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Effects of GABA receptor antagonists injected spinally on antinociception induced by opioids administered supraspinally in mice

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Abstract

The present study was designed to investigate the modulatory effects of blockade of spinal $GABA_A$ and $GABA_B$ receptors on antinociception induced by supraspinally administered μ- and ϵ-opioid receptor agonists. The effects of intrathecal (i.t.) injections with $GABA_A$ and $GABA_B$ receptor antagonists, SR 95531 [2-(3-carboxypropyl)-3-amino-6-(4-mehylphenyl)pyridazinium bromide] and 5-aminovaleric acid, respectively, on the antinociception induced by morphine (a μ-opioid receptor agonist) and β-endorphin (an ϵ-opioid receptor agonist) injected intracerebroventricularly (i.c.v.) were studied. Antinociception was assayed using the tail-flick test. The i.t. injection of SR 95531 (0.04–0.16 nmol) and 5-aminovaleric acid (32.5–130 nmol), administered alone did not affect the latencies of the tail-flick response, but selectively antagonized the inhibition of the tail-flick response induced by muscimol (a $GABA_A$ receptor agonist) and baclofen (a $GABA_B$ receptor agonist), respectively. The i.t. injection of SR 95531 attenuated dose-dependently the inhibition of the tail-flick response induced by i.c.v. administered morphine, without affecting the i.c.v. administered β-endorphin-induced response. 5-Aminovaleric acid attenuated dose-dependently the inhibition of the tail-flick response induced by β-endorphin, without affecting the response to i.c.v. administered morphine. Our results indicate that $GABA_A$ but not $GABA_B$ receptors located at the spinal cord appears to be involved in the antinociception induced by morphine administered supraspinally whereas $GABA_B$ but not $GABA_A$ receptors located at the spinal cord may be involved in the antinociception induced by supraspinally administered β-endorphin, supporting further the hypothesis that morphine and β-endorphin administered supraspinally produce their antinociception via the activation of different descending pain inhibitory systems.

Keywords: β-Endorphin; Morphine; SR 95531; 5-Aminovaleric acid; Tail-flick inhibition

1. Introduction

γ-Amino butyric acid (GABA) may play an important role in modulating opioid-induced antinociception. Systemic administration of GABAergic agents enhances the antinociception induced by opioids (reviewed by Sawynok, 1984, 1987; Sivam and Ho, 1985), although central administration (i.c.v.) or direct microinjection into the periaqueductal gray or nucleus raphe dorsalis paradoxically reduces morphine-induced antinociception (Zonta et al., 1981; Zambotti et al., 1982; Romandini and Samanin, 1984). Furthermore, GABAergic agents themselves produce antinociception (reviewed by Sawynok, 1984, 1987; Sivam and Ho, 1985).

The antinociception induced by morphine and β-endorphin applied supraspinally is mediated by stimulation of different types of opioid receptors, followed in turn by the activation of different descending pain control systems which utilize different neurotransmitters and receptors in the spinal cord. The antinociception induced by morphine is mediated by the stimulation of μ-opioid receptors and release of norepinephrine and serotonin (5-HT) acting on α_2-adrenoceptors and 5-HT receptors in the spinal cord (Kuraishi et al., 1978, 1979; Yaksh, 1979; Suh et al., 1988, 1989; Jung et al., 1994). The antinociception induced by β-endorphin is mediated by the stimulation of ϵ-opioid receptors and subsequent release of [Met^5]enkephalin acting on δ_2-opioid receptors in the spinal cord (Tseng and Fujimoto, 1984, 1985; Suh et al., 1988, 1989; Tseng and Suh, 1989; Suh and Tseng, 1990a,b, 1992).

In recent studies, Fujimoto and his colleagues have demonstrated that the spinal GABA receptors are involved in the antinociception induced by the stimulation of supraspinal δ-opioid receptors or by cold-water swimming-induced stress (Holmes and Fujimoto, 1994; Rady and Fujimoto, 1995; Killian et al., 1995). However, an

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involvement of spinal GABA receptors in supraspinally administered morphine- and β-endorphin-induced antinociception has not been characterized. The present studies were thus designed to study the modulatory roles of the blockade of spinal $GABA_A$ and $GABA_B$ receptor by i.t. injection of SR95531 and 5-aminovaleric acid, respectively, at doses which injected alone did not affect nociceptive latencies, on the antinociception induced by i.c.v. administered morphine and β-endorphin.

2. Materials and methods

2.1. Experimental animals

Male ICR mice (23–25 g) were used for all the experiments. The animals were housed 5 per cage in a room maintained at 22 ± 0.5°C with an alternating 12-h light-dark cycle. Food and water were available ad libitum. The animals were used only once.

2.2. Assessment of antinociception

Antinociception was evaluated in the tail-flick test (D'Amour and Smith, 1941). For the measurement of the latency of the tail-flick response, mice were gently held with one hand with the tail positioned in the apparatus (EMDIE Instrument, Maidens, VA; Model TF6) for radiant heat stimulation. The tail-flick response was elicited by applying radiant heat to the dorsal surface of the tail. The intensity of the heat stimulus was adjusted so that the animal flicked its tail within 3–5 s. The tail-flick latencies were measured before (T_0) and after (T_1) the injection of opioid agonists. The inhibition of the tail-flick response was expressed as 'percentage maximal possible effect (% MPE)' which was calculated as $[(T_1 - T_0)/(T_2 - T_0)] \times 100$, where the cut-off time (T_2) was set at 10 s for the tail-flick test.

2.3. Intracerebroventricular (i.c.v.) and intrathecal (i.t.) injections

I.c.v. injections were made according to the procedure of Haley and McKormick (1957). I.t. administration was performed following the method of Hylden and Wilcox (1980) using a Hamilton syringe with 30-gauge needle. The i.c.v. or i.t. injection volume was 5 μl and the injection sites were verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the ventricular space or in the spinal cord. The dye injected i.c.v. was found to be distributed through the ventricular spaces and to have reached the ventral surface of the brain and upper cervical portion of the spinal cord. The dye injected i.t. was distributed both rostrally and caudally but over a short distance (about 1 cm) and no dye was found in the brain. The experimenters were trained, using an injection of dye in the beginning of the experiments, to achieve a 95% or more accuracy for i.t. and i.c.v. injections in other mice.

2.4. Experimental protocol

In the first group of experiments, mice were injected i.t. with saline (5 μl), SR 95531 (0.04–0.16 nmol) or 5-aminovaleric acid (32.5–130 nmol) 10 min prior to i.t. administration of a fixed dose of muscimol (20 nmol) or baclofen (1.6 nmol). In the second group of experiments, mice were injected i.t. with saline (5 μl), SR 95531 (0.04–0.16 nmol) or 5-aminovaleric acid (32.5–130 nmol) 10 min prior to i.c.v. administration of morphine (6 nmol) or β-endorphin (0.3 nmol). In the third group of experiments, mice were injected i.t. with saline (5 μl) or with a fixed dose of SR95531 (0.16 nmol) or 5-aminovaleric acid (130 nmol) 10 min prior to i.c.v. administration of various doses of morphine or β-endorphin. The tail-flick response was tested 30 min after the administration of morphine, β-endorphin, muscimol or baclofen. This time chosen was based on preliminary time course studies showing that inhibition of the tail-flick response reached a maximum after the injection of morphine, β-endorphin, muscimol or baclofen.

2.5. Statistics

The data are presented as the mean ± S.E.M. One-way analysis of variance (ANOVA) was used for statistical evaluation. The median antinociceptive doses (ED_{50}) and their 95% confidence intervals were calculated according to the method described by Litchfield and Wilcoxon (1949), with the aid of a computer program described by Tallarida and Murray (1981). P values of < 0.05 were considered to indicate statistical significance.

2.6. Drugs

Morphine hydrochloride was purchased from Sam-Sung Pharm. (Seoul, Korea). β-Endorphin was purchased from the Peninsula Laboratory (Belmont, CA). SR 95531, muscimol, baclofen and 5-aminovaleric acid were purchased from Research Biomedicals (Natick, MA). Drugs were dissolved in sterile saline (0.9% NaCl solution).

3. Results

3.1. Effect of SR 95531 and 5-aminovaleric acid injected i.t. on the inhibition of the tail-flick response induced by muscimol and baclofen administered i.t.

Mice were injected i.t. with SR 95531 (0.04–0.16 nmol) or 5-aminovaleric acid (32.5–130 nmol) 10 min prior to the i.t. administration of muscimol (20 nmol) or baclofen (1.6 nmol). The tail-flick response was measured at 30 min

after the i.t. injection of muscimol or baclofen. The effects of i.t. pretreatment with various doses of SR 95531 and 5-aminovaleric acid on inhibition of the tail-flick response induced by muscimol or baclofen given i.t. were studied. The i.t. injection of muscimol or baclofen inhibited the tail-flick response in mice pretreated i.t. with saline (Fig. 1). The i.t. pretreatment with SR 95531 attenuated dose-dependently the inhibition of the tail-flick response induced by i.t. administered muscimol, without affecting the response to i.t. administered baclofen (Fig. 1a,b). On the other hand, i.t. pretreatment with 5-aminovaleric acid dose-dependently reduced inhibition of the tail-flick response induced by i.t. administered baclofen, without affecting the response to i.t. administered muscimol (Fig. 2a,b). The tail-flick latency in mice injected with SR 95531 or 5-aminovaleric acid was not significantly different from that in mice injected with saline (Fig. 1, Fig. 2).

3.2. Effect of SR 95531 and 5-aminovaleric acid injected i.t. on the inhibition of the tail-flick response induced by morphine administered i.c.v.

Mice were injected i.t. with SR 95531 (0.16 nmol) or 5-aminovaleric acid (130 nmol) 10 min prior to the i.c.v. injection of morphine or β-endorphin. The tail-flick response was measured at 30 min after the i.c.v. injection of morphine or β-endorphin. The effects of i.t. pretreatment with various doses of SR 95531 and 5-aminovaleric acid on inhibition of the tail-flick response induced by morphine given i.c.v. were studied. As shown in Fig. 3, the i.c.v. injection of 6 nmol of morphine produced a profound inhibition of the tail-flick response in mice pretreated i.t. with saline. The i.t. pretreatment with SR 95531 at doses of 0.04–0.16 nmol attenuated dose-dependently the inhibition of the tail-flick response induced by i.c.v. administered morphine (Fig. 3a). However, i.t. pretreatment with 5-aminovaleric acid at doses of 32.5–130 nmol was not effective to reduce the inhibition of the tail-flick response induced by i.c.v. administered morphine (Fig. 3b).

The effects of i.t. pretreatment with a fixed dose of SR 95531 and 5-aminovaleric acid on inhibition of the tail-flick response induced by morphine given i.c.v. were also studied. Morphine at doses of 1.5–6 nmol administered i.c.v. caused dose-dependent inhibition of the tail-flick response in mice pretreated i.t. with saline (Fig. 4). The inhibition of the tail-flick response produced by i.c.v. administered morphine was attenuated by i.t. injection of SR 95531; the

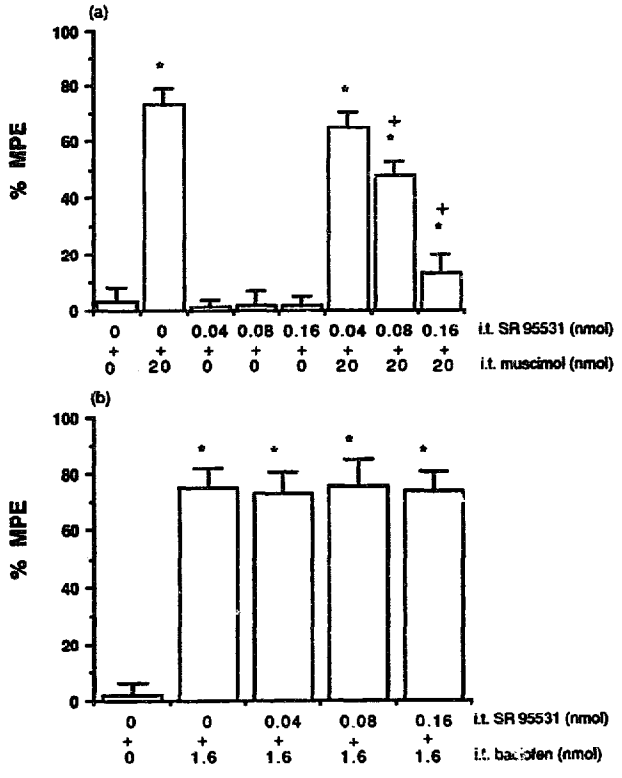

Fig. 1. Dose-dependent effects of SR 95531 injected i.t. on inhibition of the tail-flick response induced by muscimol (a) and baclofen (b) administered i.t. 10 min after the mice were pretreated i.t. with various doses of SR 95531 (0.04–0.16 nmol), either muscimol (20 nmol) or baclofen (1.6 nmol) was administered i.t. and the tail-flick response was measured 30 min after muscimol or baclofen injection. The vertical bars denote the S.E.M. The number of animals used for each group was 8–10. $^{*,+}$ $P < 0.05$ compared with the group of mice injected with saline and muscimol alone, respectively, administered i.t.

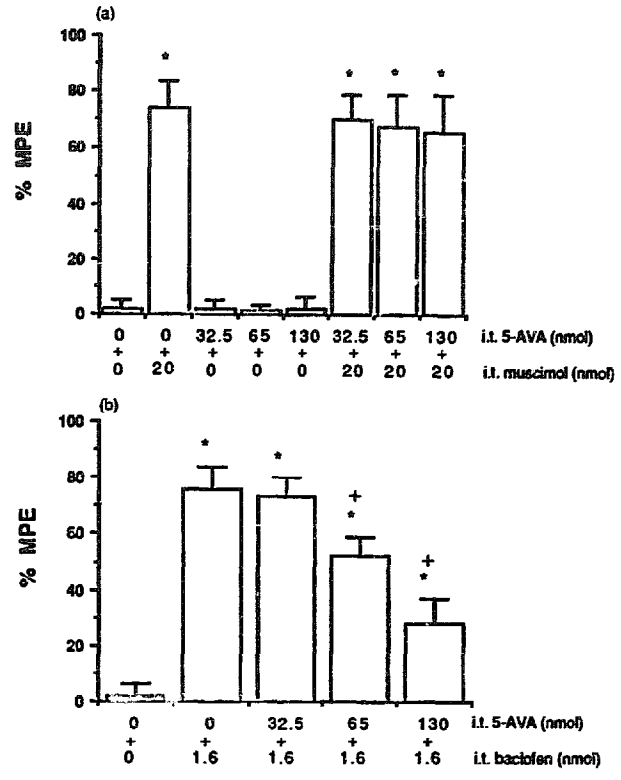

Fig. 2. Dose-dependent effects of 5-aminovaleric acid injected i.t. on inhibition of the tail-flick response induced by muscimol (a) and baclofen (b) administered i.t. 10 min after the mice were pretreated i.t. with various doses of 5-aminovaleric acid (5-AVA, 32.5–130 nmol), either muscimol (20 nmol) or baclofen (1.6 nmol) was administered i.t. and the tail-flick response was measured 30 min after muscimol or baclofen injection. The vertical bars denote the S.E.M. The number of animals used for each group was 8–10. $^{*,+}$ $P < 0.05$ compared with the group of mice injected with saline and baclofen alone, respectively, administered i.t.

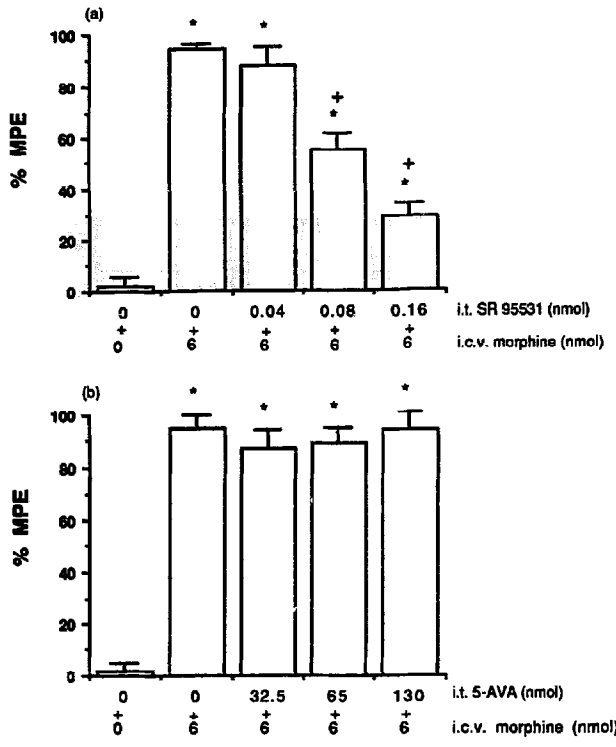

Fig. 3. Dose-dependent effects of SR 95531 (a) and 5-aminovaleric acid (b) injected i.t. on inhibition of the tail-flick response induced by morphine administered i.c.v. 10 min after the mice were pretreated i.t. with various doses of SR 95531 (0.04–0.16 nmol) or 5-aminovaleric acid (5-AVA, 32.5–130 nmol), 6 nmol of morphine was administered i.c.v. and the tail-flick response was measured 30 min after i.c.v. injection. The vertical bars denote the S.E.M. The number of animals used for each group was 8–10. $^{*,+}$ $P < 0.05$ compared with the group of mice injected with saline and morphine alone, respectively, administered i.c.v.

dose-response line for the morphine-induced inhibition of the tail-flick response in mice pretreated with SR 95531 was shifted significantly (5-fold) to the right (Table 1). In contrast, the tail-flick inhibition induced by i.c.v. adminis-

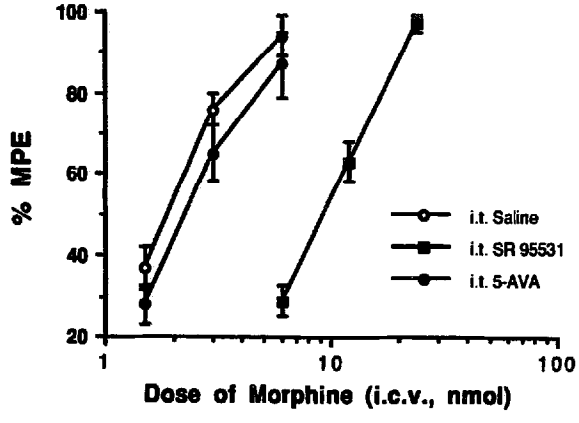

Fig. 4. Effects of SR 95531 and 5-aminovaleric acid injected i.t. on inhibition of the tail-flick response induced by morphine administered i.c.v. 10 min after the mice were pretreated i.t. with SR 95531 (0.16 nmol) or 5-aminovaleric acid (5-AVA, 130 nmol), various doses of morphine were administered i.c.v. and the tail-flick response was measured 30 min after the i.c.v. injection. The vertical bars denote the S.E.M. The number of animals used for each group was 8–10.

Table 1
Effect of SR 95531 and 5-aminovaleric acid pretreatment on ED_{50} values for morphine and β-endorphin for tail-flick inhibition

	ED_{50} (nmol/mouse) [a]		
	i.t.		
	Saline	SR 95531	5-aminovaleric acid
i.c.v. Morphine	1.8 (1.0–3.7) [b]	9.0 (3.2–18.7) [c]	2.0 (1.1–3.9)
i.c.v. β-Endorphin	0.13 (0.07–0.21)	0.12 (0.06–0.23)	0.38 (0.27–0.56) [c]

[a] ED_{50} values were calculated from Figs. 4 and 6.
[b] Numbers in parentheses indicate the 95% confidence interval.
[c] Significantly different from saline control ($P < 0.05$).

tered morphine was not affected by i.t. injection of 5-aminovaleric acid (Figs. 3 and 4, Table 1).

3.3. Effect of SR 95531 and 5-aminovaleric acid injected i.t. on the inhibition of the tail-flick response induced by β-endorphin administered i.c.v.

The effects of i.t. pretreatment with various doses of SR 95531 and 5-aminovaleric acid on inhibition of the tail-flick

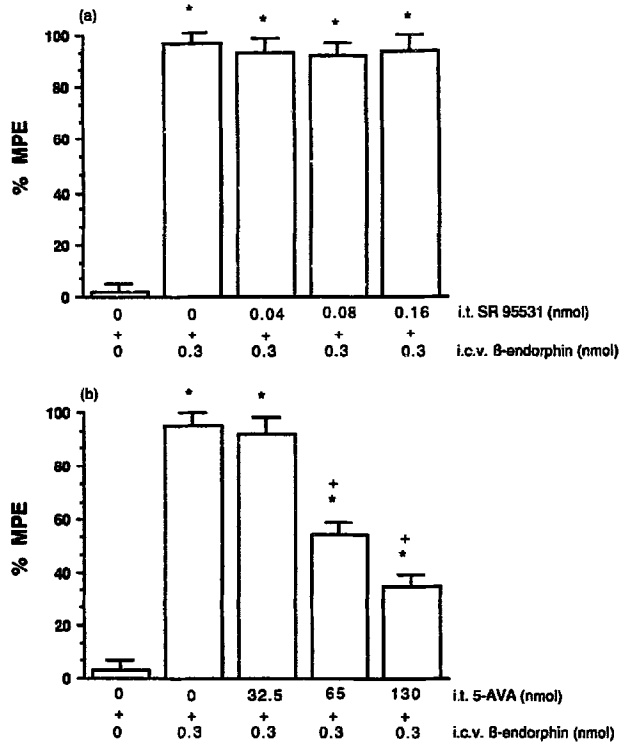

Fig. 5. Dose-dependent effects of SR 95531 (a) and 5-aminovaleric acid (b) injected i.t. on inhibition of the tail-flick response induced by β-endorphin administered i.c.v. 10 min after the mice were pretreated i.t. with various doses of SR 95531 (0.04–0.16 nmol) or 5-aminovaleric acid (5-AVA, 32.5–130 nmol), 0.3 nmol of β-endorphin was administered i.c.v. and the tail-flick response was measured 30 min after the i.c.v. injection. The vertical bars denote the S.E.M. The number of animals used for each group was 8–10. $^{*,+}$ $P < 0.05$ compared with the group of mice injected with saline and β-endorphin alone, respectively, administered i.c.v.

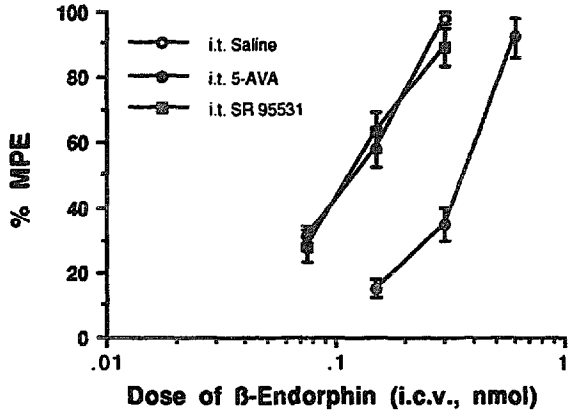

Fig. 6. Effects of SR 95531 and 5-aminovaleric acid injected i.t. on inhibition of the tail-flick response induced by β-endorphin administered i.c.v. 10 min after the mice were pretreated i.t. with SR 95531 (0.16 nmol) or 5-aminovaleric acid (5-AVA, 130 nmol), various doses of β-endorphin were administered i.c.v. and the tail-flick response was measured 30 min after the i.c.v. injection. The vertical bars denote the S.E.M. The number of animals used for each group was 8–10.

response induced by β-endorphin given i.c.v. were studied. The i.c.v. injection of 0.3 nmol of β-endorphin produced profound inhibition of the tail-flick response in mice pretreated i.t. with saline (Fig. 5). I.t. pretreatment with 5-aminovaleric acid at doses of 32.5–130 nmol attenuated dose-dependently the inhibition of the tail-flick response induced by i.c.v. administered β-endorphin (Fig. 5a). However, i.t. pretreatment with SR95531 at doses of 0.04–0.16 nmol was not effective to reduce the inhibition of the tail-flick response induced by i.c.v. administered morphine (Fig. 5b).

As shown in Fig. 6, β-endorphin at doses of 0.08–0.3 nmol administered i.c.v. caused dose-dependent inhibition of the tail-flick response in mice pretreated i.t. with saline. The inhibition of the tail-flick response produced by i.c.v. administered β-endorphin was attenuated by i.t. injection of 5-aminovaleric acid; the dose-response line for β-endorphin-induced inhibition of the tail-flick response in mice pretreated with 5-aminovaleric acid was shifted significantly (3-fold) to the right (Table 1). In contrast, the tail-flick inhibition induced by i.c.v. administered β-endorphin was not affected by i.t. injection of SR 95531 (Figs. 5 and 6, Table 1).

4. Discussion

Fujimoto and his colleagues have reported that the spinal GABA receptors participate in the antinociception induced by supraspinally administered δ-opioid receptor agonists (Holmes and Fujimoto, 1994; Rady and Fujimoto, 1995). Since the antinociceptive effects induced by supraspinally administered morphine and β-endorphin are mediated by the stimulation of different opioid receptors and activation of different descending pain control systems which utilize different neurotransmitters and their receptors, the findings of Fujimoto's group promptly led us to speculate that the blockade of spinally located $GABA_A$ and $GABA_B$ receptors may modulate differently the antinociception induced by morphine and β-endorphin administered supraspinally. We found in the present study that the blockade of $GABA_A$ but not $GABA_B$ receptors by i.t. injection of SR 95531 and 5-aminovaleric acid, respectively, which injected i.t. alone did not affect baseline pain sensitivity of nociception, attenuated the antinociception induced by morphine administered i.c.v. On the other hand, the blockade of $GABA_B$ receptors by 5-aminovaleric acid, but not of $GABA_A$ receptors by SR 95531, attenuated the antinociception induced by β-endorphin administered i.c.v. The results indicate that $GABA_A$ but not $GABA_B$ receptors appear to be involved in the antinociception induced by a μ-opioid receptor agonist, whereas $GABA_B$ but not $GABA_A$ receptors may be involved in the antinociception induced by an ε-opioid receptor agonist administered supraspinally.

Although not directly comparable, the results of the present study are partially consistent with the results obtained by Yoneda et al. (1976). They reported that bicuculline injected intraperitoneally (i.p.) inhibited the antinociception induced by morphine administered i.p. Furthermore, systemic injection of GABA receptor agonists enhances the antinociception induced by morphine administered systemically (Biggio et al., 1977; Christensen et al., 1978; Sivam and Ho, 1983). We found in the present study that 5-aminovaleric acid, a $GABA_B$ receptor antagonist, does not affect the antinociception induced by morphine administered supraspinally. However, Kaarianen and Vikberg (1976) have previously reported that subcutaneous (s.c.) injection of a $GABA_B$ receptor agonist, baclofen, enhances the antinociception induced by an opioid administered s.c.

GABA receptors are divided into at least two subtypes, $GABA_A$ and $GABA_B$ receptors (Enna and Karbon, 1986; Bormann, 1988; Bowery, 1989). Several lines of evidence have demonstrated that stimulation of spinal $GABA_A$ and $GABA_B$ receptors by i.t. injection of and muscimol and baclofen, respectively, produces antinociception (Wilson and Yaksh, 1978; Sawynok et al., 1984; Hammond and Drower, 1984). In addition, systemic injection of muscimol or baclofen produces antinociception (Sawynok and LaBella, 1982). Although it cannot be compared directly, a role of spinal GABA receptors in the production of antinociception induced by stimulation of descending pain inhibitory systems has been demonstrated. McGowan and Hammond (1993a,b) have reported that i.t. injection with bicuculline (a $GABA_A$ receptor antagonist) or phaclofen (a $GABA_B$ receptor antagonist) effectively attenuates the antinociception induced by glutaminergic stimulation of neurons in the nucleus raphe magnus and nucleus reticularis gigantocellularis pars α, suggesting that both $GABA_A$ and $GABA_B$ receptors located at the spinal cord level are

involved in the effect of supraspinally microinjected L-glutamate. In addition, Holmes and Fujimoto (1994) have reported that i.t. injection of $GABA_A$ or $GABA_B$ receptor antagonists reduces the antinociception induced by [D-Pen^2-D-Pen^5]enkephalin (a δ-opioid receptor agonist) administered supraspinally. Furthermore, Killian et al. (1995) have demonstrated that the antinociception induced by cold-water swimming stress is mediated by the activation of supraspinal δ_2-opioid receptors and the subsequent stimulation of spinal $GABA_A$ receptors.

McLaughlin et al. (1975) and Barber et al. (1982) have reported that GABA appears to be present primarily in interneurons of the dorsal horn, especially lamina II and III regions, of the spinal cord. In addition, Reichling and Basbaum (1990) and Blessing (1990) have observed that some spinal GABAergic neurons also originate from descending fibers. They found that GABAergic descending neurons primarily originate in the medulla region of the brain and project to the spinal cord. Although we found in the present study that GABAergic receptors in the spinal cord are involved in the antinociception induced by morphine and β-endorphin administered supraspinally, the origin of the GABA neurons, spinal interneuron or descending neurons is not yet known. Further studies are needed for delineation of this point.

The differential effect of SR 95531 and 5-aminovaleric acid injected spinally on the antinociception induced by supraspinally administered morphine and β-endorphin provides additional evidence to support the hypothesis that antinociception induced by morphine and β-endorphin is mediated by different neuronal mechanisms. It is hypothesized that the antinociception induced by morphine- and β-endorphin given supraspinally is mediated by the stimulation of μ- and ϵ-opioid receptors, respectively (Suh et al., 1988; Suh and Tseng, 1990a,b). The stimulation of μ-opioid receptors by morphine results in the activation of the descending 5-HT and noradrenergic pathways and, in turn, stimulation of the 5-HT receptors or α_2-adrenoceptors in the spinal cord results in the production of antinociception (Suh et al., 1989). On the other hand, the antinociception induced by β-endorphin is mediated by the stimulation of supraspinal ϵ-opioid receptors and sequential release of [Met^5]enkephalin from the spinal cord. The stimulation of opioid receptors in the spinal cord also produces antinociception (Suh et al., 1989).

The differential involvement of GABAergic receptors in opioid-induced antinociception is not limited to the spinal cord regions. Recent studies have demonstrated that GABAergic receptors modulate the antinociception induced by morphine and β-endorphin administered supraspinally. Both muscimol (a $GABA_A$ receptor agonist) and baclofen (a $GABA_B$ receptor agonist) injected supraspinally effectively attenuate the antinociception induced by β-endorphin administered supraspinally while only muscimol, but not baclofen, blocks the morphine-induced response (Mantegazza et al., 1979; Zonta et al., 1981; Zambotti et al., 1982; Suh et al., 1995), suggesting that both $GABA_A$ and $GABA_B$ receptors are involved in β-endorphin-induced antinociception, while only $GABA_A$, but not $GABA_B$, receptors are involved in morphine-induced antinociception at the supraspinal level. Therefore, it is suggested that GABA receptors located at both spinal and supraspinal levels appear to be differentially involved in the antinociception induced by morphine and β-endorphin administered supraspinally.

Acknowledgements

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