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PROSTAGLANDIN E2 RECEPTOR (EP2 SUBTYPE) CONTRIBUTES TO JEJUNAL DYSMOTILITY IN *Schistosoma mansoni* INFECTED MICE

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ABSTRACT

Prostaglandin E_2 (PGE_2) is increased in inflammation and modulated intestinal peristalsis in the small intestine. However, the receptor mechanisms involved during parasitic inflammation are unknown. The aim of the present study was to investigate the role of EP_2 receptors involved in the mechanisms underlying post-inflammatory changes during inflammation. Experiments were performed in male Swiss mice 4- and 8-wks following infection with *S. mansoni* and the results compared to those of uninfected control mice. Jejunal contraction was assessed using a modified Trendelenburg type preparation to study motor complexes (MCs). Infection had great effects on jejunal motility that reflected by an increase of amplitude and a decrease of interval between MCs. EP_2 receptor agonist AH13205 (10μM) produced a significant inhibition of MCs which appeared as a decrease in amplitude and an increase in intervals in control, 4- and 8-wks infected animals. EP_1 & EP_2 receptors antagonist AH6809 (30μM) attenuated intestinal peristalsis by a significant inhibition followed by contraction in control, 4- and 8-wks infected animals. In the presence of EP_2 receptor antagonist AH6809, EP_2 receptor agonist AH13205 failed to alter the peristaltic motor activity in control, 4- and 8-wks infected jejunum. These data demonstrated temporal differences in motor function during the intensity of inflammation. EP_2 receptor plays a potential role in modulating jejunal motility of control animals and altered motility triggered by parasitic inflammation.

Keywords: Jejunal motility; parasitic inflammation; EP_2 Receptor Agonist AH13205; EP_2 Receptor AH6809 Antagonist.

INTRODUCTION

Prostaglandins (PGs) are known to modulate a variety of actions in various tissues and cells (Martin and Wallace, 2006). They modify gastrointestinal (GI) motility by producing relaxation or contraction of smooth muscles (Shahbazian *et al.*, 2002). PGE_2 effect is attributed to its binding to four different subtypes of EP receptors (EP_1, EP_2, EP_3 and EP_4) that in turn propagate signals through alteration in the intracellular calcium (Ca^{2+}) or cyclic adenosine monophosphate (cAMP) levels. These events lead to activation of a range of kinases that modulate diverse cellular functions. The EP_1 receptor mediates a PGE_2-induced elevation of the free Ca^{2+} concentration in experimental animal cells (Katoh *et al.*, 1995). EP_1 regulates Ca^{2+} channel gating *via* an unidentified G protein (Sugimoto and Narumiya, 2007). The EP_2 and EP_4 receptors couple to G protein and mediate increases in cAMP concentrations. The EP_3 receptors are unique in their ability to couple to multiple G proteins (Sugimoto and Narumiya, 2007). The major signalling pathway of the EP_3 receptor is inhibition of adenylate cyclase *via* G protein (Dey *et al.*, 2006). However, it must be recognized that the EP receptors do not couple exclusively to one of these pathways but, often to more than one G protein and signal transduction pathway (Dey *et al.*, 2006).

PGE_2 receptors are expressed in the gut of several species including humans (An *et al.*, 1993; Morimoto *et al.*, 1997 and Sugimoto and Narumiya, 2007). Several studies indicated that EP receptor subtypes (EP_1, EP_2, EP_3 and EP_4) have been also found to be expressed in mouse tissues including the GI tract (Honda *et al.*, 1993, Sugimoto *et al.*, 1992 and Kato *et al.*, 2005). EP_2 receptor is localized in the external muscle layer of the rat and mouse GI tract and within myenteric neurons of guinea-pig (Lawrence *et al.*, 1992, Narumiya *et al.*, 1999 and Northey *et al.*, 2000). EP_1 receptor is expressed in all muscularis mucosal layer of the GI tract and may be involved in the local movement of the mucosa (Morimoto *et al.*, 1997). EP_3 receptor was found in smooth muscle cells in the longitudinal muscle layer of the GI tract, but not in the circular muscle layer and within neurons of the myenteric ganglia (Narumiya *et al.*, 1999). Pharmacological investigations conclude that EP_1 and EP_3 receptors mediate contraction when activated by agonists, while relaxation was brought on by EP_2 receptors on myenteric neurons (Lawrence *et al.*, 1992, Sametz *et al.*, 2000, Shahbazian *et al.*, 2002 and Grasa *et al.*, 2006). EP_4 is highly expressed in the glands of the gastric antrum, implicating this receptor in PGE_2-mediated secretion. However, a differential expression of EP receptors on the GI mucosa may

occur during inflammation. Cosme *et al.* (2000) showed differences of mucosal expression of the EP_4 receptors between cells in normal human colonic lamina propria and those from the inflamed human colon. Mucosal EP_4 receptor expression was upregulated in T lymphocytes. In human ulcerative colitis, the levels of EP_2 and EP_3 receptors were obvious in epithelial cells (Takafuji *et al.*, 2000). To date, the distribution levels and the role of EP_2 receptor on contractile activity in the small intestine during parasitic infections were not established.

Therefore, the aim of the present study was to characterize the EP receptors involved in the contractile activity using the synthetic ligands EP_2 receptor selective agonist AH13205 and antagonist AH6809 in normal and *S. mansoni* induced hyper contractile activity associated with the severe pain during parasitic inflammation.

METHODS

Schistosoma mansoni Infection

The maintenance of the *S. mansoni* life cycle and the transcutaneous infection of mice with *S. mansoni* were carried out according to the methods of Bogers *et al.*, (2000). Male Swiss mice (age 8 wks) were transcutaneously infected using treated water containing about 100 infectious cercariae of a *Biomphalaria alexandrina* strain of *S. mansoni*. The sarcariae were allowed to penetrate during 30 min after which the water was removed and checked for the remaining cercariae. Control and infected mice were sacrificed by cervical dislocation after 4- and 8-wks of infection and the contractile activity of isolated segments from jejunum were investigated. All experiments were approved by the Ethic Committee of King Fahad medical research centre (KFMRC).

Tissue Preparation

Before sacrificed, mice were fasted for 24 h with free access to water in order to ensure that the GI tract did not contain remnants of food. Segments of proximal jejunum were rapidly excised and cleared of any mesenteric connective tissue; the lumen was flushed with Krebs solution. Two jejunal segments ~5 cm in length were prepared from each animal, and mounted horizontally in separate 20mL perfusion chambers filled with Krebs solution, maintained at 37° C and aerated with a mixture of 5% CO_2 and 95% O_2.

Recording of Peristalsis

The oral and aboral ends of each segment were secured to two metal catheters fixed at either end of the chamber and adjusted to maintain the segments at their resting length. For each segment, the oral end was connected to a perfusion pump for intrajejunal infusion of Krebs solution at a rate of 0.16 ml/min, and the aboral end was attached to a pressure transducer (NL108T2) to record contractile activity as changes in intraluminal pressure under isovolumetric conditions. Tissues were maintained at 37°C, perfused with Krebs solution at a rate of 5 ml/min, and allowed to equilibrate for at least 30 min before experiments started. The experimental setup was standardized by routinely infusing Krebs solution into the closed segment to an initial intraluminal pressure of 2-3 cmH_2O in the mice. Regular aborally propagating waves of contraction (MCs) developed under these conditions and could be maintained for several hours. The output from the pressure transducers was relayed to a Neurolog (NL900D-Digitimer, UK) and to a data-acquisition system (CED 1401-Cambridge Electronic Design, Cambridge, UK) and from there to a computer running Spike 4 software (CED), which displayed the two channel pressure recordings online and also stored the data for subsequent offline analysis.

Experimental Protocol

Only preparations in which regular motor complexes (MCs) were maintained were used for subsequent experiments. Drugs or the appropriate vehicle were added to the chambers 20 min after stopping perfusion. The recording continued for a further 20 min before washing out the drug and reinstating perfusion.

Drugs

The following drugs were used. Atropine sulphate, hexamethonium, AH13205 [*Trans*-2-4-(1-Hydroxyhexyl) phenyl-5-oxocyclopentaneheptanoic acid] and AH6809 [6-Isopropoxy -9-oxanthene-2-carboxylic acid] were purchased from Sigma Chemical (USA). The drugs were dissolved with appropriate media, the concentrations given here in referring to the stock solutions. Atropine sulphate (1μM) was dissolved in saline (0.9% NaCl), hexamethonium (1μM) was dissolved in distilled water, AH13205 (10μM) and AH6809 (30μM) in dimethyl sulphoxide. All drugs were stored at -20°C. Freshly diluted aliquots were maintained on ice during the course of the experiments and added to the bath in micro liter volumes.

Data Analysis

The contractile activity of isolated jejunum of 4- and 8-wks infected mice was compared with the contractility of isolated segments from age-matched control mice. Motor complex (MCs) were measured in terms of their peak amplitude above baseline (cmH_2O) and interval (second) between them. Baseline values were taken during the 20 min before drug application and the response in 20 min following application. The effect of each drug was quantified by calculating the interval between MCs in the 20 min period before and after drug administration. Responses are expressed as absolute values ± SE, with *n* being the number of animals. Paired data were compared using Student's *t*-test as appropriate. Grouped data from control and infected animals were compared using independent *Mann Whitney U*- test. In all cases, a probability values of $P < 0.05$ was regarded significant.

RESULTS

Motor Complexes (MCs) in Control Jejunum

Luminal distension of isolated segments of mice jejunum evoked a regular pattern of contractile activity. The activity consisted of periodic increases in intraluminal pressure separated by periods of relative quiescent (Fig. 1). Contractile activity in isolated mice jejunum in a sample of 12 control tissues had a mean amplitude of 5.40 ± 0.5 cmH_2O and was separated by a mean interval of 43.34 ± 1 s (Fig. 2B).

MCs in Infected Mice Jejunum

Contractile activity in isolated 4-wks infected jejunum followed a similar pattern to that observed in control. In 4-wks infected animals, the mean amplitude was 4.44 ± 1 cmH_2O and separated by mean interval of 41.15 ± 1s. However, 8-wks post-infection the interval significantly decreased to 35.50 ± 1s, P< 0.001, *n*= 12) while the amplitude significantly increased to 20.60 ± 3 cmH_2O, (P<0.001) compared to control (*n*=12., Fig 2, A+B)

Effects of Muscarinic and Nicotinic Receptor Antagonists on MCs in Mice Jejunum

The administration of atropine (1μM) and hexamethonium (1μM) for 20 min completely abolished MCs in control, 4- and 8-wks infected mice (*n*=6, Fig. 3 A+B). This effect was developed within 1 min and sustained in its continue presence.

Effect of EP_2 Agonist on MCs in Mice Jejunum

The selective EP_2 receptor agonist AH13205 (10μM) produced a significant increase in the interval between MCs in control and 4-wks infected jejunum (39.04 ± 2 *vs* 51.17 ± 2s, P<0.001, and 39.38 ± 3 *vs* 52.45 ± 4s, P< 0.01, *n*=6 respectively). However, the amplitude in control and 4-wks infected jejunum were decreased from 5.04 ± 0.8 to 2.85 ± 0.6 cmH_2O, P<0.001, and from 4.69 ± 0.5 to 2.37 ± 1 cmH_2O, P<0.01, *n*=6, respectively, Fig. 4 A+B. In 8-wks post-infection, AH13205 had no significant effect on MCs interval while there was a significant decreased in amplitude (32.55 ± 4 *vs* 43.67 ± 16s, P>0.05 and 17.67 ± 1 *vs* 12.46 ± 0.7 cmH_2O, P<0.05, *n*=6, Fig. 4 B, Table 1).

Effect of EP_2 Antagonist on MCs in Mice Jejunum

EP_1 & EP_2 receptors antagonist AH6809 (30μM) produced an inhibition followed by a contraction (Fig. 5 A, Table 2). AH6809 triggered a significant decrease in amplitude in control, 4- and 8-wks infected animals (5.25 ± 0.7 *vs* 2.75 ± 0.9 cmH_2O, P<0.05, 4.87 ± 0.8 *vs* 2.46 ± 0.7 cmH_2O, P<0.05, and 17.78 ± 4 to 6.76 ± 2 cmH_2O, P<0.01, *n*=6, respectively Fig. 5 B). The inhibitory effect of AH6809 remained for 3 min. This inhibition was followed by a contraction that sustained with continue presence. In terms of intervals, there was a significant increase of MC interval in control, 4- and 8-wks compared to pre drug control (43.01 ± 1 *vs* 64.30 ± 2s, P<0.001, 41.64 ± 2 *vs* 75.11 ± 7s, P<0.01, and 36.77 ± 2 *vs* 61.76 ± 8s, P<0.05, *n*=6 respectively, Fig. 5 B).

Table 1: Effect of EP_2 agonist AH13205 on contractile activity in control and infected mice.

	Amplitude		Intervals		
	Baseline	AH13205		Baseline	AH13205
Control	5.04±1	2.85±1***	Control	39.04±2	51.17±2***
4 weeks	4.69±1	2.37±1**	4 weeks	39.38±3	52.45±4**
8 weeks	17.67±1	12.46±0.7*	8 weeks	32.55±4	43.66±16

* = P<0.05, **= P<0.01 and ***= P<0.001 *vs* baseline.

Table 2: Effect of EP_2 receptor antagonist AH6809 on contractile activity in control and infected mice.

	Amplitude		Intervals		
	Baseline	AH6809		Baseline	AH6809
Control	5.25±0.7	2.75±0.9*	Control	43.01±1	64.30±2***
4 weeks	4.87±0.7	2.46±0.7*	4 weeks	41.64±2	75.11±7**
8 weeks	17.78±4	6.76±2**	8 weeks	36.77±2	61.76±8*

* = P<0.05, **= P<0.01 and ***= P<0.001 *vs* baseline.

Table 3: Magnitude of inhibition between the agonist AH13205 (10μM) alone and the agonist in the presence of antagonist AH6809 (30μM) in control, 4 and 8 weeks infected animals.

	Amplitude	Amplitude
	Agonist	Agonist + Antagonist
Control	4.37±0.5	1.03±1**
4 weeks	4.65±1	1.79±.04**
8 weeks	5.25±1	1.48±0.7*

* = P<0.05, **= P<0.01 and ***= P<0.001 *vs* baseline.

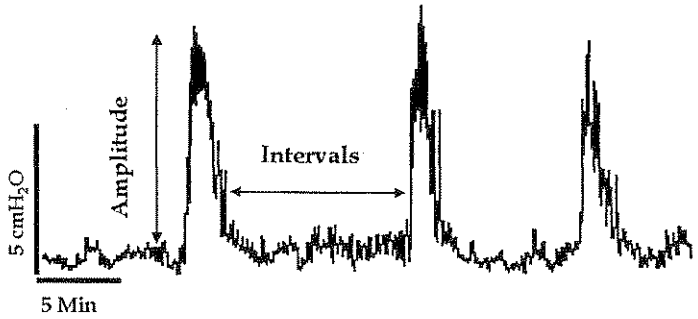

Fig. 1: Migrating motor complexes (MCs) were measured in terms of their peak amplitude (cmH_2O) above the baseline and the intervals (second) between them.

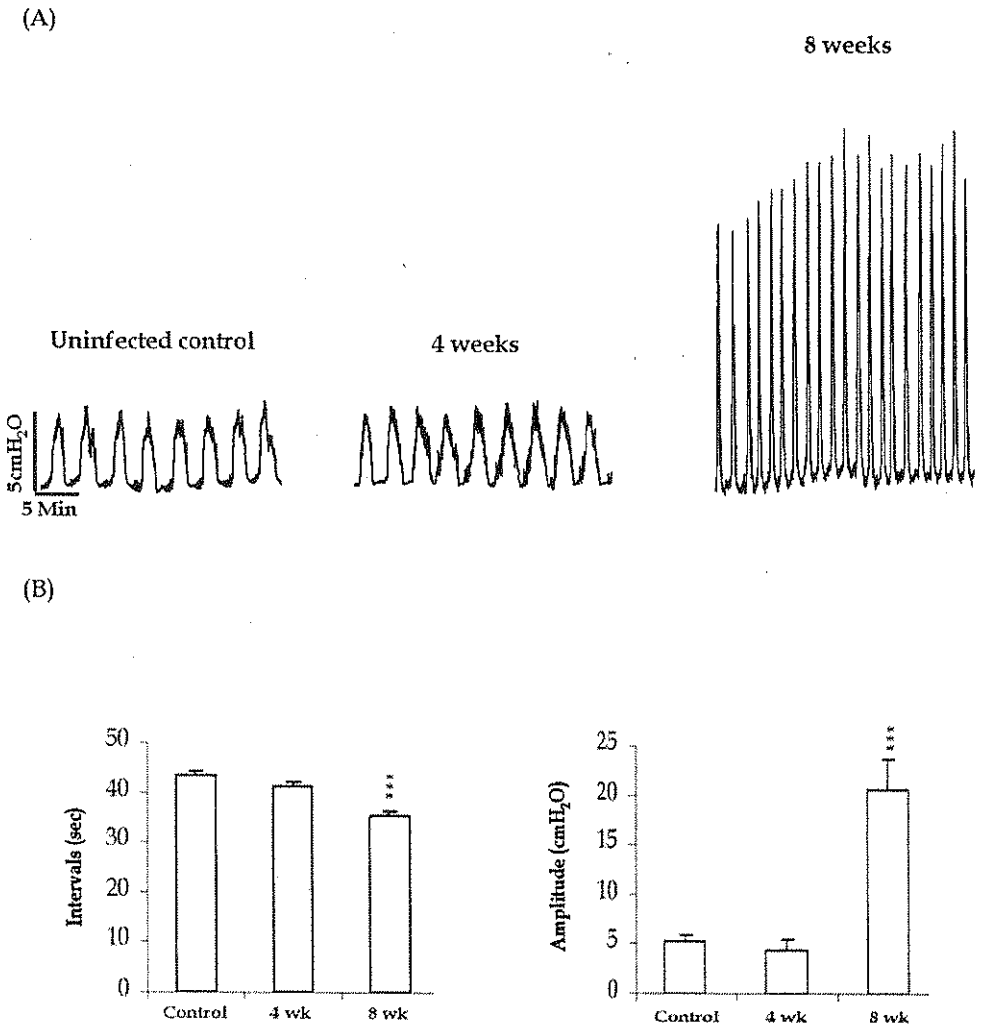

Fig. 2: Contractile Activity in Control and Infected Mice Jejunum
(A) Representative traces showing the contraction of isolated jejunum from control, 4- and 8-wks infected mice. **(B)** Histograms showing the amplitude and interval between MCs in control, 4- and 8-wks infected mice jejunum. ***=P <0.001 compared to control animals (n= 12).

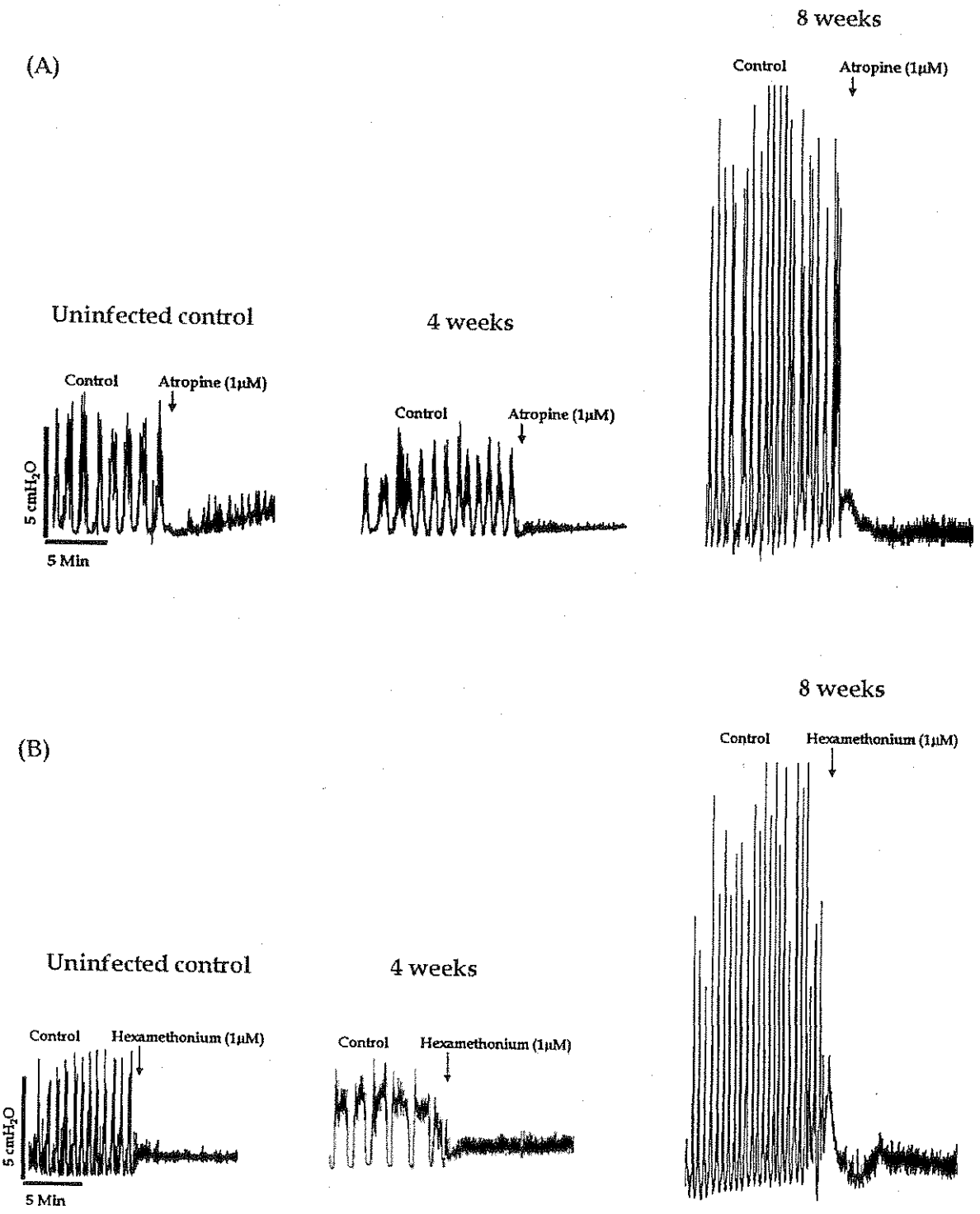

Fig.3: Effect of Muscarinic Receptor Antagonist on MCs in Mice Jejunum
(A) Representative traces showing the absence of MCs by atropine (1μM, *n*=6) in control, 4- and 8-wks infected mice. **(B)** Representative traces showing the elimination of MCs by Hexamethonium (1μM, *n*=6) in control, 4- and 8-wks infected mice.

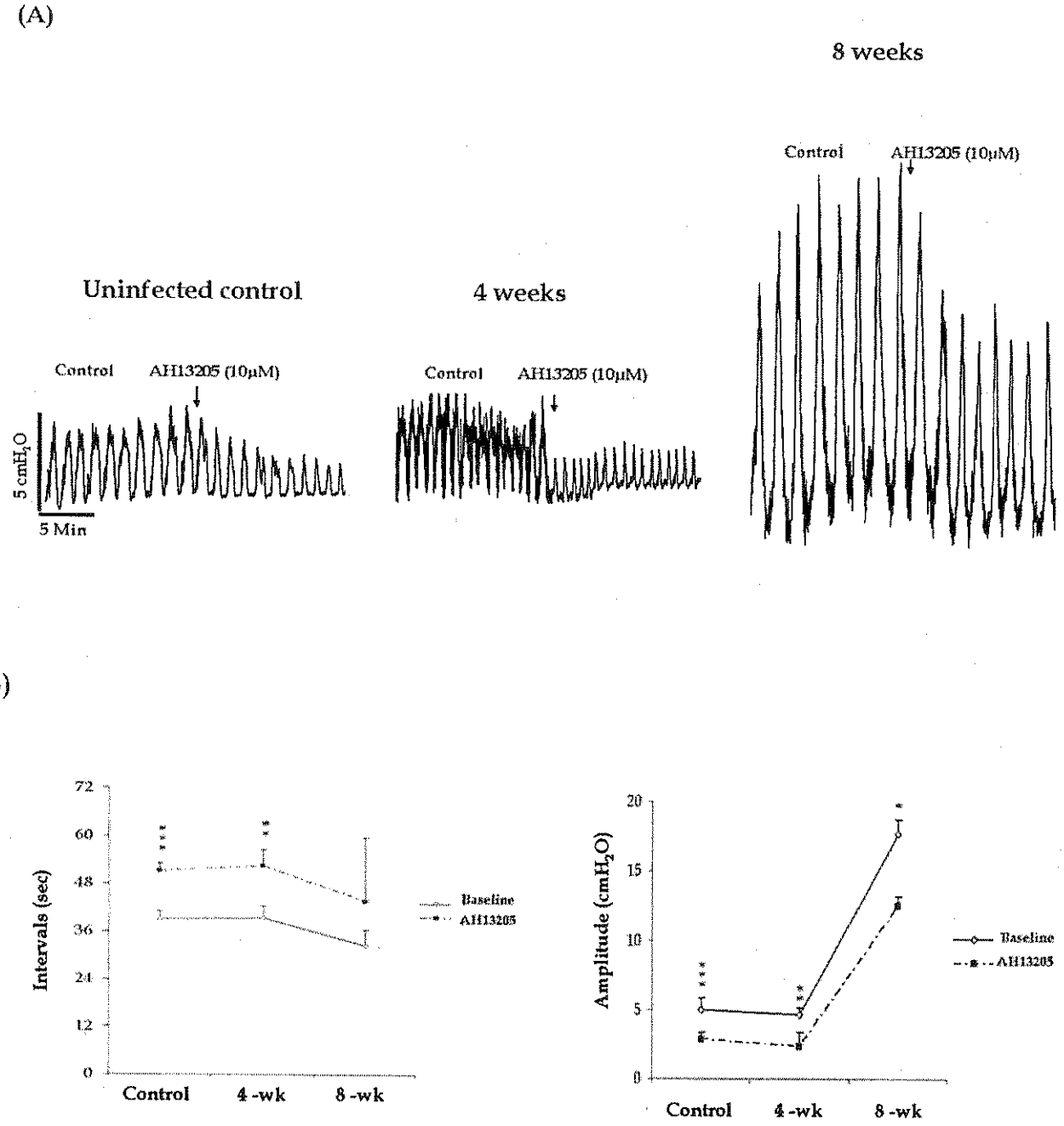

Fig. 4: Effect of EP_2 Receptor Agonist AH13205 on MCs
(A) Representative traces showing the increase in the interval and the decrease in amplitude produced by AH13205 (10μM) in control, 4- and 8-wks infected animals. **(B)** Effect of AH13205 on amplitude and interval between MCs (10μM, n=6). *=P <0.05, **=P <0.01 and ***=P <0.001 compared to pre-drug control.

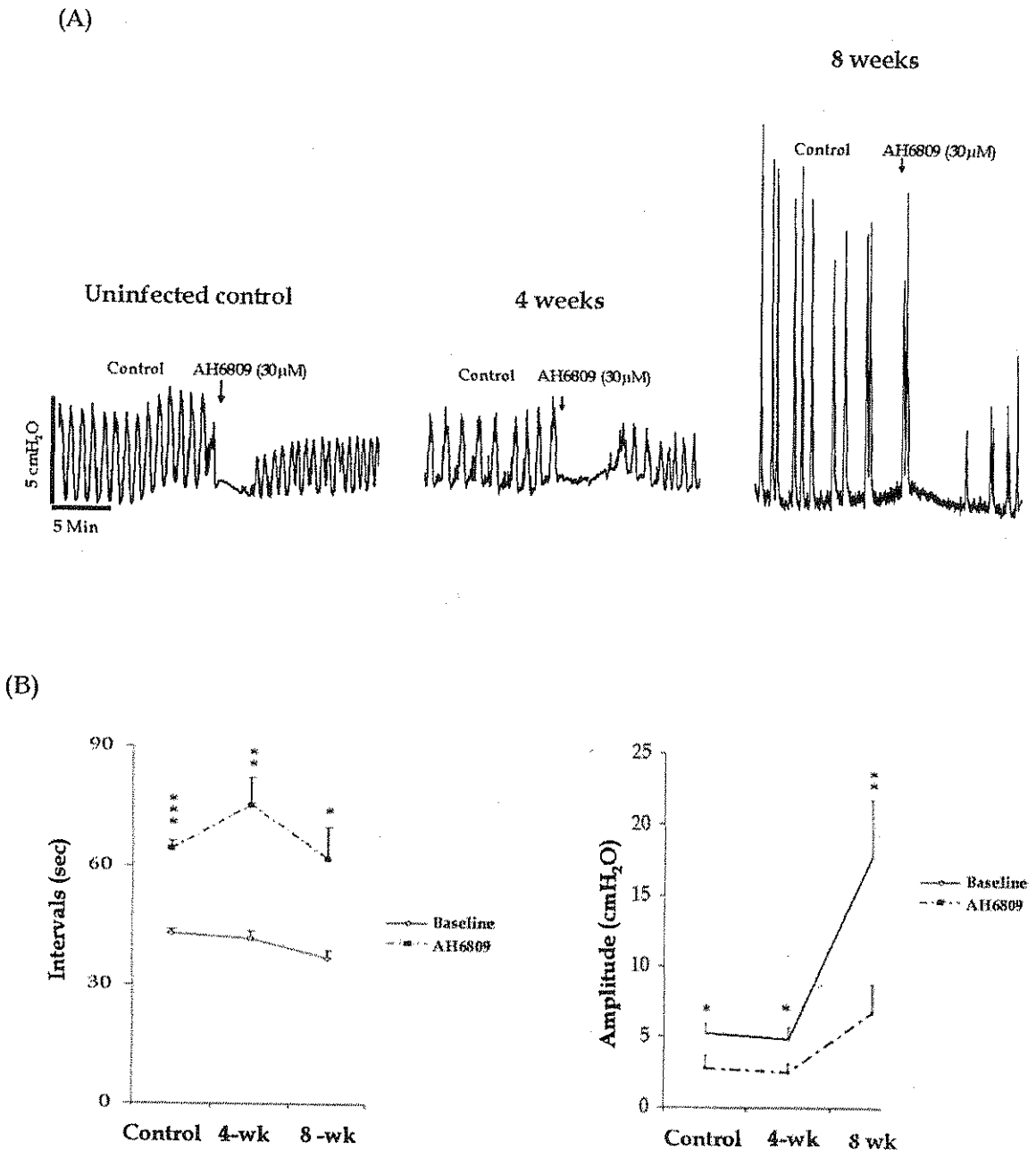

Fig. 5: Effect of EP_2 Receptor Antagonist AH6809 on MCs

(A) Representative traces showing the inhibition followed by contraction produced by AH6809 (30μM) in control, 4- and 8-wks infected animals. **(B)** Effect of AH6809 which is reflected by the increase of interval between MCs and the decrease of amplitude before and after addition of AH6809 (30μM, n=6). *=P< 0.05, **=P< 0.01 and ***=P< 0.001 compared to pre-drug control.

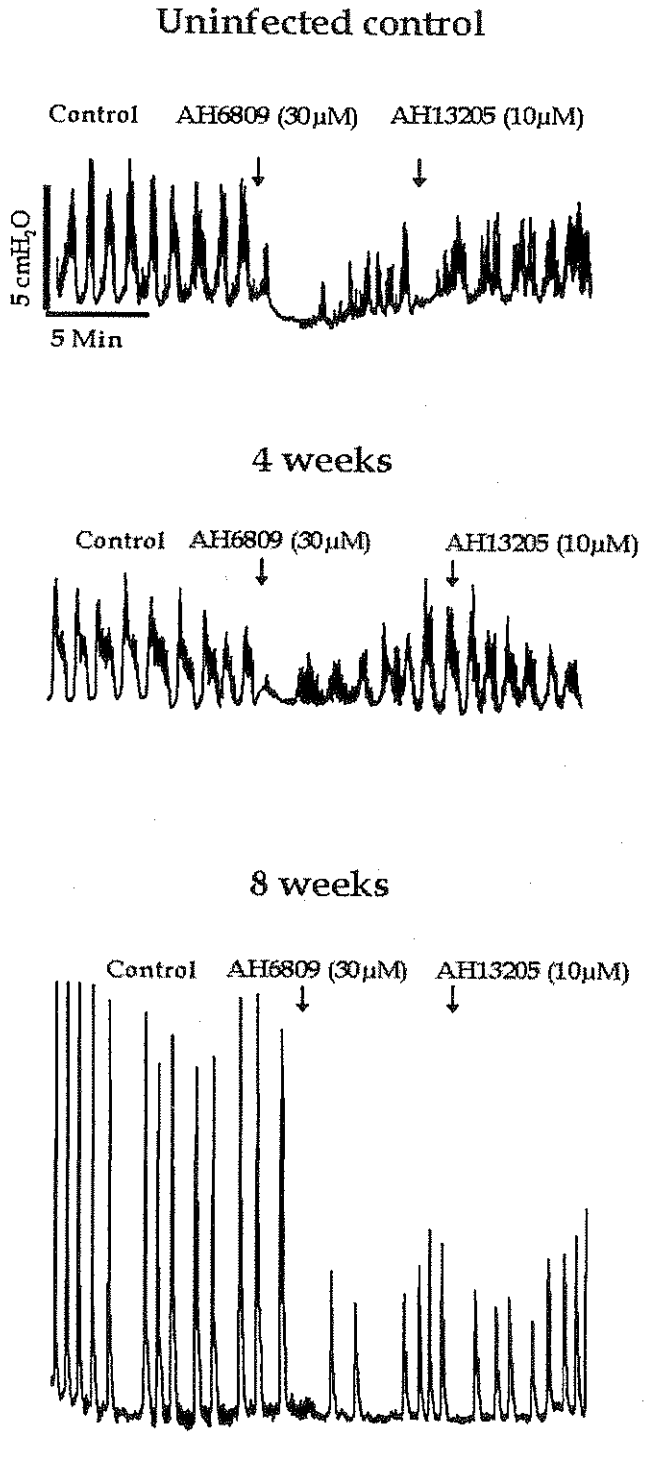

Fig. 6 (A) : Effect of EP_2 Agonist in Presence of EP_2 Antagonist

Representative traces showing the inhibition followed by contraction produced by EP2 antagonist AH6809 (30μM, *n*=6). This contraction remains unchanged after the addition of EP_2 agonist AH13205 (10μM, *n*=6) in control, 4- and 8-wks infected animals.

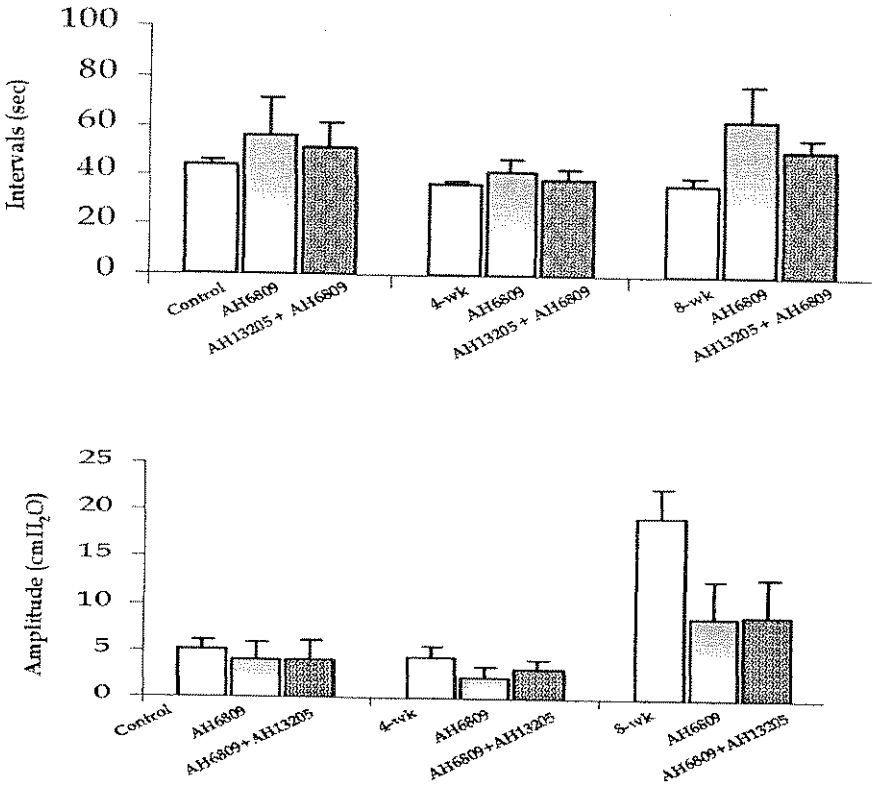

Fig. 6 (B): Effect of EP_2 Agonist in Presence of EP_2 Antagonist
Histograms illustrating the effect of AH13205 (10μM) in the presence of AH6809 (30μM, n= 6) on intervals (upper histogram) and amplitude (lower histogram) in control, 4- and 8-wks infected animals.

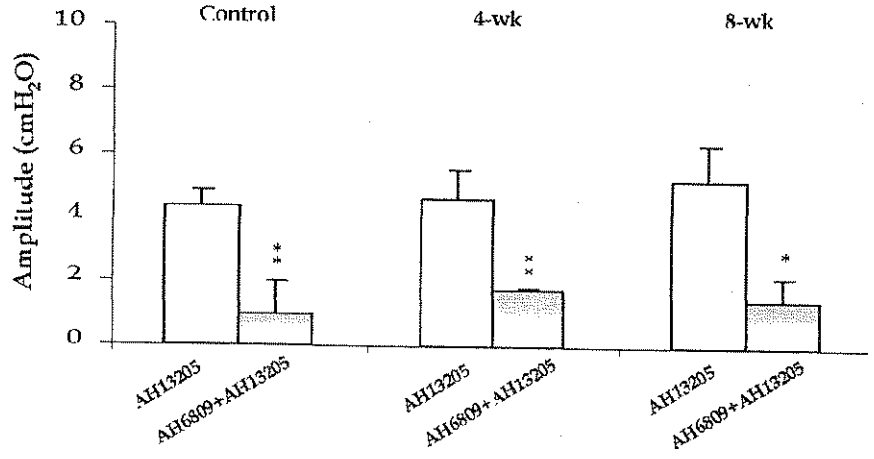

Fig. 6 (C): Effect of EP_2 Agonist in Presence of EP_2 Antagonist
The magnitude of the inhibition of EP_2 agonist AH13205 (10μM) alone and EP_2 agonist in the presence of antagonist AH6809 (30μM, n=6) in control, 4- and 8-wks infected animals.

Effect of EP_2 Agonist in the Presence of EP_2 Antagonist on MCs in Mice Jejunum

Selective EP_2 receptor agonist AH13205 (10μM) did not produce any significant effect in control, 4- and 8-wks infected animals when added to the organ bath in the presence of EP_2 receptor antagonist AH6809 for 10 min period (Fig. 6 A). The data in Fig. 5 A showed that EP_2 receptor antagonist AH6809 (30μM) produced inhibition followed by contraction. This contraction remained unchanged after the addition of EP_2 receptor agonist AH13205 (10μM) in control, 4- and 8-wks infected animals (Fig. 6A+B). In control jejunum, the effect of EP_2 receptor agonist AH13205 in the presence of antagonist AH6809 on MCs interval was 56.11 ± 16 *vs* 51.06 ± 11s, n=6 (P>0.05) and the amplitude was 3.99 ± 2 *vs* 4.01 ± 2 cmH_2O (P>0.05, Fig 6B). The same effects were observed in 4-wks infected animals, the interval was 42.15 ± 5 *vs* 39.15 ± 4 s, n=6 (P>0.05) while the amplitude was 2.37 ± 1 *vs* 3.19 ± 1 cmH_2O (P>0.05). A similar effect was also noticed in 8-wks post infection. The interval was 64.06 ±14 *vs* 51.92 ± 5s, n=6 (P>0.05) while MCs amplitude was 8.72 ± 4 *vs* 8.90 ± 4 cmH_2O (P>0.05, Fig. 6 B). The lack of effect of agonist in the presence of antagonist can be shown in (Fig. 6 C) where the magnitude of the inhibition of the agonist alone differs significantly from the magnitude of inhibition of the agonist in the presence of antagonist compared to baseline control (Table 3).

DISCUSSION

Intestinal peristalsis elicited by distension of the intestinal wall involved ascending excitatory and descending inhibitory reflexes within the enteric nervous system (Costa *et al.*, 2000), These reflexes result in aborally moving waves of coordinated contractions of longitudinal and relaxations of the circular smooth muscles (Grider, 2003). In the present study, the pattern of contractile activity observed in isolated segments of control mice jejunum was similar to the motor complexes observed by Bush *et al.* (2000); Abdu *et al.* (2002); Tanovic *et al.* (2006) and Bertrand (2006) . It consists of aborally propagating waves of activity separated by periods of serenity and these were dependent on cholinergic mechanisms because they were blocked by nicotinic and muscarinic blockers (Bush *et al.*, 2000 and Abdu *et al.*, 2002).

S. mansoni infection induce inflammation which results in disturbed enteric plexus and lead to smooth muscle hypercontractility, nausea and vomiting (El Zawawy *et al.*, 2006). The present study showed that the effect of intestinal *schistosomiasis* on contractile activity of isolated mice jejunum 4 wks post infection was similar to that observed in control animals. This observation might be attributed to the absence of abnormal changes on structural level in the smooth muscle layers and to the stability of the neurotransmitters release during this early period of inflammation (De Man *et al.*, 2002). However, 8-wks post infection, a significant decrease in intervals and an enormous increase in amplitude were noticed which provided a direct sign of the release of inflammatory mediators and imply to the structural changes in the mynteric plexus that lead to hypercontractility. This proposal was similar to those of Bogers *et al.* (2000), De Man *et al.* (2001) and De Jonge *et al.* (2003), where the hypercontractility during this period of infection was attributed to the smooth muscle layers thickness of the small intestine and to the granulomas that were abundantly present in the mucosal and smooth muscle layers of the small intestine after 8-wks of infection and onwards.

In the present study, MCs in control, 4- and 8-wks infected animals were abolished by the muscarinic receptor antagonist atropine and the nicotinic receptor antagonist hexamethonium, implicating the cholinergic enteric nerves in controlling intestinal peristalsis during inflammation. This postulation is based on other studies by Frisby *et al.* (2007), De Man *et al.* (2003) and Bertrand (2006) where the spontaneous activity and the enhanced contractile activity during inflammation were completely blocked by atropine indicating that these contractions were cholinergic in origin. De Man *et al.* (2001) also pointed out to the disturbances of enteric cholinergic neurotransmission during *schistosomiasis* which explained the increased of intestinal contractility. This explanation was strengthen by Linden *et al.* (2004) who revealed that intestinal inflammation produced an excitation of both AH and S neurons found in the myenteric plexus and provided neuronal electrical and synaptic activation.

This study showed that the selective EP_2 receptor agonist AH13205 inhibited the contractile activity in control, 4- and 8-wks infected animals. Furthermore, this EP_2 receptor agonist AH13205 failed to inhibit the peristaltic motor response in the presence of EP_2 receptor antagonist AH6809 in jejunum of control, 4- and 8-wks infected animals. Thus, it is likely that the inhibitory effect of AH13205 on MCs was mediated *via* the activation of EP_2 receptor in the jejunum myenteric neurons. This conclusion is consistent with Spiller, (2002) hypothesis that intestinal inflammation induced an increase of neurotransmitters release including PGE_2. The excess amount of PGE_2 in the intestine during this period of inflammation might activate EP_2 receptor in the enteric neurons and induced inhibition of peristalsis. This explanation was also similar to the opinion of Lawrence *et al.* (1992) and Shahbazian *et al.* (2002) who suggested that the EP_2 receptors inhibited contraction through the enteric nerve plexus of guinea-pig small intestine. Interestingly, in the present model, no significant changes were observed in the magnitude of the inhibition between control tissues and those tissues from 4- and 8-wks infected animals in response to EP_2 receptor agonist which may provide a sign of similar expression of EP_2 receptor in control and infected animals during inflammation, although, a differential expression of EP_2 receptors on the GI mucosa may occur during inflammation (Takafuji *et al.*, 2000).

In the current study, the $EP_{1,2}$ receptors antagonist AH6809 inhibited the contraction in control, 4- and 8-wks infected jejunum. This inhibition was followed by contraction, which was less pronounced as compared to control before adding the drug. One explanation for this was that the antagonist works first as a blocker of EP_1 receptors involved in the local movement of the mucosa (Morimoto *et al.*, 1997) and worked as a blocker of EP_2 receptors at a second period of its effect. Thus, in this model, EP_1 and EP_2 were functionally involved in modulating peristalsis in control and inflammatory condition. Similarly, the EP_2 receptor antagonist AH6809 attenuated intestinal peristalsis in control guinea-pig ileum (Shahbazian *et al.*, 2002). In Manning *et al.*, (2002) model EP_2 receptor antagonist AH6809 attenuated PGE_2-evoked depolarization in guinea-pig colonic myenteric ganglia. This may reflect redundancy of EP_2 receptor mechanism that influences intestinal peristalsis in normal and inflammatory conditions. Indeed, the fact that the selective EP_2 receptor agonist AH13205 failed to inhibit the peristaltic motor response in the presence of EP_2 receptor antagonist AH6809 in control, 4- and 8-wks infected jejunum, implicated EP_2 receptor in modulating smooth muscle motor activity induced by luminal distension in normal and inflammatory conditions.

In conclusion, infection with *S. mansoni* disturbs PGE_2-mediated modulation of intestinal peristalsis produced by intraluminal distension. This action implies to different receptor mechanisms. EP_2 receptor agonist AH13205 is a potent inhibitor of intestinal peristalsis involved in normal and inflammatory conditions. However, because its effcet was on both interval and amplitude, it would be possible that A H13205 in this preparation acts *via* neural or/and neuromuscular pathways.

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