Vasoactive intestinal peptide is involved in the inhibitory effect of interleukin-1\(\beta\) on the jejunal contractile response induced by acetylcholine

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**SUMMARY**

Although previous studies have shown that interleukin-1\(\beta\) (IL-1\(\beta\)) decreases acetylcholine (ACh)-induced intestinal contraction by an action on the enteric nervous system, the neuromediator(s) involved are still unknown.

**Aim** — To determine the role of nitric oxide (NO), vasoactive intestinal peptide (VIP) and/or adenosine triphosphate (ATP) in mediating this inhibitory effect.

**Methods** — The effects of NO synthase inhibitors, VIP and ATP antagonists on motor response to the ACh were investigated before and after 90-min exposure of a rat preparation of jejunal longitudinal muscle-myenteric plexus to IL-1\(\beta\). NG-nitro-L-arginine methyl ester, No-nitro-L-arginine and No-monomethyl-L-arginine were used to inhibit NO synthase, VIP (10-28) and \[D-p-Cl-Phe6, Leu17\] VIP to block VIP receptors, and suramin to block ATP receptors.

**Results** — NO synthase inhibitors failed to block the inhibitory effect of IL-1\(\beta\) on ACh-contracted jejunum smooth muscle. Suramin also failed to affect IL-1\(\beta\)-induced inhibition, whereas VIP antagonists abolished it. Moreover, the action of IL-1\(\beta\) was partly reproduced by VIP.

**Conclusions** — While neither NO nor ATP accounts for the inhibitory effect of IL-1\(\beta\) on ACh-contracted jejunum, VIP seems to be a key-mediator of this effect.

Key words: Interleukin-1\(\beta\), Intestinal motility, Vasoactive intestinal peptide. Rat. In vitro.

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Among the possible candidates, nitric oxide (NO) is a major inhibitory neurotransmitter of the non-adrenergic non-cholinergic (NANC) nerves of the myenteric plexus which induce relaxation of intestinal muscle [5-8]. The IL-1\(\beta\) relaxing effect on vascular contraction in the rat is blocked by a potent inhibitor of NO synthase [9]. Moreover, IL-1\(\beta\) up-regulates inducible NO synthase in rat intestinal myenteric neurons, which suggests that this enzyme could be the intermediate protein involved in the IL-1\(\beta\) motor effect [4, 10]. No-nitro-L-arginine (L-NNA), No-nitro-L-arginine-methylster (L-NNAME) and No-monomethyl-L-arginine (L-NMMA) are analogues of L-arginine, which is the specific substrate used by NO synthase to produce NO. These L-arginine analogues are used as NO synthase inhibitors [11]. The efficiency of these compounds differs and their rank order of potency can vary between tissues [12-15]. In the cat colon, L-NNA caused greater inhibition of off-contractions evoked by electrical field stimulation compared with L-NNAME or L-NMMA. In vascular tissues, L-NNA is considerably less potent than the other NO synthase inhibitors [14]. Since the efficiency of those

[^1]: Interleukin 1\(\beta\) (IL-1\(\beta\)) is one of the key mediators involved in inflammatory reactions. A significant increase of IL-1\(\beta\) has been found in the distal colonic mucosa of rats with experimentally induced colitis and in the intestine of patients with Crohn’s disease [1-3]. Intestinal inflammation in humans and animals is accompanied by motility changes. IL-1\(\beta\) might be involved in these alterations since colonic motor abnormalities observed in experimental models of colitis were reduced by preliminary administration of IL-1 receptor antagonist [2]. We have previously shown in vitro that IL-1\(\beta\) decreased ACh-induced contraction of rat jejunum [4]. This effect was abolished by tetrodotoxin and cycloheximide treatment, suggesting the involvement of the enteric nervous system and the role of a new synthesized protein. However, the neurotransmitters potentially involved could not be identified.

[^2]: An anti-inflammatory action of VIP has been shown in the distal colonic mucosa of rats with inflammatory bowel disease [16]. This effect is calcium-dependent and results from the activation of voltage-dependent Ca\(^{2+}\)-channels on intestinal smooth muscle [16]. VIP is also a potent vasodilator and has been shown to be released in inflammatory reactions. A significant increase of IL-1\(\beta\) [10] and IL-6 [17] has been found in the distal colonic mucosa of rats with experimental colitis. The administration of VIP reduces the severity of experimental colitis in rats [18]. The full text of this article is available in English, free of charge, on the web on: www.e2med.com/gcb

[^3]: The structural formula of the VIP antagonist is shown in the figure.
inhibitors varies in a same tissue, we decided to test three NO synthase inhibitors (L-NNA, L-NAME and L-NMMA) on the IL-1β jejunal effect.

Vasoactive intestinal polypeptide (VIP), a 28 amino-acid peptide, is another NANC inhibitory mediator of the enteric nervous system [16-19]. In almost all gastrointestinal smooth muscle preparations, VIP displays a relaxing effect through direct action on smooth muscle [18, 19]. However, in the guinea-pig ileum, neurogenic contractions have been elicited by VIP [20].

Though NO and VIP are generally considered to be the main inhibitory neurotransmitters acting on the gut, adenosine 5′-triphosphate (ATP) also participates in NANC neurotransmission [21]. Previous studies have indicated that ATP induces relaxation in certain tissues such as the duodenum, jejunum and colon [18, 22, 23].

The purpose of this study was to investigate the mechanism of IL-1β inhibition on intestinal contraction and more specifically the role of NO, VIP and/or ATP as potential neurotransmitters in this effect.

Material and methods

Study protocol

ANIMALS AND APPARATUS

Male Wistar rats (250-300 g) were sacrificed by cervical dislocation. One to 1.5 cm long segments of jejunum were rapidly removed, opened along the mesenteric border, and cleared of their intraluminal contents in a Krebs-bicarbonate solution (pH 7.4) composed of (mmol/L) 128 NaCl, 4.5 KCl, 2.5 CaCl2, 1.18 MgSO4, 1.18 KH2PO4, 125 NaHCO3 and 5.55 D-glucose. Longitudinal muscle-myenteric plexus (LM-MP) was peeled from the underlying circular muscle. These preparations were then suspended under 1 g of tension in a 10-mL organ bath containing continuously oxygenated (5% CO2, 95% O2) Krebs-bicarbonate solution. The preparations were then allowed to equilibrate for 60 min. The isometric longitudinal mechanical activity of the segments was recorded using a force-transducer (Basile No. 7005, Comerio, VA, Italy), as previously described [24]. At the beginning of each experiment, acetylcholine chloride (ACh, 10–6 M) was applied and served as a reference measure of maximal contraction. The viability of each preparation was checked at the end of each experiment by reassessment of spontaneous mechanical activity and the response to 10–5 M ACh. Results were expressed as tension (g) and normalized for cross-sectional area (CS), which was determined using the following equation [25]:

\[
CS (\text{mm}^2) = \frac{\text{tissue wet weight (mg)}}{\text{tissue length (mm)} \times \text{density (mg/mm}^2\text{)}}.
\]

A density of 1.05 was used according to Vermillion et al. [25].

EXPERIMENTAL DESIGN

In a first set of experiments, the effect of IL-1β was studied on ACh-induced contraction. A dose-response curve to ACh (10–9 to 10–4 M) was obtained in a cumulative manner after 90-min incubation with or without IL-1β (10 ng/mL). To exclude any possible effect caused by endotoxin contamination, IL-1β (10 ng/mL) was boiled for 20 min before being added to the tissue. In a second set of experiments, the effect of potential inhibitory agents on LM-MP jejunal motor response to ACh (10–5 M) was investigated after 90-min exposure to IL-1β (10 ng/mL) or saline. The following drugs were used: L-NNA (3 × 10–5 M), L-NAME (3 × 10–4 M) and L-NMMA (3 × 10–4 M). Nω-substituted L-arginine analogues to inhibit NO synthase, VIP (10–8) (10–5 M), an α carbon sequence of VIP and [D-p-Cl-Phe6, Leu17] VIP (10–5 M), a substituted analogue of VIP as VIP receptor antagonists, and suramin (3 × 10–4 M), a P2-purinoceptor antagonist, to block ATP effect. NO synthase inhibitors and antagonist concentrations and time points were chosen on the basis of preliminary studies. L-NNA, L-NAME or L-NMMA was applied for 15 min before IL-1β or saline, and VIP antagonists and suramin were added to the bath 5 min before application of IL-1β or saline. In a third set of experiments, when ACh (10–5 M)-induced contraction had reached a plateau, VIP (10–6 M) was added to the bath. Stock solutions of VIP, VIP (10–28) and [D-p-Cl-Phe6, Leu17] VIP were prepared in advance and stored frozen (-80°C). In control experiments, a volume of saline solution equal to the volume of the agents used was added to the bath. All tested substances were administered in a volume which did not exceed 1% of the total bath volume.

Data analysis

All data are presented as means ± SEM. Significance among groups was tested by one-way analysis of variance (ANOVA). A P value < 0.05 was considered statistically significant.

Chemicals

Drugs and chemicals were purchased from Sigma Chemical Co (L’Isle-d’Abeau-Chesnes, Saint-Quentin-Fallavier Cedex, France). Human recombinant IL-1β was obtained from Pepro Tech (Le Perray-en-Yvelines, Paris, France).

Results

As expected, exposure of the jejunal preparation to IL-1β (10 ng/mL) for 90 min resulted in a significant (P < 0.05, n = 8) reduction of ACh (10–7 M)-induced contraction. Inhibition after 90 min with 10 ng/mL was 35.9 ± 3%. The dose-response curve to ACh obtained after 90-min exposure to IL-1β (figure 1) shows that inhibition was significant at a dose of 10–6 M ACh up to the maximal dose of 10–3 M ACh. Preincubation of LM-MP preparations with 10 ng/mL of boiled IL-1β did not affect ACh (10–5 M)-induced jejunal response (data not shown).

The NO synthase inhibitors L-NNA, L-NAME and L-NMMA (3 × 10–4 M) did not prevent the reduction of ACh (10–5 M)-induced jejunal contraction caused by IL-1β (10 ng/mL) but significantly (P < 0.05, n = 6) increased the ACh (10–5 M) response of smooth muscle when given alone (figure 2).

The P2x-purinoceptor antagonist, suramin (3 × 10–4 M), failed to affect the inhibition induced by IL-1β (10 ng/mL (P > 0.05, n = 6)) and did not modify the contractile effect of ACh (10–7 M) on rat jejunal LM-MP preparations when given alone (P = 0.83, n = 6) (figure 3).

Blockade of VIP receptors by VIP (10–8) (10–5 M) and [D-p-Cl-Phe6, Leu17] VIP (10–5 M) suppressed the inhibition of ACh (10–5 M)-induced jejunal contraction caused by IL-1β (10 ng/mL) (figure 4). In contrast, VIP antagonists given alone did not alter ACh-induced jejunal contraction (P = 0.91, n = 6 and P = 0.86, n = 6 respectively) (figure 4). Moreover, VIP (10–5 M) caused a significant decrease (P < 0.05, n = 4) of ACh (10–5 M)-induced jejunal contraction in rat jejunal LM-MP preparations, partially reproducing the effect of IL-1β (10 ng/mL) (figures 5 and 6).
Fig. 1 – Dose-response curves of rat jejunal longitudinal muscle-myenteric plexus preparations to acetylcholine (ACh) alone and after 90-min incubation with interleukin-1β (IL-1β, 10 ng/mL). Each point represents the mean (± SEM) of values obtained from 6 rats. *P < 0.05 versus ACh alone.

Courbe dose-réponse à l’acétylcholine de préparations de plexus myentérique-muscle longitudinal de jéjunum de rat après 90 minutes d’incubation en présence ou non d’interleukine-1β (10 ng/mL). Chaque point représente la moyenne (± ESM) des valeurs obtenues à partir de 6 rats. *P < 0.05 versus ACh seul.

Fig. 2 – Lack of effect of N G -nitro-L-arginine (L-NNA, 3 × 10⁻⁴ M), N G -nitro-L-arginine methyl ester (L-NAME, 3 × 10⁻⁴ M) and N G -monomethyl-L-arginine (L-NMMA, 3 × 10⁻⁴ M) on the inhibitory effect of interleukin-1β (IL-1β, 10 ng/mL) for 90 min on 10⁻⁵ M acetylcholine (ACh)-induced contractile response on rat jejunal longitudinal muscle-myenteric plexus preparations. Each column represents the mean (± SEM) of values obtained from 6 rats. *P < 0.05 versus ACh alone. # P < 0.05 versus ACh alone from control.

Les inhibiteurs de la NO synthase: N G -nitro-L-arginine (L-NNA, 3 × 10⁻⁴ M), N G -nitro-L-arginine methyl ester (L-NAME, 3 × 10⁻⁴ M) et N G -monomethyl-L-arginine (L-NMMA, 3 × 10⁻⁴ M) sont sans effet sur la réponse contractile à l’acétylcholine (10⁻⁵ M) de préparations de plexus myentérique-muscle longitudinal de jéjunum de rat après 90 minutes d’incubation avec l’interleukine-1β (10 ng/mL). Chaque colonne représente la moyenne (± ESM) des valeurs obtenues à partir de 6 rats. *P < 0.05 versus ACh seul. # P < 0.05 versus ACh seul du groupe « Control ».

Fig. 3 – Lack of effect of suramin (3 × 10⁻⁴ M), an ATP antagonist, on the inhibitory effect of interleukin-1β (IL-1β, 10 ng/mL) for 90 min on the 10⁻⁵ M acetylcholine (ACh)-induced contractile response on rat jejunal longitudinal muscle-myenteric plexus preparations. Each column represents the mean (± SEM) of values obtained from 6 rats. *P < 0.05 versus ACh alone.

La suramine (3 × 10⁻⁴ M), antagoniste des récepteurs de l’ATP, est sans effet sur la réponse contractile à l’acétylcholine (10⁻⁵ M) de préparations de plexus myentérique-muscle longitudinal de jéjunum de rat après 90 minutes d’incubation avec l’interleukine-1β (10 ng/mL). Chaque colonne représente la moyenne (± ESM) des valeurs obtenues à partir de 6 rats. *P < 0.05 versus ACh seul.

Fig. 4 – Suppression by the vasoactive intestinal peptide (VIP) antagonists, VIP (10-28) 10⁻⁵ M and [D-p-Cl-Phe⁶, Leu¹⁷] VIP 10⁻⁵ M, of the inhibitory effect of interleukin-1β (10 ng/mL) for 90 min on the 10⁻⁵ M acetylcholine (ACh)-induced contractile response on rat jejunal longitudinal muscle-myenteric plexus preparations. Each column represents the mean (± SEM) of values obtained from 6 rats. *P < 0.05 versus ACh alone.

Effet de deux antagonistes des récepteurs du VIP: le VIP (10-28) 10⁻⁵ M et le [D-p-Cl-Phe⁶, Leu¹⁷] VIP 10⁻⁵ M, sur la réponse contractile de préparations de plexus myentérique-muscle longitudinal de jéjunum de rat induite par l’acétylcholine (10⁻⁵ M) après 90 minutes d’incubation avec l’interleukine-1β (10 ng/mL). Chaque colonne représente la moyenne (± ESM) des valeurs obtenues à partir de 6 rats. *P < 0.05 versus ACh seul.
The results of this investigation show that VIP antagonists abolished the decreasing effect of IL-1β on ACh-induced contraction of rat jejunal LM-MP preparations, whereas VIP partly reproduced it. In contrast, neither NO synthase inhibitors nor a P2-purinoceptor ATP antagonist did affect the inhibitory action of IL-1β.

The inhibitory effect of IL-1β observed in our experimental conditions was not due to contamination by endotoxin (e.g. lipopolysaccharides) since it was heat-sensitive [26]. The ability of IL-1β to decrease ACh-induced jejunal contraction corroborates previous reports showing that IL-1β inhibits the in vitro motility of gastric, ileal and other smooth muscles, including vascular and airway ones [9, 27-29].

In some tissues, NO synthase may be the intermediate protein involved in the IL-1β effect [4]. Beasley et al. found that the relaxation of vascular contraction induced by IL-1β was blocked by an NO synthase inhibitor [9]. In our study, however, application of three competitive inhibitors of L-arginine, namely L-NNA, L-NAME or L-NMMA, failed to affect the action of IL-1β on jejunal contraction. Our findings are consistent with other observations in which NO synthase blockade produced no change in the IL-1β-induced potentiation of ileal electrofield transmural stimulation-evoked release of [H3]ACh or IL-1β-induced relaxation of the proximal stomach [27, 30]. It is noteworthy that pretreatment with L-NNA, L-NAME or L-NMMA alone increased ACh-induced contractile response similarly and significantly, suggesting that NO synthase inhibition was effective with all three substances. These results are consistent with an earlier finding showing that NO synthase inhibition, through blockade of NO synthesis, enhanced the carbachol induced contraction of rat ileal smooth muscle [31]. Moreover, the
behavior of L-NNA, L-NAME or L-NMMA in our study was similar qualitatively and quantitatively to that reported in previous studies of gastrointestinal smooth muscle [32-34].

ATP mediation of the inhibitory effect of IL-1β was considered possible since ATP is one of the three main inhibitory neurotransmitters of the enteric nervous system [35-36]. It has also been shown that ATP plays a role as a relaxant agent in rat jejunum [18]. However, in our study, suramin, an ATP antagonist, failed to modify the IL-1β-induced decrease in contractile response to ACh, which suggests that ATP is not involved in this effect. In addition, suramin had no effect on the magnitude of ACh-induced intestinal contraction as it was previously reported in guinea-pig intestine [37].

The fact that the inhibitory effect of IL-1β on intestinal motility was abolished by prior administration of the VIP antagonists VIP (10-28) and [D-p-Cl Phe6, Leu7] VIP supports the role of VIP mediation. Moreover, exogenous VIP applied to jejunal LM-MP preparations decreased ACh-induced contraction, thus partially mimicking the VIP effect and treatments with VIP antagonists abolished the IL-1β inhibitory effect on jejunal longitudinal smooth muscle in rat [18, 38]. In a previous work, we found that the effect of IL-1β requires the synthesis of an intermediate protein and is mediated through the enteric nervous system [4]. VIP, which is one of the major NANC inhibitory enteric neurotransmitters, could be that intermediate protein. Indeed, IL-1β regulates VIP synthesis in chromaffin cells from bovine adrenal medulla and increases VIP expression in rat superior cervical ganglion [39, 40]. Alternatively, IL-1β increase may up regulates the expression of VIP receptors at the membrane of smooth muscle cells, thus producing a larger relaxing effect in response to the VIP released in their vicinity. However, IL-1β could have induced VIP release in LM-MP preparations through action on another cell type. For instance, macrophage-like cells have been proposed as cellular mediators of the IL-1β effect on sympathetic neurotransmission in the rat jejunum myenteric plexus [41].

In our study, VIP partially reproduced the IL-1β inhibitory effect and treatments with VIP antagonists abolished the IL-1β effect, which strongly supports the notion that another member of the NANC member of VIP family, i.e. putative adenylate cyclase activating peptide (PACAP) may also be involved [42, 43]. Indeed, IL-1β induced PACAP expression in neurons of rat hypothalamus-pituitary-adrenocortical axis [44].

While previous in vitro studies have shown that ATP, NO and VIP can mediate NANC relaxation in longitudinal jejunal muscle in rat, our study indicates that only VIP seems to be involved in the inhibitory effect of IL-1β on ACh (10-5 M)-induced contraction in the rat jejunum [18, 45]. This result is not unexpected since NO has been shown not to be involved in the IL-1β effect on fundic contractile activity although it is considered as NANC mediator in the rat fundus [27]. Our results do not exclude that NO and ATP may be involved in the effect of IL-1β at other intestinal sites. Indeed, NO and ATP but not VIP were shown to be the mediators of inhibitory NANC neurotransmission in longitudinal muscle of the rat ileum and colon [23, 46]. Finally, although Fargeas et al. have reported that the effect of IL-1β on intestinal motility is mainly attributable to a central action, our results indicate that a peripheral action may also contribute to the intestinal motor alterations observed in some pathological conditions such as inflammatory bowel diseases [47].

In conclusion, our results suggest that IL-1β could inhibit the ACh-induced-contractile response of rat jejunum through a mechanism involving VIP mediation but independent of NO and ATP mediation.


