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Rectal antinociceptive properties of alverine citrate are linked to antagonism at the 5-HT_{1A} receptor subtype

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Abstract

Serotonin (5-HT) is considered as a major mediator causing hyperalgesia and is involved in inflammatory reactions and irritable bowel syndrome. Alverine citrate may possess visceral antinociceptive properties in a rat model of rectal distension-induced abdominal contractions. This study was designed to evaluate the pharmacological properties of alverine citrate in a rat model of rectal hyperalgesia induced by 5-HTP (5-HT precursor) and by a selective 5-HT_{1A} agonist (8-OH-DPAT) and to compare this activity with a reference 5-HT_{1A} antagonist (WAY 100635). At 4 h after their administration, 5-HTP and 8-OH-DPAT increased the number of abdominal contractions in response to rectal distension at the lowest volume of distension (0.4 mL). When injected intraperitoneally before 8-OH-DPAT and 5-HTP, WAY 100635 (1 mg kg^{-1}) blocked their nociceptive effect, but also reduced the response to the highest volume of distension (1.6 mL). Similarly, when injected intraperitoneally, alverine citrate (20 mg kg^{-1}) suppressed the effect of 5-HTP, but not that of 8-OH-DPAT. However, when injected intracerebroventricularly (75 μg/rat) alverine citrate reduced 8-OH-DPAT-induced enhancement of rectal distension-induced abdominal contractions. In-vitro binding studies revealed that alverine citrate had a high affinity for 5-HT_{1A} receptors and a weak affinity for 5-HT_3 and 5-HT_4 subtypes. These results suggest that 5-HTP-induced rectal hypersensitivity involves 5-TH_{1A} receptors and that alverine citrate acts as a selective antagonist at the 5-HT_{1A} receptor subtype to block both 5-HTP and 8-OH-DPAT-induced rectal hypersensitivity.

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Introduction

Alverine citrate is considered as an antispasmodic drug and is commonly used in the treatment of spastic and painful affections of the colon and in the treatment of irritable bowel syndrome (IBS) in association with simethicone (Barthet et al 1996; Danne et al 1996). Data from the literature indicate that, in-vitro, alverine citrate is able to abolish the contractions of the guinea-pig ileum after acetylcholine injection (Ireson et al 1972) and to block the contraction of the rabbit jejunum induced by electrical stimulation of cholinergic nerve fibres (Levy & Apfel 1967). Moreover, in-vivo, the systemic administration of alverine citrate can inhibit the spontaneous and neurally evoked contractions of rabbit proximal colon (Bouvier et al 1992). A recent report from Abysique et al (1999) has shown that alverine citrate can act on vagal sensory endings located in the intestine where it affects the responses of mechanoreceptors to both mechanical and chemical stimuli. The exact mechanism by which alverine citrate can affect these functions is not known. The antispamodic effects of alverine citrate on the digestive tract are probably mediated

by complex processes involving smooth muscle cells and the extrinsic nervous system, as well as the blockade of calcium channels (Lemann et al 1995). Further studies proposed that alverine citrate could act as papaverine, which is known to block calcium entry through membrane channels of vascular smooth muscle cells (Bouvier et al 1992). In the same way, a recent report from Abysique et al (1999) proposed that the marked decrease observed in the vagal responses to chemical stimuli after alverine citrate application can be explained by a direct action on specific mechanosensitive channels (e.g. potassium, sodium, calcium channels), thus enhancing the neuronal response and the muscle tone.

Recently, alverine citrate has been demonstrated to possess visceral antinociceptive properties in a model of rectal distension-induced abdominal contractions in rats, an effect more pronounced under inflammatory conditions (Labie et al 1998). Calcium-channel blockers, such as pinaverium bromide or nifedipine, also reduce the response of vagal afferent neurons to chemical stimuli, such as cholecystokinin, substance P and serotonin (5-HT), substances known to activate vagal afferents (Lucchini et al 1995). Clinical studies tend to suggest a direct action of alverine citrate on sensitive innervation of the distal digestive tract, responsible for reducing some IBS symptoms (Barthet et al 1996; Danne et al 1996). In fact, the antinociceptive effect of alverine citrate could be explained by a direct action on primary afferent fibres projecting to the spinal cord, but, until now, no previous work has evaluated the exact mechanisms of action on primary afferents.

Serotonin and its receptors are widely present in smooth muscle and enteric nervous system of the gastrointestinal tract (Gershon & Erde 1981) and in the central nervous system. Serotonin is considered as a major inflammatory mediator causing hyperalgesia following nerve damage by acting directly on receptors located on primary nociceptive afferent endings, involving particularly 5-HT_1 and 5-HT_3 receptor subtypes (Levine & Taiwo 1994). At the visceral level, both 5-HT_{1A} and 5-HT_3 receptors are involved in inflammatory hyperalgesia (Morteau et al 1994; Coelho et al 1998).

This work was performed to determine the effect of alverine citrate on visceral nociceptive responses (allodynia) elicited by both 5-HTP (a precursor of 5-HT) and 5-HT_{1A} specific agonist, using a model of visceral pain (rectal distension) in conscious rats, to evaluate its site of action (central vs peripheral) on 5-HT_{1A} activation-induced hyperalgesia and to evaluate the affinity of alverine citrate for 5-HT receptor binding sites (5-HT_{1A}, 5-HT_3 and 5-HT_4) using in-vitro binding studies.

Material and Methods

In-vivo pharmacological studies

Animal preparation

Male Wistar rats, 200–250 g, were surgically prepared for electromyography, according to a previously described technique (Ruckebusch & Fioramonti 1975). Rats were anaesthetized by intraperitoneal injection of acepromazine (Calmivet; Vetoquinol, Lure, France) and ketamine (Imalgene 1000; Rhône-Mérieux, Lyon, France) at doses of 0.6 and 120 mg kg^{-1}, respectively. Three groups of three electrodes of nichrome wire (60 cm in length, 80 μm in diam.) were implanted bilaterally in the abdominal external oblique musculature, just superior to the inguinal ligament. Electrodes were exteriorized on the back of the neck and protected by a glass tube attached to the skin. Animals were individually housed in polypropylene cages and kept in a temperature-controlled room (21°C). They were allowed free access to water and food (UAR pellets; Epinay, France). All protocols were approved by the Local Animal Care and Use Committee of Institut National de la Recherche Agronomique.

Chronic intracerebroventricular cannula

Immediately after implantation of abdominal electrodes, animals were prepared for intracerebroventricular injections with two small polyethylene catheters (0.7 mm o.d., 0.3 mm i.d.) inserted into lateral ventricles of the brain (Stewart et al 1978) using a stereotaxis apparatus (Kopf, Los Angeles, CA). The bone reference marks were determined as follows (coordinates from the bregma): anteroposterior, 0.6 mm; lateral, 2 mm; ventral, 4 mm. Two screws were inserted in the bone surface. Catheters and screws were then secured to the skull with a dental cement. Injections were delivered with a 10-μL Hamilton syringe.

Electromyographic recording

Electromyographic recordings began five days after surgery. The electrical activity of abdominal striated muscles was recorded with an electroencephalograph machine (Mini VIII; Alvar, Paris, France) using a short time constant (0.03 s) to remove low-frequency signals (< 3 Hz) and with a paper speed of 3.6 cm min^{-1}.

Rectal distension procedure

Rats were placed in plastic tunnels (25 cm in length, 6 cm in diam.) where they could not move, escape or turn around, to prevent damage to the balloon. They had been accustomed to this procedure during the three

days before rectal distension to minimize stress reactions during experiments. The balloon used for distension was an arterial embolectomy catheter (Fogarty, Edwards Laboratories, Inc, Santa Ana). Rectal distension was performed by insertion of the balloon (2 cm in length, 2 mm in diam.) into the rectum, 1 cm from the anus. The catheter was fixed to the tail with adhesive tape. It was progressively inflated from 0 to 1.6 mL in 0.4-mL steps, each inflation step lasting 5 min. To detect possible leakage, the volume of water introduced in the balloon was checked by complete removal with a syringe at the end of the distension period.

Chemicals

5-Hydroxy-L-tryptophane (5-HTP, L-2-amino-3-[5-hydroxyindolyl]-propionic acid) was purchased from Sigma Aldrich (Saint Quentin Fallavier, France). Alverine citrate and WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide oxalate) were kindly supplied by Mayoly Spindler (Chatou, France) and Fabre (Castres, France) laboratories, respectively. 8-OH-DPAT (8-hydroxy-2-(di-*n*-(propylamino)tetralin) and 2-methyl 5-HT (2-methyl 5-hydroxytryptamine maleate) were obtained from RBI (Natik, MA). All drugs were dissolved in saline (0.9% NaCl). In all experiments, intraperitoneal injection of drugs or vehicles were given in a volume of 1 mL kg^{-1}.

Experimental procedure

Effect of alverine citrate on 5-HTP response

Two groups of animals were used to evaluate the effect of alverine citrate on the 5-HTP nociceptive response. Group 1 (n = 8) received 5-HTP (0.1 mg kg^{-1}, i.p.) or vehicle, preceded (5 min) by vehicle (1 mL kg^{-1} saline, i.p.) as control. Group 2 (n = 7) received alverine citrate or vehicle (1 mL kg^{-1}, i.p.) at an intraperitoneal dose of 20 mg kg^{-1}, followed (5 min) by 5-HTP (0.1 mg kg^{-1}, i.p.) or vehicle administration. In all groups, rectal distension was performed 4 h after 5-HTP administration. The dose of 5-HTP was previously found to be effective in a model of rectal sensitivity and the time of 4 h before performing rectal distension corresponds to the maximal effect on rectal sensitivity (Coelho et al 1998).

Effect of WAY 100635 and alverine citrate on 8-OH-DPAT response

In a first series of experiments, two groups of rats were used. Group 1 (n = 8) was injected with 8-OH-DPAT (1 mg kg^{-1}, i.p.) preceded (10 min) by WAY 100635 (1 mg kg^{-1}, i.p.) or its vehicle. Group 2 (n = 8) was injected with 8-OH-DPAT (1 mg kg^{-1}, i.p.) preceded (10 min) by alverine citrate (20 mg kg^{-1}, i.p.) or its vehicle. Rectal distension was performed before (−1 h) and 4 h after 8-OH-DPAT administration. The doses of 8-OH-DPAT and WAY 100635 were previously found to be effective in a model of rectal distension (Coelho et al 1998).

In a third series of experiments, a group of 7 animals received intracerebroventricular alverine citrate at a dose of 75 μg/rat, or its vehicle (2 μL/rat), 10 min before 8-OH-DPAT (1 mg kg^{-1}, i.p.). Rectal distension was performed before (−1 h) and 4 h after agonist administration.

In-vitro receptor-binding studies

Cell culture and membrane preparation

Rat cerebral cortical membranes expressing 5-HT_{1A} receptors were freshly prepared for each experiment, according to the method of Hall et al (1985). Male Sprague-Dawley rats (250–300 g; Charles River) were kept for at least 7 days in a controlled environment (21°C) and were allowed free access to water and food (UAR pellets, Epinay, France) before experiments. Animals were killed by cervical dislocation and decapitation, and their brains rapidly removed at 4°C. Tissues were homogenized in 40 vols (v/w) ice-cold 50 mM Tris-HCl (pH 7.4 at 23°C) using a Polytron disrupter and centrifuged at 40 000 *g* for 20 min. The supernatant was discarded and the pellet washed twice by resuspension in 40 vols Tris-HCl and centrifugation, homogenized in 40 vols Tris-HCl, and incubated at 37°C for 10 min to remove endogenous 5-HT. Membranes were then collected by centrifugation and washed twice before final resuspension in 10 vols 50 mM Tris-HCl (pH 7.4). Binding assays were performed using 50-μL aliquots (equiv. 0.25–0.3 mg protein).

According to the method of Hoyer & Neijt (1988), mouse neuroblastoma cells of the clone N1E-115, expressing 5-HT_3 receptors, were grown in Dulbecco's modified Eagle's medium with HEPES (7.6 mM) and sodium bicarbonate (30 mM). The antibiotics penicillin (100 IU mL^{-1}) and streptomycin (100 μg mL^{-1}) were supplemented, together with 7.5% fetal calf serum (Gibco) and the following amino acids (mM): cysteine-hydrochloride, 0.30; L-alanine, 0.40; asparagine, 0.45; L-aspartic acid, 0.40; L-proline, 0.40; and L-glutamic acid, 0.40. Cells were cultured at 37°C in closed tissue culture roller bottles (850 cm^2, 0.75 rpm; Falcon), gassed with CO_2, fed every second day and subcultured every 5

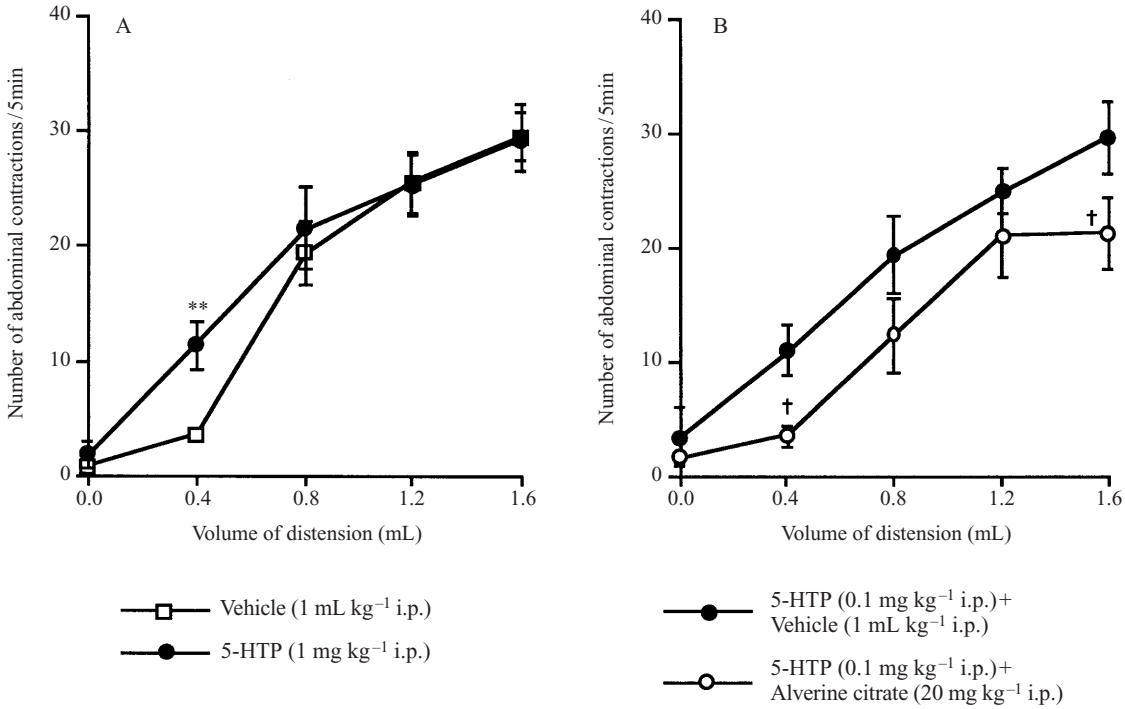

Figure 1 Effect on the enhancement of abdominal contractions in response to rectal distension induced by 5-HTP (0.1 mg kg^{-1}, i.p.) (A) of alverine citrate (20 mg kg^{-1}, i.p.) (B), in conscious rats. Values are expressed as means ± s.e.m., n = 7–8. $^{**}P < 0.01$, significantly different compared with corresponding value for vehicle; $\dagger P < 0.05$, significantly different compared with corresponding values for 5-HTP/vehicle.

days. The cells were grown to a density of $8–15 \times 10^7$ cells/bottle (log phase sparse culture) and harvested by vigorous shaking. Harvested cells were centrifuged at 900 *g* at 4°C for 5 min. The supernatant was discarded and the cell pellet was resuspended in Tris buffer (20 mM, pH 7.5) containing 154 mM NaCl, and homogenized with a Polytron. The homogenate was centrifuged again at 900 *g*. The pellet was discarded and the supernatant was used for direct binding studies.

Guinea-pig striatum membranes, expressing 5-HT_4 receptors, were freshly prepared for each experiment, according to the method of Grossman et al (1993). Briefly, Dunkin Hartley guinea-pigs (approx. 400 g) were killed by cervical dislocation and the brain removed and dissected. Pooled striatal brain tissue was placed in 15 vols HEPES buffer (50 mM, pH 7.4, 4°C), homogenized for 12 s and subsequently centrifuged at 48 000 *g* at 4°C. The resulting pellet was resuspended in HEPES buffer to make a homogenate at 30 mg mL^{-1} for striatum.

Binding assays

In the 5-HT_{1A} binding assays, cerebral cortical membranes were incubated in Tris-HCl buffer with [^{3}H]8-OH-DPAT (0.5 nM) for 30 min at 22°C. Non-specific binding was defined in rat cortical membranes with 8-OH DPAT (10 μM). In the 5-HT_3 binding assays, N1E-115 cell membranes were incubated in buffer or test compound, and [^{3}H]BRL 43694 (1 nM, final concn). Non-specific binding was defined by addition of metoclopramide (100 μM). Membrane suspensions were incubated for 180 min at 4°C. In the 5-HT_4 binding assays, guinea-pig striatum membranes were incubated with [^{3}H]GR 113808 (0.1 nM) for 30 min at 37°C. Non-specific binding was defined using serotonin (30 μM). In binding assays, alverine citrate was tested at two concentrations (10 and 100 μM) and competition (displacement) experiments were carried out with eight concentrations of reference molecules (8-OH-DPAT for 5-HT_{1A} receptors, MDL 72222 for 5-HT_3 receptors and serotonin for 5-HT_4 receptors). In a second series of 5-HT_{1A} binding assays, the same protocol was performed as described above and competition experiments were carried out with alverine citrate (from 10^{-8} to 10^{-5} M) and WAY 100635 (from 3×10^{-11} to 10^{-7} M). All experiments were carried out in duplicate for two series of binding essays.

Reactions were terminated by rapid vacuum filtration

through Whatman GF/B filters (Packard or Filtermat A; Wallac), presoaked in buffer. Filters were washed 3 times with ice-cold buffer using a filtrate system Cell Harvester (Packard or Tomtec). Filters were placed into scintillation solution (Microscint 0; Packard) or solid scintillation liquid (Meltilex B/HS; Wallac) and radioactivity was measured with a scintillation counter (Topcount; Packard or Betaplate; Wallac).

Statistical analysis

Statistical analysis of the number of abdominal contractions occurring during each 5-min period of rectal distension was performed by analysis of variance followed by Student's paired *t*-test for each series of experiments. In each series, values observed under treatment (5-HTP, 8-OH-DPAT) were compared with controls and treatment plus alverine. Values are expressed as mean ± s.e.m. Differences were considered significant for $P < 0.05$. In binding studies, values of IC50 were the mean of two measures for two series of binding (n = 4) and represented the inhibitory percentages of specific control binding.

Results

Antagonism of 5-HTP-induced rectal allodynia

As previously shown by Morteau et al (1994), gradual rectal distension increased the frequency of abdominal contractions in a volume-dependent manner from 0.8 to 1.6 mL, with 0.8 mL characterizing the threshold volume with a significant increase in the number of abdominal contractions. 5-HTP significantly increased ($P < 0.05$) the number of abdominal contractions only for the lowest volume of distension (0.4 mL) 4 h after injection (11.4 ± 2.1 vs 3.5 ± 1.4 contractions/5 min for control) (Figure 1A). When administered 5 min before 5-HTP, alverine citrate suppressed ($P < 0.05$) the number of contractions at the volume of 0.4 mL (3.6 ± 0.9 vs 10.9 ± 2.2) evoked by 5-HTP, but also significantly reduced the number of abdominal contractions for the highest volume of distension (1.6 mL) (Figure 1B).

Antagonism of 8-OH-DPAT-induced rectal allodynia

When administered 10 min before 8-OH-DPAT, WAY 100635 suppressed the abdominal response elicited by the 5-HT_{1A} agonist with a significant attenuation of the abdominal response to 1.6-mL rectal distension (Table 1). Alverine citrate (20 mg kg^{-1}) did not significantly affect the increase of abdominal contractions observed

Table 1 Effect of systemic (i.p.) administration of WAY 100635 and alverine citrate on the number of abdominal contractions induced by rectal distension at 0.4 and 1.6 mL, 4 h after administration of 8-OH-DPAT (1 mg kg^{-1}).

Treatment	Volume of rectal distension	
	0.4 mL	1.6 mL
Vehicle (1 mL kg^{-1})	11.9 ± 2.1	31.2 ± 3.6
WAY 100635 (1 mg kg^{-1})	3.3 ± 2.5*	21.3 ± 2.7*
Vehicle (1 mL kg^{-1})	10.2 ± 2.1	30.3 ± 2.9
Alverine citrate (20 mg kg^{-1})	13.2 ± 3.4	28.6 ± 3.1

Values correspond to the number of abdominal contractions occurring during 5-min periods at the given volume of distention (mean ± s.e.m., n = 6–8). WAY 100635 significantly reduced the number of abdominal contractions elicited by 8-OH-DPAT. *$P < 0.05$, significantly different compared with the corresponding vehicle.

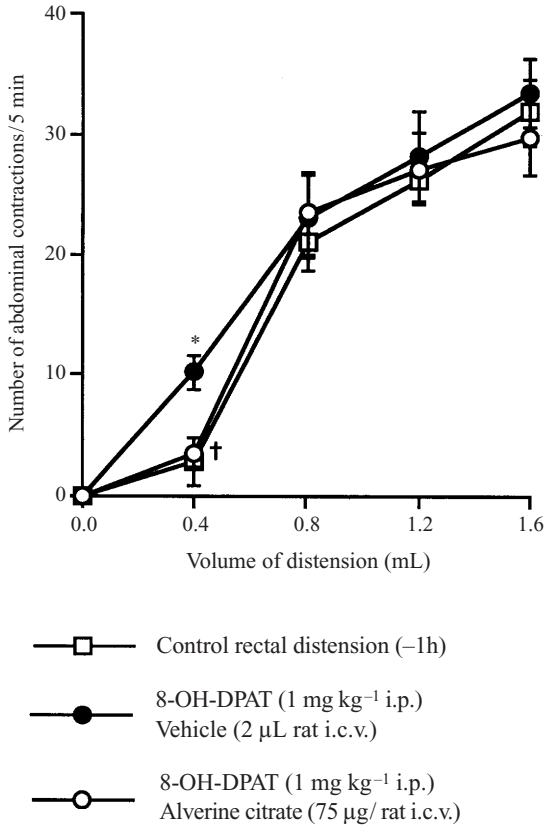

Figure 2 Effect of intracerebroventricular administration of alverine citrate (75 μg/rat) on 8-OH-DPAT (1 mg kg^{-1} i.p.)-induced enhancement (4 h) of abdominal response to rectal distension in conscious rats. Values are expressed as means ± s.e.m., n = 7. *$P < 0.05$, significantly different compared with corresponding control value; †$P < 0.05$, significantly different compared with corresponding values for 8-OH-DPAT/vehicle.

Table 2 Effect of alverine citrate on specific binding of radioligand at serotonergic receptors and affinity values (IC50) of drug references.

		5-HT_{1A}	5-HT_3	5-HT_4
% Inhibition	Alverine citrate (10 μM)	97	15	17
	Alverine citrate (100 μM)	100	41	81
IC50 (nM)	8-OH-DPAT	1.7 ± 0.3	–	–
	MDL 72222	–	14 ± 3	–
	Serotonin	–	–	68 ± 9
	Alverine citrate	101 ± 13	$> 10^4$	$> 10^3$
	WAY 100635	4.9 ± 0.5	–	–

Results for % inhibition are expressed as a percentage of specific binding control inhibition (means of two values).

4 h after 8-OH-DPAT (Table 1). In contrast, when injected intracerebroventricularly at 10 min before 8-OH-DPAT, alverine citrate (75 μg/rat) suppressed the enhancement of abdominal contractions at the threshold volume of distension (0.4 mL) triggered by peripheral administration of 8-OH-DPAT (3.6 ± 1.2 vs 10.3 ± 1.4 abdominal contractions/5 min) (Figure 2).

Receptor binding affinity

When tested at concentrations of 10 and 100 μM, alverine citrate potently inhibited [^{3}H]8-OH-DPAT binding to 5-HT_{1A} receptors expressed on rat brain membranes, this percentage rising to 97% for 10 μM alverine citrate. For the two other serotonergic receptors (5-HT_3 and 5-HT_4), alverine citrate inhibited [^{3}H]BRL 43964 binding to 5-HT_3 receptors expressed in N1E-115 neuroblastoma cells with an IC50 value greater than 100 μM, and [^{3}H]GR 113808 binding to 5-HT_4 receptors expressed in guinea-pig striatum membranes with an IC50 of between 10 and 100 μM (Table 2). In a second series of experiments, with an extended range of concentrations, it was found that alverine citrate had an IC50 value of 101 nM on 5-HT_{1A} receptors vs 4.9 nM for WAY 100635, a selective and potent antagonist of 5-HT_{1A} receptors. 8-OH-DPAT had an IC50 value of 0.7 nM in the same brain preparation membranes.

Discussion

This study shows that alverine citrate inhibits 5-HTP rectal allodynia, similar to WAY 100635, when injected peripherally in rats. It also blocks the allodynic effect of 8-OH-DPAT, a selective 5-HT_{1A} agonist, on rectal distension, but only when injected centrally. This suggests that the effect of 8-OH-DPAT on rectal sensitivity is centrally mediated and that alverine citrate does not cross the blood–brain barrier. Thus, it is probable that blockade of 5-HTP-induced rectal allodynia by alverine as well as by WAY 100635 is peripherally mediated.

Serotonin is considered as a major mediator in both somatic and visceral pain by direct excitation followed by sensitization of peripheral nociceptive fibers (Besson & Chaouch 1987; Rueff & Dray 1992). In the first part of our experiment, we confirmed that selective activation of 5-HT_{1A} receptors enhances visceral nociception similarly to 5-HTP, a precursor of 5-HT. When injected intraperitoneally, 8-OH-DPAT induced a time-delayed lower pain threshold to rectal distension (allodynia) observed approximately 4 h after its administration, similar to 5-HTP. These results are in agreement with our previous report showing that 5-HT_{1A} receptors are involved in gut inflammation and mast cell degranulation-induced visceral hypersensitivity (Coelho et al 1998). Both the delay and the duration of these effects may result from a spinal cord facilitation of nociceptive message transmission following local gut immune reactions (Simone et al 1991; Willis 1993).

Our data show that preventive administration of WAY 100635 blocks the 8-OH-DPAT-induced time-delayed lower pain threshold to rectal distension. Alverine citrate was able to reduce this occurrence only when centrally administered. 5-HT_{1A} receptors are present in high concentrations in both the spinal cord dorsal horn and in the cerebral structures involved in nociceptive processing (Pazos et al 1988). Moreover, the same receptors have been found in intestinal myenteric neurons (Galligan et al 1988; Kirchgessner et al 1996). Consequently, the hyperalgesic action of 8-OH-DPAT may be centrally or peripherally mediated, since this compound can cross the blood–brain barrier. An in-vitro study has shown that serotonin-evoked activation of previously sensitized peripheral fibres involves 5-HT_1-like receptors (Rueff & Dray 1992). However, hypersensitivity to noxious mechanical stimulation has been observed after spinal treatment with selective 5-HT_{1A} agonists such as 8-OH-DPAT (Murphy et al 1992), and the site of action of the 5-HT_{1A} agonist to enhance pain perception may correspond to a reduction of the activity of descending pathways, rather than a facilitation of spinal pain transmission (Zemlan et al 1994). This hypothesis agrees with our data showing that 8-OH-DPAT affects only the threshold of sensitivity to rectal distension. Furthermore, 8-OH-DPAT has also been shown to act centrally to modulate the threshold of pain sensation to gastric distension (Rouzade et al 1998). Our data suggest that these effects are centrally mediated, since alverine citrate, which is unable to cross

the blood–brain barrier, is active only when administered intracerebroventricularly. In contrast, WAY 100635, which easily crosses the blood–brain barrier (Mathis et al 1994), blocks the effect of 8-OH-DPAT when injected peripherally. Therefore, we suppose that the action of 8-OH-DPAT is centrally mediated. The radioligand binding experiments confirmed that alverine citrate interacts selectively with the 5-HT_{1A} receptor subtype with an IC50 of 101 nM. On rat cerebral cortex membranes, this affinity was 100- to 1000-times greater than observed for 5-HT_4 and 5-HT_3 receptor subtypes, respectively, confirming the selectivity to 5-HT_{1A} receptors, even though this affinity was largely lower than that described on similar rat cerebral cortex membranes for WAY 100635 (IC50 = 4.9 nM).

Our experiments showed that alverine citrate blocks 5-HTP-induced allodynia when administered systemically. Since alverine citrate does not cross the blood–brain barrier, we can speculate that the 5-HT_{1A} receptor is involved at the peripheral level in the mechanism of delayed allodynia (4 h) induced by 5-HTP. Both 5-HT_{1A} and 5-HT_3 receptors have been described on several cells involved in the sensitization of primary afferents in the gut (Rueff & Dray 1992). Mast cells and/or enterochromaffin cells express both 5-HT_{1A} receptors as well as primary afferents. Mast cell degranulation is known to produce a delayed rectal allodynia, also blocked by 5-HT_{1A} receptor antagonists (Coelho et al 1998). Thus, we can hypothesize that the delayed allodynia evoked by 5-HTP may be linked to mast cell degranulation, which, in turn, activates a cascade of events involving activation of peripheral 5-HT_{1A} receptors. This hypothesis may explain the efficacy of 5-HT_{1A} antagonist to prevent 5-HT-induced visceral pain. Nevertheless, alverine citrate may interact with other receptors involved in the cascade of events initiated by 5-HTP and we cannot exclude that the antagonism of 5-HTP-induced allodynia is linked to another mechanism.

Since the release of serotonin in the gut by enterochromaffin cells and platelets is a common feature in local inflammatory processes, also associated with immunocyte activation, our results suggest that alverine citrate, as well as other 5-HT_{1A} antagonists, may be as potent as 5-HT_3 receptor antagonists to prevent visceral pain in various functional and organic gastrointestinal disorders.

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