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Reversal of Inflammatory and Non-Inflammatory Visceral Pain by Central or Peripheral Actions of Sumatriptan

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Abstract

Background and Aims—Sumatriptan is used specifically to relieve headache pain. The possible efficacy of sumatriptan was investigated in two models of visceral pain.

Methods—Pancreatic inflammation was induced by intravenous injection of dibutyltin dichloride. Non-inflammatory irritable bowel syndrome was induced by intracolonic instillation of sodium butyrate. The effects of systemic sumatriptan on referred hypersensitivity were tested in both models. Effects of sumatriptan within the rostral ventromedial medulla (RVM), a site of descending modulation of visceral pain, was determined by (a) testing the effects of RVM administration of 5HT_{1B/D} antagonists on systemic sumatriptan action and (b) determining whether RVM application of sumatriptan reproduced the actions of systemic drug administration.

Results—Systemic sumatriptan elicited a dose- and time-related blockade of referred hypersensitivity in both models that was blocked by systemic administration of either 5HT_{1B} or 5HT_{1D} antagonists. Sumatriptan administered into the RVM similarly produced dose- and time-related blockade of referred hypersensitivity in both visceral pain models. This was blocked by local microinjection of the 5HT_{1B} antagonist, but not the 5HT_{1D} antagonist. Microinjection of 5HT_{1B} or 5HT_{1D} antagonists into the RVM did not block the effects of systemic sumatriptan.

Conclusions—Our findings suggest that sumatriptan suppresses either inflammatory or non-inflammatory visceral pain, most likely through peripheral 5HT_{1B/1D} receptors. Actions at 5HT_{1B} receptors within the RVM offer an additional potential site of action for the modulation of visceral pain by triptans. These studies offer new insights into the development of strategies which may improve therapy of visceral pain conditions using already available medications.

Introduction

Visceral pain is difficult to treat and often requires the use of opioids. While widely used, the severe dose-limiting side-effects of opioids often results in diminished efficacy¹.

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Additionally, opioids carry the risk of abuse and physical dependence, and induce constipation and other unwanted side-effects which diminish quality of life^{2, 3}. For this reason, improved treatments for visceral pain are highly desirable. Triptans are widely used in the treatment of migraine headache⁴ and are thought to be specific in their pain relieving actions for this condition. Since the triptans act at 5HT_{1B} and 5HT_{1D} receptors, and as these receptors are widely distributed throughout the peripheral and central nervous system^{5, 6}, the reasons for the proposed specificity of triptans for headache pain are not understood.

Very few clinical reports have investigated the actions of sumatriptan in visceral pain conditions. In patients with dyspepsia, a painful condition, sumatriptan produces relaxation of the gastric fundus, allowing for larger volumes to be accommodated, and therefore reducing the threshold for gastric perceptions^{7, 8}. Recently, sumatriptan has been used in IBS patients to regulate anorectal function and increase the threshold for first sensation in this population of patients⁹. However, to our knowledge, no preclinical reports are available on the possible efficacy of triptans for treatment of visceral pain.

In our study, we examined the possible effects of sumatriptan in two established rodent models of visceral pain. One model resembles some aspects of pancreatitis by producing inflammation of the pancreas and referred cutaneous hypersensitivity of the abdominal area¹⁰. Pain from pancreatitis can be referred to somatic structures in humans¹¹ and in animal models^{10, 12, 13}. Measuring the degree of referred somatic hypersensitivity has become a useful approach to evaluate visceral hypersensitivity and has been applied to study persistent pain associated with inflammation of the pancreas^{14, 15}. An increase in the area of referred hypersensitivity is also observed in patients with pancreatitis.¹¹ Recently, a novel model of colonic hypersensitivity has been developed¹⁶ and has been suggested to mimic some aspects of irritable bowel syndrome (IBS). This model elicits cutaneous hypersensitivity in the lumbar dermatomes of rodents similar to reports of hypersensitivity in patients with IBS.¹⁷ Additionally, this novel model induces hypersensitivity without producing injury or apparent inflammation of the colon, similar what is seen in patients with IBS.¹⁸

In the present study, we explored the possible actions and mechanisms of triptans in the modulation of visceral pain. For migraine, triptans are thought to act on blood vessels of the meningeal vasculature,¹⁹ and in the trigeminal ganglion²⁰. Nonetheless, the receptors upon which sumatriptan exerts its effects are widely expressed in the peripheral nervous systems, suggesting possible activity of the triptans in visceral pain states. In addition to peripheral expression, triptan receptors are found within the central nervous system including areas of pain modulation such as the RVM⁵. Previous studies have implicated the RVM in descending modulation of visceral pain. Electrical stimulation of the RVM produces biphasic modulation of spinal cord responses to acute colorectal distention (CRD)²¹ and of CRD-induced nociceptive reflexes²². Microinjection of lidocaine into the RVM reduced spontaneous activity and responses of spinal neurons to CRD²². The RVM also has a facilitatory role on persistent visceral pain. Microinjection of lidocaine into the RVM attenuated referred visceral hypersensitivity induced by pancreatic inflammation²³. Collectively, these studies suggest the possibility that triptans may act within the RVM to modulate visceral pain. Our data reveal that sumatriptan inhibits both inflammatory and non-

inflammatory visceral pain through peripheral sites and additionally suggest a potential central site of action within the RVM.

Materials and Methods

Animals

Adult, male Sprague Dawley rats (Harlan, Indianapolis, IN), weighing 150–200 g were maintained in a climate-controlled room with ad libitum food and water on a 12-h light/dark cycle (lights on at 07:00 hours). All procedures followed the policies of the International Association for the Study of Pain and the NIH guidelines for the handling and use of laboratory animals. Studies were approved by the Institutional Animal Care and Use Committee of the University of Arizona.

Drugs

Dibutyltin dichloride (DBTC) was obtained from Sigma Aldrich (Milwaukee, WI) and dissolved in 100% ethanol to a concentration of 8 mg/kg²⁴. Sumatriptan succinate was obtained from Toronto Research Chemicals (ON, Canada) and dissolved in saline to 1, 3 and 10 µg in 0.5 µl for RVM microinjections. Sumatriptan was given intraperitoneally at doses of 100, 200 and 300 µg/kg²⁵. The 5HT_{1B} antagonist isamoltane²⁶ and the 5HT_{1D} antagonist BRL15722²⁷ were obtained from Tocris (Ellisville, MO). Isamoltane was dissolved in saline to a concentration of 3 µg in 1 µl for RVM microinjection and injected systemically at 4 mg/kg²⁸. BRL15722 was dissolved in 10% DMSO to a concentration of 3 µg in 1 µl for RVM microinjection and injected systemically at 0.3 mg/kg²⁸.

Experimental design

For microinjection of drugs into the RVM, rats underwent surgeries to implant bilateral cannulae aimed at the RVM. After 5 days, rats received either intravenous injection of DBTC to induce pancreatitis, or intracolonic injection of sodium butyrate to induce colonic hypersensitivity. Animals were monitored for development of referred visceral hypersensitivity on subsequent days. On day 6 after either induction of pancreatitis or colonic hypersensitivity, rats underwent baseline behavioral measurements and were then microinjected with graded doses of sumatriptan into the RVM. Behavioral thresholds were monitored for 2 hr after RVM sumatriptan. Serotonin receptor antagonists were microinjected either alone or concurrently with RVM sumatriptan and behavioral responses again monitored for the subsequent 2 hrs.

For systemic drug administration, 6 days after induction of pancreatitis or colonic hypersensitivity, rats underwent baseline behavioral measurements and then received graded doses of intraperitoneal (i.p.) sumatriptan. Behavioral thresholds were monitored every 20 min for 2 hr after injection. Serotonin receptor antagonists, were injected i.p. alone or immediately after the injection of sumatriptan. Other rats were injected with i.p. sumatriptan and microinjected concurrently with the antagonists in the RVM. Animals were tested for behavioral signs of hypersensitivity every 20 min for 2 hr after the end of the injections. Other rats were injected with the highest effective dose of RVM sumatriptan by the

intravenous (i.v.) route on day 6 after induction of the visceral pain models and behavior was monitored for the next 2 hr.

Visceral pain models

Pancreatitis was produced by a tail vein injection of dibutyltin dichloride (DBTC, Aldrich, Milwaukee, WI, 0.25 ml) dissolved in 100% ethanol at a dose of 8 mg/kg under isofluorane anesthesia as previously reported²³. Control animals were injected with the vehicle solution only (100% ethanol, 0.25 ml).

Colonic hypersensitivity was induced by enemas of a sodium butyrate solution (110 mg/ml) twice daily for 3 days¹⁶. For each enema, a catheter made of P100 polyethylene tube was placed into the colon at 7 cm from the anal opening, and the animals received 1 mL of sodium butyrate at neutral pH. Care was taken to avoid damage of the colonic wall by insertion of the catheter.

Behavioral measures

Referred abdominal hypersensitivity in the pancreatitis model was quantified by measuring the number of withdrawal events (determined by either abdominal withdrawal, licking of the abdominal area, or whole body withdrawal) evoked by application of a calibrated 4 g von Frey filament. Rats were placed inside Plexiglas boxes on an elevated fine fiberglass screen mesh and acclimated for 30 min before testing. The 4 g von Frey filament was applied from underneath through the mesh floor, to the abdominal area at different points on the surface. A single trial consisted of 10 applications of this filament applied once every 10 sec to allow the animals to cease any response and return to a relatively inactive position. The mean occurrence of withdrawal events in each trial is expressed as the number of responses to 10 applications as previously described¹⁰.

Referred lumbar hypersensitivity in the colonic hypersensitivity model was quantified by applying von Frey filaments to the lumbar dermatomes of rats as previously described by Bourdu and colleagues¹⁶. Rats were shaved on the lumbar dermatomes before any manipulation and acclimated inside Plexiglas boxes for 30 min on the day of testing. Calibrated von Frey filaments of increasing diameter were applied 5 times for 1 sec, ranging from 0.04 to 6 g. The mechanical threshold corresponded to the force of the von Frey filament which induced lumbar skin wrinkling which was followed by escape behavior from the filament. If rats did not respond to any of the von Frey filaments, a cutoff value of 6 g was assigned to the rat.

Surgeries and microinjection procedures

Rats were anesthetized with ketamine/xylazine (100 mg/kg) and placed in a stereotaxic headholder. For the RVM cannula implantation procedure, the skull was exposed and two 26 ga guide cannula separated by 1.2 mm (Plastics One Inc., Roanoke, VA), were directed at the lateral portions of the RVM (anteroposterior, -11.0 mm from bregma; lateral, -0.6 mm from midline; dorsoventral, -8.5 mm from the cranium and secured to the skull with dental cement as previously described²⁹. After recovery (5 days), animals were injected with i.v. DBTC to induce pancreatitis or given intracolonic injections of sodium butyrate to induced

colonic hypersensitivity. On day 6 after DBTC injection or initiation of the sodium butyrate enemas, animals received microinjection of drugs into the RVM. Drug administration, using a Hamilton syringe, was performed slowly expelling 0.5 μ l bilaterally of drug solution through a 33 ga injection needle inserted through the guide cannula and protruding an additional 1 mm into fresh brain tissue to prevent backflow. Animals were euthanized at the end of the experiments and the brain was harvested for confirmation of cannula placement and only data from animals with appropriately placed cannula were used in the analysis. Pancreatic tissue and the colon were examined for signs of inflammation by performing hematoxylin and eosin staining visualization of the tissues under the light microscopy. Examination of pancreatic tissue demonstrated signs of inflammation as previously described^{10, 23} (data not shown). No signs of inflammation were observed in the colon consistent with previously reports¹⁶ (data not shown).

Statistical procedures

Data were analyzed using a two-factor ANOVA follow by the Fishers Least Significance Difference post-hoc test to determine differences between experimental groups for the behavioral test across time. One-factor ANOVA was used to detect significant differences in behavioral outcomes within each experimental group over time. A linear regression analysis was used to detect the dose-dependency of the effects of sumatriptan and to determine the A50, i.e., the dose producing a 50% response. Significance was established at the $p < 0.05$ level.

Results

Systemic sumatriptan reduces referred hypersensitivity in visceral pain models

In the experimental pancreatitis model, following i.v. DBTC, rats showed significantly increased withdrawal frequency to mechanical stimulation of the abdomen compared to rats injected with vehicle, indicating development of pancreatitis and associated referred abdominal hypersensitivity as previously described¹⁰ ($p < 0.05$, Figure 1A, DBTC group treated with saline). On day 6 after i.v. injection of DBTC, i.p. administration of sumatriptan reduced the frequency of withdrawals in DBTC-injected rats in a time- and dose-dependent manner (Figures 1A and 1B respectively). The A50 dose (and 95% C.L.) for i.p. sumatriptan was 172.4 (124.5–386.7) μ g/kg. Systemic sumatriptan was active up to 100 min post-injection and the effect dissipated at the 120 min time-point (Figure 1A). Systemic administration of sumatriptan did not alter responses to abdominal stimulation in vehicle injected control rats (Figure 1A).

In the colonic hypersensitivity model, rats demonstrated reduced mechanical thresholds when compared to vehicle-treated rats (Figure 1C, butyrate-saline treated group) indicating the development of referred lumbar hypersensitivity. Systemic sumatriptan increased the mechanical threshold of rats injected with sodium butyrate in a time and dose-dependent manner (Figures 1C and 1D respectively); the A50 (and 95% C.L.) dose for i.p. sumatriptan was 232.6 (182.2 – 322.8) μ g/kg. Systemic sumatriptan was active for 60 min post-injection and the effect dissipated by the 100 min timepoint (Figure 1C). Systemic administration of

sumatriptan did not modify the behavior of rats previously injected with intracolonic vehicle (Figure 1C).

Systemic actions of sumatriptan on visceral pain models are mediated by both the 5HT_{1B} and the 5HT_{1D} receptor

In the experimental pancreatitis model, i.v. injection of DBTC produced referred abdominal hypersensitivity as indicated by increased frequency of withdrawals (Figure 2A, pancreatitis-saline group). As demonstrated above, i.p injection of sumatriptan (300 µg/kg) reduced the frequency of withdrawals (Figure 2A, DBTC-sumatriptan group). Concurrent systemic (i.p.) injection of the 5HT_{1B} antagonist isamoltane (4 mg/kg) blocked the effects of sumatriptan ($p < 0.05$). Likewise, concurrent i.p. injection of the 5HT_{1D} antagonist BRL15722 (0.3 mg/kg) with systemic sumatriptan also blocked the effect of sumatriptan (Figure 2A). The antagonists injected alone did not produce any effects in either vehicle- or DBTC-treated rats (data not shown).

In the colonic hypersensitivity model, colonic injection of sodium butyrate produced referred lumbar hypersensitivity as indicated by a reduction in mechanical threshold to muscle contraction and escape behavior from von Frey stimulation (Figure 2B, butyrate-saline group). As demonstrated previously, i.p. injection of sumatriptan (300 µg/kg) increased the mechanical threshold (Figure 3B, butyrate-sumatriptan group). Concurrent systemic (i.p.) injection of the 5HT_{1B} antagonist isamoltane (4 mg/kg) blocked the effect of systemic sumatriptan ($p < 0.05$). Likewise, concurrent systemic (i.p.) injection of the 5HT_{1D} antagonist BRL15722 (0.3 mg/kg) blocked the effect of systemic sumatriptan (Figure 2B). The antagonists injected alone did not produce any effects in either vehicle- or sodium butyrate treated rats (data not shown).

Sumatriptan acts in the RVM to reduced referred hypersensitivity in visceral pain models

In the experimental pancreatitis model, RVM administration of sumatriptan attenuated the increased frequency of withdrawals associated with referred abdominal hypersensitivity in a time and dose-dependent manner (Figures 3A and 3B respectively). The A50 dose (and 95% C.L.) for RVM sumatriptan was 4.3 (3.1–16.2) µg. The effects of RVM sumatriptan endured for approximately 60 min and dissipated by 100 min post-injection (Figure 3A). Sumatriptan microinjected into the RVM did not alter responses to abdominal stimulation in vehicle injected rats (Figure 3A).

In the colonic hypersensitivity model, RVM administration of sumatriptan elicited a time and dose-dependent attenuation of lumbar hypersensitivity as indicated by an increase in lumbar dermatome mechanical threshold (Figures 3C and 3D respectively). The A50 (and 95% C.L.) dose for RVM sumatriptan was 3.2 (2.0–12.5) µg. The effects of RVM sumatriptan endured for approximately 60 min and dissipated by 100 min post-injection (Figure 3C). Microinjection of RVM sumatriptan did not modify the behavior of rats previously injected with intracolonic vehicle (Figure 3C).

Sumatriptan acts through the 5HT_{1B} receptor in the RVM to inhibit referred hypersensitivity in visceral pain models

In the experimental pancreatitis model, i.v. injection of DBTC produced referred abdominal hypersensitivity as indicated by increased frequency of withdrawals (Figure 4A, DBTC-saline group). RVM microinjection of sumatriptan (10 µg) reduced the frequency of withdrawals in rats with experimental pancreatitis (Figure 4A, DBTC-sumatriptan group). Concurrent microinjection of the 5HT_{1B} antagonist isamoltane (3 µg) into the RVM blocked the effect of sumatriptan, with number of withdrawals observed in this group being equivalent to that seen in the saline treated group (Figure 4A). In contrast, concurrent microinjection of the 5HT_{1D} antagonist BRL15722 (3 µg) did not block the effect of RVM sumatriptan (Figure 4A) as the frequency of withdrawals after application of BRL15722 did not differ from the frequency presented by rats receiving RVM sumatriptan alone in rats with pancreatitis. Microinjection of either antagonist alone did not produce any effects in control of pancreatitis rats (data not shown).

In the colonic hypersensitivity model, lumbar hypersensitivity as indicated by a reduction in mechanical threshold to muscle contraction and escape behavior from von Frey stimulation was observed (Figure 4B, butyrate-saline group). Microinjection of sumatriptan (10 µg) into the RVM increased the mechanical threshold (Figure 4B, butyrate-sumatriptan group). Concurrent microinjection of the 5HT_{1B} antagonist isamoltane (3µg) blocked the effect of RVM sumatriptan with observed mechanical thresholds equivalent to the sodium butyrate treated group (Figure 4B). In contrast, concurrent microinjection into the RVM of the 5HT_{1D} antagonist BRL15722 (3 µg) did not block the effect of RVM sumatriptan (Figure 4B). Microinjection of either antagonist alone did not modify the mechanical threshold of rats treated with sodium butyrate (data not shown).

The RVM is not a site of action for systemically applied sumatriptan

In the experimental pancreatitis model, i.v. injection of DBTC produced referred abdominal hypersensitivity as indicated by increased frequency of withdrawals (Figure 5A, DBTC-saline group). As before, i.p. injection of sumatriptan (300 µg/kg) reduced the frequency of withdrawals (Figure 5A, DBTC-sumatriptan group). Concurrent microinjection of the 5HT_{1B} antagonist isamoltane (3µg) into the RVM did not modify the effect of systemic sumatriptan. Likewise, concurrent microinjection of the 5HT_{1D} antagonist BRL15722 (3µg) with systemic sumatriptan did not block the effect of sumatriptan (Figure 5A).

In the colonic hypersensitivity model, colonic injection of sodium butyrate produced referred lumbar hypersensitivity as indicated by a reduction in mechanical threshold to muscle contraction and escape behavior from von Frey stimulation (Figure 5B, butyrate-saline group). As above, i.p. injection of sumatriptan (300 µg/kg) increased the mechanical threshold (Figure 5B, butyrate-sumatriptan group). Concurrent microinjection of the 5HT_{1B} antagonist isamoltane (3µg) into the RVM did not modify the effect of systemic sumatriptan. Likewise, concurrent microinjection into the RVM of the 5HT_{1D} antagonist BRL15722 (3µg) did not block the effect of systemic sumatriptan (Figure 5B).

RVM sumatriptan does not act systemically

As an additional control, rats received intravenous (i.v.) injection of the highest dose of sumatriptan tested in the RVM (10 µg) and monitored the rats for signs of referred hypersensitivity (data not shown). Rats with previous injection of DBTC to induce pancreatitis presented increased frequency of abdominal withdrawals which was not altered by i.v. injection of 10 µg of sumatriptan at any of the time points investigated (up to 120 min post injection). Similarly, rats with previous injection of sodium butyrate presented with reduced threshold to respond to mechanical stimulation of the lumbar dermatomes and i.v. sumatriptan (10 µg) did not reverse the reduction in threshold at any of the time points investigated (up to 120 min post injection).

Discussion

The present study investigated the effects of the antimigraine drug, sumatriptan, in two different visceral pain models. Here, we demonstrate that 1) both systemic and RVM administration of sumatriptan significantly inhibits the referred somatic hypersensitivity observed after induction of pancreatitis or colonic hypersensitivity; 2) within the RVM, sumatriptan mediates its anti-hypersensitivity effects through selective activity of the 5HT_{1B} receptor; and 3) systemic application of sumatriptan blocks referred somatic hypersensitivity from visceral pain through activity at peripheral 5HT_{1B} and 5HT_{1D} receptors. These studies demonstrate that sumatriptan is (a) active in the modulation of inflammatory and non-inflammatory visceral pain and (b) can exert antihyperalgesic actions within the RVM in modulation of pain. These findings reveal a novel potential application and multiple sites and mechanisms of action for triptans in the modulation of visceral pain. These studies provide a basis for further investigation of possible therapeutic application of a widely used class of drugs currently used almost exclusively for the treatment of migraine and headache pain, either alone or as adjuncts to other therapies in visceral pain conditions.

One of the main characteristics of visceral pain is that it is referred to somatic dermatomes receiving innervation from the same areas of the CNS which innervate visceral structures³⁰. Such referred hypersensitivity can be reproduced in animal models of visceral pain^{10, 12, 13, 31}. In the present study, we measured referred somatic hypersensitivity in two established models of persistent visceral pain. Experimental pancreatitis is an inflammatory persistent visceral pain state which is characterized by referred abdominal hypersensitivity that can be measured by increase frequency of withdrawals to stimulation with von Frey filaments. Enhanced responsiveness in this model is inhibited by opioids¹⁰, NK-1 antagonists³², and manipulations which interfere with descending facilitation originating in the RVM²³. The second model we used is a recently established model of non-inflammatory colonic hypersensitivity which appears to mimic some aspects of IBS. One of the main characteristics of this model is the development of somatic hypersensitivity in the absence of inflammation of the colon which is referred to the lumbar dermatomes¹⁶. Somatic hypersensitivity was used as an indication of ongoing persistent visceral pain.

Peripheral 5HT_{1B} receptors have been shown to be present in meningeal blood vessels, representing a possible site of action of triptans in headache pain.³³ However, both the 5HT_{1B} and the 5HT_{1D} receptor are found in trigeminal and dorsal root ganglia^{34, 5, 35, 36}

suggesting that triptan actions at these receptors may also be a site of action for headache pain. As the distribution of 5HT_{1B/D} receptors is not unique to the trigeminal system, the apparent specific actions of triptans in headache pain²⁰ is puzzling. Consistent with the apparent distribution of 5HT_{1B/D} receptors, systemic administration of sumatriptan produced a time- and dose-related reduction in referred somatic hypersensitivity originating from inflammatory pancreatitis or non-inflammatory colonic hypersensitivity.

The receptor and site of action of sumatriptan was confirmed by studies with antagonists to the 5HT_{1B} and the 5HT_{1D} receptors. These antagonists had no effect alone in either control animals or in animals with experimental visceral pain. The observed antagonism of i.p. sumatriptan activity by systemic 5HT_{1B} or 5HT_{1D} antagonists suggests that the activity of triptans occur through peripheral 5HT_{1B} and 5HT_{1D} receptors. In this regard, the 5HT_{1B} receptor has been localized to the enteric neurons in the gastrointestinal tract³⁷ but the presence of this receptor in the pancreas is unknown. It is possible to speculate that the 5HT_{1B} receptor is found in the vasculature in the pancreas, similar to its distribution in other organ systems where it may play a role in regulation of vasculature contractility, maintenance of pancreatic inflammation and subsequent pain³⁸. The 5HT_{1D} receptor is localized in trigeminal afferents where its activation results in inhibition of neurotransmitter release^{39, 40}, and is also found in primary afferent terminals in the spinal cord and cell bodies of the DRG³⁶. Thus, triptans could act at this receptor and block neurotransmitter release and the transmission of evoked noxious stimuli. This concept warrants further study in the context of visceral pain states.

Our previous studies have demonstrated that enhanced descending facilitation arising in the RVM plays an important role in the maintenance of persistent visceral pain²³. For this reason, possible activity of sumatriptan within the RVM was explored. Like the activity observed following i.p. sumatriptan, RVM microinjection produced both dose- and time-related antihyperalgesic actions in both models of persistent visceral pain. To confirm that the observed actions were within the RVM and not the result of the RVM injection gaining access to the general circulation, we demonstrated that intravenous injection of the highest effective dose of RVM sumatriptan failed to attenuate the visceral hypersensitivity in both models. These data suggest that the RVM is a potential site of action for sumatriptan in persistent visceral pain. Both 5HT_{1B} and 5HT_{1D} mRNA have been demonstrated in the RVM⁴¹ and 5HT_{1B} receptor binding sites have been reported in the RVM⁵. Our data show that the antinociceptive effect of RVM sumatriptan was blocked by concurrent microinjection of the 5HT_{1B} receptor antagonist isamoltane, not microinjection the 5HT_{1D} receptor antagonist BRL15722. In our study we used antagonists at doses known to block their respective receptors with high affinity using in vitro assays^{26, 27}. While isamoltane has been reported to have activity at beta-2 adrenergic receptors, the possibility that this mechanism may mediate the observed antihyperalgesic actions of sumatriptan appear unlikely due to the reported absence of these receptors within the RVM^{42, 43}. It is also possible that BRL15722 might have antagonized the actions of RVM sumatriptan if the antagonist were given at a higher dose. However, interpretation of this result would be difficult due to the possibility of non-selective actions at other 5HT receptors at higher doses.

Data from the microinjection studies suggest that sumatriptan may act within the RVM to attenuate visceral pain through activation of 5HT_{1B} receptor. However, the RVM administration of the antagonists failed to block the anti-hyperalgesic effects of sumatriptan injected systemically, suggesting that systemically applied sumatriptan does not reach the RVM to exert its antihyperalgesic effects. This conclusion is in agreement with studies that report poor blood brain barrier-penetrating ability of sumatriptan⁴⁴. Other, more brain penetrant triptans or triptan-like molecules might have greater access to the RVM raising the possibility that the RVM could be an additional site of action to elicit efficacy in visceral pain states.

It has been suggested that triptans act exclusively in the trigeminal system to abort migraine pain⁴⁵. However, a few studies suggest that sumatriptan might have activity in other pain states. Thermal hypersensitivity induced by intraplantar injection of carrageenan is attenuated by sumatriptan injected systemically at similar doses used in the present study⁴⁶. Sumatriptan also inhibits capsaicin-induced hyperemia in the sciatic nerve⁴⁷ and the evoked release of CGRP from the rat isolated spinal cord⁴⁸. Interestingly, sumatriptan also has antinociceptive efficacy in acetic acid-induced abdominal writhing in mice^{49, 50}, which has a visceral pain component. These studies suggest that the triptans might be effective in treatment of a broader spectrum of pain states besides headache pain. Together with the data from the current study, it seems possible that triptans may find a role in the direct or adjunctive treatment of persistent inflammatory or non-inflammatory visceral pain. This possibility will require future clinical evaluation.

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Figure 1. Effects of systemic sumatriptan in experimental visceral pain in rats

A) Time course of the effects of sumatriptan in rats with pancreatitis (DBTC) or without pancreatitis (vehicle). B) Dose-response curve of sumatriptan 40 min after i.p. injection in rats with experimental pancreatitis. C) Time course of the effects of sumatriptan in rats with colonic hypersensitivity (butyrate) or controls (saline). D) Dose-response curve of sumatriptan 40 min after i.p. injection in rats with experimental colonic hypersensitivity (N=8 per dose).

Figure 2. Effects of systemic serotonin antagonists on the effects of systemic sumatriptan

A) In rats with experimental pancreatitis (DBTC-injected), sumatriptan (300 μ g/kg; i.p.) attenuated the frequency of withdrawals compared with rats receiving i.p. saline (# denotes $P < 0.05$ vs. saline group). The 5HT_{1B} antagonist isamoltane (4 mg/kg; i.p.) reduced the effects of sumatriptan. The 5HT_{1D} antagonist BRL15722 (0.3 mg/kg; i.p.) also reduced the effects of systemic sumatriptan (* denotes $P < 0.05$ vs. sumatriptan group). B) In rats with experimental colonic hypersensitivity (butyrate-injected), sumatriptan (300 μ g/kg; i.p.) increased the mechanical threshold compared to rats receiving saline (# denotes $P < 0.05$ vs. saline group). The 5HT_{1B} antagonist isamoltane (4 mg/kg; i.p.) reduced the effects of sumatriptan. The 5HT_{1D} antagonist BRL15722 (0.3 mg/kg; i.p.) also reduced the effects of systemic sumatriptan. (* denotes $P < 0.05$ vs. control group with no colonic hypersensitivity; $N = 8$ per experimental group).

Figure 3. Effects of microinjection of sumatriptan into the RVM in experimental visceral pain in rats

A) Time course of the effects of RVM sumatriptan in rats with pancreatitis (DBTC) or without pancreatitis (vehicle). B) Dose-response curve of sumatriptan 40 min after microinjection into the RVM of rats with experimental pancreatitis. C) Time course of the effects of RVM sumatriptan in rats with colonic hypersensitivity (butyrate) or in controls (saline). D) Dose-response curve of sumatriptan 40 min after microinjection into the RVM of rats with experimental colonic hypersensitivity (N=8 per dose).

Figure 4. Effects of RVM serotonin antagonists on the antinociceptive effects of RVM sumatriptan

A) In rats with experimental pancreatitis (DBTC-injected), sumatriptan (10 μ g) microinjected in the RVM attenuated the frequency of withdrawals compared with rats receiving saline in the RVM (# denotes $P < 0.05$ vs. saline group). The 5HT_{1B} antagonist isamoltane (3 μ g) blocked the effects of sumatriptan (* denotes $P < 0.05$ vs. control group with no pancreatitis). The 5HT_{1D} antagonist BRL15722 (3 μ g) did not have any effects. B) In rats with experimental colonic hypersensitivity (butyrate-injected), sumatriptan (10 μ g) microinjected in the RVM increase the mechanical threshold compared with rats receiving

saline in the RVM (# denotes $P < 0.05$ vs. saline group). The 5HT_{1B} antagonist isamoltane (3 μ g) blocked the effects of sumatriptan (* denotes $P < 0.05$ vs. control group with no colonic hypersensitivity; N=8 per experimental group). The 5HT_{1D} antagonist BRL15722 (3 μ g) did not have any effects.

Figure 5. Effects of serotonin antagonists microinjected in the RVM on the effects of systemic sumatriptan

A) In rats with experimental pancreatitis (DBTC-injected), sumatriptan (300 $\mu\text{g}/\text{kg}$; i.p.) attenuated the frequency of withdrawals compared with rats receiving saline (# denotes $P < 0.05$ vs. saline group). The 5HT_{1B} antagonist isamoltane (3 μg) in the RVM failed to antagonize the effects of sumatriptan. The 5HT_{1D} antagonist BRL15722 (3 μg), in the RVM, failed to antagonize the effects of systemic sumatriptan. B) In rats with experimental colonic hypersensitivity (butyrate-injected), sumatriptan (300 $\mu\text{g}/\text{kg}$; i.p.) increased the mechanical threshold compared to rats receiving saline (# denotes $P < 0.05$ vs. saline group). The 5HT_{1B} antagonist, isamoltane (3 μg) in the RVM failed to antagonize the effects of sumatriptan. The 5HT_{1D} antagonist BRL15722 (3 μg) failed to antagonize the effects of systemic sumatriptan. (* denotes $P < 0.05$ vs. control group with no colonic hypersensitivity; $N = 8$ per experimental group).