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Short communication

Involvement of the midbrain periaqueductal gray 5-HT_{1A} receptors in social conflict induced analgesia in mice

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Abstract

Recent results from our laboratory have shown that 30-bites social conflict in mice produces a high-intensity, short-term analgesia which is attenuated by systemically injected 5-HT_{1A} receptor agonists, such as BAY R 1531 (6-methoxy-4-(di-*n*-propylamino)-1,3,4,5-tetrahydrobenz(*c,d*)indole hydrochloride) and gepirone. The present study investigated the effects of these drugs, as well as the 5-HT_{1A} receptor antagonist WAY 100135 (*N-tert*-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide) injected into the midbrain periaqueductal gray matter of mice on 30-bites analgesia. Four to five days after guide-cannula implantation, each mouse received microinjection of gepirone (30 nmol/0.2 μl), BAY R 1531 (10 nmol/0.2 μl), WAY 100135 (10 nmol/0.2 μl), saline (0.9% NaCl) or vehicle (saline + 4% Tween 80) 5 min before either an aggressive (30 bites) or a non-aggressive interaction. Nociception was assessed by the tail-flick test made before as well as 1, 5, 10 and 20 min after social interaction. The full 5-HT_{1A} receptor agonist BAY R 1531 blocked, whereas, WAY 100135 and gepirone intensified 30-bites analgesia. Neither non-aggressive interaction, per se, nor the three compounds given after this type of social interaction significantly changed nociception. These results indicate that 5-HT_{1A} receptors in the periaqueductal gray inhibit analgesia induced by social conflict in mice. © 1998 Elsevier Science B.V.

Keywords: Social conflict; Analgesia; 5-HT_{1A} receptor; BAY R 1531; Gepirone; WAY 100135; Periaqueductal gray

1. Introduction

The role of the periaqueductal gray matter in analgesia has been thoroughly investigated since Reynolds (1969) demonstrated a marked analgesia induced by electrical stimulation of this brain structure (for a review, see Depaulis and Bandler, 1991). The periaqueductal gray has also been implicated in the expression of defensive behavior and in the elaboration of emotional states such as fear and anxiety (Fanselow, 1991; Graeff, 1990). A relationship between fear and analgesia has been suggested by many (Bolles and Fanselow, 1980; Rodgers and Randall, 1988; Siegfried et al., 1990). In particular, a state of fear/anxiety is thought to mediate the analgesia induced by defeat in mice (Canto de Souza et al., 1997; Rodgers and Cole, 1993; Rodgers and Randall, 1986).

Stimulation of 5-HT_{1A} receptors in the periaqueductal gray of the rat has been shown to inhibit aversion and escape behavior generated by electrical or chemical stimulation of the same brain area (Beckett et al., 1992; Nogueira and Graeff, 1995). Earlier results have suggested that stimulation of presynaptic 5-HT_{1A} receptors inhibits, whereas, that of postsynaptic 5-HT_{1A} receptors increase aggressive interaction-induced analgesia (Canto de Souza et al., 1997; Rodgers and Shepherd, 1989). The present study explores the role of postsynaptic 5-HT_{1A} receptors of the periaqueductal gray in aggressive interaction-induced analgesia. For this, we measured the effects of microinjection into the periaqueductal gray of drugs acting on 5-HT_{1A} receptors on 30-bites social conflict-induced analgesia in Swiss albino mice. The following drugs were used: the full

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agonist BAY R 1531 (6-methoxy-4-(di-*n*-propylamino)-1,3,4,5-tetrahydrobenz(*c*,*d*)indole hydrochloride), the partial agonist gepirone, and the antagonist WAY 100135 (*N*-*tert*-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide) (De Vry et al., 1991; Glaser et al., 1987; Griebel, 1995).

2. Materials and methods

Male Swiss albino mice (School of Pharmaceutical Sciences, Paulista State University, UNESP) weighting 23–25 g, were stereotaxically implanted under sodium pentobarbital (50 mg/kg, 0.1 ml/10 g body weight, i.p.) anesthesia with a stainless steel guide cannula (gauge 26), according to the method described by Miczek et al. (1985). The cannula was permanently fixed to the skull with acrylic cement an its tip was positioned 1.0 mm ventral to the skull surface and directly dorsal to the target site of injection. A stylet was inserted into the guide cannula to avoid obstruction, which was removed before injection. Mice were allowed at least 4 days to recover from surgery before testing. For intracerebral injection, a 33-gauge needle was inserted into the guide cannula. The length of the injection needle exceeded the tip of the guide cannula by 2.0 mm. The needle was attached by PE-10 tubing to a 5-μl Hamilton syringe mounted on an infusion pump. The following drugs were used: gepirone hydrochloride (Bristol-Myers), WAY 100135 (Wyeth) and BAY R 1531 (Bayer). All compounds were dissolved in physiological saline (0.9% NaCl), except for WAY 100135 that was suspended in saline with 4% Tween 80, as vehicle. The drugs were injected in 120 s into the periaqueductal gray in a volume of 0.20 μl. The movement of a small air bubble inside the PE-10 tubing during the injection confirmed the delivery of solution. Nociception was assessed by the tail-flick test (Canto de Souza et al., 1997) three times at 5-min intervals to ensure stability. Another four measurements were carried out at the 1st, 5th, 10th and 20th min after the aggressive interaction (see below). A cut-off time of 6 s was applied for non-reactive animals. Each tail-flick latency (TFL) was normalized by calculating an index of analgesia (IA):

$$\mathrm{IA} = \frac{(\mathrm{testTFL}) - (\mathrm{average\ baseline\ TFL})}{6 - (\mathrm{average\ baseline\ TFL})}.$$

Aggressive interaction involved exposing a Swiss albino mouse 5 min after periaqueductal gray injection to 30 attack bites by a socially isolated (4 weeks), trained aggressive mouse of the same strain, as described previously (Canto de Souza et al., 1997). Stress exposure lasted 2–3 min and then the aggressive mouse was removed, so that the intruder mouse stayed into the aggressors home cage until the end of nociception test. The control mice were exposed for 2 min to a non-aggressive group-housed Swiss albino mouse. One day after completion of the experiments, animals were intracerebrally injected with 0.2 μl of a 1% methylene blue solution according to the procedure described above. They were then sacrificed by decapitation and their brains were removed and stored in a 10% formalin solution. Three days later, serial 55-m brain sections were cut using a microtome (Cryocut 1800, Reichert). Sections were inspected for correct injection sites by visualizing the dispersion of the dye under a dissecting microscope. Data were analysed by a two-way between-within analysis of variance (ANOVA) followed by the Duncan test (between group comparisons) or Dunnet test (within group comparisons).

3. Results

Analysis of data was carried out only for those animals in which the tip of the injection cannula was located within the borders of the periaqueductal gray.

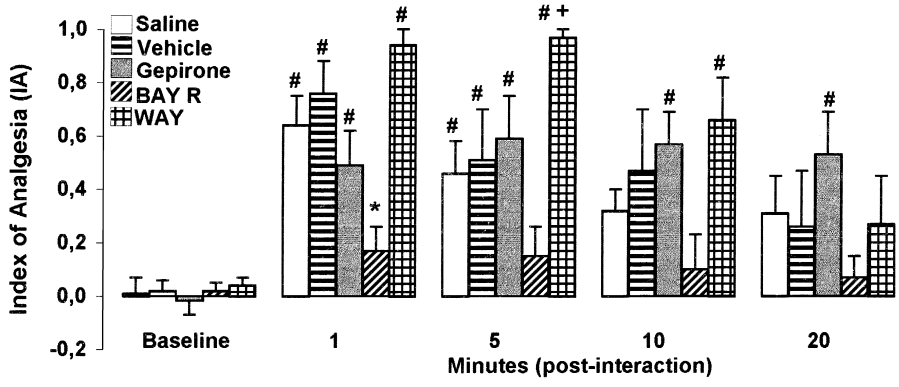

Fig. 1. Index of analgesia (IA) recorded pre-(baseline) and post-(1, 5, 10 and 20 min) 30 bites aggressive interactions in mice injected into the periaqueductal gray with saline ($n = 10$), vehicle (saline + Tween 80, $n = 7$), gepirone (30 nmol/0.2 μl, $n = 8$), BAY R 1531 (10 nmol/0.2 μl, $n = 10$) and WAY 100135 (10 nmol/0.2 μl, $n = 9$). Data are presented as mean (±S.E.) of IA. $^{\#}P < 0.05$ compared with baseline; $^{*}P < 0.05$ compared with saline and $^{+}P < 0.05$ compared with vehicle.

Table 1
Index of analgesia recorded pre- (baseline) and 1, 5, 10 and 20 min post-non-aggressive interaction (0-bite) in mice treated intra-periaqueductal gray with saline ($n = 6$), vehicle (saline + 4% Tween 80, $n = 5$), gepirone (30 nmol/0.2 μl, $n = 8$), BAY R 1531 (10 nmol/0.2 μl, $n = 7$) and WAY 100135 (10 nmol/0.2 μl, $n = 5$)

Treatment	Baseline	Minutes (post-interaction)			
		1	5	10	20
Saline	-0.06 ± 0.07	-0.04 ± 0.07	0.14 ± 0.17	-0.10 ± 0.06	-0.02 ± 0.12
Vehicle	-0.04 ± 0.08	0.03 ± 0.08	0.27 ± 0.19	-0.17 ± 0.05	0.06 ± 0.12
Gepirone	0.0 ± 0.04	0.16 ± 0.08	0.14 ± 0.07	0.06 ± 0.10	0.09 ± 0.06
BAY R 1531	-0.02 ± 0.07	-0.15 ± 0.11	-0.09 ± 0.07	-0.11 ± 0.08	-0.01 ± 0.08
WAY 100135	0.02 ± 0.02	-0.04 ± 0.11	-0.04 ± 0.13	0.04 ± 0.11	0.19 ± 0.20

Data are presented as mean ($\pm$S.E.) of index of analgesia.

Fig. 1 shows the IA recorded 5 min before (baseline) and 1, 5, 10 and 20 min after 30-bites aggressive interaction in mice treated intra-periaqueductal gray with saline (0.9% NaCl), vehicle (saline + 4% Tween 80), gepirone, BAY R 1531 or WAY 100135. ANOVA showed significant effects of time ($F(4,156) = 29.86$, $P < 0.001$), treatment ($F(4,39) = 4.68$, $P = 0.003$) and treatment vs. time interaction ($F(16,156) = 2.79$, $P = 0.001$). Within-group comparisons along time (baseline vs. post-interaction) indicated that aggressive interaction significantly increased the IA when compared with baseline in all groups, except for the BAY R 1531 group (Saline and Vehicle: 1st and 5th min; Gepirone: 1st to 20th min; WAY 100135: 1st to 10th min). Between-group comparisons at each time revealed that BAY R 1531 reduced significantly the IA at the 1st min when compared to saline. In addition, WAY 100135 produced a significant increase in the IA in the 5th min post-interaction as compared with vehicle. Between-group comparisons also revealed that mice treated with either saline or vehicle did not show any significant difference in IA along the whole experiment.

Table 1 shows the IA recorded 5 min before (baseline) and 1, 5, 10 and 20 min after non-aggressive interaction (0-bite) in mice treated intra-periaqueductal gray with saline (0.9% NaCl), vehicle (saline + 4%Tween 80), gepirone, BAY R 1531 or WAY 100135. ANOVA showed neither significant effects of time ($F(4,104) = 2.20$, $P = 0.074$) nor of treatment ($F(4,26) = 1.29$, $P = 0.299$).

4. Discussion

The results of a recently reported study (Canto de Souza et al., 1997) indicate that the analgesia induced by 30-bites social conflict in Swiss albino mice does not involve opioid and GABA (γ-aminobutyric acid)-benzodiazepine mechanisms, since this analgesia was not affected by either naloxone or diazepam. In contrast, the 5-HT_{1A} receptor agonists BAY R 1531 and gepirone reduced this type of aggressive interaction-induced analgesia. Because the highest dose of the full 5-HT_{1A} receptor agonist BAY R 1531 (0.1 mg/kg, i.p.) was ineffective, in contrast to the lowest doses of the drug (0.01 mg/kg), the suggestion was made that pre-synaptic 5-HT_{1A} receptors would mediate the antianalgesic drug effect, whereas stimulation of post-synaptic 5-HT_{1A} receptors would enhance social conflict-induced analgesia. Accordingly, gepirone being a full pre-synaptic 5-HT_{1A} receptor agonist and a partial postsynaptic 5-HT_{1A} receptor agonist caused an antianalgesic effect within a broader range of doses (0.3 and 3.0 mg/kg) than BAY R 1531.

The results of the present experiment do not support the above hypothesis, at least in the periaqueductal gray, since local administration of BAY R 1531 antagonized aggressive interaction-induced analgesia, whereas blockade of 5-HT_{1A} receptors with WAY 100135 enhanced the analgesic response to social conflict. The present results further show that the 5-HT_{1A} receptor partial agonist gepirone not only failed to decrease aggressive interaction-induced analgesia, but actually prolonged the duration of such analgesia. The last results, thus, suggest that in the present experimental conditions gepirone behaves more like a 5-HT_{1A} receptor antagonist than like a receptor agonist. Moreover, the proanalgesic effect of WAY 100135 shown by the present results indicates that endogenous 5-HT tonically inhibits aggressive interaction-induced analgesia through stimulation of 5-HT_{1A} receptors located in the periaqueductal gray. That these mechanisms are called upon by social conflict, alone, is testified by the failure of the 5-HT_{1A} ligands to change nociception when given after a non-aggressive social interaction.

In view of the apparent discrepancy between the above results obtained with systemic as compared to intra-periaqueductal gray drug administration, two possibilities should be considered: first, the hypothesis of a presynaptic mediation of the antianalgesic effect of 5-HT_{1A} receptor agonists is to be rejected. Second, the periaqueductal gray is not a critical site of the antianalgesic action of systemically injected 5-HT_{1A} receptor agonists.

On the other hand, the present results with intra-periaqueductal gray injection are compatible with the view that analgesia induced by defeat is related to fear (Rodgers and Randall, 1986; Rodgers and Randall, 1988; Siegfried et al., 1990), since experiments in the rat suggest that 5-HT

inhibits defense in the dorsal periaqueductal gray by stimulating postsynaptic 5-HT_{1A} receptors (Beckett et al., 1992; Nogueira and Graeff, 1995). Thus, the analgesia induced by social conflict may be viewed as part of the defense repertoire, having the adaptive function of inhibiting pain-elicited behaviors that are likely to interfere with effective fight or flight reactions (Bolles and Fanselow, 1980; Siegfried et al., 1990).

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