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European Journal of Pharmacology 528 (2005) 59 – 64
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Antinociception: Mechanistic studies on the action of MD-354 and clonidine. Part 1. The 5-HT_3 component

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Received 2 June 2005; received in revised form 18 October 2005; accepted 25 October 2005

Abstract

MD-354 (*m*-chlorophenylguanidine) is a 5-HT_3/α_{2B}-adrenoceptor ligand. Both receptors play a role in antinociception. In the mouse tail-flick assay, subcutaneously administered MD-354 was inactive as an analgesic. However, a combination of an inactive dose of clonidine (0.25 mg/kg) with an inactive dose of MD-354 (6 mg/kg) produced a substantial antinociceptive effect (maximal possible effect = 66%). Considering the 5-HT_3 receptor partial agonist properties of MD-354, the analgesia enhancing effect of MD-354 on clonidine might be associated, at least in part, with its 5-HT_3 receptor agonist or antagonist activity. Combinations of an inactive dose of clonidine (0.25 mg/kg) with 5-HT_3 receptor antagonists (tropisetron, zacopride and ondansetron) were examined. Saline-like doses of tropisetron, zacopride and ondansetron significantly enhanced the antinociceptive effect of clonidine (combinations: maximal possible effect = 86%, 82% and 79% respectively), suggesting that MD-354 may enhance the analgesic actions of clonidine, at least in part, through a 5-HT_3 receptor antagonist mechanism.

Keywords: α_2-Adrenoceptor; 5-HT_3 receptor; MD-354 (*m*-chlorophenylguanidine); Antinociception; In vivo model

1. Introduction

MD-354 (*m*-chlorophenylguanidine) enhances the antinociceptive effect of an inactive dose of the α_2-adrenoceptor agonist clonidine (Wesolowska et al., 2004). MD-354 is a fairly selective ligand with comparable affinity for 5-HT_3 receptors and α_{2B}-adrenoceptors (K_i = 35 nM and 25 nM, respectively) (Dukat et al., 1996; Wesolowska et al., 2004). Several in vitro and in vivo assays characteristic for 5-HT_3 receptor agonists and antagonists indicate that MD-354 possesses both agonist (Dukat et al., 1996, 2000) as well as antagonist (Dukat et al., 1996) character. Overall, the data suggest that MD-354 is a 5-HT_3 receptor partial agonist. Thus, the observed (enhancement of clonidine-induced antinociception) effect might be due either to 5-HT_3 receptor agonist or antagonist activity, or to the action of MD-354 on α_2-adrenoceptors. In this study we examine the hypothesis that 5-HT_3 receptors might be involved.

The significance of 5-HT_3 receptors in nociception in animals and humans is well documented (reviewed by Dukat, 2004; Farber et al., 2004). Ondansetron, granisetron, and tropisetron, 5-HT_3 receptor antagonists, are highly effective antiemetics used clinically in the treatment of nausea and vomiting associated with cancer chemotherapy (Riering et al., 2004). More recent clinical studies with ondansetron and another 5-HT_3 receptor antagonist, alosetron, imply a promising role for 5-HT_3 receptor antagonists in the treatment of pain associated with irritable bowel syndrome (reviewed by Costall and Naylor, 2004). Patients with systemic inflammatory pain responded to granisetron, while chronic low-back pain and cervical pain was treated with tropisetron (Israili, 2001). Nonetheless, conflicting experimental reports indicate a role for both stimulation and blockade of 5-HT_3 receptors in nociception. In the rat tail-flick assay Glaum et al. (1990) showed that intrathecal administration of the 5-HT_3 receptor agonist 2-methylserotonin mimicked the antinociceptive effect of 5-HT and that the effect was blocked by 5-HT_3 receptor antagonists tropisetron (formally ICS 205-930) and MDL 72222. Other investigators using similar antinociceptive assays in rats (Crisp et al., 1991; Giordano, 1991) and mice (Alhaider et al., 1991) reported similar

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doi:10.1016/j.ejphar.2005.10.037

findings. In contrast, Ali et al. (1996) reported that intrathecal microinjection of the 5-HT_3 receptor agonist m-CPBG (*m*-chlorophenylbiguanide) or the 5-HT_3 antagonist tropisetron and ondansetron did not alter nociceptive responses in the rat tail-flick assay. 5-HT_3 receptor antagonists, when administered to mice via the subcutaneous route, were also inactive as analgesics in thermal and mechanical pain tests (Giordano and Dyche, 1989).

Lack of readily brain penetrating 5-HT_3 receptor agonists prompted us to test two 5-HT_3 receptor antagonists: zacopride and tropisetron, on the MD-354 potentiaton of clonidine in the tail-flick assay. Previously we showed that pretreatment with zacopride (0.5 and 1.0 mg/kg i.p.) failed to attenuate the potentiation effect (maximal possible effect=66%) in the tail-flick latency produced by subcutaneous administration of MD-354 (6 mg/kg) in combination with clonidine (0.25 mg/kg) (Wesolowska et al., 2004). Herein more detailed studies involving a possible 5-HT_3 serotonergic component of action are presented.

2. Materials and methods

2.1. Animals

Male ICR mice (24–28 g) obtained from Harlan Laboratories (Indianapolis, IN) were used throughout the study. Mice were housed in groups of five, with free access to food and water. Animals were housed in an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) approved facility, and the study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. Mice were allowed to adapt to the testing environment for at least 2 h prior to any treatment. Animals were weighed on the day of the experiment(s) for the calculation of drug dosages.

2.2. Drugs

Clonidine hydrochloride and tropisetron hydrochloride were purchased from Sigma-Aldrich Chemical Company, Inc. (Milwaukee, WI). MD-354 nitrate was resynthesized as reported previously (Dukat et al., 1996). Zacopride hydrochloride was purchased from Tocris (Ballwin, MO). Ondansetron hydrochloride "Zofran" (GlaxoSmithKline) for injection (2 mg/ml) was purchased from the MCVH-Pharmacy. All drugs were dissolved in physiological saline (0.9% sodium chloride) and given in a total volume of 10 ml/1000 g body weight for subcutaneous (s.c.) and intraperitoneal (i.p.) injections to mice.

2.3. Behavioral assays

2.3.1. Antinociception

2.3.1.1. Tail-flick test. Antinociception was assessed by the tail-flick method of D'Amour and Smith (1941) as modified by Dewey et al. (1970) using a Columbus Tail-Flick Analgesia Meter. A control response (1.7–4.0 s) was determined for each mouse before treatment, and a test latency was determined after drug administration. In order to minimize tissue damage, a maximum latency of 10 s was imposed. The antinociceptive response was calculated as percent maximum possible effect, where % maximal possible effect = [(test − control)/(10 − control)] × 100. Groups of six to nine animals were used for each dose and for each treatment.

The experimental protocol for testing the effects of drugs was as follows: 15 min prior to injection of drugs, baseline tail-flick was determined for each mouse. The animal then was injected with zacopride, tropisetron, or ondansetron using a 50-min pretreatment interval. Zacopride was administered i.p. or s.c. 5 min prior to a combination test of MD-354 with clonidine. Tropisetron was administered s.c. 5 min prior to the combination test of MD-354 with clonidine. In combination tests using i) MD-354 with clonidine, MD-354 was injected 25 min prior to the clonidine dose and 45 min before the test, ii) zacopride with clonidine, zacopride s.c. or i.p. was injected 30 min prior to the clonidine, iii) tropisetron with clonidine, tropisetron was injected 30 min prior to the clonidine, iiii) ondansetron with clonidine, ondansetron was injected 30 min prior to the clonidine. The order and timing of administration of clonidine (20 min—the time of peak effect) (Spaulding et al., 1979; Kameyama et al., 1986) ensured that the times of its peak antinociceptive effects coincided.

2.3.2. Spontaneous activity

Mice were placed into individual *Tru Scan Infrared Locomotor Activity System* (Coulbourn Instruments, Allentown, PA) photocell activity cages (40 cm^3) after s.c. administration of either 0.9% saline, zacopride (0.01 mg/kg), tropisetron (0.2 mg/kg), clonidine (0.25 mg/kg), combinations of i) MD-354 (6 mg/kg) with clonidine (0.25 mg/kg); ii) zacopride (0.01 mg/kg) with clonidine (0.25 mg/kg); iii) tropisetron (0.2 mg/kg) with clonidine (0.25 mg/kg). Ambulatory movement was measured by the number of times the animal interrupted the infrared beams traversing the cage for a period of 15 min. Measurements were taken 20, 35 and 50 min following drug treatment.

The analysis was focused only on main measures (3 main measures: total moves, move time, move distance) of activity to determine whether zacopride and tropisetron (n=6/dose) depressed this action relative to saline (n=6) control. Similarly, in the case of combinations the analysis was focused on 3 main measures of activity as well to determine whether MD-354 with clonidine (n=6/dose), zacopride with clonidine (n=6/dose) and tropisetron with clonidine (n=6/dose) depressed this action relative to clonidine (n=6) control.

2.4. Statistical analysis

Data were analyzed statistically by an analysis of variance (ANOVA) followed by the Newman–Keuls test for multiple post-hoc comparison test. The null hypothesis was rejected at the 0.05 level. For the time-course studies, each animal was used once. Data were analyzed by one-way or a two-factor

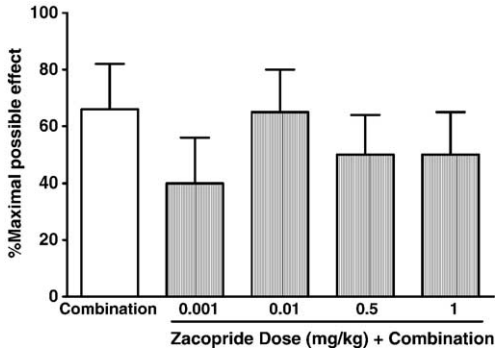

Fig. 1. Effect (±S.E.M.) of zacopride (i.p.) on the antinociceptive actions of a clonidine/MD-354 combination (clonidine (0.25 mg/kg) and MD-354 (6 mg/kg)) in the tail-flick assay (n=7–8 mice/treatment).

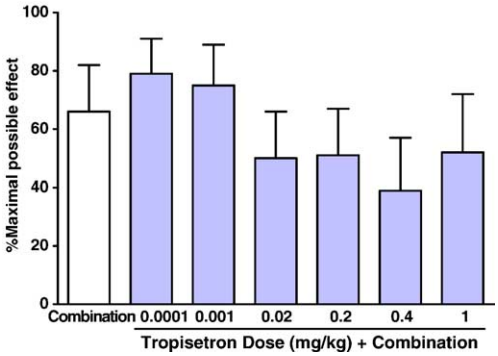

Fig. 3. Effect (±S.E.M.) of tropisetron (s.c.) on the antinociceptive actions of a clonidine/MD-354 combination (clonidine (0.25 mg/kg) and MD-354 (6 mg/kg)) in the tail-flick assay (n=7–8 mice/treatment).

ANOVA as applicable. The results are expressed as mean values±S.E.M.

3. Results

3.1. Antinociceptive activity

3.1.1. Effect of selected doses of zacopride (i.p.) on antinociceptive action of clonidine

Administration of zacopride (0.5 and 1 mg/kg, i.p.; maximal possible effect <27%) in combination with clonidine (0.25 mg/kg; maximal possible effect=13%) had no effect on the antinociceptive action of clonidine (maximal possible effect <26%). Although, an additional lower dose of zacopride (0.01 mg/kg, i.p.; maximal possible effect=1%) produced a slight increase in antinociception (maximal possible effect=42%) when administered with clonidine, the effect was not statistically significant (data not shown).

3.1.2. 5-HT_3 antagonists do not attenuate MD-354-enhanced clonidine-antinociception

Neither i.p. administration of the 5-HT_3 receptor antagonist zacopride (doses 0.01–1.0 mg/kg; 1–27% maximal possible effect) nor s.c. zacopride (doses 0.0001–2 mg/kg; 0–7% maximal possible effect) produced a statistically significant antinociceptive effect in the tail-flick assay when administered alone (data not shown). Tropisetron (doses 0.0001–1.0 mg/kg, s.c.; 0–5% maximal possible effect) and ondansetron (doses 0.02–2 m/kg, s.c.; 1–4% maximal possible effect) given alone had no analgesic effect as well (data not shown). Pretreatment with zacopride i.p. (doses 0.001–1 mg/kg) or s.c. (doses 0.00001–1 mg/kg) failed to significantly attenuate the increase (maximal possible effect=66%) in tail-flick latency produced by s.c. administration of MD-354 (6.0 mg/kg) in combination with clonidine (0.25 mg/kg) (Figs. 1 and 2). Similarly, no significant antagonistic effect of the above potentiation was observed when tropisetron (0.0001–1 mg/kg) was administered prior to the combination of MD-354 (6.0 mg/kg) with clonidine (0.25 mg/kg) (Fig. 3). As control, combination of MD-354 (6 mg/kg) with doses of zacopride (0.01, 0.5 and 1 mg/kg, i.p.) or with doses of tropisetron (0.2 and 1 mg/kg, s.c.), like saline, failed to produce >3% maximal possible effect (data not shown).

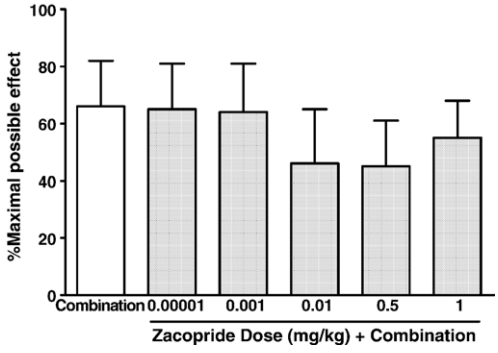

Fig. 2. Effect (±S.E.M.) of zacopride (s.c.) on the antinociceptive actions of a clonidine/MD-354 combination (clonidine (0.25 mg/kg) and MD-354 (6 mg/kg)) in the tail-flick assay (n=7–8 mice/treatment).

3.1.3. 5-HT_3 antagonists enhance the antinociceptive effect of clonidine

A combination of an "inactive" dose of clonidine (0.25 mg/kg, maximal possible effect=13%) plus an "inactive" dose of

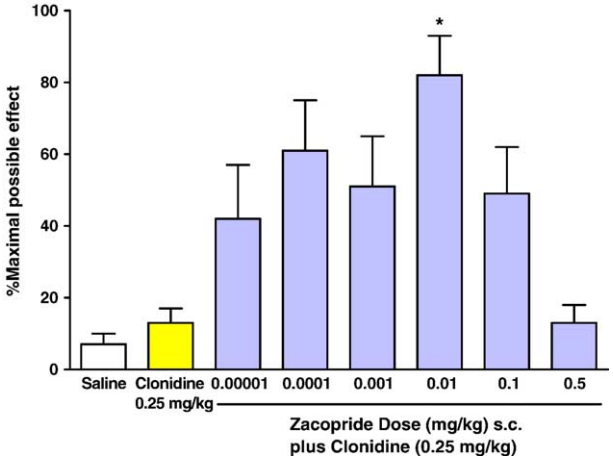

Fig. 4. Potentiation of the antinociceptive actions (±S.E.M.) of clonidine (0.25 mg/kg) by zacopride (s.c.) in the tail-flick assay (n=8–9 mice/treatment). Asterisk denotes a significant difference compared to control group; *P<0.01; one way ANOVA ($F_{7,58}$=5.06) followed by Newman–Keuls post hoc test.

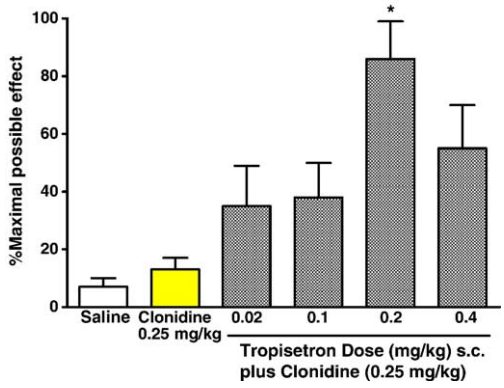

Fig. 5. Potentiation of the antinociceptive actions (±S.E.M.) of clonidine (0.25 mg/kg) by tropisetron (s.c.) in the tail-flick assay (n=7–9 mice/treatment). Asterisk denotes a significant difference compared to control group; *P<0.001; one way ANOVA ($F_{5,40}$=6.01) followed by Newman–Keuls post hoc test.

zacopride (0.01 mg/kg, maximal possible effect=4%) augmented the antinociceptive effect of clonidine in mice (maximal possible effect=82%) when administered by the s.c. route (Fig. 4). Similarly, other "inactive" doses (0.00001, 0.0001, 0.001, and 0.1 mg/kg, s.c.) of zacopride in combination with an "inactive", dose of clonidine (0.25 mg/kg; maximal possible effect=13%) produced substantial antinociception (42–61% maximal possible effect) in a dose-dependent manner (Fig. 4) except that % maximal possible effect decreased with zacopride doses higher than 0.01 mg/kg. An inactive dose of the more selective 5-HT_3 antagonist tropisetron (i.e., 0.2 mg/kg; maximal possible effect=1%) in combination with an "inactive" dose of clonidine (0.25 mg/kg; maximal possible effect=13%) elevated the analgesic effect in the mouse tail-flick assay (maximal possible effect=86%) (Fig. 5). The 5-HT_3 antagonist ondansetron (i.e., 0.1 and 0.2 mg/kg; maximal possible effect=<4%), similar to zacopride and tropisetron when administered in combination with clonidine (0.25 mg/kg; maximal possible effect=13%), produced a significant increase in antinociception (maximal possible effect=68–79%) (Fig. 6).

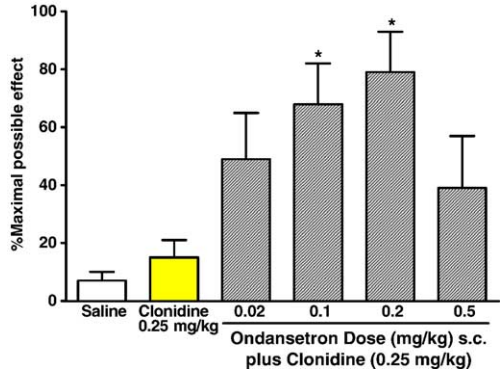

Fig. 6. Potentiation of the antinociceptive actions (±S.E.M.) of clonidine (0.25 mg/kg) by ondansetron (s.c.) in the tail-flick assay (n=7–8 mice/treatment). Asterisk denotes significant differences compared to control group; *P<0.05; one way ANOVA ($F_{5,38}$=3.93) followed by Newman–Keuls post hoc test.

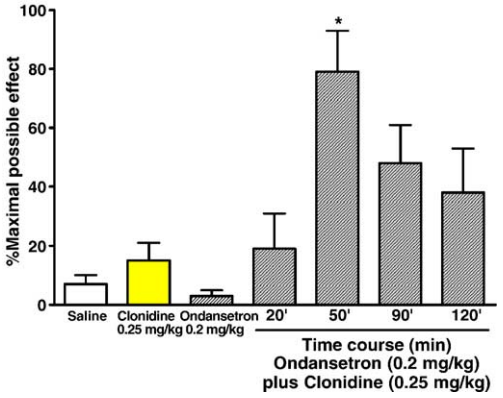

Fig. 7. Time course of potentiation of the antinociceptive action (±S.E.M.) of clonidine (0.25 mg/kg) by ondansetron (s.c., 0.2 mg/kg) in the tail-flick assay (n=7–9 mice/treatment). Asterisk denotes a significant difference compared to the clonidine control group; *P<0.05; one way ANOVA ($F_{6,46}$=5.12) followed by Newman–Keuls post hoc test.

3.1.4. Time course for ondansetron potentiation of clonidine

To determine duration of the potentiation effect of ondansetron on clonidine antinociception, a combination of an ondansetron (i.e., 0.2 mg/kg) dose with clonidine (0.25 mg/kg) was examined using various pretreatment times. Shorter (i.e., 20 min) and longer (i.e., 90- and 120-min) pretreatment times resulted in a diminished antinociceptive effect than that obtained with a 50-min pretreatment time (Fig. 7).

3.2. Spontaneous activity

Subcutaneous administration of 0.01 mg/kg of zacopride after 20, 35 and 50 min produced saline-like effects in all three measurements. For example, after 50 min the measure of i) *total movement* was recorded as 387±18 and 383±12 for saline and zacopride, respectively; ii) *total movement time* was 1719±117 s for saline and 1766±109 s for zacopride; iii) *total movement distance* was 5414±580 cm and 6175±687 cm for saline and zacopride, respectively. Similarly, subcutaneous administration of 0.2 mg/kg of tropisetron after 20, 35 and 50 min also produced saline-like effects in all three measures. Here also after 50 min the measure of i) *total movement* was recorded as 341±17 and 340±21 for saline and tropisetron, respectively; ii) *total movement time* was 1568±147 s for saline and 1474±113 s for tropisetron; iii) *total movement distance* was 5766±1036 cm and 4919±622 cm for saline and tropisetron, respectively.

Table 1
Effect of clonidine (0.25 mg/kg) and its combination with MD-354 (6 mg/kg), zacopride (0.1 mg/kg) and tropisetron (0.2 mg/kg) in the mouse spontaneous activity assay

Treatment	Total movement	Total movement time (s)	Total movement distance (cm)
Clonidine	123±22	404±76	1287±272
MD-354/clonidine	92±13	287±43	773±119
Zacopride/clonidine	99±10	359±35	1179±189
Tropisetron/clonidine	80±11	264±56	887±252

The values are means±S.E.M. There was no statistical difference between the effects of clonidine and the clonidine/antagonist combinations.

In combination studies of i) MD-354 (6 mg/kg) with clonidine (0.25 mg/kg); ii) zacopride (0.01 mg/kg) with clonidine (0.25 mg/kg); iii) tropisetron (0.2 mg/kg) with clonidine (0.25 mg/kg) after 20, 35 and 50 min, all three combinations produced clonidine-like effects in all three measures (Table 1).

4. Discussion

MD-354 potentiates the antinociceptive actions of clonidine by a mechanism that has yet to be defined. Attempts to block potentiation (maximal possible effect=66%) of the antinociceptive effect of an inactive dose of clonidine (0.25 mg/kg, maximal possible effect=13%) and MD-354 (6 mg/kg, maximal possible effect=0%) by the 5-HT_3 receptor antagonist zacopride (i.p., doses 0.5 and 1 mg/kg) were unsuccessful (Wesolowska et al., 2004). MD-354 is a 5-HT_3 receptor partial agonist. If potentiation of the antinociceptive effect of clonidine by MD-354 is due to its 5-HT_3 receptor antagonist character, 5-HT_3 receptor antagonists might not attenuate the potentiaton effect, but they might potentiate the antinociceptive action of clonidine. We had previously examined two doses of zacopride as a possible antagonist of the MD-354/clonidine combination; in the present study we examined the same two doses of zacopride (i.p., 0.5 mg/kg and 1 mg/kg) in combination with an inactive dose of clonidine (0.25 mg/kg; maximal possible effect=13%), and the obtained results did not show that clonidine's antinociceptive action was altered (maximal possible effect=26% and 24%, respectively). However, although a combination of a lower dose of zacopride (0.01 mg/kg) with clonidine (0.25 mg/kg) slightly elevated (42% maximal possible effect) the antinociceptive effect of clonidine, the results were not statistically significant.

Failure to potentiate the antinociceptive action of clonidine by zacopride prompted us to reevaluate our thinking. The effect of MD-354 on clonidine antinociception might be related to the 5-HT_3 receptor agonist character, not the antagonist character, of the partial agonist MD-354. Hence, zacopride should block the potentiaton effect of clonidine by MD-354. But it did not. Zacopride is a fairly potent (Kilpatrick et al., 1990; Young and Johnson, 1991) and high affinity (Kilpatrick et al., 1990) 5-HT_3 receptor antagonist. However, 5-HT_3 receptor antagonists are known to frequently produce their effect in a characteristic bell-shaped dose–response manner, and this phenomenon is observed in a very low dosage range (Farber et al., 2004). Thus, we examined additional, lower doses of zacopride (i.p., 0.01 and 0.001 mg/kg) on the antinociceptive effect of the MD-354/clonidine combination. Here too, the results suggested a lack of antagonism by these lower zacopride doses (Fig. 1). Before concluding that the potentiating effect of MD-354 on clonidine antinociception is not mediated through a 5-HT_3 serotonergic mechanism, there was one other question that would require an answer: is the lack of effect related to the route of drug administration? Consequently, we conducted similar studies to the above using a subcutaneous route of administration for zacopride.

Similarly to the intraperitoneal studies described above, here also subcutaneously administered zacopride plus the MD-354/clonidine combination did not suppress the antinociceptive effect produced by the MD-354/clonidine combination (Fig. 2). Because zacopride possesses both 5-HT_3 antagonist and 5-HT_4 receptor agonist character it was of interest to examine a 5-HT_3 receptor antagonist with a different chemical structure and pharmacological profile. Tropisetron is an indole analog with high affinity and better selectivity for 5-HT_3 receptors than zacopride (Kilpatrick et al., 1990; Israili, 2001). As seen with zacopride, subcutaneously administered tropisetron failed to antagonize the antinociceptive effect produced by the MD-354/clonidine combination (Fig. 3). Combined results from our antagonist studies suggest that the potentiation of clonidine antinociceptive action by MD-354 is unlikely related to its 5-HT_3 agonist character. For that reason, further work with the antagonist hypothesis of MD-354 action on clonidine's antinociception was thought justified.

If MD-354, when administered in combination with clonidine in the mouse tail-flick assay, behaves as a 5-HT_3 antagonist, other 5-HT_3 antagonists in combination with clonidine might mimic this action. All tropisetron doses examined potentiated the antinociceptive effect of 0.25 mg/kg of clonidine (Fig. 5). Here also, the observed potentiation is not simply additive because the effect of an inactive dose of clonidine (0.25 mg/kg, maximal possible effect=13%) in combination with tropisetron (e.g., 0.2 mg/kg, maximal possible effect=0%) is greater (maximal possible effect=86%) than the sum of the two treatments when the agents were examined alone. This prompted a re-examination of zacopride via the subcutaneous route. Subcutaneously injected zacopride doses of 0.00001 to 0.1 mg/kg all enhanced the antinociceptive effect of 0.25 mg/kg of clonidine (Fig. 4). The observed effect is produced in a manner as seen with the MD-354/clonidine combination.

It seems unlikely that the observed enhancement of clonidine's effects by zacopride and tropisetron is attributable to a general central depressant effect, because both 5-HT_3 antagonists failed to influence locomotor activity as determined in the spontaneous motor assay. Furthermore, all three combinations (MD-354/clonidine, zacopride/clonidine, tropisetron/clonidine) did not further depress the locomotor activity evoked by clonidine (0.25 mg/kg) administered alone (Table 1). These data suggest that the effect of MD-354 and 5-HT_3 antagonists (zacopride and tropisetron) on the antinociceptive action of clonidine is functionally selective and unlikely related to clonidine's sedation mechanism.

To eliminate the possibility of coincidental action of zacopride and tropisetron on clonidine's antinociceptive action we tested our hypothesis with one additional clinically used 5-HT_3 antagonist, ondansetron ("Zofran"). Ondansetron represents yet another chemical class of 5-HT_3 antagonists widely used in the treatment of emesis associated with cancer chemotherapy and is a very selective and high affinity ligand (Israili, 2001). As expected, all ondansetron doses enhanced the antinociceptive effects of clonidine (0.25 mg/kg) (Fig. 6). For example, a 0.25 mg/kg dose of clonidine (maximal possible effect=13%) in combination with a 0.2 mg/kg dose of ondansetron (maximal possible

effect=3%) produced an antinociceptive effect (maximal possible effect=79%) that was greater than the sum of the effect of either of the drugs administered individually. The time course of ondansetron/clonidine antinociceptive action indicated that peak antinociception is related to the peak action of ondansetron and that the effect diminishes with time (Fig. 7).

Zacopride was found to be ineffective via the i.p. route in enhancing the antinociceptive action of clonidine, whereas it was effective when administered via the s.c. route. No implication is made that zacopride might not have been effective had additional doses been examined via the i.p. route.

The results provided herein argue that at a dose of 6 mg/kg MD-354 enhances the antinociceptive action of an inactive dose of clonidine by a mechanism that does not involve 5-HT_3 agonism. That is, the effect of this combination was not antagonized by either zacopride or tropisetron. A working hypothesis is that MD-354 might produce its effect via a 5-HT_3 antagonist mechanism. This hypothesis is supported by the demonstration that standard 5-HT_3 antagonists from three different structural classes also enhanced the antinociceptive action of clonidine. The possibility exists, however, that MD-354 and the 5-HT_3 antagonists work via entirely different (dependent or independent) mechanisms. That is because MD-354 also binds at α_{2B}-adrenoceptors, an adrenergic mechanism accounting for the action of MD-354 cannot be discounted. Studies focusing on any adrenergic involvement in the effect of MD-354 on clonidine antinociception are currently underway in our laboratories.

The results also show, for the first time, that low doses of clinically used 5-HT_3 antagonists (e.g., tropisetron, ondansetron) also are able to enhance the antinociceptive actions of clonidine. Because clonidine is often used to control pain in cancer patients, and because 5-HT_3 antagonists are used to suppress chemotherapy-induced emesis, the present findings strongly suggest that the effect of 5-HT_3 antagonists on clonidine's analgesic action be investigated in a clinical setting.

Acknowledgements

We are grateful to Prof. Richard A. Glennon for helpful discussions and proof reading of the manuscript. This study was supported by IRG-73-001-28 from the American Cancer Society.

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