ELSEVIER

European Journal of Pharmacology 337 (1997) 279–282

ejp

Short communication

Effects of nematode infection on sensitivity to intestinal distension: Role of tachykinin NK_2 receptors

Peter G. McLean, Céline Picard, Rafael Garcia-Villar, Jean Moré, Jean Fioramonti, Lionel Buéno *

Department of Pharmacology–Toxicology, INRA, 180 chemin de Tournefeuille, BP3, 31931 Toulouse, France

Received 19 June 1997; revised 4 August 1997; accepted 15 August 1997

Abstract

Distension of the rat intestine causes a depressor response which is predictive of nociception. This study investigated the effects of previous infection with *Nippostrongylus (N.) brasiliensis* on the sensitivity to intestinal distension and the role of tachykinin NK_2 receptors. The tachykinin NK_2 receptor antagonist, SR48968 (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) inhibited the nociceptive response ($ED_{50} = 0.7$ mg/kg) in control rats. In post-*N. brasiliensis*-infected rats sensitivity to intestinal distension was increased which was accompanied by an increase in the apparent potency value of SR48968 ($ED_{50} = 0.1$ mg/kg). The hypersensitivity was limited to areas of hypermastocytosis. It is concluded that the post-inflammatory changes that occur in post-infected rats increase visceral sensitivity and the apparent potency of tachykinin NK_2 receptor antagonists. © 1997 Elsevier Science B.V.

Keywords: Intestinal distension; Visceral hyperalgesia; Visceral nociception; Mast cell; Tachykinin; Tachykinin NK_2 receptor

1. Introduction

In anaesthetised rats, distension of the gastrointestinal tract causes a cardiovascular depressor response which is considered to be predictive of visceral nociception (Ness and Gebhart, 1990). To date, knowledge of the pharmacology of this reflex is somewhat limited. 5-HT_3 receptors have been shown to be involved (Moss and Sanger, 1990) and the reflex is abolished in capsaicin-treated rats (Lembeck and Skofitsch, 1982). Therefore, the purpose of the current study was to extend this knowledge via an investigation into the involvement of tachykinins, specifically tachykinin NK_2 receptors. The involvement of tachykinins was chosen for investigation since they have been implicated in the transmission of somatic nociception (e.g. Seguin et al., 1995) acting via tachykinin NK_1 and NK_2 receptors. Tachykinin NK_1 and NK_2 receptors have also been implicated in visceral nociception (Julia et al., 1994).

The study also aimed to investigate whether the post-inflammatory, neuroimmune changes, that persist in rats previously infected with *Nippostrongylus (N.) brasiliensis* (Stead, 1992) affect sensitivity to intestinal distension and if so to determine whether this hypersensitivity is limited to the areas of hypermastocytosis or extended to other unaffected regions. The possible involvement of tachykinin NK_2 receptors in the hypersensitivity was also investigated.

2. Materials and methods

2.1. Surgical and experimental procedure

Control or post-*N. brasiliensis*-infected (30 days post-infection) male Wistar rats (330–400 g) were used. Infection was achieved via subcutaneous injection of 5000 third stage infective larvae of *N. brasiliensis* in 0.5 ml sterile saline inoculated into the flank of the rats. Rats were anaesthetised with pentobarbitone sodium (60 mg/kg, s.c.). The trachea was cannulated and mean systemic blood pressure was recorded via the left common carotid artery.

Jejunal (7 cm from the ligament of Treitz) or colonic (distal colon, 3 cm from the rectum) distension was performed by the rapid inflation of a 5 cm long latex balloon

* Corresponding author. Tel.: (33-5) 6128-5141; Fax: (33-5) 6128-5310.

0014-2999/97/$17.00 © 1997 Elsevier Science B.V. All rights reserved.

connected to a Statham pressure transducer (P23ID) to record intraluminal pressure. For the jejunal experiments a cut was made at one end of the selected segment of jejunum on the antimesenteric side to introduce the deflated latex balloon which was loosely secured by cotton thread. For the colonic distension experiments the deflated latex balloon was introduced into the rectum.

Pressure–response curves to jejunal or colonic distension were constructed using application of graded pressures (12.5–75 mmHg, 25 s every 5 min) and compared in control and post-infected rats.

2.2. Effects of tachykinin NK_2 receptor antagonism

Prior infection with *N. brasiliensis* was shown to cause a jejunum-specific hypersensitivity and as such the antagonist was only tested against the jejunal distension-induced depressor response to determine whether the altered jejunal sensitivity involved changes to apparent antagonist potency.

A modification of the method of Moss and Sanger (1990) was used as follows: Repeated jejunal distension (75 mmHg, 25 s every 5 min) was applied until a constant depressor response was achieved. The involvement of tachykinins was investigated via the use of the non-peptide tachykinin NK_2 receptor selective antagonist SR48968 (Emonds-Alt et al., 1992). SR48968 (0.1–10 mg/kg) or an equivalent volume of vehicle were given i.p. and the effect observed over the subsequent 30 min.

2.3. Histochemical assessment and myeloperoxidase activity

Samples of jejunum and colon were fixed in Carnoy's fluid. Slides were routinely stained by hemalun and eosin; mast cells were stained with Alcian Blue-safranin as described previously (Enerback et al., 1985).

Intestinal myeloperoxidase activity was measured to ensure that intestinal inflammation had resolved in 30 day post-infected rats. Myeloperoxidase activity was measured in intestinal samples according to the method of Bradley et al. (1982).

2.4. Data analysis

Responses were compared using Student's paired or unpaired *t*-test. The criterion for statistical significance was $P < 0.05$. The equation as described by Ness and Gebhart (1988a,b): $(\Delta\text{blood pressure})^2 = (\text{slope} \times \text{intestinal distension}) + \text{intercept}$ allowed a transformation of the graded pressure–response function. Distension thresholds were calculated from the pressure that would evoke a change in blood pressure of 10 mmHg.

The responses to jejunal distension following SR48968 administration were expressed as a percentage of the response immediately prior to dosing. The maximal effects observed over the test period were used to calculate apparent potency values expressed as ED_{50} values with 95% confidence limits (95% C.L.) all other values are represented as mean ± S.E.M.

2.5. Drugs

SR48968 (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) was dissolved in dimethyl sulfoxide and made up to volume with an equivalent volume of 0.9% saline and was kindly supplied by Dr. Emonds-Alt, Sanofi Laboratories (Montpellier).

3. Results

3.1. Histological and myeloperoxidase analysis

Inflammatory sites were not detected macroscopically or microscopically in intestinal tissue samples from control ($n = 9$) and post-infected rats ($n = 9$). However, significant hypermastocytosis was observed in the jejunum (control, 73 ± 30 mast cells/mm^2 tissue, $n = 9$; post-infected, 317 ± 109 mast cells/mm^2 tissue, $n = 9$; $P < 0.05$) but not in the colon (control, 82 ± 52 mast cells/mm^2 tissue, $n = 9$; post-infected, 129 ± 63 mast cells/mm^2 tissue; $P > 0.05$) in post-infected rats.

Myeloperoxidase activity values were not significantly different between control (jejunum, 68 ± 11 IU/mg; colon, 120 ± 55 IU/mg, $n = 9$) and post-infected (jejunum, 111 ± 31 IU/mg; colon, 144 ± 31 IU/mg, $n = 9$) rats ($P > 0.05$).

3.2. Distension studies

Jejunal or colonic distension (12.5–75 mmHg, 25 s every 5 min) elicited a pressure-dependent depressor response (Fig. 1). The depressor responses to jejunal distension were significantly greater in post-infected rats (Fig. 1A, $P < 0.05$) which lead to a 5-fold decrease in the calculated threshold pressure (22.5 mmHg, control; 4.3 mmHg, post-infected). However, the colonic distension-induced responses were not significantly affected (Fig. 1B, $P > 0.05$, threshold values: 16.8 mmHg (control) and 15.1 mmHg (post-infected).

SR48968 (0.1–10 mg/kg) produced a dose-dependent inhibition of the depressor response to jejunal distension giving rise to an ED_{50} of 0.7 mg/kg (95% C.L., 0.4–1.5, $n = 20$) in control rats (Fig. 2). In post-infected rats the apparent potency value of SR48968 was significantly greater than in control rats ($ED_{50} = 0.1$ mg/kg (95% C.L., 0.05–0.22, $n = 13$, $P < 0.05$, Fig. 2).

The administration of SR48968 did not modify the volume of air required to obtain a constant intestinal pressure of 75 mmHg in the repeated intestinal pressure

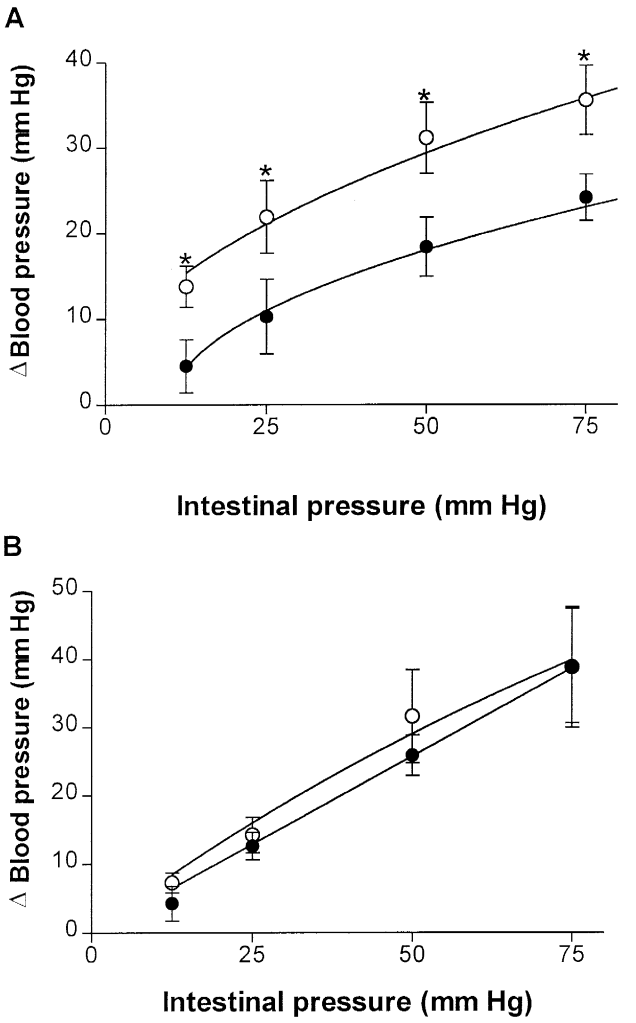

Fig. 1. Depressor response to graded (A) jejunal and (B) colonic distension (12.5–75 mmHg for 25 s) in anaesthetised rats. Responses are compared in control (●, $n = 9$ (jejunum), $n = 5$ (colon) or post-infection with *N. brasiliensis* (○, $n = 9$ (jejunum), $n = 5$ (colon). Results are expressed as means ± S.E.M. $^{*}P < 0.05$ post-infected vs. control rats (Student's *t*-test).

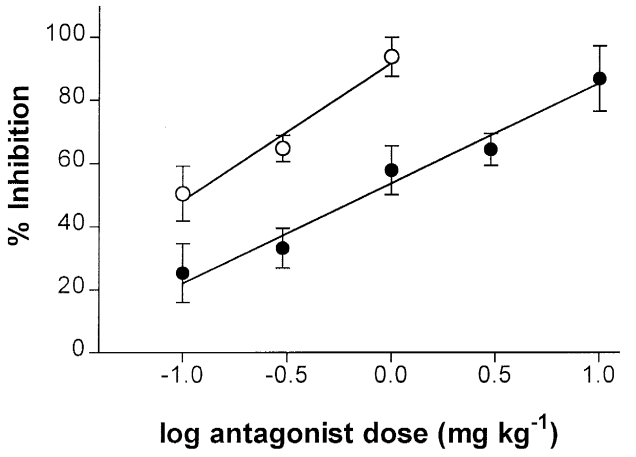

Fig. 2. Regression analysis of the effect of SR48968 (0.1–10 mg/kg) on the depressor response induced by jejunal distension (75 mmHg, 25 s every 5 min) in control (●) or post-infected (○) anaesthetised rats. Each point represents the mean ± S.E.M. of at least 4 data points.

experiments nor did infection modify the relationship between volume and intestinal pressure in the graded intestinal pressure experiments. This indicates that intestinal compliance was not affected.

4. Discussion

This study has shown that the chronic changes which occur in rat jejunum post-infection with *N. brasiliensis*, at a time when intestinal inflammation, as assessed by myeloperoxidase activity, has resolved cause increased sensitivity to distension an effect which was limited to the areas of hypermastocytosis. The data obtained with SR48968 show, for the first time, that tachykinin NK_2 receptor antagonists, inhibit nociception in response to noxious jejunal distension in anaesthetised rats, an effect which is possibly related to tachykinin NK_2 receptor antagonism and that changes to tachykinin NK_2 receptor-mediated nociception are possibly involved in the visceral hyperalgesia observed following intestinal infection.

The tachykinin NK_2 receptor antagonist SR48968 (Emonds-Alt et al., 1992), without significantly affecting resting blood pressure, inhibited the jejunal distension-induced depressor response in a dose-dependent fashion. These data are in line with previous studies in which tachykinin NK_2 receptor antagonists have been shown to have antinociceptive properties in various animal models of somatic pain (Santucci et al., 1993; Seguin et al., 1995). Tachykinin NK_2 receptor antagonists have also been shown to inhibit visceral nociception in rats (Julia et al., 1994). Further, the apparent in vivo potency of SR48968 determined in the present study is similar to other in vivo reports (Santucci et al., 1993; Poncelet et al., 1993).

The observation that the hypersensitivity to distension was limited to the areas of hypermastocytosis (i.e. the jejunum) suggests that mast cell activation is involved in the post-infection hypersensitivity, however further studies are required to fully investigate the involvement of mast cells. The hypersensitivity can not be attributed to acute inflammatory changes as myeloperoxidase activity, a marker of intestinal inflammation, had returned to control levels in 30 day post-infected rats. The fact that the hypersensitivity was observed at both noxious and innocuous pressures suggests also that the pressures used are relevant to pathophysiological conditions. However, one potential disadvantage of the present study is that only jejunal hypersensitivity was observed which is arguably not directly relevant to irritable bowel syndrome, a disorder which predominantly affects the colon, but jejunal hypersensitivity is observed in irritable bowel syndrome (Evans et al., 1996) and as such the mechanisms involved in jejunal hyperalgesia probably relate to the generalised hyperalgesia seen in irritable bowel syndrome.

The post-inflammatory changes which occur in rat jejunum at day 30 post-infection with *N. brasiliensis* have

previously been shown to include an increase in mucosal mast cell number and substance P levels, nerve re-modeling and a shift in the enteric nervous system away from cholinergic to tachykininergic regulation (Stead, 1992; Masson et al., 1996). The change in substance P levels possibly explains the altered apparent antagonist potency observed in the present study but is also possibly due to changes in tachykinin NK_2 receptor number following infection or a combination of both factors. Further studies are required to fully explore these possibilities, however, it is unlikely that the affinity of the antagonist for the tachykinin NK_2 receptor is affected. The increase in mast cells putatively parallels the pathophysiology of irritable bowel syndrome, as suggested recently (Weston et al., 1993; Pang et al., 1996). However, mastocytosis also occurs in other conditions in which visceral hyperalgesia is observed such as infection and inflammatory bowel disorders (Mayer and Raybould, 1993). Nevertheless, as mast cells are in intimate contact with peptidergic nerves in the gut (Stead et al., 1989) changes in the relationship between mast cells and enteric neurons may affect the sensitivity of visceral afferents and be responsible for the change in sensitivity observed in the present study.

In summary, the apparent link observed in this study between mast cell number, apparent tachykinin NK_2 receptor antagonist potency and visceral sensitivity suggests that hypermastocytocis and/or changes in tachykinin neurotransmission, in the absence of inflammation, may be partly responsible for visceral hypersensitivity.

References

Bradley, P.P., Priebat, D.A., Christensen, R.D., Rothstein, G., 1982. Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. J. Invest. Dermatol. 78, 206–209.

Emonds-Alt, X., Vilain, P., Goulaouic, P., Proietto, V., Van broeck, D., Advenier, C., Naline, E., Neliat, G., Le fur, G., Breliere, J.C., 1992. A potent and selective non-peptide antagonist of the neurokinin A (NK_2) receptor. Life Sci. 50, PL101–106.

Enerback, L., Kolset, S.O., Kusche, M., Hjerpe, A., Lindahl, U., 1985. Glycosaminoglycans in rat mucosal mast cells. Biochem. J. 227, 661–668.

Evans, P.R., Bennett, E.J., Bak, Y.T., Tennant, C.C., Kellow, J.E., 1996. Jejunal sensorimotor dysfunction in irritable bowel syndrome: Clinical and psychosocial features. Gastroenterology 110, 393–404.

Julia, V., Morteau, O., Bueno, L., 1994. Involvement of neurokinin 1 and 2 receptors in viscerosensitive response to rectal distension in rats. Gastroenterology 107, 94–102.

Lembeck, F., Skofitsch, G., 1982. Visceral pain reflex after pretreatment with capsaicin and morphine. Naunyn-Schmiedeberg's Arch. Pharmacol. 321, 116–122.

Masson, S.D., McKay, D.M., Stead, R.H., Agro, A., Stanisz, A., Perdue, M.H., 1996. *Nippostrongylus brasiliensis* infection evokes neuronal abnormalities and alterations in neurally regulated electrolyte transport in rat jejunum. Parasitology 113, 173–182.

Mayer, E.A., Raybould, H.E. (Eds.), 1993. Basic and Clinical Aspects of Chronic Abdominal Pain. Elsevier, New York, NY.

Moss, H.E., Sanger, G.J., 1990. The effects of granisetron, ICS 205-930 and ondansetron on the visceral pain reflex induced by duodenal distension. Br. J. Pharmacol. 100, 497–501.

Ness, T.J., Gebhart, G.F., 1988a. Colorectal distension as a noxious visceral stimulus: Physiologic and pharmacologic characterization of pseudoaffective reflexes in the rat. Brain Res. 450, 153–169.

Ness, T.J., Gebhart, G.F., 1988b. Visceral pain: A review of experimental studies. Pain 41, 167–234.

Pang, X., Boucher, W., Triadafilopoulos, G., Sant, G.R., Theoharides, T.C., 1996. Mast cell and substance P-positive nerve involvement in a patient with both irritable bowel syndrome and interstitial cystitis. Urology 47, 436–438.

Poncelet, M., Gueudet, C., Emonds-Alt, X., Breliere, J.C., Le Fur, G., Soubrie, P., 1993. Turning behavior induced in mice by a neurokinin A receptor agonist: Stereoselective blockade by SR 48968, a non-peptide receptor antagonist. Neurosci. Lett. 149, 40–42.

Santucci, V., Gueudet, C., Edmonds-Alt, X., Breliere, J.C., Soubrie, P., Le Fur, G., 1993. The NK2 receptor antagonist SR 48968 inhibits thalamic responses evoked by thermal but not mechanical nociception. Eur. J. Pharmacol. 237, 143–146.

Seguin, L., Le Marouille-Girardon, S., Millan, M.J., 1995. Antinociceptive profiles of non-peptidergic neurokinin1 and neurokinin2 receptor antagonists: A comparison to other classes of antinociceptive agent. Pain 61, 325–343.

Stead, R.H., 1992. Nerve remodelling during intestinal inflammation. Ann. N.Y. Acad. Sci. 664, 443–455.

Stead, R.H., Dixon, M.F., Bramwell, N.H., Riddell, R.H., Bienenstock, J., 1989. Mast cells are closely apposed to nerves in the human gastrointestinal mucosa. Gastroenterology 97, 575–585.

Weston, A.P., Biddle, W.L., Bhatia, P.S., Miner Jr., P.B., 1993. Terminal ileal mucosal mast cells in irritable bowel syndrome. Dig. Dis. Sci. 38, 1590–1595.