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$GABA_B$ receptor mechanism and imipramine-induced antinociception in ligated and non-ligated mice

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Abstract

This study concerned the effects of $GABA_B$ receptor agents on imipramine-induced antinociception in ligated and non-ligated mice in hot-plate test. The data showed that different doses of morphine (3, 6 and 9 mg/kg) induced a dose-dependent antinociception in non-ligated or ligated mice. However, the opioid response was decreased in the ligated animals. Intracerebroventricular (i.c.v.) administration of imipramine (5, 10, 20 and 40 μg/mouse) did not induce antinociception in either non-ligated or ligated mice. However, the response induced in the ligated mice was less than that induced in the non-ligated animals. Intraperitoneal (i.p.) administration of imipramine (10, 20, 30 and 40 mg/kg) induced antinociception in both ligated and non-ligated animals. The responses to the drug were not significantly different in the two groups. Administration of baclofen either i.c.v. (0.125, 0.25 and 0.5 μg/mouse) or i.p. (0.5, 1, 2 and 4 mg/kg) induced antinociception. The response to the drug was not significantly different in ligated and non-ligated mice. I.c.v. administration of a lower dose of baclofen (0.125 μg/mouse) with different doses of imipramine (2.5, 5 and 10 mg/kg) potentiates the response of imipramine. This effect was reduced by i.c.v. injection of $GABA_B$ receptor antagonist, CGP35348 [*P*-(3-aminopropyl)-*p*-diethoxymethyl-phosphinic acid] (20 μg/mouse). The higher dose of antagonist (20 μg/mouse) also decreased the response induced by baclofen or imipramine. CGP35348 itself (2.5, 5, 10 and 20 μg/mouse) induced dose-dependent antinociception with no significant difference in the ligated and non-ligated mice. It is concluded that a GABA receptor mechanism(s) may modulate the antidepressant-induced antinociception.

Keywords: Baclofen; Imipramine; Ligation; Hot-plate test; (Mouse)

1. Introduction

Constriction injury of a peripheral nerve, induces hyperalgesia and may be used as an animal model of neuropathic pain. The method has been employed in the study of neurogenic pain mechanisms and its pharmacological treatment (Cui et al., 1997). Neuropathic pain is a chronic and disabling condition, arising from peripheral nerve injury which is a disorder often refractory to conventional analgesics such as opiates and non-steroidal anti-inflammatory drugs (Max et al., 1988; Tanelian and Brose, 1991). The mechanisms underlying neuropathic pain are complex and appear to involve various peripheral and central components (Fields and Rowbotham, 1994).

Baclofen, which is a prototypic agonist for the $GABA_B$ receptors (Bowery, 1993), produces analgesia in a variety of available tests (Sawynok, 1987; Zarrindast and Djavdan, 1989; Zarrindast and Moghadampour, 1991). Baclofen elicits its effect through both spinal and supraspinal sites of action (Sawynok, 1987). The drug has major clinical use as an antispastic agent (Young and Delwaide, 1981), reduces pain associated with spasticity (Pinto et al., 1972), and is useful in the treatment of trigeminal neuralgia (Fromm et al., 1984). The involvement of neurotransmitters in pharmacological effects of baclofen is supported by the results of biochemical studies which have shown significant effects of baclofen on the content, release and turnover of these agents in various brain regions (for review, see Sawynok, 1989). $GABA_B$ receptors are associated with many brain functions, and as such may be targets for drugs with central effects including antidepressants (Knight and Bowery, 1996). $GABA_B$ binding sites may be

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upregulated by administration of antidepressants (Lloyd et al., 1985). GABA has been shown to play a role in depression (Petty et al., 1995a,b). We had also shown that a GABA receptor mechanism(s) may modulate antidepressant-induced antinociception (Sabetkasai et al., 1999). Antidepressants are probably the most commonly prescribed drugs for the treatment of chronic pain (Feinmann, 1985). Since ligation is a model of neuropathic pain (Ardid and Guilbaud, 1992), the present experiments evaluated the possible involvement of a $GABA_B$ receptor mechanism(s) in antinociception induced by tricyclic antidepressants in non-ligated and ligated mice.

2. Materials and methods

2.1. Animals

Male NMRI mice weighing 20–25 g were used in all experiments. The animals were housed in groups of 10 under conditions of constant temperature (21 ± 2°C) and a light-controlled room (light period, 07h00 min–19h00 min). Animals had free access to food and water except during the experiments.

2.2. Surgical procedure for nerve ligation

The unilateral peripheral mononeuropathy was produced on the right hind limb, based on the method of Seltzer et al. (1990), except that the animals were anaesthetized with sodium thiopental (40 mg/kg) and a copper wire was used for ligation. The animal's right sciatic nerve was exposed, and a 2–3 nm long nerve segment was then dissected. The only one ligature with fine metal wire was made around the dissected nerve. All the control animals were sham-operated.

2.3. Chronic guide canula implantation

Stainless-steel guide cannulas (23 gauge) were stereotaxically (David Koff Instruments, USA) implanted under anaesthesia with sodium thiopental (40 mg/kg, i.p.). The guide cannulas were implanted in the right lateral ventricle at the following coordinates based on the method of Jiang et al. (1990) with a minor modification: 1.5 mm lateral and 0.9 mm caudal to bregma at a depth of 3 mm.

2.4. Antinociception measurement

Pain sensitivity was assessed in hot-plate test according to the method of Eddy and Liembach (1953), with a minor modification. Briefly, the animal was placed on a circular surface (diameter 19 cm) maintained at 55 ± 0.2°C and surrounded by a Plexiglass wall 12 cm high. The apparatus (Harvard, England) was equipped with a timer and a thermocouple to maintain a constant temperature. Licking the forepaws, lifting hindpaws or jumping from the surface was used as the end point for the determination of response latencies (Pick et al., 1992). Failure to respond by 45 s resulted in the termination of the test (cut-off). Fourteen days after nerve ligation of animals, the antinociception induced by drugs was measured. Antinociception was quantified as the Area Under the Curve (AUC) of response latencies (s) on 15, 30, 45, 60 min after drug administration for each animal. AUC [time(min) · latency(s)] was calculated as drug response (s) plotted against time (min) using the trapezoidal rule (Coutinho et al., 1998).

2.5. Drugs

The chemicals used were: morphine sulphate (Temad, Iran), sodium thiopental (Specia, Paris), baclofen, CGP35348 (*P*-[3-aminopropyl]-*p*-diethoxymethyl-phosphinic acid) and imipramine (Ciba-Geigy, Switzerland). All drugs were dissolved in saline. Morphine was injected subcutaneously (s.c.), and the other drugs were injected intracerebroventricularly (i.c.v.) or intraperitoneally (i.p.) in a volume of 10 ml/kg, respectively.

2.6. Statistical analysis

Analysis of variance (ANOVA) followed by Newman–Keuls test was used for analysis of the data. Differences between means were considered statistically significant if $P < 0.05$. Each point is the means ± S.E.M. for nine mice.

3. Results

3.1. Antinociception induced by morphine or imipramine in non-ligated or ligated mice in hot-plate test

Effects of different doses of morphine are shown in Fig. 1. The animals were either ligated or non-ligated. Subcutaneous (s.c.) administration of morphine (3, 6 and 9 mg/kg) to mice induced antinociception in the non-ligated [one-way ANOVA; $F(3,32) = 18.1$, $P < 0.0001$] and ligated mice [$F(3,32) = 33.2$, $P < 0.0001$] as compared with saline-treated animals. The antinociceptive response to the drug was dose-dependent and the greater response was obtained with 9 mg/kg of the opioid. Two-way ANOVA showed that morphine-induced antinociception [$F(3,64) = 34.7$, $P < 0.0001$] with a difference between non-ligated and ligated-animals [$F(1,64) = 26.3$, $P < 0.0001$] and ligation elicited an interaction with the morphine response [$F(3,64) = 3.1$, $P < 0.05$]. Post hoc analysis indicates that the antinociceptive response to morphine was decreased in ligated animals.

Effects of different doses of imipramine are shown in Fig. 2A and B. Intraperitoneal (i.p.) administration of imipramine (10, 20, 30 and 40 mg/kg) to mice induced

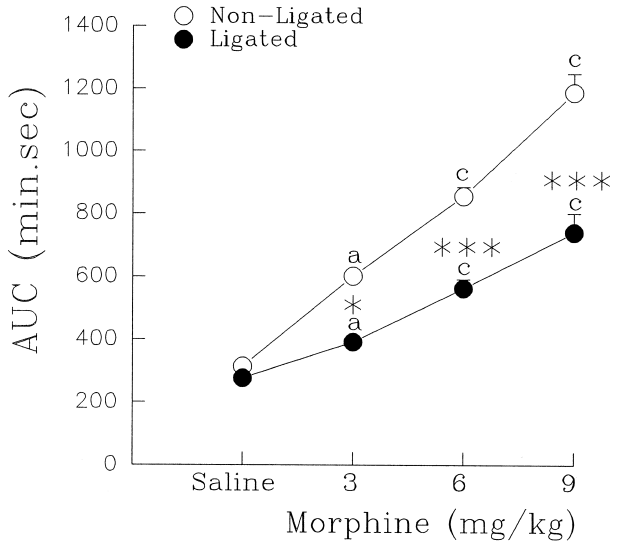

Fig. 1. Antinociceptive effect of different doses of morphine in non-ligated (○) or ligated mice (●) in the hot-plate test. Mice were injected subcutaneously (s.c.) with saline (10 ml/kg) or different doses of morphine (3, 6 and 9 mg/kg). Antinociception was recorded 15, 30, 45 and 60 min after drug injection. Each point is the mean ± S.E.M. of area under the curve (AUC) for nine mice. $^{*}P < 0.05$, $^{***}P < 0.001$ different from respective non-ligated control groups. $^{a}P < 0.05$, $^{c}P < 0.001$ different from respective saline control groups.

dose-dependent antinociception in the non-ligated [one-way ANOVA; $F(4,40) = 44.2$, $P < 0.0001$] or ligated mice [one-way ANOVA; $F(4,40) = 65.6$, $P < 0.0001$]. The greater antinociceptive effect was obtained with 40 mg/kg of the drug in both non-ligated and ligated animal. Two-way ANOVA showed that ligation [Factor 1; $F(1,80) = 9.78$, $P < 0.01$] and i.p. injection of different doses of imipramine (Factor 2; $F(4,80) = 96.5$, $P < 0.0001$) showed no interactions [$F(4,80 = 0.47$, $P > 0.05$). Post hoc analysis indicated no significant difference between the responses to imipramine in non-ligated and ligated animals (Fig. 2A). Intracerebroventricular (i.c.v.) administration of imipramine (5, 10, 20 and 40 μg/mouse) did not induce antinociception in the non-ligated [one-way ANOVA; $F(4,40) = 1.1$, $P > 0.05$] or ligated mice [one-way ANOVA $F(4,40) = 0.3$, $P > 0.05$] (Fig. 2B). Two-way ANOVA also showed that imipramine did not induce any antinociceptive response [$F(4,80) = 0.15$, $P > 0.05$]. There was a significant difference between the response to the drug in the ligated and non-ligated animals [$F(1,80) = 50.4$, $P < 0.0001$] indicating that ligation induced a hyperalgesic effect. No interaction was found between response to the drug and ligation [$F(4,80) = 0.8$, $P > 0.05$].

3.2. Effects of baclofen or CGP35348 on antinociception in non-ligated or ligated mice

The effects of baclofen are shown in Fig. 3A and B. I.p. injection of baclofen (0.5, 1, 2 and 4 mg/kg) also induced antinociception in both non-ligated [one-way ANOVA; $F(4,40) = 4.8$, $P < 0.005$] and ligated animals [one-way ANOVA; $F(4,40) = 10.4$, $P < 0.0001$]. A greater effect was obtained with 4 mg/kg of the drug. Two-way ANOVA showed that ligation [Factor 1; $F(1,80) = 2.8$, $P > 0.5$] and baclofen [Factor 2; $F(4,80) = 15.5$, $P < 0.0001$] did not elicit interactions [$F(4,80) = 0.86$, $P > 0.05$], which shows that ligation is not able to alter the drug response (Fig. 3A).

I.c.v. administration of baclofen (0.125, 0.25 and 0.5 μg/mouse) induced significant antinociception in the non-ligated [one-way ANOVA; $F(3,32) = 24.3$, $P < 0.0001$] or ligated mice [one-way ANOVA; $F(3,32) = 91.6$,

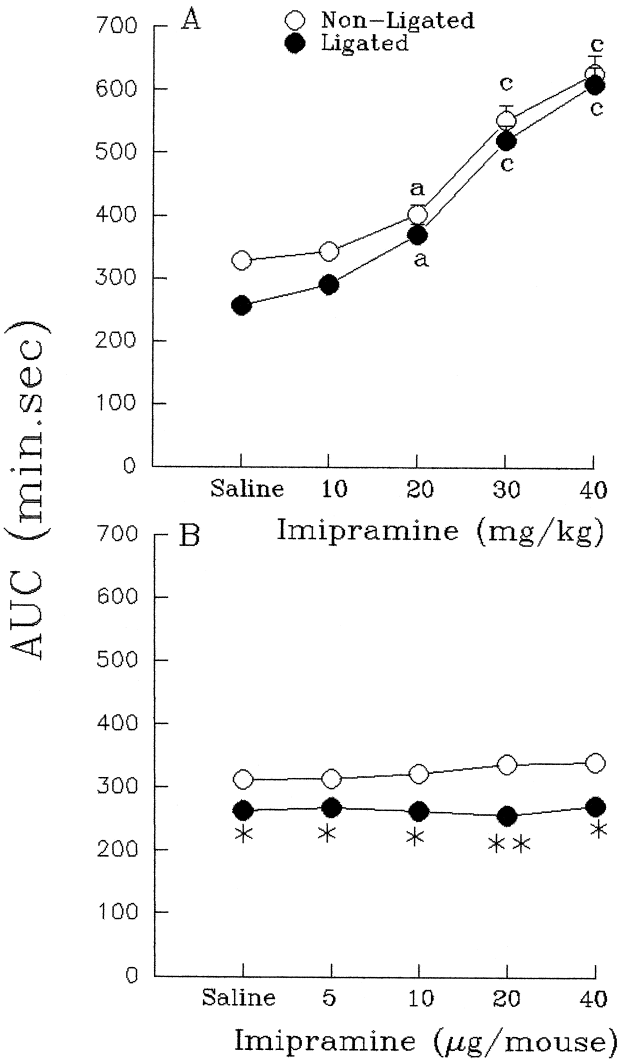

Fig. 2. Antinociceptive effect of different doses of imipramine in the non-ligated (○) and ligated animals (●) in the hot-plate test. One group of mice (panel A) received intraperitoneally (i.p.) different doses of imipramine (10, 20, 30 and 40 mg/kg). The animals in the second group (panel B) received an intracerebroventricular (i.c.v.) injection of different doses of imipramine (5, 10, 20 and 40 μg/mouse). Antinociception was recorded 15, 30, 45 and 60 min after drug injection. Each point is the mean ± S.E.M. of AUC for nine mice. $^{*}P < 0.05$, $^{**}P < 0.01$ different from respective non-ligated control groups. $^{a}P < 0.05$, $^{c}P < 0.001$ different from respective saline control groups.

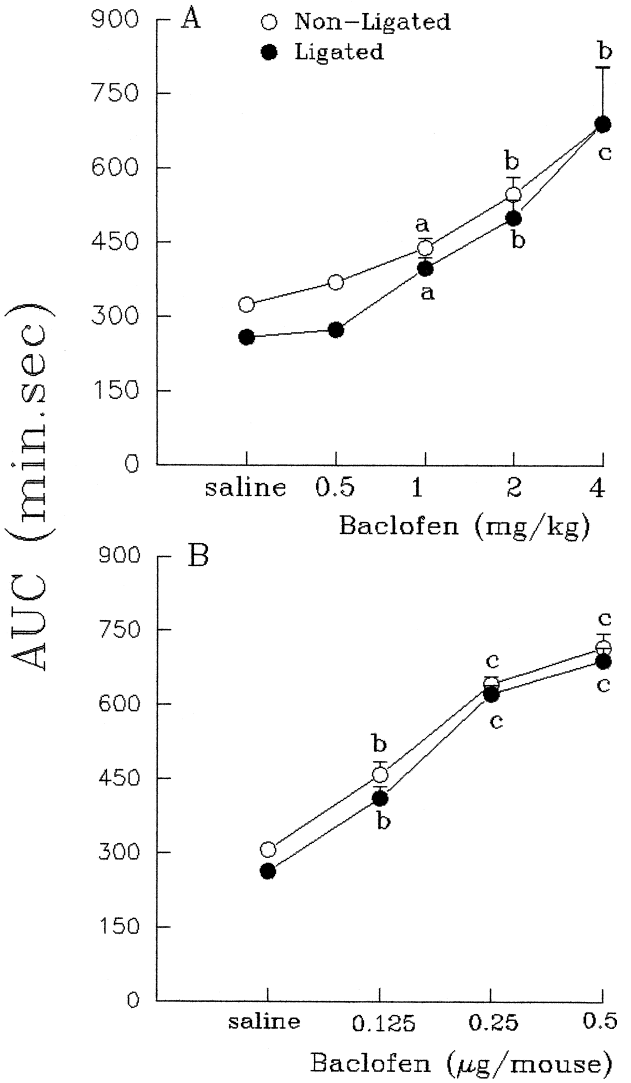

Fig. 3. Antinociceptive effect of different doses of baclofen in the non-ligated (○) and ligated animals (●) in the hot-plate test. One group of mice (panel A) received intraperitoneally (i.p.) different doses of baclofen (0.5, 1, 2 and 4 mg/kg). The animals in the second group (panel B) received an intracerebroventricular (i.c.v.) injection of different doses of baclofen (0.125, 0.25 and 0.5 μg/mouse). Antinociception was recorded 15, 30, 45 and 60 min after drug injection. Each point is the mean ± S.E.M. of AUC for nine mice. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ different from respective saline control groups.

$P < 0.0001$]. The dose of 0.5 μg/mouse produced a greater response. Two-way ANOVA showed that ligation [Factor 1; $F(1,64) = 2.1$, $P > 0.5$] and baclofen [Factor 2; $F(3,64) = 69.9$, $P < 0.0001$] did not elicit interactions [$F(3,64) = 0.12$, $P > 0.5$] indicating that the response induced by i.c.v. administration of baclofen is not altered by ligation (Fig. 3B).

Various doses of CGP35348 (2.5, 5, 10 and 20 μg/mouse) also induced antinociception in the non-ligated [one-way ANOVA; $F(4,40) = 13.5$, $P < 0.0001$] or ligated animals [$F(4,40) = 29.2$, $P < 0.0001$]. The response was dose-dependent and the higher effect was achieved with 20 μg/mouse of the drug.

Two-way ANOVA showed that ligation [Factor 1; $F(1,80) = 13.8$, $P < 0.0001$] and the response to i.c.v. administration of different doses of CGP35348 [Factor 2; $F(4,80) = 33.5$, $P < 0.0001$] had no interactions [$F(4,80) = 0.35$, $P > 0.05$]. Further analysis showed that the response to CGP35348 was not altered by ligation (Fig. 4).

The effect of CGP35348 on the baclofen response is shown in Fig. 5A and B. Two-way ANOVA indicates that CGP35348 (Factor 1; 1,64 = 102.1, $P < 0.0001$) and baclofen [Factor 2; 3,64) = 24.4, $P < 0.0001$] elicited a significant interaction [$F(3,64) = 82$, $P < 0.0001$] in non-ligated mice. Further analysis showed that CGP35348 reduced the baclofen response in the non-ligated animals (Fig. 5A). Two-way ANOVA also showed that CGP35348 [$F(1,64) = 96.1$, $P < 0.0001$] and baclofen [$F(3,64) = 22.5$, $P < 0.0001$] caused an interaction [$F(3,64) = 93.1$, $P < 0.0001$] in ligated mice. Post hoc analysis showed that CGP35348 decreased the baclofen effect in the ligated animals (Fig. 5B).

3.3. Effects of CGP35348 on antinociception induced by imipramine or imipramine plus baclofen

The effect of the $GABA_B$ receptor antagonist, CGP35348, in the presence or absence of imipramine is shown in Fig. 6A and B. Fig. 6A shows the response to CGP35348 in the presence or absence of imipramine in non-ligated mice. Two-way ANOVA showed that i.p. administration of imipramine [Factor 1; $F(1,80) = 0.34$, $P > 0.05$] and antinociceptive effect of different doses of CGP35348 (2.5, 5, 10 and 20 μg/mouse) [Factor 2; $F(4,80) = 20.3$, $P < 0.0001$] had an interaction [$F(4,80)$

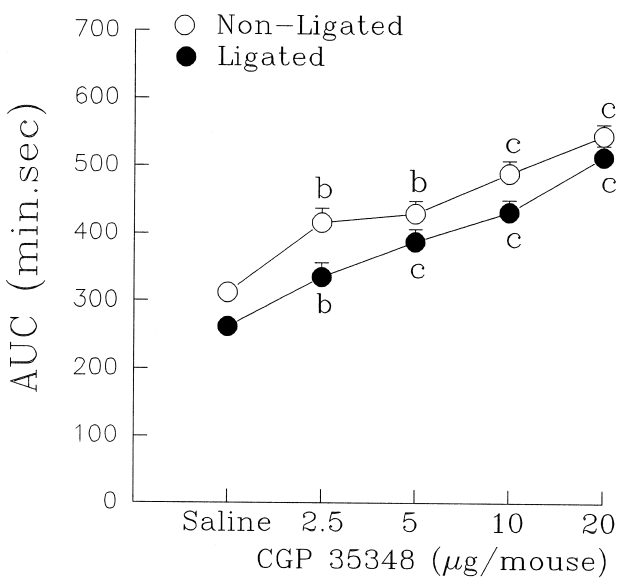

Fig. 4. Effect of CGP35348 in non-ligated (○) or ligated mice (●). Mice were injected i.c.v. with different doses of CGP35348 (2.5, 5, 10 and 20 μg/mouse) and antinociception was recorded 15, 30, 45 and 60 min after the drug injection in each animal. Each point is the mean ± S.E.M. of AUC for nine mice. $^{b}P < 0.01$, $^{c}P < 0.001$ different from respective saline control groups.

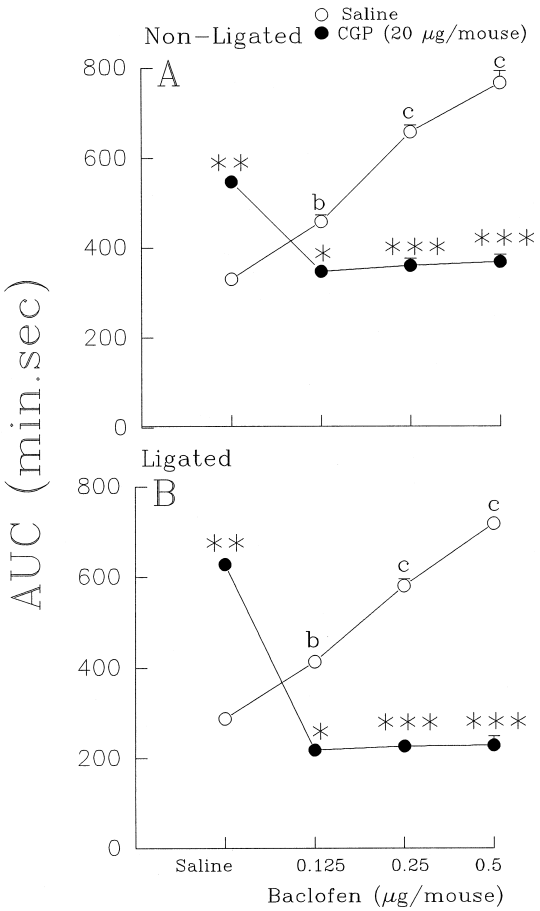

Fig. 5. Effect of baclofen in the presence or absence of CGP35348. Non-ligated (panel A) or ligated (panel B) animals were given (i.c.v.) different doses of baclofen (0.125, 2.5 and 0.5 μg/mouse) or CGP35348 (20 μg/mouse) 15 min before baclofen injection. Antinociception was recorded 15, 30, 45 and 60 min after baclofen administration. Each point is the mean ± S.E.M. of AUC for nine mice. $^{a}P < 0.05$, $^{c}P < 0.001$ different from respective saline control groups. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ different from respective baclofen groups.

$= 7.9,\ P < 0.0001$]. Further analysis indicated that CGP35348 induced antinociception and that a higher dose (20 μg/mouse) of the antagonist reduced the imipramine response in the non-ligated animals.

Fig. 6B shows the response to CGP35348 in the presence or absence of imipramine in ligated mice. Two way ANOVA showed that i.p. injection of imipramine [Factor 1; $F(1,80) = 13,\ P < 0.0001$] and the effect of CGP35348 [$F(4,80) = 40.3,\ P < 0.0001$] had an interaction [$F(4,80) = 13.1,\ P < 0.0001$]. Post hoc analysis indicates that CGP35348 induced antinociception in ligated animals and a higher dose of CGP35348 decreased the antinociception induced by imipramine.

The effect of baclofen or CGP35348 on the imipramine response is shown in Fig. 7. Two-way ANOVA indicated that baclofen [Factor 1; $F(1,64) = 20.7,\ P < 0.0001$], and different doses of imipramine [Factor 2; $F(3,64) = 10.3$] induced an interaction [$F(3,64) = 7.6,\ P < 0.0001$]. Further analysis showed that baclofen potentiated the imipramine effect in non-ligated animals. When a higher dose of CGP35348 (20 μg/mouse) [Factor 1; $F(1,64) = 116.9,\ P < 0.0001$] was challenged against imipramine (2.5-10 mg/kg) plus baclofen (0.125 μg/mouse) [Factor 2; $F(3,64) = 10.0,\ P < 0.0001$], the response induced by the combination of the latter drugs was decreased in the non-ligated animals [$F(3,64) = 3.6,\ P < 0.0001$].

Two-way ANOVA also showed that baclofen [Factor 1; $F(1,64) = 383.9,\ P < 0.0001$] and different doses of imipramine [Factor 2; $F(3,64) = 18.2,\ P < 0.0001$] induced an interaction [$F(3,64) = 10.0,\ P < 0.0001$]. Post hoc analysis showed that baclofen potentiated the imipramine effect in ligated animals. When CGP35348 (20 μg/mouse) [Factor 1; $F(1,64) = 186.6,\ P < 0.0001$] was challenged against imipramine (2.5-10 mg/kg) plus baclofen (0.125 μg/mouse) [Factor 2; $F(3,64) = 14.7,\ P <$

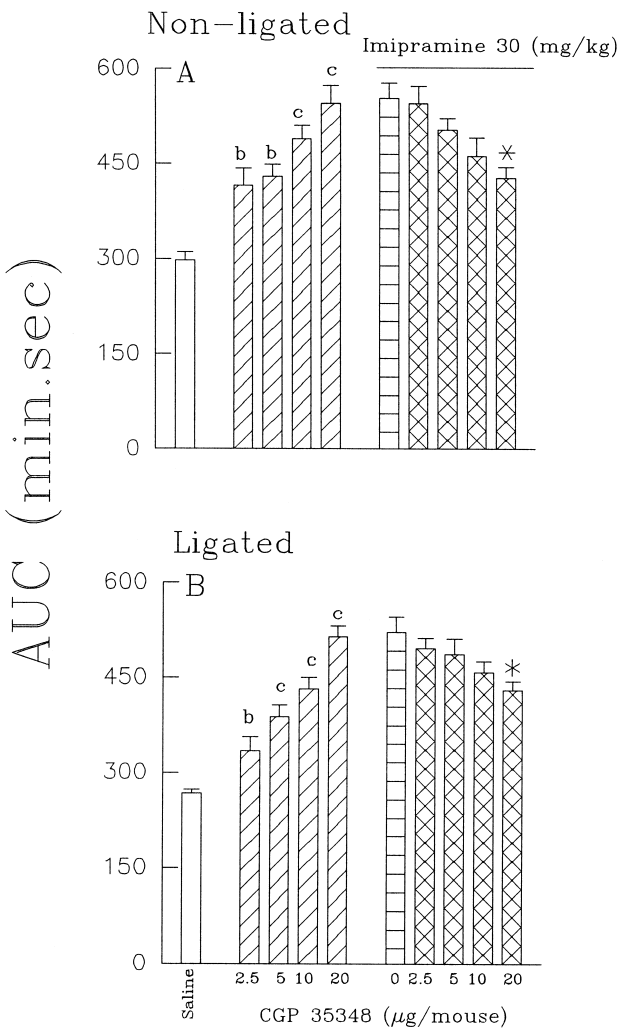

Fig. 6. Effect of CGP35348 in the presence or absence of imipramine. Non-ligated (panel A) or ligated (panel B) animals were given (i.c.v.) either different doses of CGP35348 (2.5, 5, 10 and 20 μg/mouse) alone or CGP35348 plus imipramine (30 mg/kg, i.p.) 15 min before imipramine injection. Antinociception was recorded 15, 30, 45 and 60 min after imipramine or CGP35348 injection. Each point is the mean ± S.E.M. of AUC for nine mice. $^{*}P < 0.05$ different from respective imipramine group. $^{b}P < 0.01$, $^{c}P < 0.001$ different from saline control group.

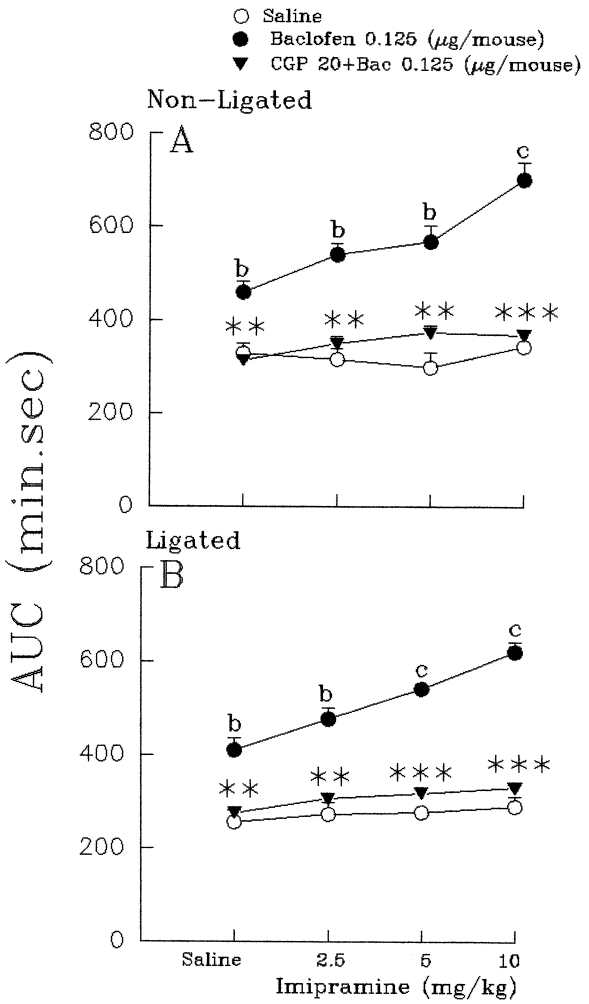

Fig. 7. Effects of $GABA_B$ receptor agonist and antagonist on antinociception induced by imipramine. Mice were treated (i.p.) with imipramine (2.5, 5 and 10 mg/kg), imipramine plus baclofen (0.125 μg/mouse, i.c.v.), or CGP35348 (20 μg/mouse, i.c.v.) plus imipramine and baclofen. CGP35348 was injected 30 min and imipramine 15 min prior to baclofen administration. Antinociception was recorded 15, 30, 45 and 60 min after baclofen injection. Each point is the mean ± S.E.M. of AUC for 9 mice. $^{b}P < 0.01$, $^{c}P < 0.001$ different from respective saline control groups. $^{**}P < 0.01$, $^{***}P < 0.001$ different from respective baclofen control groups.

0.0001], the response induced by the combination of the latter drugs was decreased in the non-ligated animals [$F(3,64) = 4.7,\ P < 0.0001$].

4. Discussion

Sciatic nerve ligation is now a widely used model of neuropathic pain. The model may mimic important characteristics of chronic neurogenic pain in patients following peripheral nerve injury (Cui et al., 1997). In the present study, the effect of imipramine in the presence or absence of a $GABA_B$ receptor agonist and antagonist was evaluated in the non-ligated or ligated mice in the hot-plate test. The morphine response also was tested for comparison.

Opioids have been used for many years for moderate to severe pain (Arner and Meyerson, 1988; Fields, 1988; Jadad et al., 1992; Kupers et al., 1991; Rowbotham et al., 1991). A limiting factor in the clinical administration of opioids for pain relief is that repeated doses may lead to development of tolerance and physical dependence upon them.

In the present study, we replicated the experimental model of neuropathy and found that different doses of morphine-induced antinociception in the hot-plate test. It has been shown that pain-related disorders are maximal 14 days after nerve ligation (Filliatreau et al., 1994). Our data showed that ligated mice exhibited an exaggerated response to thermal stimulus in the hot-plate test, indicating that ligation of the sciatic nerve decreases the response to morphine in mice, 14 days after nerve ligation. This is in agreement with other findings that nerve ligation (using four ligatures) as a model of neuropathic pain can reduce morphine-induced antinociception (Mao et al., 1995). However, we used one copper-wire ligature for ligation. The mechanisms underlying the decrease in morphine response in ligated animals is thought to be complex. Neuronal plasticity and interference with the morphine effect, increases in spinal level of cholecystokinin (see Suzuki et al., 1999), upregulation of cholecystokinin-B receptors in spinal cord (Wiesenfeld-Hallin et al., 1997) and activation of spinal cord excitatory amino acids, particularly NMDA subtype receptors (Seltzer et al., 1991) all have been shown to be implicated in reduced morphine analgesia. Intraperitoneal (i.p.), but not intracerebroventricular (i.c.v.), administration of imipramine also induced antinociception in both non-ligated and ligated animals. The response to imipramine was not significantly different in non-ligated and ligated animals. This may imply that a supraspinal mechanism is not a possible site of drug action. Our results are supported by reports that antidepressants produce antinociception in chronic pain (Feinmann, 1985; Ardid and Guilbaud, 1992) and that imipramine may be often more effective than opioid analgesics in treating neuropathic pain (Max et al., 1992). The present data may indicate that the antinociception induced by morphine and imipramine is mediated through different mechanisms. Since the antinociception by morphine is mediated through both spinal and supraspinal sites and that by imipramine cannot be induced by i.c.v. administration, the difference between the responses to the two drugs in ligated animals may indicate that tolerance to the morphine response is mediated at least partially through supraspinal site. However, tricyclic antidepressants increase monoamines levels at supraspinal sites and monoamines have been proposed to be involved in antinociception at both supraspinal and spinal sites (Sawynok, 1987).

The complex association between pain and depression has created some confusion. There is still some dispute as to whether antidepressants exert their effects via analgesic or antidepressant pathways (Feinmann, 1985). The work of

Feinmann et al. (1984) implies that pain relief obtained with tricyclic antidepressants is independent of the antidepressant effect.

There is increasing evidence to suggest that GABA may play a role in affective disorders such as depression. Alterations in GABAergic activity have been associated with clinical depression (Berrettini et al., 1983; Gerner and Hare, 1981). Monoamines appear to be involved in the antinociceptive effect of the $GABA_B$ receptor agonist, baclofen (Young and Delwaide, 1981; Bowery et al., 1983), which seems to act at both supraspinal and spinal sites (Sawynok, 1987). In the present study, different doses of baclofen produced a dose-dependent antinociceptive effect. However, as with imipramine, no difference was found between responses to the drug in the non-ligated and ligated animals. The response to baclofen was reduced by a $GABA_B$ receptor antagonist, CGP35348. The antinociceptive effect of baclofen has also been described previously (Sawynok, 1987; Zarrindast and Djavdan, 1989; Zarrindast and Sabetkasai, 1992; Sabetkasai and Zarrindast, 1993). There is much evidence that the phenomenon of peripheral hypersensitivity, with allodynia and hyperalgesia, is a result of central sensitization which reflects a loss of tonic GABA-mediated inhibition as well as an increase in excitatory neurotransmitters in the spinal dorsal horn (Woolf and Doubell, 1994; Devor, 1996). It has been suggested that in the spinal cord inhibition of the release of excitatory neurotransmitters/neuromodulators from primary afferent fibres accounts for the intrathecally injected baclofen-induced antinociception, and that this is prevented by $GABA_B$ receptor antagonists (Aran and Hammond, 1991).

Our data showed that the combination of a lower dose of baclofen with doses of imipramine that do not elicit antinociception produced a high antinociceptive effect. Thus, there may be an interaction between baclofen and imipramine responses. These results may support our present data which shows that the selective $GABA_B$ receptor antagonist, CGP35348 (Olpe et al., 1990), reduced the antinociceptive effect of baclofen plus impramine. This would suggest that the antinociceptive effect of imipramine is mediated through a $GABA_B$ receptor mechanism. A GABA receptor mechanism has been shown to be involved in depression (Petty et al., 1995a,b). The hypothesis that $GABA_B$ receptors are associated with many brain functions, and as such may be targets for drugs with analgesic and antidepressant effects (Bowery, 1993) supports this idea. Whether the antinoception responses of imipramine and baclofen are mediated through a similar mechanism should be clarified.

The involvement of dopamine, noradrenaline and 5-hydroxytryptamine (5-HT) mechanisms in the antinociceptive effect of baclofen has been described previously (for review, see Sawynok, 1989) and the existence of $GABA_B$ receptors regulating the release of noradrenaline (Bowery et al., 1980), dopamine (Bowery et al., 1980; Reimann et al., 1982), serotonin (Bowery et al., 1980; Gray and Green, 1987) and glutamate (Potashner, 1979) was also reported in the central nervous system.

Analgesia can also be effected through modulation of monoamine activity with 5-HT or noradrenaline uptake inhibitors such as trycyclic antidepressants (Magni, 1991). On the other hand, imipramine non-selectively blocks the re-uptake of serotonin (Sindrup et al., 1992; Gram, 1983). It has been proposed that 5-HT_3 receptors in the spinal cord induce antinociception possibly through GABA release (Alhaider et al., 1991). This represents a possible mechanism of a serotonin/GABA receptors mechanism for producing antinociceptive effects, and may be consistent with our data that a $GABA_B$ receptor antagonist, CGP35348, reduce imipramine's antinociceptive effect.

It has been shown that CGP35348 increases the release of GABA from electrically stimulated cortical slices (Bittiger et al., 1990). This action of CGP35348 may account for the antinociception induced by the antagonist itself in the present study.

More experiments are needed to clarify the possible involvement of a GABA receptor mechanism in the antinociception induced by antidepressants.

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