5-HT$_3$ RECEPTOR ANTAGONISM BY ALOSETRON DOES NOT AFFECT COLONIC HAEMODYNAMICS AND ISCHEMIA-REPERFUSION DURING NITRIC OXIDE SYNTHASE BLOCKADE-INDUCED HYPERTENSION IN RATS

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Introduction

Alosetron has been shown to be effective in female diarrhea-predominant IBS patients (Camilleri *et al*. 1999). Despite marked symptom improvements, the use of alosetron has been limited because of a small number of cases of ischemic colitis in patients receiving treatment (Friedel *et al*. 2001). Potential contributing factors include altered blood flow regulation, constipation and pre-disposition in the patient group. Hypertension may also be a risk factor for ischemic colitis (Medina *et al*. 2004). Mesenteric vasoconstrictor responses to 5-HT are potentiated in hypertensive animals (Su and Uruno, 1984). To date there have been no detailed studies on the long term effects of 5-HT$_3$ receptor blockade on colonic haemodynamics. Our aim was to investigate the effect of alosetron on baseline haemodynamics and reactive hyperaemia under both control conditions, and after blocking NOS in order to compromise vasodilation and induce hypertension.

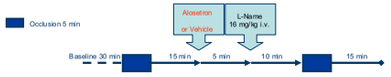
Methods

Experiments were performed in pentobarbitol (60-80 mg/kg) anaesthetized rats following institutional animal care guidelines. The following parameters were measured:

- Mesenteric blood flow (MBF) using a Transonic ultrasonic flow meter on the terminal superior mesenteric artery.
- Serosal perfusion (P[s]) using a laser Doppler probe.
- Tissue oxygen (pO_2) from a serosal oxygen electrode.
- Mean arterial pressure (MAP) from the carotid artery, and heart rate derived from the triggered pressure pulses.
- Intraluminal pressure (LP) was also recorded.
- Data are presented as mean ± SEM and were analyzed statistically using Student's t-test or ANOVA with post-hoc Tukey's test as appropriate (n≥ 6).

Experimental protocols

Alosetron (30 µg/kg i.v.) was tested on baseline and occlusion / reperfusion haemodynamics:



Results

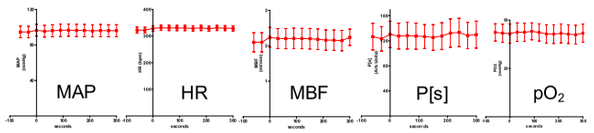


Figure 1 - Short term effects of alosetron on baseline haemodynamics - Alosetron (30 µg/kg) had no immediate effect on colonic haemodynamics. Alosetron (100 µg/kg) had no effects on colonic baseline dynamics for up to 1 hr post-treatment (unpublished observations).

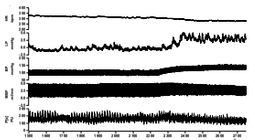
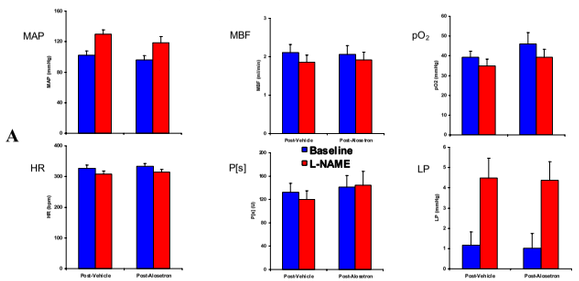


Figure 2 - Effect of L-NAME - Representative trace in a vehicle-treated animal.



B

	MAP	HR	MBF	P[s]	pO2	LP
Post-V *versus* Post-A	p=0.55	p=0.98	p=0.45	p=0.39	p=0.81	p=0.95

Figure 3 - Effect of L-NAME in vehicle– and alosetron-treated animals - A - Histograms showing mean change in baseline parameters following treatment with L-NAME in vehicle- and alosteron-treated animals. **B** - Summary statistics showing that changes following L-NAME were not different in vehicle- and alosteron-treated rats (unpaired Student's t-test).

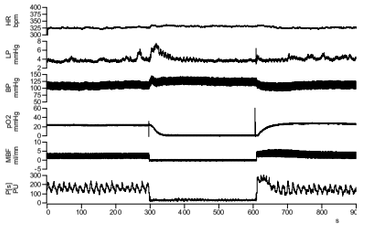


Figure 4 - Response to occlusion and reperfusion - Representative trace showing the haemodynamic responses to a 5min period of occlusion and subsequent reperfusion response

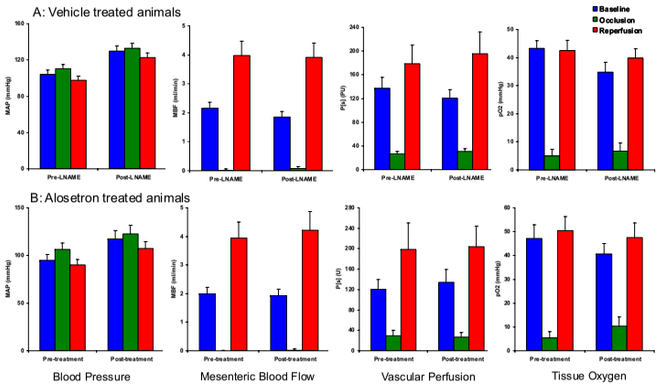


Figure 5 - Mean haemodynamic parameters during occlusion and following reperfusion.

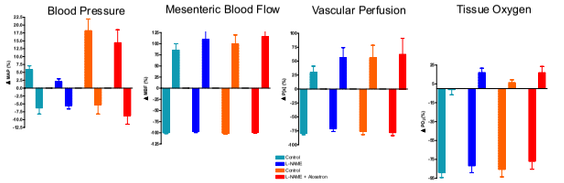


Figure 6 - Haemodynamic changes expressed as % of baseline - during occlusion (left bars) and reperfusion (right bars in) before and after either L-NAME and vehicle or before and after L-NAME and alosetron.

Summary statistics for the occlusion response (normalized to baseline) in animals treated with vehicle and L-NAME or alosetron and L-NAME.				
ANOVA →	MAP (p<0.0001)	MBF (p=0.71)	P[s] (p=0.57)	pO2 (p=0.69)
Pre-V *versus* Post-V	N.S.	N.S.	N.S.	N.S.
Pre-A *versus* Post-A	N.S.	N.S.	N.S.	N.S.
Pre-V *versus* Pre-A	p<0.05	N.S.	N.S.	N.S.
Post-V *versus* Post-A	p<0.05	N.S.	N.S.	N.S.

Summary statistics for the reperfusion response (normalized to baseline) in animals treated with vehicle and L-NAME or alosetron and L-NAME.				
ANOVA	MAP (p=0.70)	MBF (p=0.58)	P[s] (p=0.60)	pO2 (p<0.05)
Pre-V *versus* Post-V	N.S.	N.S.	N.S.	N.S.
Pre-A *versus* Post-A	N.S.	N.S.	N.S.	N.S.
Pre-V *versus* Pre-A	N.S.	N.S.	N.S.	N.S.
Post-V *versus* Post-A	N.S.	N.S.	N.S.	N.S.

Fig 7 - Occlusion / reperfusion summary statistics - The increase in MAP during occlusion was higher in the alosetron group both before and after treatment with L-NAME and/or alosetron. Alosetron had no effect on the magnitude of the colonic haemodynamic response to occlusion or reperfusion.

Summary

- Alosteron (30µg/kg) had no effect on baseline colonic haemodynamics.
- Alosetron had no effect on L-NAME-induced changes to MAP or colonic haemodynamics compared to vehicle.
- Alosetron had no effect on the magnitude of the occlusion / reperfusion response in animals treated with L-NAME.

Conclusions

5-HT$_3$ receptor antagonism by alosetron does not affect either baseline colonic haemodynamics or ischemia-reperfusion during L-NAME-induced hypertension.

References: Camilleri M, *et al.* Aliment Pharmacol Ther 1999; 13: 1149-59. Friedel D, Thomas R, Fisher RS. Gastroenterol 2001; 120: 557-560. Medina C, *et al.* Dis Colon Rectum 2004; 47(2): 180-4. Su C and Uruno T. Eur J Pharmacol. 1984; 106:283-90.

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