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Effects of cannabinoid receptor-2 activation on accelerated gastrointestinal transit in lipopolysaccharide-treated rats

[1,3]Ronald Mathison, [1,2,3]Winnie Ho, [2,3]Quentin J. Pittman, [1,2]Joseph S. Davison & *,[1,2,3]Keith A. Sharkey

[1]Gastrointestinal Research Group, Department of Physiology and Biophysics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada; [2]Neuroscience Research Group, Department of Physiology and Biophysics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada and [3]Mucosal Inflammation Research Groups, Department of Physiology and Biophysics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada

1 The biological effects of cannabinoids (CB) are mediated by CB_1 and CB_2 receptors. The role of CB_2 receptors in the gastrointestinal tract is uncertain. In this study, we examined whether CB_2 receptor activation is involved in the regulation of gastrointestinal transit in rats.

2 Basal and lipopolysaccharide (LPS)-stimulated gastrointestinal transit was measured after instillation of an Evans blue-gum Arabic suspension into the stomach, in the presence of specific CB_1 and CB_2 agonists and antagonists, or after treatment with inhibitors of mediators implicated in the transit process.

3 In control rats a CB_1 (ACEA; $1\,mg\,kg^{-1}$), but not a CB_2 (JWH-133; $1\,mg\,kg^{-1}$), receptor agonist inhibited basal gastrointestinal transit. The effects of the CB_1 agonist were reversed by the CB_1 antagonist AM-251, which alone increased basal transit. LPS treatment increased gastrointestinal transit. This increased transit was reduced to control values by the CB_2, but not the CB_1, agonist. This inhibition by the CB_2 agonist was dose dependent and prevented by a selective CB_2 antagonist (AM-630; $1\,mg\,kg^{-1}$).

4 By evaluating the inhibition of LPS-enhanced gastrointestinal transit by different antagonists, the effects of the CB_2 agonist (JWH-133; $1\,mg\,kg^{-1}$) were found to act *via* cyclooxygenase, and to act independently of inducible nitric oxide synthase (NOS) and platelet-activating factor. Interleukin-1β and constitutive NOS isoforms may be involved in the accelerated LPS transit.

5 The activation of CB_2 receptors in response to LPS is a mechanism for the re-establishment of normal gastrointestinal transit after an inflammatory stimulus.
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Abbreviations: CB_1, cannabinoid receptor-1; CB_2, cannabinoid receptor-2; IL, interleukin; LPS, lipopolysaccharide; NO, nitric oxide; NOS, nitric oxide synthase; cNOS, constitutive NOS; eNOS, endothelial NOS; iNOS, inducible NOS; nNOS, neuronal NOS; PAF, platelet activating factor

Introduction

Cannabinoid research has evolved rapidly over the last decade with the development of specific cannabinoid receptor ligands (Herkenham *et al*., 1990; Palmer *et al*., 2002; Pertwee & Ross, 2002), the identification of endogenous cannabinoids (Devane *et al*., 1992), and the isolation of a cannabinoid receptor from brain (Matsuda *et al*., 1990) and another one from the spleen (Munro *et al*., 1993). It is generally accepted that cannabinoid-1 (CB_1) receptors exist primarily on central and peripheral neurons, their major function being to modulate neurotransmitter release, whereas the cannabinoid-2 (CB_2) receptors are found mainly on immune cells (Jeon *et al*., 1996; Pertwee & Ross, 2002).

Cannabinoids were first demonstrated to inhibit contractions of the rat small intestine (Rosell *et al*., 1976), and this was confirmed later when delta 9-tetrahydrocannabinol was found to reduce the frequency of intestinal contractions and the transit of food in the small intestine, without altering basal tone (Shook & Burks, 1989). Specificity for cannabinoid action on the intestine was established with the identification of CB_1 receptors in the guinea-pig intestine (Pertwee *et al*., 1996), and the selective antagonism of CB_1 inhibition of gastrointestinal motility, propulsion and transit in mice (Pinto *et al*., 2002). CB_1 receptor agonists decrease, whereas antagonists increase, intestinal secretion, fluid accumulation and defecation (Izzo *et al*., 1999a; Tyler *et al*., 2000; MacNaughton *et al*., 2004). CB_1 receptors are distributed widely on fibers of the myenteric plexus in several species (Coutts *et al*., 2002), where the inhibitory effects of CB_1 agonists occur mainly through prejunctional inhibition of acetylcholine release (Lopez-Redondo *et al*., 1997). CB_2 receptors have been found predominately in the peripheral immune system and dorsal root ganglion cells (Ross *et al*., 2001; Pertwee & Ross, 2002), and are believed to participate in the modulation of local

*Author for correspondence at: Department of Physiology and Biophysics, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 4N1; E-mail: ksharkey@ucalgary.ca
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thermal nocioception (Malan *et al*., 2001), nerve growth factor-dependent hyperalgesia (Farquhar-Smith *et al*., 2002) and nerve growth factor-induced mast cell granulation and neutrophil migration (Rice *et al*., 2002). However, mRNA for the CB_2 receptor has also been isolated from rat fundus (Storr *et al*., 2002) and guinea-pig whole gut preparations (Griffin *et al*., 1997), and several studies point to a functional CB_2 receptor in the gastrointestinal tract. A CB_2 agonist inhibits defecation in mice (Hanes *et al*., 1999), and a CB_2 antagonist increases nerve stimulation-elicited relaxation of the rat fundus (Storr *et al*., 2002). Nonetheless, the functional and mechanistic features of the CB_2 receptors in the gastrointestinal tract remain poorly characterized.

These observations led us to explore a potential function and mechanism of action of CB_2 receptors in the modulation of intestinal motility in the rat gastrointestinal tract. In this study, we demonstrate a functional activity in preventing the increase in gastrointestinal transit elicited by exposure to the inflammatory stimulus lipopolysaccharide (LPS), through mechanisms possibly involving nitric oxide synthase (NOS), cyclooxygenase metabolites and interleukin (IL)-1β.

Methods

Animals

In all, 200 male Sprague–Dawley rats, weighing 180–220 g, were purchased from Charles River Laboratory (Montreal, PQ, Canada). They were maintained with lights on from 7:00 to 19:00 h, and provided food and water *ad libitum*. All experiments were carried out in accordance with the Canadian Council on Animal Care guidelines, and received prior approval from the University of Calgary Animal Care Committee.

Gastrointestinal transit

Gastrointestinal transit was evaluated by gavage feeding of a nonabsorbable marker (0.2 ml of 5% Evans blue (Fisher Scientific, Fair Lawn, NJ, U.S.A.) and 5% gum Arabic (Sigma-Aldrich, St Louis, MO, U.S.A.) in 0.9% saline with an orogastric tube to 18 h fasted rats, lightly anesthetized with halothane. Rats recovered in 1–2 min and were returned to their home cages. After 30 min, the rats were killed by cervical dislocation, and at laparotomy the small intestine from the gastric pylorus to the caecum was carefully removed. Gastrointestinal transit was expressed as a percentage of the distance from the oral end of the intestine to the leading front of colored distal sites, relative to the total length of the intestine.

Drug treatment protocols

Basal transit The effects of cannabinoid agonists and antagonists on basal gastrointestinal transit were determined by treating an animal with a drug at 0 min, administration of the transit marker at 10 min and measuring transit at 40 min, after allowing gastrointestinal transit to proceed for 30 min. A different protocol was used to evaluate the effects of LPS on gastrointestinal transit.

LPS-stimulated transit Previously, we established that 65 $\mu g\,kg^{-1}$ of intravenously administered LPS maximally perturbed myoelectric activity of intestinal longitudinal muscle for approximately 2 h (Tan *et al*., 2000). To avoid the anesthetic procedures required for intravenous administration of LPS, we gave the LPS intraperitoneally at 65 $\mu g\,kg^{-1}$ of LPS. In preliminary experiments, gastrointestinal transit was evaluated at 30 min, 120 min and 19 h after injection of LPS. Optimal enhancement of transit was seen at 120 min, and this time was used for subsequent experiments. To evaluate the contribution of cannabinoids, the following protocol was used: LPS was injected at 0 min, a drug (cannabinoids and/or antagonists of NOS, PAF, cyclooxygenase and IL-1β) was added at 80 min, the transit marker was given at 90 min and transit was measured at 120 min. This protocol was used to allow time for the inflammatory actions of LPS, and permitted evaluation of the effects of cannabinoid drugs on gastrointestinal transit rather than the development of the inflammation. The interactions between CB_2 receptor activation and neurotransmitter or autocoid inhibition were also examined by treating the animals with JWH-133 at 70 min after LPS injection. Two exceptions to this protocol occurred. Indomethacin was given concurrently with LPS injection at 0 min, and the IL-1β receptor antagonist Anakinra was administered 60 min before LPS. All drugs, other than LPS, were administered subcutaneously.

Drugs

The drug doses used were identified from the literature (cited below) as those providing significant inhibitory effects, but minimal nonspecific actions. The following compounds were obtained from Tocris (Ellisville, MO, U.S.A.): CB_1 agonist – arachidonyl-2¢-chloroethylamide (ACEA; Hillard *et al*., 1999); CB_1 antagonist – *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM-251; Gatley *et al*., 1996); CB_2 agonist – (6a*R*,10a*R*)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[b,d]pyran (JWH-133; Huffman *et al*., 1999); CB_2 antagonist – 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone (AM-630; Hosohata *et al*., 1997); constitutive NOS (cNOS) inhibitor – N^G-nitro-L-arginine (LNNA; Boer *et al*., 2000); inducible NOS (iNOS) inhibitor – *S*-(2-aminoethyl)isothiourea dihydrobromide (SATU; Southan *et al*., 1995); neuronal NOS (nNOS) inhibitor – N^w-propyl-L-arginine (NPA; Zhang *et al*., 1997); platelet-activating factor (PAF) antagonist – 1,4-dihydro-2,4,6-trimethyl-3,5-pyridinedicarboxylic acid methyl 2-(phenylthio)ethyl ester (PCA 4248; Ortega *et al*., 1990). The cyclooxygenase inhibitor indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole; Walters & Willoughby, 1965) and lipopolysaccharide (*Salmonella typhosa* (lot number: 78H4059)) were purchased from Sigma-Aldrich (St Louis, MO, U.S.A.). Anakinra (Kineret), a recombinant version of the human interleukin-1β receptor antagonist (IL-1ra), which blocks the biologic activity of IL-1 by competitively inhibiting IL-1 binding to the interleukin-1 type I receptor (Arend *et al*., 1998), was purchased from Amgen (Thousand Oaks, CA, U.S.A.).

All drugs were administered at a dose of 1 $mg\,kg^{-1}$ except for indomethacin (4 $mg\,kg^{-1}$), PCA 4248 (2 $mg\,kg^{-1}$) and Anakinra (10 & 100 $mg\,kg^{-1}$). Indomethacin was dissolved in 2.5% sodium bicarbonate. SATU and NPA were dissolved in 0.9% saline. All other drugs were dissolved in 100% ethanol to a concentration of 10 $mg\,ml^{-1}$ and diluted in 0.9% saline to

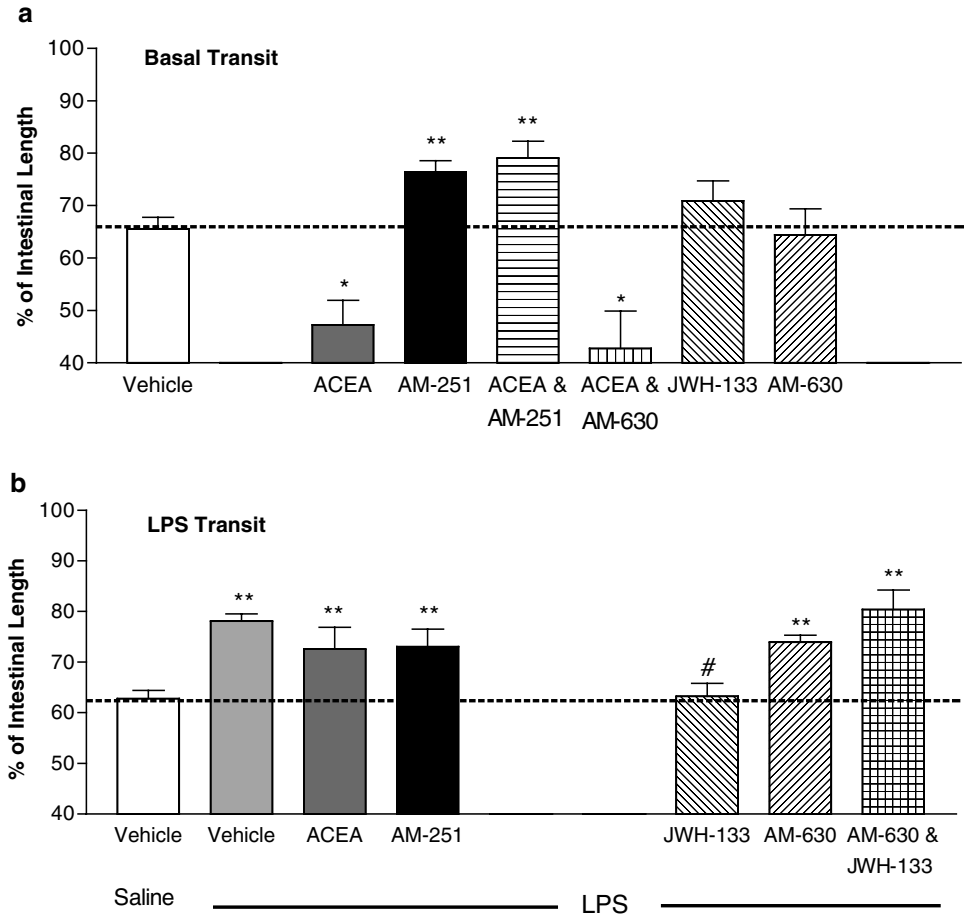

Figure 1 Cannabinoid receptors and gastrointestinal transit. (a) Basal transit (Vehicle) was reduced by the CB_1 agonist ACEA ($1\,mg\,kg^{-1}$). This inhibition was prevented by the CB_1 antagonist AM-251 (1 and $2\,mg\,kg^{-1}$), which alone increased basal transit at the higher dose ($2\,mg\,kg^{-1}$). The CB_2 antagonist AM-630 ($1\,mg\,kg^{-1}$) did not alter the effects of ACEA. The CB_2 agonist (JWH-133, $1\,mg\,kg^{-1}$) and antagonist (AM-630, $1\,mg\,kg^{-1}$) did not affect basal gastrointestinal transit. (b) Intraperitoneal injections with lipopolysaccharide (Vehicle/LPS, $65\,\mu g\,kg^{-1}$) increased transit relative to control rats. This increase in gastrointestinal transit elicited by LPS was not modified by CB_1 agonist (ACEA) or antagonist (AM-251, $1\,mg\,kg^{-1}$). The CB_2 agonist, JWH-133, prevented the LPS-evoked increase in transit, an action that was blocked by the CB_2 antagonist AM-630. Significance with $P<0.05$: * Less than vehicle control; ** Greater than vehicle control; #Less than LPS and AM-630 with JWH-133. $N\geqslant 5$ per group.

$1\,mg\,ml^{-1}$ immediately prior to use. The vehicle was prepared as 10% ethanol in 0.9% saline and was administered at 0.1 ml per 100 g rat body weight, which is equivalent to the volume used for the administration of all the other drugs used in the study.

Data analysis

Data are expressed as the mean values $\pm$ s.e.m. of at least five different experiments. Statistical significance was evaluated using one-way analysis of variance (ANOVA) with identification of differences between pairs using Dunnett's test (PRISM, version 3.0; GraphPad Software Inc., San Diego, CA, U.S.A.). Probability values of <0.05 were considered significant.

Results

Basal gastrointestinal transit

In normal, vehicle-treated rats, gastrointestinal transit 30 min after gavaging a gum Arabic-Evans blue mixture in the stomach was $\sim$65% of the length of the small intestine (Figure 1a). This transit was inhibited by the CB_1 agonist ACEA, an effect that was completely reversed by the CB_1 antagonist AM-251 ($1\,mg\,kg^{-1}$), which at this dose did not affect transit ($67.4\pm3.5\%$ of intestinal length in relation to a control value of $65.5\pm2.5\%$). However, a $2\,mg\,kg^{-1}$ dose of AM-251 significantly increased transit to $76.6\pm2.3\%$ (Figure 1a). Neither the selective CB_2 agonist JWH-133 nor the CB_2 antagonist AM-630 affected basal transit (Figure 1a). AM-630 did not alter ACEA-induced slowing of transit, confirming the specificity of the response.

LPS and gastrointestinal transit

To identify an optimal time to study LPS modification of gastrointestinal transit, a time course study was performed. At 30 min after intraperitoneal injection of LPS, gastrointestinal transit was not modified relative to vehicle-injected animals (68.0 ± 2.5 and $63.1\pm1.7\%$), respectively. However, after 2 h, gastrointestinal transit increased from 61.1 ± 2.3 to $77.0\pm1.1\%$, an increase of an additional 16% of the length of the

intestine. LPS no longer modified gastrointestinal transit when measured 19 h after LPS injection. In subsequent studies, gastrointestinal transit was evaluated at 2 h after intraperitoneal injection of LPS.

Neither the CB_1 receptor agonist ACEA nor the antagonist AM-251 ($1\,mg\,kg^{-1}$) significantly reduced gastrointestinal transit in the LPS-treated rats (Figure 1b). However, the CB_2 receptor agonist JWH-133 reduced the stimulated gastrointestinal transit back to control values, and this inhibition was completely prevented by the CB_2 receptor antagonist AM-630, which itself was without effect. JWH-133 dose-dependently inhibited LPS-stimulated gastrointestinal transit with 0.7 and $1.0\,mg\,kg^{-1}$ showing significant inhibition (Figure 2).

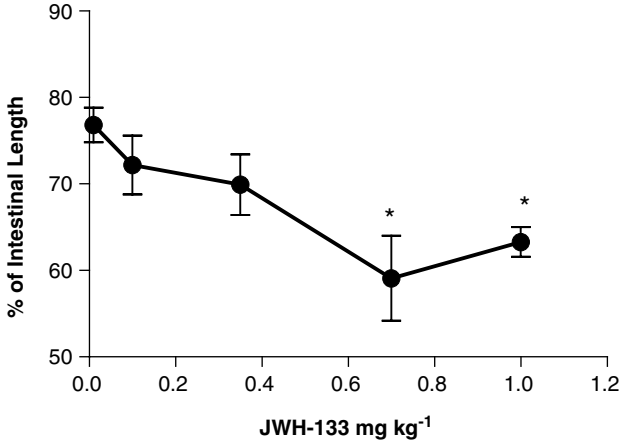

Figure 2 Dose–response relationship for inhibition of LPS-stimulated gastrointestinal transit by subcutaneously administered CB_2 agonist, JWH-133. In the absence of LPS, basal transit was approximately 60% of intestinal length. Significance with $P<0.05$. *Less than LPS-treated without JWH-133. $N\geqslant 6$ per group.

Mediators of CB_2 receptor activation

To examine the role of putative mediators that may be involved in the inhibition of LPS-stimulated increase in gastrointestinal transit by CB_2 receptors, we tested the actions of several antagonists in the absence and presence of JWH-133.

Gastrointestinal transit after inhibition of cyclooxygenase with indomethacin did not affect the LPS-stimulated increase in gastrointestinal transit. Indomethacin completely abrogated the inhibitory effect of JWH-133 (Figure 3). Thus, CB_2 alteration of gastrointestinal transit has a cyclooxygenase component. In contrast, inhibition of PAF with PCA 4248 was $70.8\pm2.9\%$, and not different from that seen with LPS alone ($77.5\pm1.3\%$), and the combination of CB_2 receptor activation with JWH-133 and PAF inhibition re-established transit to $55.2\pm3.5\%$, which was not different from transit of $61.8\pm1.4\%$ seen in control animals (Figure 3). These results suggest that PAF is not involved in mediating either the increased transit in response to LPS or the CB_2 alteration in transit.

Enhanced expression of IL-1 is a feature of LPS activation. Anakinra ($10\,mg\,kg^{-1}$), an IL-1ra, inhibited the LPS-induced increase in transit to levels seen in control animals ($59.0\pm3.9\%$), and no further inhibition in transit occurred if Anakinra and JWH-133 were used together ($64.5\pm1.9\%$). A higher dose of Anakinra ($100\,mg\,kg^{-1}$) reduced transit to below control levels (data not shown) and was not tested with the CB_2 agonist. CB_2 receptor activation may involve the IL-1 receptor, but in the absence of an accelerated response to modify we cannot draw conclusions at this time.

The role of NOS in the inhibitory actions of CB_2 agonists on LPS-induced gastrointestinal transit was then evaluated (Figure 4). A significant cNOS component (nNOS and eNOS) to LPS-stimulated transit was apparent, since the cNOS inhibitor LNNA (Boer *et al*., 2000) reduced transit

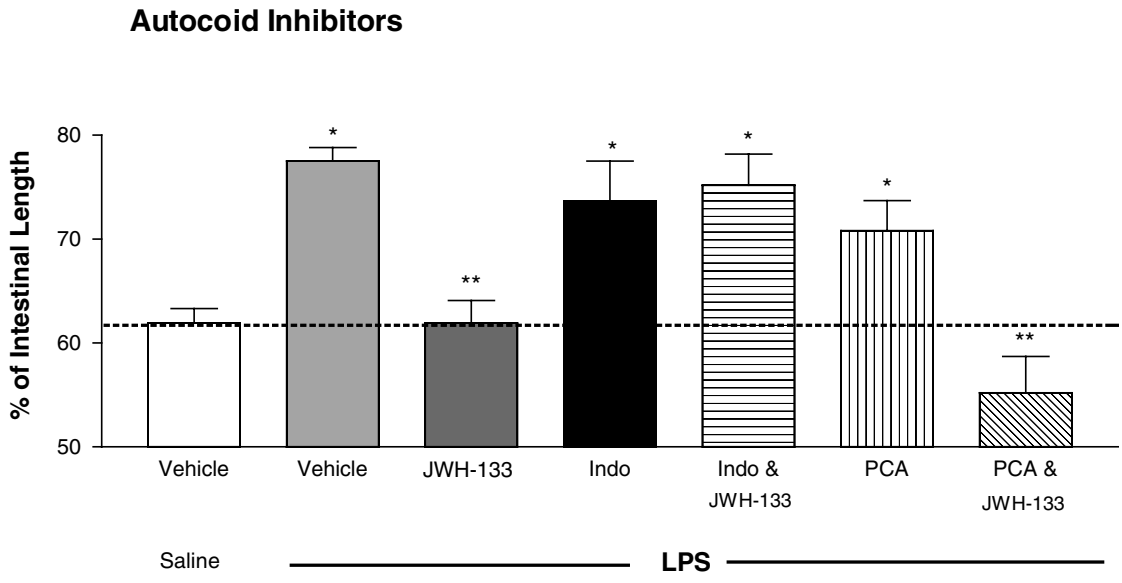

Figure 3 Effects of inhibition of cyclooxygenase (Indo – indomethacin; $4\,mg\,kg^{-1}$) and PAF (PCA 4248; $2\,mg\,kg^{-1}$) on LPS-stimulated gastrointestinal transit in the absence and presence of JWH-133. Vehicle controls received 0.9% saline i.p., and all other animals received $65\,\mu g\,kg^{-1}$ of LPS i.p. Significance with $P<0.05$ * Greater than vehicle control; ** Less than LPS-treated (Vehicle/LPS). $N\geqslant 5$ per group.

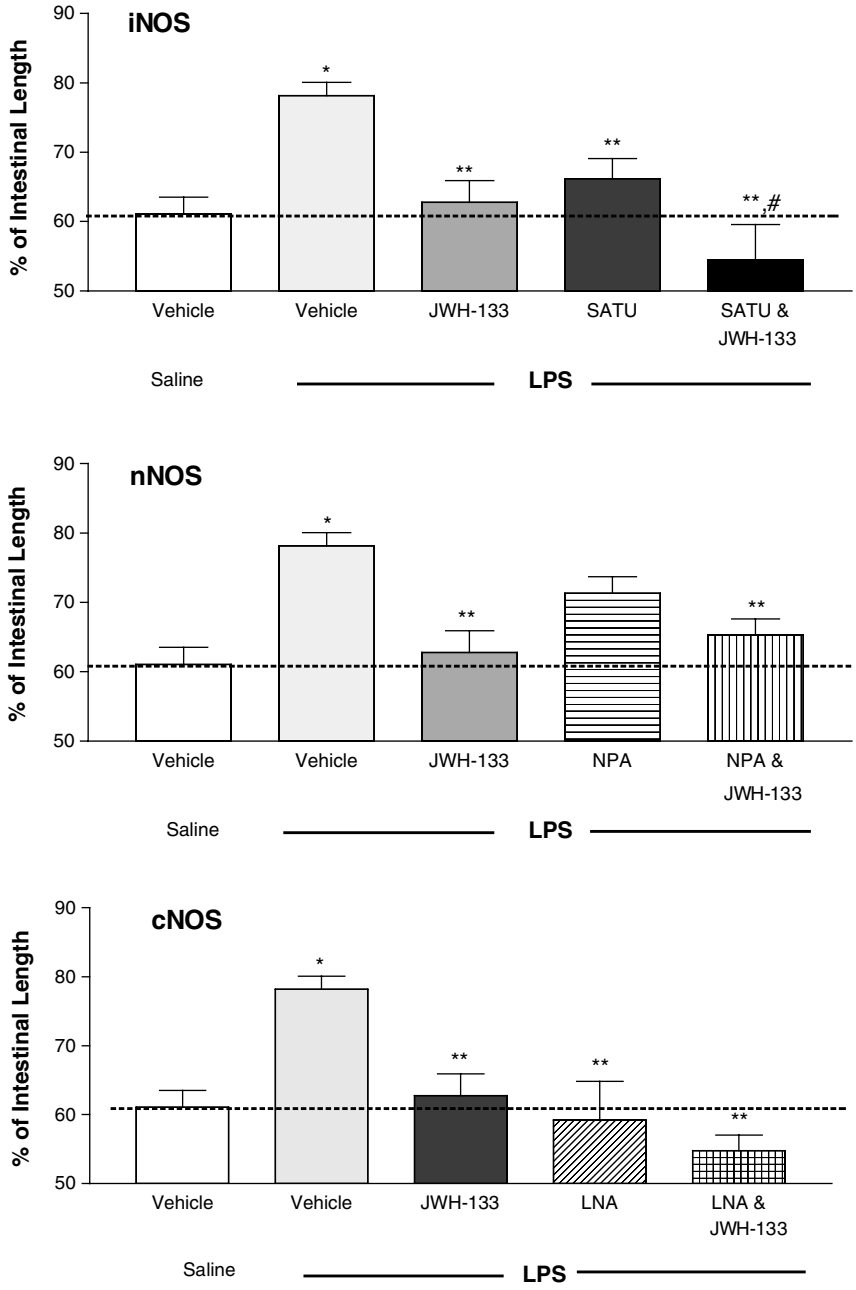

Figure 4 Effects of inhibition of iNOS with SATU (1 mg kg^{-1}), nNOS with NPA (1 mg kg^{-1}) and cNOS with LNNA (1 mg kg^{-1}) on LPS-stimulated gastrointestinal transit in the absence and presence of JWH-133. Vehicle controls received 0.9% saline i.p., and all other animals received 65 μg kg^{-1} of LPS i.p. Significance with $P<0.05$. * Greater than vehicle control; ** Less than LPS-treated (Vehicle/LPS); $^{\#}$Less than SATU alone. $N\geqslant 5$ per group.

to control levels (59.0$\pm$5.6%), and this response was not further reduced when LNNA and JWH-133 were given together. These experiments suggest an NOS component to LPS-stimulated gastrointestinal transit that may be predominately cNOS mediated. Independently, LPS activation may lead to iNOS production; therefore, we examined the effects of an iNOS inhibitor, SATU (Southan *et al*., 1995). SATU reduced LPS-enhanced gastrointestinal transit to 66.2$\pm$2.9% from the 78.2$\pm$1.9% occurring with LPS alone. This was similar to the effects of the CB_2 agonist alone. However, the combination of iNOS inhibition with CB_2 activation reduced transit a further 12% of intestinal length to 54.5$\pm$5.1%. Thus, CB_2 receptor activation appears to be additive with iNOS inhibition in reducing gastrointestinal transit, suggesting inhibition of transit by independent mechanisms. To further assess the role of the cNOS components, we also examined the inhibition of nNOS with the selective antagonist NPA (Zhang *et al*., 1997). NPA did not significantly reduce LPS-stimulated transit (73.0$\pm$3.3%) relative to LPS alone, although the simultaneous treatment with JWH-133 and NPA decreased transit to 65.2$\pm$1.6%, close to the transit occurring with JWH-133 alone (62.6$\pm$2.4%). This result implies that the cNOS contribution to LPS-stimulated transit involves predominantly eNOS and

that there is only a minor, if any, nNOS involvement in the actions of the CB_2 agonist.

Discussion

In this study, we show that CB_2 receptor activation inhibits the increase in gastrointestinal transit elicited by an intraperitoneal injection of LPS. The gastrointestinal functions that are modified by cannabinoids clearly show a role for the CB_1 receptor (see Introduction for references), and in our experiments the gastrointestinal transit of a transportable marker in normal rats was modified by CB_1 agonists only. In keeping with a previously demonstrated increase in basal transit by a CB_1 antagonist (SR141716A; Izzo *et al*., 1999b), AM-251 ($2\,mg\,kg^{-1}$) also increased transit, suggesting that AM-251 has inverse-agonist properties similar to SR141716A (Shire *et al*., 1999). However, the CB_1-mediated reduction in basal transit was absent in rats treated intraperitoneally with LPS, being replaced by a CB_2-mediated inhibition of stimulated transit as evidenced by the dose-dependent actions of the agonist JWH-133, which were prevented by the selective CB_2 antagonist AM-630. Since the CB_2 receptor antagonist alone was without effect in the LPS-induced increase in transit, CB_2 receptors do not appear to be activated by endotoxin or a tonically released endogenous agonist at the time point we studied.

The lack of an effect of CB_1 receptor on LPS-stimulated gastrointestinal transit may reflect an inactivation of this receptor by this inflammatory stimulus. CB_1 receptors are dynamically regulated, being downregulated by their own activation (Hsieh *et al*., 1999), and yet upregulated by another type of inflammation, that induced by croton oil (Izzo *et al*., 2001). Similarly, CB_2 receptors show state-dependent activities, and those on macrophages undergo major modulatory changes in relation to cell activation (Carlisle *et al*., 2002). As with the modification of gastrointestinal transit by CB_2 receptors, which manifests under LPS stimulation, a CB_2 agonist normally has no effect on paw-withdrawal latencies, but becomes a powerful inhibitor of the hyperalgesia if inflammation is induced (Quartilho *et al*., 2003). The onset of CB_2 receptor regulation of LPS-stimulated transit, with inhibition at 90 min, may reflect its priming by the inflammatory stimulus, as occurs with the attenuation by CB_2 agonists of LPS-induced pulmonary inflammation (Berdyshev *et al*., 1998) and reduction in the severity of endotoxic shock (Gallily *et al*., 1997).

The inhibition of LPS-induced increase in gastrointestinal transit by CB_2 receptor activation contrasts with the observations that croton oil-induced increases in intestinal motility are associated with an increase in CB_1 receptor expression, and inhibited specifically by the CB_1 receptor antagonist SR141716A (Izzo *et al*., 2001). One possible explanation for this apparent discrepancy is that different inflammatory agents increase intestinal transit by unrelated mechanisms. Croton oil-induced increases in transit appear to involve opioid receptor (Valle *et al*., 2000), whereas that elicited by LPS involves cyclooxygenase, IL-1 and NO. This conclusion is supported by other investigations that have identified iNOS (Jeon *et al*., 1996), IL-1ra (Molina-Holgado *et al*., 2003) and arachidonic acid metabolites (Chang *et al*., 2001) as participating in CB_2 receptor activation.

A role of the prostanoids in regulating intestinal transit functions is confused by the apparent conflicting observation that a cyclooxygenase inhibitor, indomethacin, prevents LPS-induced decreases in the contractions of rabbit intestinal tissue (Rebollar *et al*., 2002) and increases migrating myoelectric complexes, but does not affect increases in intestinal transit (Hellström *et al*., 1997). The latter observation was corroborated in the present study, with the additional observation that the inhibitory actions of a CB_2 agonist depend upon the presence of an intact cyclooxygenase pathway, since indomethacin treatment eliminated the JWH-133 inhibition of LPS-stimulated gastrointestinal transit. These complex effects are probably related to NO inhibition of intestinal contractions by a noncholinergic, nonadrenergic and nonprostanoid mechanism (Martinez-Cuesta *et al*., 1996), but require cyclooxygenase-generated metabolites for priming of the CB_2 receptor. In keeping with the complex effects of fatty acid metabolites, we found that PAF antagonism did not modify LPS-induced gastrointestinal transit, even though this lipid mediator, like indomethacin, prevents LPS-induced perturbations of migrating myoelectric complexes (Pons *et al*., 1991).

Several studies, including the present one, have established that inhibition of NOS, in particular cNOS (Wirthlin *et al*., 1996; Hellström *et al*., 1997), but also iNOS (De Winter *et al*., 2002), prevents the increase in intestinal transit elicited by LPS. Since inhibition of nNOS had minimal effects on LPS-provoked increases in transit (Figure 4), it appears that, of the cNOS isoforms, eNOS may be the major contributor to LPS actions. Although there is an iNOS component to intestinal transit (De Winter *et al*., 2002; Figure 4), this NOS isoform does not participate in a major way in CB_2-mediated inhibition since JWH-133, the CB_2-agonist, provided additional inhibition over that seen with iNOS inhibition. Thus, it appears that CB_2-mediated inhibition of LPS-induced gastrointestinal transit possibly involves alteration in eNOS activity. Although the modulation of NO by CB_1 and CB_2 receptors has focused on the downregulation of iNOS in macrophages (Chang *et al*., 2001) and astrocytes (Molina-Holgado *et al*., 2002), recent studies point to a role for macrophage- (Connelly *et al*., 2003) and astrocyte- (Iwase *et al*., 2000) derived eNOS in initiating the inflammatory response. Cannabinoid receptor activation interacts with NO production, by either facilitating (Lagneux & Lamontagne, 2001) or inhibiting (Molina-Holgado *et al*., 2003) NO production. The exact nature of the interactions, which may exhibit receptor and tissue differences, remain to be defined.

IL-1 receptor antagonist, an important anti-inflammatory cytokine, blocks the colonic motor responses elicited by anaphylaxis in guinea-pigs (Theodorou *et al*., 1993), as well as the increase in gastrointestinal transit elicited by LPS observed in the present study. It has been shown that the protective actions of IL-1ra involve cannabinoid receptors since activation of CB_1 and CB_2 receptors increases LPS-induced release of IL-1ra from cultured glial cells, and IL-1ra is required for cannabinoid-induced inhibition of NO production (Molina-Holgado *et al*., 2003). A similar dependency of CB_2-receptor activation on IL-1ra that involves NO may occur in the rat myenteric plexus since an NOS component to LPS-stimulated transit has been described (see above) and confirmed by us.

In summary, we show that CB_2 receptors in the rat intestine contribute to attenuation of the gastrointestinal transit increases elicited by an endotoxic inflammation. Activation of CB_2 receptor in response to LPS is a novel mechanism for the re-establishment of normal gastrointestinal transit after this inflammatory stimulus. The inhibitory effects of CB_2 receptor activation are *via* cyclooxygenase, and independent of iNOS and PAF. IL-1β and constitutive NOS isoforms (probably eNOS) may be involved in accelerated LPS transit.

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