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Clonidine and guanfacine-induced antinociception in visceral pain: possible role of α_2/I_2 binding sites

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Abstract

Visceral pain is one of the most common forms of pain which is poorly understood. We now studied the influence of imidazoline/guanidinium compounds such as clonidine and guanfacine on visceral pain in the presence or absence of yohimbine and benazoline. To produce visceral pain-related behaviours, formalin (10%) was administered by inserting a fine cannula into the colon via the anus. Each experiment took 1 h. Clonidine (0.001, 0.01 and 0.1 mg/kg, i.p.) and guanfacine (2.5, 5 and 10 mg/kg, i.p.) produced analgesia dose dependently. The clonidine response was inhibited by yohimbine (0.2 mg/kg, i.p.). On the other hand, benazoline (5 mg/kg, i.p.) blocked the antinociceptive effect of guanfacine (5 mg/kg). Benazoline (2.5 and 5 mg/kg) itself also induced analgesia in inflammatory colonic pain. In this study, we used morphine to ensure that the behavioural responses were pain-related. Our results showed that morphine (2.5, 5 and 10 mg/kg, s.c.) produced a dose-dependent antinociception. The morphine (7 mg/kg, s.c.) response was reduced by naloxone (2 mg/kg, i.p.). However, we concluded that both imidazoline (I_2) and α_2-adrenoceptors may play a role in producing analgesia in visceral pain.

Keywords: Clonidine; Guanfacine; Benazoline; Visceral pain; Formalin; Imidazoline receptor

1. Introduction

Visceral pain is one of the most frequent reasons for patients to seek medical care. Since abdominal visceral pain is difficult to treat clinically, there is a need for further studies aimed at understanding visceral pain and finding suitable agents to relieve pain.

Over recent years, it has become clear that α_2-adrenoceptor ligands with an imidazoline or guanidine moiety, such as clonidine and guanfacine, bind not only to α_2-adrenoceptors but also to a novel class of binding sites termed imidazoline receptors (Bousquet et al., 1992; Molderings et al., 1992). In the past decades, there has been considerable interest in the effects of imidazoline/guanidine compounds on pain.

However, it has been reported that clonidine as an antihypertensive drug produces analgesia in animals (Millan et al., 1994; Shannon and Lutz, 2000; Yaksh, 1985) and in clinical studies (Eisenach et al., 1995; Mendez et al., 1990). Besides, some work has supported the possibility that guanfacine, a guanidinium derivative, also has an analgesic effect in animals (Millan et al., 1994; Ossipov et al., 1984; Parale and Kulkarni, 1985; Smith et al., 1992).

During recent years, there has been a controversy over the involvement of imidazoline binding sites in the modulation of pain. Data remain controversial as to whether imidazoline (I_2) receptors enhance or suppress nociception (Diaz et al., 1997; Houghton and Westlund, 1996; Sanchez-Blazquez et al., 2000) and whether imidazoline (I_1) binding sites have any

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role in producing analgesia (Millan, 2002). In 2001, Laird et al. proposed a new model of visceral pain based on the intracolonic instillation of capsaicin and mustard oil via the anus in mice (Laird et al., 2001). We now tested the pain promoting effects of formalin when administered into the colon in mice. Behaviours were then recorded for 1 h. Morphine and naloxone were used to ensure that the behavioural patterns reflected pain. Benazoline is reported to be a ligand for imidazoline (I_2) receptors; however, its pharmacological effects are still being debated (Bruban et al., 2000; Greney et al., 2000; Polidori et al., 2000).

In this study, our aim was to investigate the antinociceptive action of clonidine and guanfacine in the presence or absence of yohimbine and benazoline in visceral pain and also to clarify the agonist or antagonist action of benazoline. Throughout the work, the guidelines proposed by the Committee on Research and Ethical Issues of IASP (Zimmermann, 1983) for investigations in experimental pain in animals were followed.

2. Materials and methods

2.1. Animals

Male NMRI mice (Institute Pasteur, Iran) weighing 25–30 g were used for all studies. The animals were housed in groups of 4 or 5 mice per cage at room temperature (22±1 °C) with a 12-h light–dark cycle (light on 8:30 a.m. to 8:30 p.m.). The animals had free access to food and water prior to the experiments. Testing took place between 9 a.m. and 3 p.m. The mice were habituated to the test environment for 30 min on the day before testing, and again for 1 h before the test started. The animals were fasted before the beginning of the experiment, used only for one procedure and were humanely killed on completion of the experiment.

2.2. Nociceptive stimulus

In 1994, Miampamba et al. suggested that intracolonic injection of formalin caused visceral pain in rats (Miampamba et al., 1994). During recent years, some scientists have shown that intracolonic administration of capsaicin and mustard oil produced inflammatory colonic pain in mice (Laird et al., 2001). In this experiment, the stimulus was formalin (10%). Under minimum restraint, 10 μl of formalin (10%) was administered by introducing a fine cannula with a rounded tip (external diameter=0.61 mm; 4 cm long) into the colon via the anus. To avoid stimulation of somatic areas by contact with the irritant chemicals, petroleum jelly (Vaseline) was applied in the perianal area.

2.3. Experimental protocol and drug administration

The study was conducted in two parts, each including control groups (i.e. intracolonic administration of saline and formalin without antinociception agent). The first part was designed to evaluate the antinociceptive effects of clonidine (0.001, 0.01 and 0.1 mg/kg, i.p.), guanfacine (2.5, 5 and 10 mg/kg, i.p.) and morphine (2.5, 5 and 10 mg/kg, s.c.) on inflammatory colonic pain induced by formalin.

In the second part of the study, yohimbine (0.1 and 0.2 mg/kg, i.p.) and benazoline (2.5 and 5 mg/kg, i.p.) were injected in mice 15 min prior to clonidine (0.01 mg/kg) and guanfacine (5 mg/kg) but naloxone (0.2, 2 mg/kg, i.p.) was administered 2 min before morphine (7 mg/kg). In both parts of the study, saline (7 ml/kg) was injected intraperitoneally for controls.

2.4. Nociceptive behaviour assessment

Selective behaviours had previously been reported to reflect visceral pain: licking of abdomen or perineal area (Abelli et al., 1989), stretching (Koster et al., 1959) and contraction of the flanks (Siegmund et al., 1957). These behaviours were observed in the present experiment after intracolonic instillation of formalin. We now tested the pain promoting effects of 10 μl formalin 10% when administered into the colon via the anus. Behavioural responses to formalin stimulation were then recorded for an hour. Each animal was kept in an individual Plexiglas observation chamber (30×30×50 cm) 20 min before the test was started. As soon as the intracolonic administration of formalin was completed, the rat was placed in its observation chamber. For an hour, the animal displayed various pain-related behaviours ranked by their order of intensity: (1) licking and grooming (L), (2) hiccups (H), (3) stretching and whole body contraction (C).

The nociceptive response (S) was then calculated for each 5-min time block, using the formula: S=1L+2H+3C.

To investigate the analgesic effects of the drugs, the area under the time-course curve ($AUC_{0–60}$) was calculated from individual scores at each time which indicated the pain score for an hour.

2.5. Drugs

The drugs were: morphine sulphate (Mac Farlan Smith, UK), clonidine hydrochloride (LEIRAS, Finland), yohimbine and guanfacine hydrochloride (Boehringer Ingelheim, Germany), naloxone hydrochloride (Tocris, UK), benazoline (Sigma, USA). All drugs were freshly dissolved in distilled water on the day of the experiment.

2.6. Statistical analysis

The results are presented as mean±S.E.M. values from six animals in each treatment group as area under the curve (AUC) which was calculated as the sum, over 1 h, of the individual values for each time point for each animal using the trapezoidal rule. The data were analysed using an

analysis of variance (ANOVA) followed by Newman–Keul's test. Significance was set at the $P<0.05$ level.

3. Results

3.1. Antinociception effect of morphine, clonidine and guanfacine on inflammatory colonic pain induced by formalin

Effects of morphine in comparison with those of saline injection are shown in Fig. 1. Subcutanoeus injection of morphine (2.5, 5 and 10 mg/kg) to mice induced dose-dependent antinociception [$F(3,20)=14.8$, $P<0.0001$] in visceral pain. Analgesic effects of clonidine are shown in Fig. 2. Intraperitoneal injection of clonidine (0.1, 0.01 and 0.001 mg/kg) produced antinociception in a dose dependent manner [$F(3,20)=20.7$, $P<0.0001$].

Effects of guanfacine on inflammatory colonic pain are shown in Fig. 3. Intraperitoneal administration of guanfacine (2.5, 5 and 20 mg/kg) increased analgesia dose dependently [$F(3,20)=43.1$, $P<0.0001$] in comparison with the effect in saline-injected animals.

3.2. Effects of naloxone on antinociception induced by morphine

In Fig. 4, naloxone (2 mg/kg) reduced the analgesia induced by morphine (7 mg/kg) significantly [$F(11,60)=5.9$, $P<0.0001$].

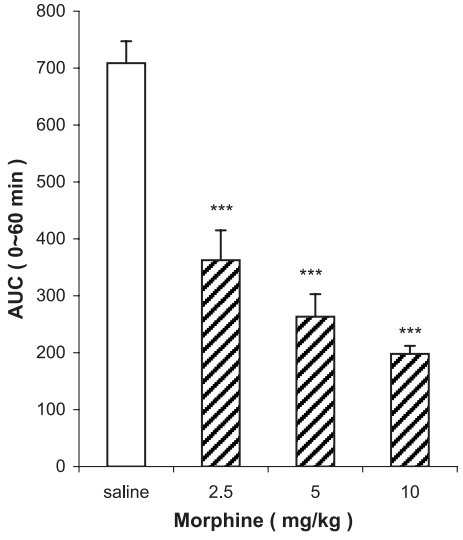

Fig. 1. Antinociceptive effect of different doses of morphine in visceral pain model. Animals were given (i.p.) saline (7 ml/kg) and (s.c.) different doses of morphine (2.5, 5 and 10 mg/kg) 15 min before formalin instillation. Antinociception was recorded 0–60 min after formalin administration. Results are expressed as area under the time curve (AUC). Each point is the mean±S.E.M. of area under the time curve of six mice. ***$P<0.001$ different from control group.

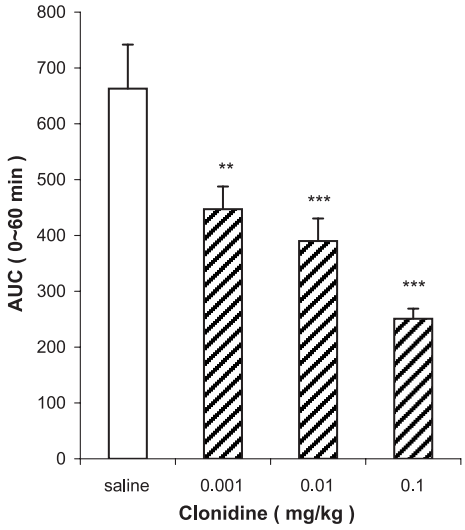

Fig. 2. Analgesic effect of different doses of clonidine on inflammatory colonic pain. Mice were treated (i.p.) either saline (7 ml/kg) or different doses of clonidine (0.001, 0.01 and 0.1 mg/kg) 15 min prior to formalin administration. Antinociception was recorded for 0–60 min after formalin instillation. Results are expressed as area under the time curve (AUC). Each point is the mean±S.E.M. of area under the time curve for six mice. **$P<0.01$, ***$P<0.001$ different from control group.

3.3. Yohimbine effects on analgesia induced by clonidine and guanfacine

In Fig. 5, the antinociceptive effects of clonidine and guanfacine are shown in the presence of yohimbine. One-way ANOVA indicated that yohimbine (0.2 mg/kg) decreased the analgesic effect of clonidine (0.01 mg/kg) significantly in

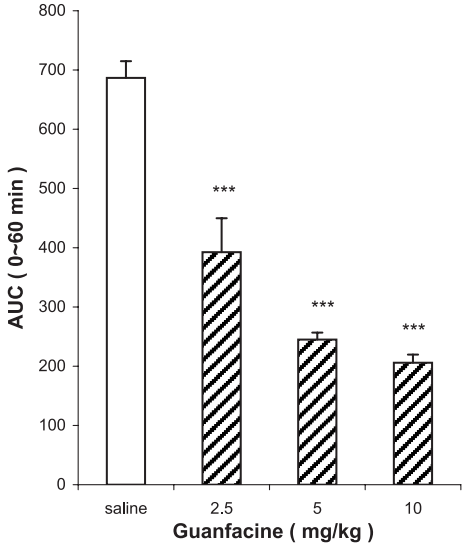

Fig. 3. Antinociception induced by different doses of guanfacine in viceral pain model. Mice were injected with (i.p.) either saline (7 ml/kg) or different doses of guanfacine (2.5, 5 and 10 mg/kg) 15 min before formalin administration. Antinociception was recorded for 0–60 min after formalin instillation. Results are presented as area under the time curve (AUC). Each point is the mean±S.E.M. of area under the time curve of six mice. **$P<0.01$, ***$P<0.001$ as compared with control group.

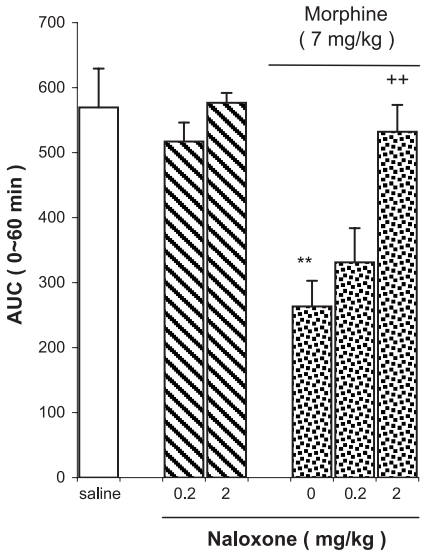

Fig. 4. Effect of naloxone on morphine-induced analgesia in visceral pain model. Mice were administered (i.p.) with saline (7 ml/kg) 30 min, (s.c.) morphine (7 mg/kg) 15 min before formalin instillation. Animals were treated (i.p.) with naloxone (0.2 and 2 mg/kg) 2 min before morphine. Results are shown as area under the time curve (AUC). Each point is the mean±S.E.M. of area under the time curve for six mice. $^{**}P<0.01$ as compared with saline control group. $^{++}P<0.001$ as compared with morphine control group.

visceral pain in mice [$F(8,45)=6.2$, $P<0.0001$] but had no effect on guanfacine action.

3.4. Effects of benazoline on antinociception induced by clonidine and guanfacine

In Fig. 6, benazoline (2.5 and 5 mg/kg) showed analgesic effects in mice with visceral pain ($P<0.01$).

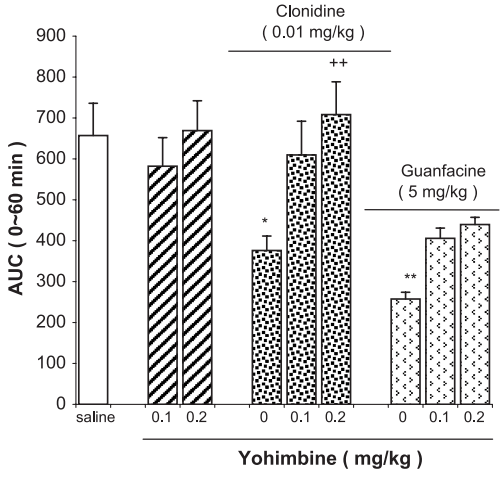

Fig. 5. Effect of yohimbine on antinociception induced by clonidine and guanfacine in visceral pain model. Animals were treated (i.p.) with saline (7 ml/kg), yohimbine (0.1 and 0.2 mg/kg) 30 min or clonidine (0.01 mg/kg) and guanfacine (5 mg/kg) 15 min before formalin administration. Results are expressed as area under the time curve (AUC). Each point is the mean±S.E.M. of area under the time curve of six mice. $^{*}P<0.05$, $^{**}P<0.01$ as compared with saline control group. $^{++}P<0.001$ as compared with clonidine control group.

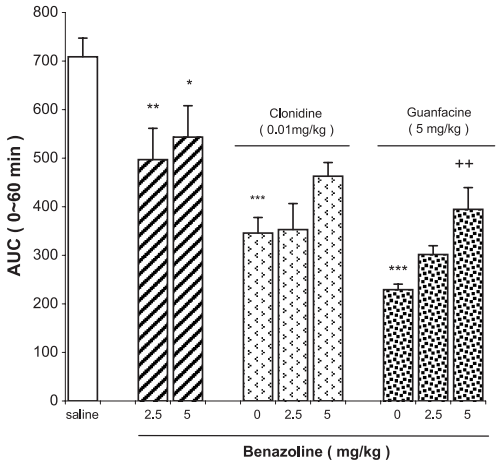

Fig. 6. Effect of benazoline on antinociception induced by clonidine and guanfacine in visceral pain model. Animals were injected (i.p.) with saline (7 ml/kg), benazoline (2.5 and 5 mg/kg) 30 min or clonidine (0.01 mg/kg) and guanfacine (5 mg/kg) 15 min prior to formalin instillation. Results are presented as area under the time curve (AUC). Each point is the mean±S.E.M. of area under the time curve for six mice. $^{*}P<0.05$, $^{***}P<0.001$ different from saline control group. $^{++}P<0.001$ different from guanfacine control group.

However, one-way ANOVA indicated that benazoline reduced the analgesic effect of guanfacine (5 mg/kg) in formalin-induced colonic pain in mice [$F(8,45)=11.1$, $P<0.0001$] but it did not decrease the antinociceptive action of clonidine.

4. Discussion

In the present study, the analgesic effects of α_2-adrenoceptor agonists, clonidine and guanfacine, in the presence or absence of yohimbine and benazoline were evaluated using the inflammatory colonic pain induced by intracolonic instillation of formalin. Most of the animal models of visceral pain require surgery or intubation of a hollow viscus (Giamberardino, 1999; Ness and Gebhart, 1990). On the other hand, for about three decades irritant chemicals have been infused into the lumen of hollow organs such as the urinary bladder (Koltzenburg and Mc Mahan, 1986; Mc Mahan and Abel, 1987) and colon (Mac Pherson and Pfeiffer, 1978) to produce an inflammatory condition. The model of visceral pain we used resembles the one elicited by intracolonic administration of other substances such as mustard oil and capsaicin via the anus (Laird et al., 2001).

The present study provided evidence that the intracolonic instillation of formalin via the anus evokes differentiated behaviours, which reflect visceral pain. All types of behaviour were dose dependently inhibited by morphine, indicating that they are pain-related (Laird et al., 2001). However, naloxone blocked the morphine response in visceral pain.

Our findings showed that clonidine in the doses employed produced antinociception in a dose-dependent manner for the inflammatory colonic pain induced by intracolonic administration of formalin. Our results are consistent with those of previous work which demonstrated the analgesic effects of clonidine in different kinds of pain models such as the formalin test (Kanui et al., 1993; Przesmycki et al., 1998; Shannon and Lutz, 2000; Tasker and Melzack, 1989), writhing test (Sierralta et al., 1996), tail-flick (Kumar et al., 1993; Ossipov et al., 1984) and visceral nociception induced by colorectal distention (Harada et al., 1995).

In addition, our data showed that yohimbine, an α_2-antagonist, did not show any analgesic effect on inflammatory colonic pain and was able to reduce antinociception induced by clonidine. These results are consistent with those of previous studies (Harada et al., 1995; Kumar et al., 1993; Mc Cleary and Leander, 1981). Moreover, pretreatment of animals with benazoline, an imidazoline (I_2) ligand (Pigini et al., 1997), did not have any effect on the antinociceptive activity of clonidine in mice. Our data may indicate that the clonidine response occurred via the activation of α_2-receptors located in lamina I and II of the dorsal horn of the spinal cord (Atweh and Kuhar, 1997), the site of afferent Aδ and C fibers entry into the central nervous system (CNS). Activation of these receptors inhibits the release of substance P (Kuraishi et al., 1985; Yaksh et al., 1980), a peptide which may play a role in the transmission of nociceptive signals from the inflamed colon (Miampamba et al., 1992). In general, some research supported the possibility that clonidine produced its antinociceptive activity via α_2-adrenoceptors rather than imidazole receptors (Harada et al., 1995; Ono et al., 1991; Sierralta et al., 1996).

Nevertheless, it has been reported that clonidine is a potent ligand of imidazoline (I_1) receptors compared to imidazoline (I_2) binding sites (Bylund et al., 1994; Hieble et al., 1995; Millan et al., 2000). Our findings showed that benazoline, an imidazoline (I_2) ligand, did not have any effect on analgesia induced by clonidine. This was consistent with results of a previous study which demonstrated that the antinociceptive activity of clonidine was resistant to I_1/I_2 antagonists but reduced by α_2-adrenoceptor antagonists devoid of affinity for I_1/I_2 sites (Millan, 2002).

Interestingly, our results showed that guanfacine, an α_2-adrenoceptor agonist,produced dose-dependent antinociception in inflammatory colonic pain induced by instillation of formalin. The analgesic effect of guanfacine had been described previously (Parale and Kulkarni, 1985; Smith et al., 1992). However, pretreatment of animals with yohimbine did not block the antinociception induced by guanfacine.

However, benazoline reduced the analgesic effect of guanfacine and it also produced antinociception when it was administered alone. This may indicate that the guanfacine response is induced via imidazoline (I_2) binding sites. There is a body of evidence that guanidine-type ligands like guanfacine and guanabenz display high affinities towards the imidazoline (I_2) receptors (Milligan and Mac Kinnon, 1997). Additionally, it may indicate that benazoline acted as a partial agonist on imidazoline (I_2) receptors. There is a need for additional studies on the involvement of imidazoline receptors in antinocoception and their interactions with other receptors.

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