Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 494 (2004) 23–29

www.elsevier.com/locate/ejphar

Effect of the $5HT_{1A}$ receptor partial agonist buspirone on colorectal distension-induced pseudoaffective and behavioral responses in the female Wistar rat

Digavalli V. Sivarao*, Kimberly Newberry, Nicholas J. Lodge

Neuroscience Drug Discovery (3CD-422), Pharmaceutical research Institute, Bristol Myers Squibb Co., 5 Research Parkway, Wallingford, CT 06067, USA

Received 19 November 2003; received in revised form 14 April 2004; accepted 21 April 2004

Abstract

In the present study, we have evaluated the visceral analgesic property of buspirone, a $5HT_{1A}$ receptor partial agonist, on colorectal distension-induced mean arterial pressure and behavioral changes in anesthetized and awake Wistar rats, respectively. The selection of the rat strain was based on the observation that anesthetized Wistar rats exhibited a more prominent mean arterial pressure change in response to colorectal distention when compared to other strains (Sprague–Dawley, Wistar–Kyoto and Spontaneously Hypertensive). Buspirone dose-dependently (0.1–1 mg/kg, i.v.) antagonized mean arterial pressure change over a range of distensions (10–90 mmHg). In parallel studies conducted in awake animals, buspirone (1–5 mg/kg, s.c.) attenuated the abdominal withdrawal response, a nociceptive behavior, in response to colorectal distension. This effect was antagonized by co-administration of the 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2- pyridinylcyclohexanecarboxamide (WAY-100635) (5 mg/kg, s.c.). We conclude that buspirone exhibits significant visceral analgesic property in two models of abdominal nociception.

Keywords: Colorectal distension; Visceral pain; Anti-nociception; 5-HT_{1A} receptor; Buspirone

1. Introduction

Chronic visceral pain in the absence of organic abnormality is a hallmark of irritable bowel syndrome and other functional bowel disorders (Mertz, 2003a). While the etiology of abdominal pain is not well understood, it is believed that an enhanced sensitivity to distension may be an important factor (Ritchie, 1973). It is not clear whether this enhancement is mediated peripherally during afferent transmission or in the central processing of that signal (Mertz, 2003b). There is evidence for modulation of both peripheral and central neuronal targets to increase visceral sensitivity. For example, an acute inflammatory insult to the colon exaggerates pelvic afferent firing to distension (Coelho et al., 2000; Su et al., 1997). Moreover, repeated application of a physical insult causes a long lasting sensitization in the second-order neurons within the spinal cord, an effect that persists long after the injury is resolved (Al-Chaer et al., 2000). In addition to physical stress, postnatal maternal separation trauma instigates a long lasting visceral hypersensitivity (Coutinho et al., 2002).

Serotonin is an important neurotransmitter within the enteric nervous system, modulating the motility and secretion functions of the gastrointestinal tract through a diversity of serotonin receptor subtypes (for a review, see Gershon, 1999). Alosetron, a selective 5-HT_3 receptor antagonist, blocks serotonin-induced depolarization of extrinsic enteric afferents (Gershon, 1999) and was effective in attenuating colonic distension-induced autonomic and neuronal responses in the anesthetized rat (Kozlowski et al., 2000). In the clinic, alosetron has been shown to help a subset of patients suffering from irritable bowel syndrome (Camilleri et al., 2001).

The $5HT_{1A}$ subtype is present on the cell bodies of afterhyperpolarization-type sensory afferents in the gut, where it mediates hyperpolarization of the cell membrane

* Corresponding author. Tel.: +1-203-677-7439; fax: +1-203-677-7569.

E-mail address: siva.digavalli@bms.com (D.V. Sivarao).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.04.034

through an increase in K^+ conductance (Galligan et al., 1988). In other myenteric neurons, through a presynaptic locus, $5HT_{1A}$ receptors inhibit the release of neurotransmitters mediating both fast and slow excitatory postsynaptic potentials (Galligan, 1996), possibly leading to an inhibition in the local gut reflexes.

Within the central nervous system, $5HT_{1A}$ receptors have been demonstrated in the superficial laminae of the spinal dorsal horn, the dorsal and medial raphe nuclei, limbic areas of the hippocampus and lateral septum, and in cingulate and entorhinal cortex (for a review, see Barnes and Sharp, 1999). When injected into the dorsal horn, 5-HT_{1A} receptor agonists depress activity of neurons involved in the transmission of nociceptive signals (Gjerstad et al., 1996). The endogenous input to the dorsal horn neurons arises in part from descending fibers of the nucleus raphe magnus (Zemlan et al., 1994).

In healthy volunteers that ingested a maximum tolerated volume of fluid, the 5-HT_{1A} receptor partial agonist buspirone decreased postprandial symptoms and nausea (Chial et al., 2003). In a preliminary clinical study (Tack et al., 1999), buspirone was useful in treating the symptoms of non-ulcer dyspepsia. Moreover, a full agonist at 5-HT_{1A} receptors, 8-OH DPAT, reduced gastric distension-induced nociception (Rouzade et al., 1998). While the mechanism of action is unknown, it is possible that the 5-HT_{1A} receptor-mediated relaxation of the fundic tone and an inhibition in sensory transmission may be involved. Thus, activation of 5-HT_{1A} receptors both peripherally and centrally appears to modulate visceral sensitivity.

In the current study, using colorectal distension-induced depressor response in mean arterial pressure as an index, we first sought to identify a sensitive strain for visceral nociception among the commonly used albino rat strains. Second, we tested the hypothesis that the 5-HT_{1A} receptor partial agonist buspirone is effective in reducing colorectal distension-induced autonomic (blood pressure) and behavioral (abdominal withdrawal response) changes in anesthetized and conscious rats, respectively.

2. Materials and methods

2.1. General

All animals were obtained from Harlan Bioproducts (Indianapolis, IN). On the night before the experiment, animals were denied food (for ~ 14 h) but not water. To identify the most suitable strain, comparison of distension-induced change in mean arterial pressure between outbred Sprague–Dawley, Wistar, inbred Wistar–Kyoto and Spontaneously Hypertensive strains, was performed in female rats of similar age (range 9–12 weeks); estrus cycle was not monitored. All experimental procedures were approved by the Bristol Myers Squibb Institutional Animal Care and Use Committee.

2.2. Surgery

On the day of the experiment, the animal was anesthetized with pentobarbital (40–60 mg/kg, i.p.) and equipped with indwelling femoral arterial and venous catheters for cardiovascular (mean arterial pressure and heart rate) monitoring and drug administration, respectively. Anesthesia was maintained by a constant pentobarbital infusion (5–8 mg/kg/h) throughout the experiment. Even though the animals breathed spontaneously, tracheotomy followed by cannulation was performed for mechanical ventilation if needed.

2.3. Colorectal distension

The colorectal distension procedure outlined here is modified from a well characterized model of abdominal nociception (for a review, see Gebhart and Sengupta, 1996). To facilitate balloon insertion, an isotonic saline enema (5–7 ml/rat) was given using flexible gavage tubing. A 6-cm-long lubricated balloon catheter, fashioned out of the middle digit of a medium-sized latex glove and PE250 tubing, was introduced into the colon such that the base of the balloon was 1-cm orad to the anus and fastened to the base of the tail. Through a three-way cannula, the balloon catheter was connected to a sphygmomanometer and a 10-ml syringe for making pressure-controlled distensions.

2.4. Subject selection

Once a stable blood pressure was established, the rat was subjected to a test distension of 90 mmHg that lasted for 20 s. The transient change in mean arterial pressure was noted and this procedure was repeated three times with a 5-min interval. The test distensions were used to verify consistency in response. If two consecutive distensions resulted in responses that differed from each other by greater than 20%, they were repeated until a consistent response was obtained. Generally, this criterion was met easily within six test distensions. A second criterion that was only applied to the Wistar rat was that the minimum change in the mean arterial pressure to a 90 mmHg test distension was required to be 15 mm or greater. Animals that did not meet this criterion were not included in the experiment. Typically, less than 20% of animals were rejected based on the above criteria.

2.5. Single distension protocol

This was done to rapidly evaluate efficacy. Once a stable baseline response to 90 mmHg distension was established, animals were subjected to regular distensions before and after vehicle and drug injections. A 10-min distribution time was allowed between intravenous injection and distension. A 5-min period between distensions allowed ample time for the distension-induced blood pressure change to return to baseline.

2.6. Graded distension protocol

In a separate group of animals, a more elaborate response to graded distensions (10–90 mmHg) was recorded before and after treatment. The post-treatment distribution time and the interval between distensions were same as above. Graded distensions were also used to identify the most sensitive strain for distension-induced change in mean arterial pressure.

2.7. Colorectal distension in conscious animals

Animals were anesthetized with an ultra-short acting barbiturate (methohexital sodium, 50 mg/kg, i.p.) and prepared for distension as outlined with the exception of surgical procedure. After balloon catheterization, animals were allowed to recover for a 60- to 90-min period as required. Each animal was administered vehicle or drug (s.c.) in a blinded design. After a 20-min period, the animal was placed on a raised platform and subjected to colorectal distensions at 10, 30, 60 and 90 mmHg. Each distension lasted for 20 s with a 3-min interval between stimuli. Abdominal withdrawal response was quantified as follows: an abdominal indentation was scored 0.5, a slight lifting of the abdomen off the platform was scored 1, a prominent lift (1 cm or more off the platform) was scored 2 and if the latter was accompanied by a convex hunching of the back, it was scored 3. An average score from three consecutive trials was recorded for each animal.

2.8. Drug treatments

Morphine sulfate, *R*(+)-8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) and *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl cyclohexane carboxamide (WAY-100635) were purchased from Sigma-Aldrich (St. Louis, MO) while buspirone was obtained from the in-house compound repository. All drugs were dissolved in normal saline. Slow intravenous dosing was performed over a period of 2 min followed by a 10 min distribution time. In the behavioral study, after the animal recovered completely from anesthesia, it was treated subcutaneously with the test agent(s) and tested for distension response 20 min after. For convenience, the seven treatment groups were broken into two separate blocks of experiments [block 1: vehicle, buspirone (1 and 3 mg/kg) and morphine (5 mg/kg); block 2: WAY100635 (5 mg/kg, s.c.), buspirone (5 mg/kg, s.c.) and WAY + buspirone (5 mg/kg each)]. All treatments were coded by individuals unconnected to the study and the code was revealed only after completion of the study.

2.9. Statistical analysis

Mean arterial pressure changes to distension following treatment for each animal was noted and pooled to arrive at group means. The means were compared to vehicle mean using a one-way analysis of variance (ANOVA) with or without repeated-measures as appropriate, followed by Tukey's (for multiple comparisons) or Dunnett's (for comparison with vehicle group) posthoc tests using a commercially available statistical software (Graphpad-Prism version 3.00, GraphPad, San Diego, CA). Statistical significance was fixed at a $P < 0.05$.

3. Results

3.1. Strain comparison

The objective of this study was to identify the most responsive rat strain for distension-mediated change in mean arterial pressure. As expected, distension produced a stimulus-dependent decrease in all strains. While qualitatively the response to distension in each strain was identical, they differed in the response magnitude. The decrease in mean arterial pressure to graded distensions, summarized in Fig. 1A, shows that Wistar rat was the most responsive and WKY the least. For example, in response to a 90 mmHg distension, Wistar rats showed a 36 ± 4 mmHg mean reduction while WKY rats showed a fall of 18 ± 2 mmHg ($P < 0.001$; one-way ANOVA with Tukey's post-test). However, when the reduction was expressed as percent of maximum response, each strain showed an superimposable stimulus–response relationship (not shown). Typical mean

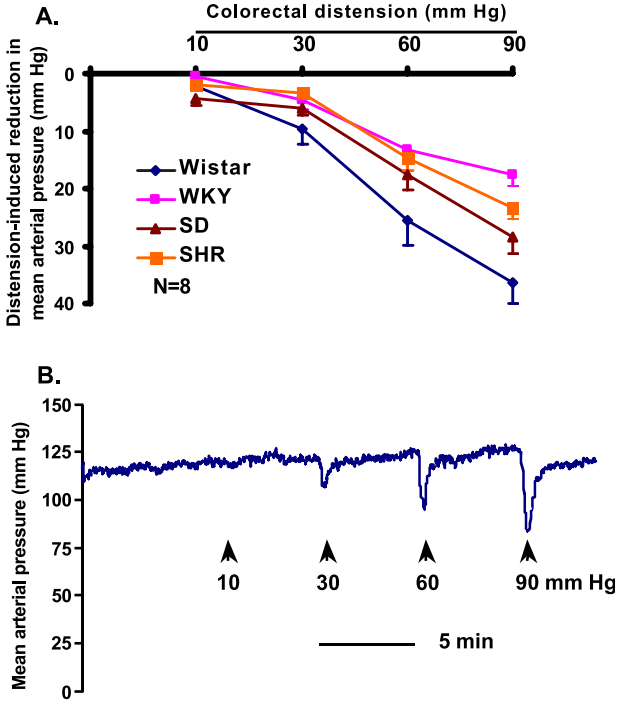

Fig. 1. (A) Colorectal distension-induced reduction in mean arterial pressure in four strains of anesthetized female rats. (B) A sample trace showing transient, distension-dependent depressor response in a female Wistar rat. Arrow head marks a 20-s distension.

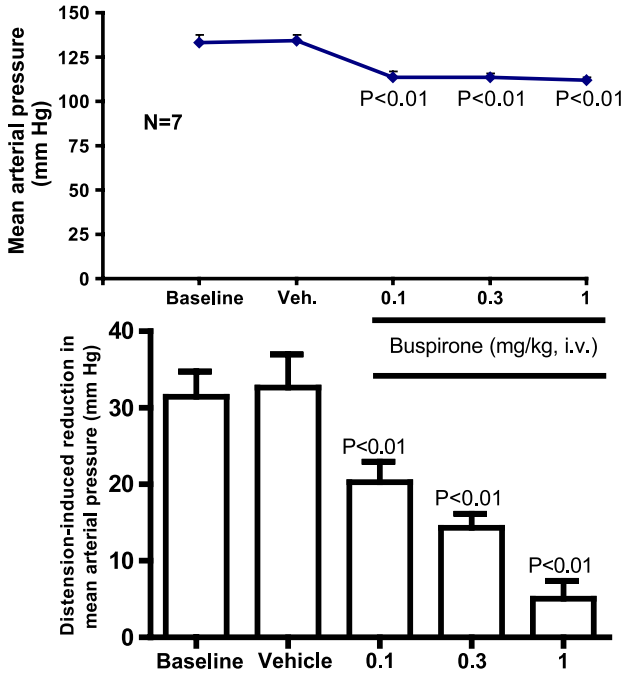

Fig. 2. (Top panel) Mean arterial pressure before and after intravenous vehicle and drug administration. A comparison between mean arterial pressure after vehicle (1 ml/kg, i.v.) and buspirone (0.1–1 mg/kg) treatments was made using a one-way ANOVA with repeated measures followed by Dunnett's post-hoc analysis. Note the "first-dose" hypotension with buspirone. (Bottom panel) Buspirone showed a dose-dependent attenuation of the depressor response elicited by a 90 mm Hg distension. Statistical analysis was the same as above.

arterial pressure changes accompanying graded colorectal distension in a Wistar rat are depicted in Fig. 1B. The resting blood pressure (mmHg) of Wistar (122 ± 4), Wistar–Kyoto (122 ± 6) and Sprague–Dawley (135 ± 5) strains were similar and significantly lower than that of the spontaneously hypertensive strain (173 ± 3) ($P < 0.001$; one-way ANOVA with Tukey's post-test). On the other hand, mean heart rate for all the strains ranged from 305 ± 15 (Wistar–Kyoto) to 319 ± 11 (Wistar) beats/min ($P > 0.05$; one-way ANOVA with Tukey's post-test). Female Wistar rats were employed in the following studies.

3.2. Effect of cumulative doses of buspirone on resting and distension-induced change in blood pressure

The resting blood pressure in anesthetized rats and the effects of vehicle and cumulative doses of buspirone are summarized in Fig. 2(top panel). Vehicle alone (1 ml/kg) had no effect on the resting pressure (before, 133 ± 4 mmHg; after, 134 ± 3 mmHg; $P > 0.05$, $N = 7$). In contrast, buspirone injection produced a "first dose" hypotensive effect; that is, the mean arterial pressure decreased from 134 ± 3 to 114 ± 3 mmHg ($P < 0.01$, $N = 7$) in response to the initial dose (0.1 mg/kg, i.v.). This decrease was sustained throughout the experiment. Subsequent doses of buspirone (up to 1 mg/kg) had no further effect on the resting blood pressure (1 mg/kg, 112 ± 2 mmHg). Whereas, the distension-mediated depressor response was dose-dependently attenuated by cumulative doses of buspirone (Fig. 2, bottom panel). Thus, buspirone at 0.1 mg/kg dose produced a 33% (% vehicle; $P < 0.01$) attenuation and at 1 mg/kg, an 86% ($P < 0.01$, $N = 7$) attenuation in the depressor response.

Pretreatment with a low dose (0.1 mg/kg, i.v.) of the 5-HT_{1A} receptor antagonist WAY-100635 partially blocked buspirone's (0.1 mg/kg, i.v.) effect on distension-induced response (% control, buspirone: 67 ± 10; WAY-100635 + buspirone: 85 ± 7; $N = 7–8$; $P > 0.05$). A higher dose of the antagonist was not tested because WAY-100635 alone at 1 mg/kg i.v., caused a marked and long-lasting (>30 min) reduction in blood pressure (data not shown).

3.3. Effect of buspirone on mean arterial pressure response to graded distensions

To further examine the buspirone effect, graded distensions (10–90 mmHg) were performed (Fig. 3). The distension response was progressively and robustly attenuated by increasing doses of buspirone. At a dose of 1 mg/kg, buspirone completely abolished colorectal distension-induced depressor response at all intensities (Fig. 3).

3.4. Effect of buspirone on behavioral response to colorectal distension in awake rats

Colorectal distension produced clear behavioral responses in conscious rats. The typical response included ceasing to move in response to the initiation of distension and thereafter showing an abdominal response of tightening and withdrawal that progressed with increased intensity of

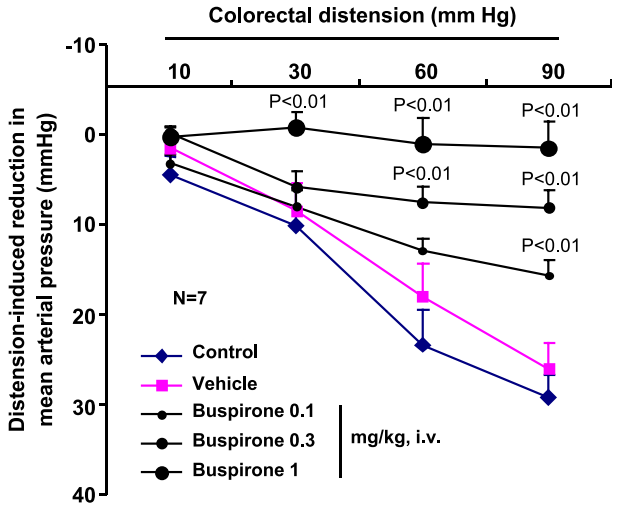

Fig. 3. Buspirone mediates a dose-dependent attenuation of distension-induced mean arterial pressure response over a range of distensions. At each distension, comparison between vehicle (1 ml/kg, i.v.) and buspirone (0.1–1 mg/kg) treated groups was made using a one-way ANOVA with repeated measures followed by Dunnett's post-hoc analysis.

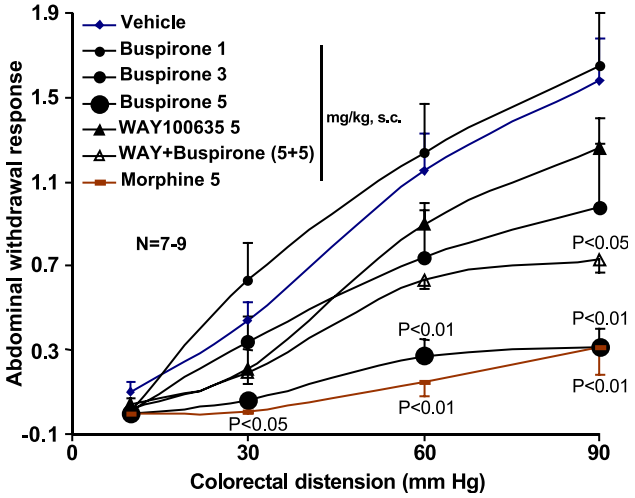

Fig. 4. Buspirone dose-dependently attenuated abdominal withdrawal response to distension in awake rats. Co-administration of WAY-100635, a selective 5-HT_{1A} antagonist, reduced buspirone's effectiveness. Vehicle (1 ml/kg, s.c.) or drug(s) was administered at time zero and animals were subjected to graded distensions 20 min after. Morphine was used as a positive control. Analysis was similar to Fig. 3 without repeated measures.

distension. Buspirone dose-dependently (1, 3, 5 mg/kg) decreased the abdominal withdrawal response to distension with the highest dose showing a significant reduction at 60 and 90 mmHg distensions (Fig. 4). Lower lip retraction was occasionally noted in the buspirone-treated animals. No other gross motor effects were observed.

The selective 5-HT_{1A} receptor antagonist, WAY-100635 (5 mg/kg) alone did not affect the distension-induced abdominal withdrawal response. In contrast, when co-administered with a high dose of buspirone (5 mg/kg), WAY-100635 (5 mg/kg) attenuated buspirone's effect by shifting the curve upwards (Fig. 4). However, a significant reduction in abdominal withdrawal response, albeit reduced in magnitude, was still observed at 90 mmHg distension. As expected, morphine (5 mg/kg), administered as a positive control, showed a marked attenuation of abdominal withdrawal response to 30 mmHg distension ($P<0.05$) and above ($P<0.01$).

4. Discussion

We identified the outbred Wistar female rat to be the most sensitive to colorectal distension-induced depressor response among the four strains of rats tested. Using this strain, we found that the 5-HT_{1A} receptor partial agonist, buspirone, dose-dependently inhibited responses to distension in two experimental models of visceral nociception.

Of the strains tested, anesthetized Wistar rats consistently showed the most robust mean arterial pressure response to distension. Conversely, anesthetized Wistar–Kyoto rats showed the least response. Interestingly, in a study that compared abdominal contraction in response to sustained colorectal distension in awake rats (Gunter et al., 2000), Wistar–Kyoto strain was the most responsive. Since this strain is known to be highly susceptible to anxiety, the heightened response to distension in awake animals may be a result of stress. Stress has been previously shown to exacerbate visceral pain response (Gue et al., 1997). Despite the difference between the response magnitude, the stimulus–response relationship was identical between strains. That is, the intensity coding of the stimulus was identical in all strains.

Buspirone (0.1 mg/kg) produced a robust drop of approximately 20 mmHg in resting blood pressure in anesthetized animals. This drop was sustained throughout the experiment. Subsequent cumulative doses of buspirone had no further effect on the resting pressure while progressively and steeply attenuating the distension-induced depressor response. Thus, the buspirone-induced attenuation of the nociceptive response was measured against a stable mean arterial pressure background. In a separate set of experiments, buspirone produced a dose-dependent inhibition of responses to the entire range of distensions, not only confirming the finding from the single distension study but also extending this to a range of stimuli.

Buspirone-induced hypotension is likely mediated through 5-HT_{1A} receptors located on the cell bodies of the sympathoexcitatory neurons in the rostroventrolateral medulla (Kubo et al., 1995). The neurons of this region project excitatory input to the sympathetic motor neurons in the intermediolateral cell column of the thoracolumbar spinal cord (Brown and Guyenet, 1984), which in turn provide the excitatory drive to the myocardium and the resistance arterioles. Agonism at 5-HT_{1A} receptor-bearing neurons of the rostroventrolateral medulla, causes a reduction in the sympathetic outflow leading to systemic hypotension (Bago and Dean, 2001).

The pharmacological basis for buspirone's attenuation of the distension-induced responses in anesthetized rats could not be further characterized as the 5-HT_{1A} antagonist WAY-100635 itself at a dose of 1 mg/kg showed prominent inhibition of the resting blood pressure. Unlike buspirone, the hypotensive effect of the antagonist is unlikely to be related to the alteration of sympathetic tone (Bago and Dean, 2001). Instead, at this dose, it is likely to be due to blockade of peripheral α_1-adrenoceptors (Villalobos-Molina et al., 2002). This illustrates a drawback of using change in mean arterial pressure as a sole index of nociception.

Lastly, we tested buspirone in awake animals using the previously characterized abdominal withdrawal response to distension as an index (Al-Chaer et al., 2000; Gebhart and Sengupta, 1996). Buspirone, at doses that showed no gross behavioral or locomotor effects, produced a dose-dependent reduction in distension-mediated response over a range of stimuli. At the highest dose tested, buspirone markedly attenuated the abdominal withdrawal response. Buspirone's efficacy was partially antagonized by pretreatment with the 5-HT_{1A} antagonist WAY-100635. WAY-100635 alone did

not interfere with the distension-mediated response. Indeed, animals treated with WAY-100635 alone responded no differently from vehicle-treated animals. If these animals were hypotensive as a result of α_1-adrenoceptor antagonism, it did not appear to affect the gross behavior of the animals or the assay.

Even though buspirone is generally regarded as a partial agonist at 5-HT_{1A} receptors, its efficacy spans a wide spectrum depending on such factors as the assay measure, receptor reserve and receptor–effector coupling. Buspirone has been shown to act as a full agonist at the somatodendritic autoreceptors in the midbrain raphe (Meller et al., 1990) while being less efficacious on hippocampal postsynaptic receptors (De Vivo and Maayani, 1986). Correspondingly, in appropriate behavioral tests, it is not unusual that buspirone shows full agonist-like efficacy. For example, in the lower lip retraction assay (Koek et al., 2000), as well as in a model of thermal nociception (Robles et al., 1996), buspirone showed an effect comparable to that of a full agonist, although with a lower potency.

That buspirone produced a first-dose hypotension that did not increase with increased doses of the drug while causing an apparent dose-dependent inhibition of the distension-related measures suggest that the hypotensive and antinociceptive effects may be mediated through separate loci; buspirone acts as a partial agonist at the hypotensive locus and as a full agonist at the antinociceptive site. In whole animal studies such as these, it is not possible to say with a measure of certainty, where exactly the locus of action is but only that buspirone was clearly efficacious in two complementary indices of visceral pain.

In summary, of the four strains of anesthetized rats tested for colorectal distension-induced pseudoaffective response, Wistar was the most responsive. In the Wistar rat, buspirone produced a dose-dependent attenuation in colorectal distension-induced changes in mean arterial pressure and abdominal withdrawal in anesthetized and conscious rats, respectively. Buspirone's effects appear to be mediated through 5-HT_{1A} receptors.

References

Al-Chaer, E.D., Kawasaki, M., Pasricha, P.J., 2000. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. Gastroenterology 119, 1276–1285.

Bago, M., Dean, C., 2001. Sympathoinhibition from ventrolateral periaqueductal gray mediated by 5-HT(1A) receptors in the RVLM. Am. J. Physiol. Regul. Integr. Comp. Physiol. 280, 976–984.

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38, 1083–1152.

Brown, D.L., Guyenet, P.G., 1984. Cardiovascular neurons of brain stem with projections to spinal cord. Am. J. Physiol. 247, 1009–1016.

Camilleri, M., Chey, W.Y., Mayer, E.A., Northcutt, A.R., Heath, A., Dukes, G.E., McSorley, D., Mangel, A.M., 2001. A randomized controlled clinical trial of the serotonin type 3 receptor antagonist alosetron in women with diarrhea-predominant irritable bowel syndrome. Arch. Intern. Med. 161, 1733–1740.

Chial, H.J., Camilleri, M., Burton, D., Thomforde, G., Olden, K.W., Stephens, D., 2003. Selective effects of serotonergic psychoactive agents on gastrointestinal functions in health. Am. J. Physiol. Gastrointest. Liver Physiol. 284, G130–G137.

Coelho, A.M., Fioramonti, J., Bueno, L., 2000. Systemic lipopolysaccharide influences rectal sensitivity in rats: role of mast cells, cytokines, and vagus nerve. Am. J. Physiol. Gastrointest Liver Physiol. 279, G781–G790.

Coutinho, S.V., Plotsky, P.M., Sablad, M., Miller, J.C., Zhou, H., Bayati, A.I., McRoberts, J.A., Mayer, E.A., 2002. Neonatal maternal separation alters stress-induced responses to viscerosomatic nociceptive stimuli in rat. Am. J. Physiol. Gastrointest. Liver Physiol. 282, G307–G316.

De Vivo, M., Maayani, S., 1986. Characterization of the 5-hydroxytryptamine1a receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. J. Pharmacol. Exp. Ther. 238, 248–253.

Galligan, J.J., 1996. Electrophysiological studies of 5-hydroxytryptamine receptors on enteric neurons. Behav. Brain Res. 73, 199–201.

Galligan, J.J., Surprenant, A., Tonini, M., North, R.A., 1988. Differential localization of 5-HT1 receptors on myenteric and submucosal neurons. Am. J. Physiol. 255, G603–G611.

Gebhart, G.F., Sengupta, J.N., 1996. Evaluation of visceral pain. In: Gaginella, T.S. (Ed.), Handbook of Methods in Gastrointestinal Pharmacology. CRC Press, Boca Raton, pp. 359–373.

Gershon, M.D., 1999. Review article: roles played by 5-hydroxytryptamine in the physiology of the bowel. Aliment. Pharmacol. Ther. 13 (Suppl 2), 15–30.

Gjerstad, J., Tjolsen, A., Hole, K., 1996. The effect of 5-HT1A receptor stimulation on nociceptive dorsal horn neurones in rats. Eur. J. Pharmacol. 318, 315–321.

Gue, M., Del Rio-Lacheze, C., Eutamene, H., Theodorou, V., Fioramonti, J., Bueno, L., 1997. Stress-induced visceral hypersensitivity to rectal distension in rats: role of CRF and mast cells. Neurogastroenterol. Motil. 9, 271–279.

Gunter, W.D., Shepard, J.D., Foreman, R.D., Myers, D.A., Greenwood-Van Meerveld, B., 2000. Evidence for visceral hypersensitivity in high-anxiety rats. Physiol. Behav. 69, 379–382.

Koek, W., Assie, M.B., Zernig, G., France, C.P., 2000. In vivo estimates of efficacy at 5-HT1A receptors: effects of EEDQ on the ability of agonists to produce lower-lip retraction in rats. Psychopharmacology (Berl.) 149, 377–387.

Kozlowski, C.M., Green, A., Grundy, D., Boissonade, F.M., Bountra, C., 2000. The 5-HT(3) receptor antagonist alosetron inhibits the colorectal distention induced depressor response and spinal c-*fos* expression in the anaesthetised rat. Gut 46, 474–480.

Kubo, T., Taguchi, K., Ozaki, S., Amano, M., Ishizuka, T., 1995. 8-OH-DPAT-induced hypotensive action and sympathoexcitatory neurons in the rostral ventrolateral medulla of the rat. Brain Res. Bull. 36, 405–411.

Meller, E., Goldstein, M., Bohmaker, K., 1990. Receptor reserve for 5-hydroxytryptamine1A-mediated inhibition of serotonin synthesis: possible relationship to anxiolytic properties of 5-hydroxytryptamine1A agonists. Mol. Pharmacol. 37, 231–237.

Mertz, H., 2003a. Review article: visceral hypersensitivity. Aliment. Pharmacol. Ther. 17, 623–633.

Mertz, H.R., 2003b. Overview of functional gastrointestinal disorders: dysfunction of the brain–gut axis. Gastroenterol. Clin. North Am. 32, 463–476.

Ritchie, J., 1973. Pain from distension of the pelvic colon by inflating a balloon in the irritable colon syndrome. Gut 14, 125–132.

Robles, L.I., Barrios, M., Del Pozo, E., Dordal, A., Baeyens, J.M., 1996. Effects of K^+ channel blockers and openers on antinociception induced by agonists of 5-HT1A receptors. Eur. J. Pharmacol. 295, 181–188.

Rouzade, M.L., Fioramonti, J., Bueno, L., 1998. Decrease in gastric sensitivity to distension by 5-HT1A receptor agonists in rats. Dig. Dis. Sci. 43, 2048–2054.

Su, X., Sengupta, J.N., Gebhart, G.F., 1997. Effects of kappa opioid recep-

tor-selective agonists on responses of pelvic nerve afferents to noxious colorectal distension. J. Neurophysiol. 78, 1003–1012.

Tack, J., Piessevaux, H., Coulie, B., Caenepeel, D., Janssens, J., 1999. A placebo-controlled trial of buspirone, a fundus relaxing drug, in functional dyspepsia: effect on symptoms and gastric sensory and motor function. Gastroenterology 116, G1423 (Abstract).

Villalobos-Molina, R., Lopez-Guerrero, J.J., Gallardo-Ortiz, I.A., Ibarra, M., 2002. Evidence that the hypotensive effect of WAY 100635, a 5-HT1A receptor antagonist, is related to vascular alpha1-adrenoceptor blockade in the adult rat. Auton. Autacoid Pharmacol. 22, 171–176.

Zemlan, F.P., Murphy, A.Z., Behbehani, M.M., 1994. 5-HT1A receptors mediate the effect of the bulbospinal serotonin system on spinal dorsal horn nociceptive neurons. Pharmacology 48, 1–10.