alosetron in anaesthetised rats is elicited by therapeutically relevant doses of the drug. This conclusion is supported by the results obtained with i.d.-administered alosetron. As the intestinal absorption rate of alosetron in anaesthetised rats has not been published, the haemodynamic effects of i.d.-administered alosetron were tested with a dose ($0.3 \text{ mg}\cdot\text{kg}^{-1}$)

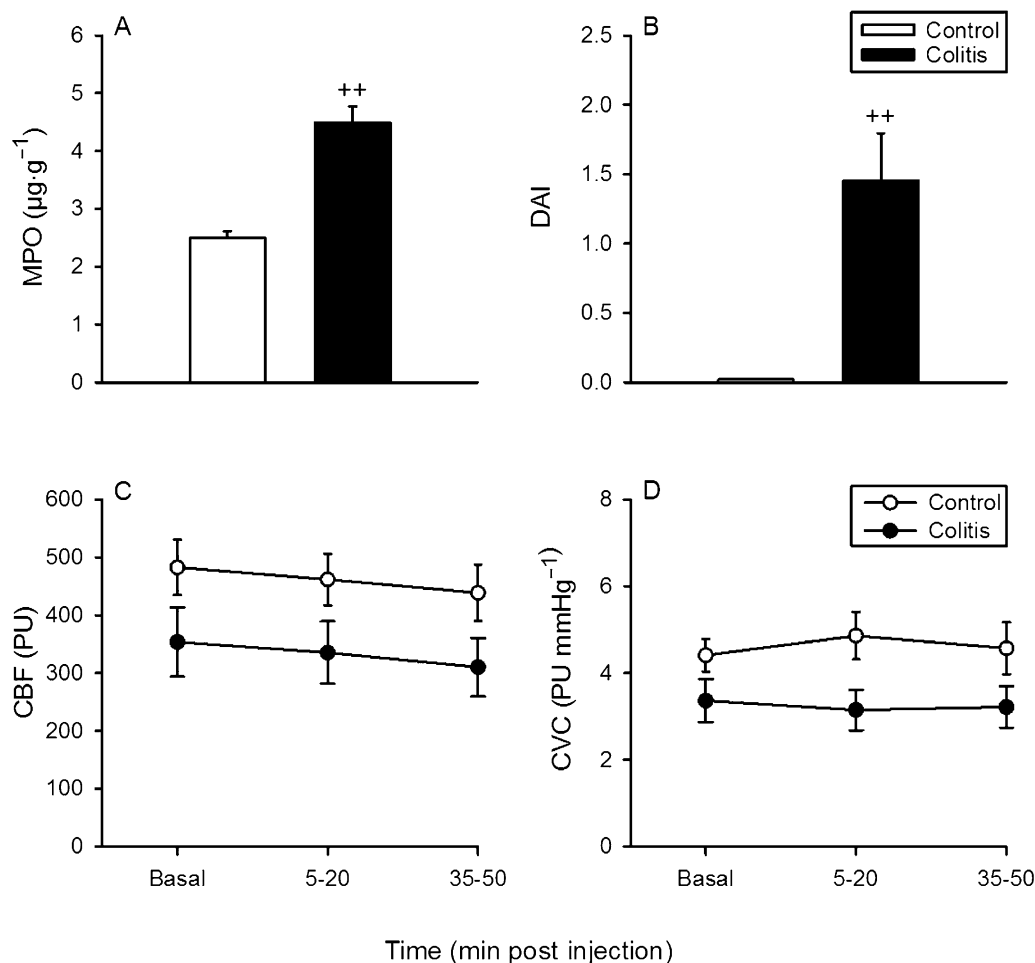


Figure 7 Effect of low-grade colitis on (A) the myeloperoxidase (MPO) content of the colonic wall, (B) the disease activity index (DAI), and the effect of i.v.-administered cilansetron ($0.3 \text{ mg}\cdot\text{kg}^{-1}$) on (C) colonic blood flow (CBF) measured by laser Doppler flowmetry and (D) colonic vascular conductance (CVC). Low-grade colitis was induced by adding dextran sulphate sodium (3%) to the drinking water for 7 days. The control animals received normal tap water. CBF and CVC were recorded and averaged during the 15–0 min period pre injection and the 5–20 min and 35–50 min periods post injection. It should be noted that the DAI for the control group in panel B was 0. Means \pm SEM, $n = 10\text{--}11$. ++ $P < 0.01$ versus control (Student's *t*-test). PU, perfusion unit.

10 times higher than that found to reduce MBF and MVC after i.v. injection.

The effect of alosetron to reduce MBF and MVC was shared by cilansetron, another 5-HT₃ receptor antagonist (Chey and Cash, 2005), which indicates that mesenteric vasoconstriction is a class effect of 5-HT₃ receptor antagonists, at least in the rat. In analysing this action, we need to consider that MBF and MVC were attenuated in the absence of any reduction of CBF and CVC. In particular, our measurements of CBF and CVC with hydrogen gas clearance and laser Doppler flowmetry show that nutrient blood flow through the colonic mucosa was not compromised by alosetron and cilansetron. This inference can be made because laser Doppler flowmetry with an endoscopic fibre probe records blood flow primarily in the mucosa, and the hydrogen gas clearance was measured with an electrode positioned close to the submucosal arteriolar plexus, which is critical to the regulation of mucosal blood flow (Granger *et al.*, 1980; Guth and Leung, 1987; Holzer *et al.*, 1991). As about two-thirds of the total resistance in the GI circulation arises from the resistance in the intra-

mural arterioles (Gore and Bohlen, 1977) and about three-quarters of the blood delivered to the GI tract is fed into the submucosal–mucosal arteriolar circuit (Bohlen *et al.*, 1978; Granger *et al.*, 1980; Guth and Leung, 1987), it is, at first sight, surprising that MVC was reduced by alosetron and cilansetron without a concomitant decrease in CVC. However, there are numerous examples that total blood flow to the GI tract and fractional blood flow to its microcirculatory circuits are differentially regulated by endocrine and neural factors as well as by pressure-flow autoregulation, capillary recruitment and opening of arteriovenous anastomoses (Holzer, 2006). As the current study suggests, this is also true for 5-HT₃ receptor-mediated modification of the splanchnic circulation.

The observation that MBF and MVC were attenuated by alosetron and cilansetron in the absence of any change of MAP and HR suggests that the haemodynamic alterations in the mesenteric arterial bed are either too small to be reflected by changes of MAP or counterbalanced by alterations in other vascular beds. It was beyond the scope of this study to

pinpoint the site of 5-HT₃ receptors that are responsible for the mesenteric vasoconstriction caused by alosetron and cilansetron. Mechanistically, this finding implies that endogenous 5-HT exerts a 5-HT₃ receptor-mediated dilator action on the mesenteric arterial bed. There is evidence for a local 5-HT-ergic system in the superior mesenteric artery of the rat (Ni *et al.*, 2008), and enteric vasodilator reflexes induced by mucosal stroking are blocked by combined 5-HT₃ and 5-HT₄ receptor blockade (Reed and Vanner, 2003). However, this finding is likely to reflect that 5-HT released from enterochromaffin cells initiates a vasodilator reflex, but is unlikely to mean that 5-HT released from enteric neurones causes submucosal vasodilatation. 5-HT₃ receptors have not yet been firmly localised to mesenteric and GI blood vessels, and little is known of their involvement, if any, in the regulation of the splanchnic circulation (Martin, 1994; Potenza *et al.*, 1998; Gul *et al.*, 2003). In the digestive tract, 5-HT₃ receptors are expressed by enteric neurones, extrinsic afferent neurones, endocrine cells and interstitial cells of Cajal (Glatzle *et al.*, 2002) and play a role in reflexes relevant to emesis, GI motility, GI secretion and GI pain (De Ponti, 2004; Gershon and Tack, 2007). It should not go unnoticed, therefore, that 5-HT₃ receptor-mediated changes in the GI circulation may be indirect consequences of other changes in GI function. One of these factors was addressed by measuring intraluminal pressure in the colon. The pertinent results indicate that the mesenteric vasoconstrictor response to alosetron and cilansetron is not associated with an increase in intracolonic pressure and, for this reason, unlikely to result from compression of GI blood vessels caused by intestinal muscle contraction.

In another set of experiments, we explored whether the mesenteric vasoconstrictor effect of alosetron differs in fasted versus non-fasted rats, given that intestinal blood flow is regulated according to digestive activity (Granger *et al.*, 1980; Holzer, 2006). As expected, baseline MBF and MVC in non-fasted rats were significantly higher than in fasted rats, which demonstrates that splanchnic blood flow in the interdigestive non-absorbing period of gut activity is reduced compared with that in the post-prandial period. As alosetron also tended to reduce MBF and MVC in non-fasted rats, we conclude that the mesenteric vasoconstrictor effect of alosetron is not related to the post-prandial/interdigestive state of gut function.

Unlike those of alosetron and cilansetron, clinically relevant doses of tegaserod (0.3–1 mg·kg⁻¹) failed to reduce MBF, MVC, CBF and CVC. On the contrary, following i.v. injection of tegaserod, a transient increase in MBF and MVC was noted in some of the experiments. In assessing the effects of this drug, its other activities, besides being a partial 5-HT₄ receptor agonist, as a 5-HT_{1B} and 5-HT_{2B} receptor antagonist need to be considered (Beattie *et al.*, 2004; Weber *et al.*, 2006). The mesenteric vasodilator action could arise from the antagonistic property of tegaserod at 5-HT_{1B} receptors that, when activated by endogenous 5-HT, cause mesenteric vasoconstriction (Gul *et al.*, 2003; Weber *et al.*, 2006). The observation that the tegaserod-evoked mesenteric vasodilation was absent in non-fasted rats may be related to the high baseline values of MBF and MVC recorded under these conditions. The absence of intracolonic pressure changes after i.v. injection of tegaserod negates the possibility that the rise of MBF and MVC seen in

our experiments was a result of stimulation of intestinal motor activity. This failure of tegaserod to alter colonic motility in our study may seem surprising in view of the ability of this drug to increase intestinal motility in other experimental paradigms and in IBS patients with constipation (Rivkin, 2003; De Ponti, 2004; Evans *et al.*, 2007). It needs, however, to be considered that our experimental set-up involves anaesthesia, laparotomy, incision and multiple instrumentation of the colon. These experimental perturbations are likely to bring about a state of post-operative ileus in which the prokinetic activity of tegaserod may be compromised. In addition, the intrinsic activity of tegaserod at 5-HT₄ receptors is only about 0.2 (De Ponti, 2004).

In additional experiments in which the haemodynamic effect of i.d.-administered tegaserod was evaluated, the dose of tegaserod was increased to 30 mg·kg⁻¹ because the oral bioavailability of tegaserod in humans is only about 10% (Rivkin, 2003; Evans *et al.*, 2007) and because we wanted to test whether even a supra-pharmacological dose of tegaserod would be without adverse effect on the splanchnic circulation. However, this dose of tegaserod also remained without effect on systemic, mesenteric and colonic haemodynamics. Similarly, experiments with isolated human and non-human primate mesenteric arteries have revealed that tegaserod is devoid of a constrictor action and fails to modify mesenteric vasodilation induced by acetylcholine, bradykinin or forskolin (Weber *et al.*, 2006). Taken together, our data show that i.v.- or i.d.-administered tegaserod even at supra-therapeutic doses does not compromise the splanchnic circulation in anaesthetised rats.

Because, in humans, alosetron and tegaserod are taken orally, another aim of our study were to mirror this situation in rats and to test whether peroral administration of the two drugs for 1 week affected the rat splanchnic circulation at baseline and following challenge with a vasoconstrictor drug. As already shown for subcutaneous treatment of rats with alosetron (0.5 mg·kg⁻¹ twice daily) for 5 days (Grundy *et al.*, 2007), short-term treatment of rats with therapy-relevant doses of alosetron or tegaserod did not interfere with the cardiovascular system of the rat in general and with the mesenteric and colonic circulation in particular. This outcome is consistent with the other results of the current study and corroborates the conclusion that the mesenteric vasoconstrictor effect of alosetron seen after acute i.v. or i.d. administration is transient and reversible. It was furthermore revealed that the mesenteric and colonic vasoconstriction elicited by L-NAME was not altered by acute i.v. administration of alosetron (Grundy *et al.*, 2007) or short-term treatment with alosetron or tegaserod (this study). Similarly, reactive hyperaemia after a transient occlusion of the superior mesenteric artery remained unabated by acute i.v. and short-term subcutaneous treatment with alosetron (Grundy *et al.*, 2007). We can thus rule out the possibility that acute and short-term exposure to alosetron or tegaserod compromised physiological vasodilator mechanisms, which subsequently resulted in vasoconstriction.

There is emerging evidence that IBS is associated with low-grade colitis (Bercik *et al.*, 2005; Spiller, 2007; De Giorgio and Barbara, 2008) and that the availability of 5-HT is increased in IBS and intestinal inflammation (Camilleri *et al.*, 2002; Coates

et al., 2004; Yeo *et al.*, 2004; Gershon and Tack, 2007). This association makes it conceivable that the splanchnic vasoconstrictor effect of 5-HT₃ receptor antagonists is exacerbated in patients with IBS. As there may be crosstalk between different 5-HT receptors (Martin, 1994), it has been speculated that, when, in patients with IBS, alosetron prevents 5-HT from binding to 5-HT₃ receptors, an excess of 5-HT may stimulate other 5-HT receptors governing intestinal vasoconstriction (Beck, 2001). However, the notion that mild inflammation may unmask a vasoconstrictor effect of 5-HT₃ receptor antagonism was refuted by the inability of low-grade colitis induced by DSS (Okayasu *et al.*, 1990) to reveal any cilansetron-induced change of CBF.

Conclusions

The present results have shown that 5-HT₃ receptor antagonists can induce a minor constriction of the mesenteric vascular bed but fail to alter haemodynamics in the colonic wall. The inability to change CBF and the transient nature of the mesenteric vasoconstrictor response to 5-HT₃ receptor antagonism do not provide any clue as to the potential of alosetron and cilansetron to enhance the incidence of ischaemic colitis in patients with IBS (Miller *et al.*, 2003; Chey and Cash, 2005; Chang *et al.*, 2006; Andresen *et al.*, 2008; Rahimi *et al.*, 2008). In addition, the current results rule out a number of factors that may pose a risk for 5-HT receptor ligands to cause splanchnic vasoconstriction, such as increased intestinal muscle tone, alterations of blood flow related to feeding and fasting, and low-grade inflammation. In view of these data, the question arises as to whether the rat is an appropriate model to study the impact of the 5-HT system on the splanchnic circulation. However, a review of the available information led Gershon and Tack (2007) to conclude that the presence of 5-HT and of 5-HT receptors in the human gut is consistent with that found in laboratory animals including the rat. It need hence be inferred that there are other factors that may shape the impact of 5-HT₃ and 5-HT₄ receptor ligands on the splanchnic circulation (Camilleri, 2007; Grundy *et al.*, 2007). The challenge is still to identify these factors in order to understand why IBS itself and the pharmacological manipulation of 5-HT₃ and 5-HT₄ receptors are associated with an increased incidence of ischaemic colitis.

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Conflict of interest

Except for the fact that this study was financially supported by Novartis Pharma, the authors state no conflict of interest.

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