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Spinal GABA Receptors Mediate Brain Delta Opioid Analgesia in Swiss Webster Mice

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RADY, J. J. AND J. M. FUJIMOTO. *Spinal GABA receptors mediate brain delta opioid analgesia in Swiss Webster mice.* PHARMACOL BIOCHEM BEHAV 51(4) 655-659, 1995.—Morphine and heroin act on supraspinal μ-opioid receptors in ICR mice to activate descending noradrenergic and serotonergic systems to inhibit the tail flick response. Antinociception induced by supraspinal [D-Pen^{2,5}]-enkephalin (DPDPE, δ agonist) involves a descending system mediated by spinal γ-aminobutyric acid, $GABA_A$ and $GABA_B$, receptors. Because in Swiss Webster mice the receptor selectivity of heroin changes to δ whereas morphine remains μ, the purpose of the present study was to determine whether this δ action of heroin was mediated spinally by $GABA_A$ and $GABA_B$ receptors. Bicuculline ($GABA_A$ receptor antagonist) and picrotoxin (chloride ion channel blocker) given intrathecally produced rightward shifts in the dose-response curves of DPDPE and heroin given intracerebroventricularly. Thus, spinal $GABA_A$ receptors were involved. Intrathecal administration of 2-hydroxysaclofen ($GABA_B$ receptor antagonist) also shifted the dose-response curves to the right. Thus, the antinociception produced by heroin, like DPDPE, by activation of δ receptors in the brain of Swiss Webster mice involved both $GABA_A$ and the $GABA_B$ receptors in the spinal cord.

Heroin DPDPE Delta opioid receptor $GABA_A$ $GABA_B$ Antinociception Descending pathway

HEROIN and morphine produce antinociception in the tail flick test in ICR mice by activating μ receptors in the brain (13,14). The μ receptor activation inhibits the tail flick response through the modulatory influence of descending serotonergic and noradrenergic systems (1,2,18,20-22). One model used to demonstrate these descending systems in mice is administration of the antinociceptive agonists intracerebroventricularly (ICV) with antagonists given intrathecally (IT) to inhibit the actions of the neurotransmitters at the spinal level (1,18,20). Administration of serotonergic (methysergide) and noradrenergic (yohimbine) antagonists IT inhibits the antinociception produced by heroin and morphine acting on supraspinal μ receptors (13,14). Antinociception produced by ICV [D-Pen^{2,5}]enkephalin (DPDPE), which is a prototypic δ receptor agonist (11), does not involve serotonergic and noradrenergic descending systems (14). Thus, another descending antinociceptive system must be involved in the action of δ agonists to inhibit the radiant heat tail flick response, which is a spinal reflex response (19).

Recently, a descending antinociceptive pathway involving γ-aminobutyric acid (GABA) receptors in the spinal cord has been described (9,10). Glutamate administered into specific sites in the brain of rats produces antinociception, which is inhibited by administration of the $GABA_A$ and $GABA_B$ receptor antagonists IT. Subsequent work in ICR mice demonstrates that ICV DPDPE-induced antinociception is inhibited by administration of $GABA_A$ and $GABA_B$ antagonists IT (6). Therefore, the purpose of the present study was to determine if the antinociception induced by heroin acting on δ receptors in the brain of Swiss Webster mice involved the descending pathway mediated by $GABA_A$ and $GABA_B$ receptors in the spinal cord. This evaluation was undertaken because when the switch occurs in receptor selectivity of heroin from μ in ICR mice to δ in Swiss Webster mice, it is not known if a corresponding shift occurs in the descending pathway. Even though it is known that the supraspinal δ agonist action of heroin in Swiss Webster mice is not mediated by noradrenergic and serotonergic descending pathways (14), it is not known if the spinal GABA system is involved.

The results demonstrated that DPDPE and heroin antinociception, after ICV administration in Swiss Webster mice, was inhibited by $GABA_A$ and $GABA_B$ antagonists given IT. The

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antinociception produced by heroin, like that of DPDPE, through δ receptors in the brain of Swiss Webster mice, was mediated by a descending system that, in the spinal cord, involves $GABA_A$ and $GABA_B$ receptors.

METHOD

Animals and Antinociception

Male Swiss Webster mice weighing 25–40 g (Hilltop Lab Animals, Scottdale, PA) were used for all experiments. Each animal was used only once. Antinociception was measured using the radiant heat tail flick test (3). The tail flick latencies were converted to percent maximum possible effect (% MPE) (4):

$$\%\ \text{MPE} = \frac{(\text{postdrug time} - \text{predrug time})\ 100}{(10 - \text{predrug time})}.$$

Statistical Analysis

The mean % MPE values (± SEM) were calculated for the various groups. Student's *t*-test was used to compare the mean of one treatment group to that of a control group, and Dunnett's test was used to compare the means of several treatment groups to the mean of one control group. Both tests used a $p \leq 0.05$ to determine significant differences (17). Dose-response data were plotted; the slopes and ED_{50} values for the treatment groups were obtained as in the previous publication (6).

Drug Sources and Protocols for Drug Administration

Picrotoxin, (+)-bicuculline, and DPDPE were purchased from Sigma (St. Louis, MO). Isoguvacine hydrochloride, (+)-baclofen and 2-hydroxysaclofen were purchased from Research Biochemicals International (Natick, MA). Morphine sulfate · $5H_2O$ was obtained from Mallinckrodt (St. Louis, MO). Heroin hydrochloride was obtained from the National Institute on Drug Abuse (Rockville, MD). The stated doses of drugs refer to the form mentioned above. DPDPE was dissolved in a 0.01% Triton X-100 solution in 0.9% saline. All other compounds were dissolved in 0.9% sodium chloride solution. For bicuculline a few drops of 0.1 M hydrochloric acid solution were added to aid in dissolving the compound. Slight heating was also necessary for dissolving bicuculline and 2-hydroxysaclofen.

The opioid agonists (DPDPE, heroin, morphine) and saline were administered ICV in 4-μl volumes according to the method of Haley and McCormick (5) under light halothane anesthesia. The $GABA_A$ agonist (isoguvacine) and antagonists (picrotoxin and bicuculline), $GABA_B$ agonist (baclofen) and antagonist (2-hydroxysaclofen), and saline were administered IT in 5-μl volumes by the method of Hylden and Wilcox (7). The doses and times of drug administration are given with each experiment. All experiments were done in compliance with the Institutional Animal Care and Use Committee (Animal Studies Subcommittee).

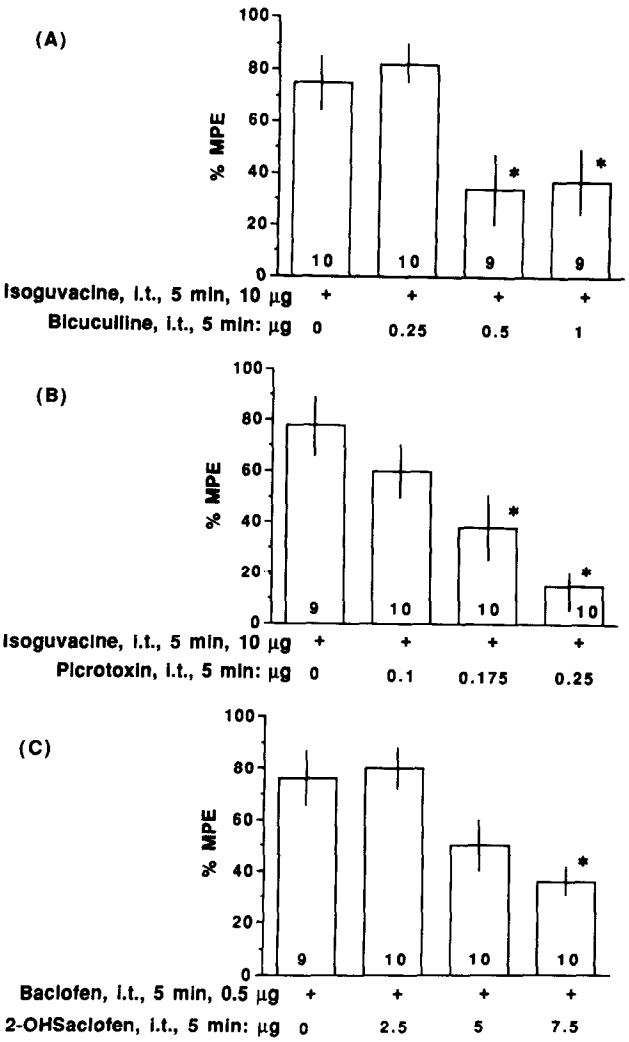

FIG. 1. Effective doses of GABA antagonists given IT were determined against GABA agonists given IT for the tail flick test in Swiss Webster mice. Isoguvacine (a $GABA_A$ agonist) administered IT produced antinociception (A,B, group 1). Groups are designated as 1, 2, etc., with the leftmost group as number 1. This antinociception was reduced by coadministration of (A) bicuculline (a selective $GABA_A$ antagonist) at 0.5 and 1 μg and (B) picrotoxin (a chloride ion channel blocker) at 0.175 and 0.25 μg. *Significantly different from isoguvacine only group using Dunnett's test; $p < 0.05$. A+ under the bar indicates that the agonist stated to the left was administered. The bars represent the mean % MPE for each group. The vertical lines at the top of the bars indicate the SEM and the numbers at the bottom of the bars indicate the number of mice in each group. (C) Administration of baclofen (a $GABA_B$ agonist) IT also produced antinociception (group 1). This antinociception was decreased by coadministration of 7.5 μg of 2-hydroxysaclofen (2-OHSaclofen). *Significantly different from the baclofen-only group using Dunnett's test; $p < 0.05$. The 5-min times for the agonists and antagonists were as in a previous study (6).

RESULTS

GABA Agonist-Induced Antinociception and Inhibition by GABA Antagonists

Administration of isoguvacine, a $GABA_A$ receptor agonist (10 μg), IT in Swiss Webster mice produced an antinociceptive response in the tail flick test that was reduced by coadministration of bicuculline, a $GABA_A$ receptor antagonist (Fig. 1A). The antinociceptive activity of isoguvacine was also attenuated by coadministration of picrotoxin, a chloride channel blocker, in a dose-dependent manner (Fig. 1B). These results indicated that the antinociception produced by the $GABA_A$ receptor agonist, isoguvacine, was inhibited by bicuculline and picrotoxin.

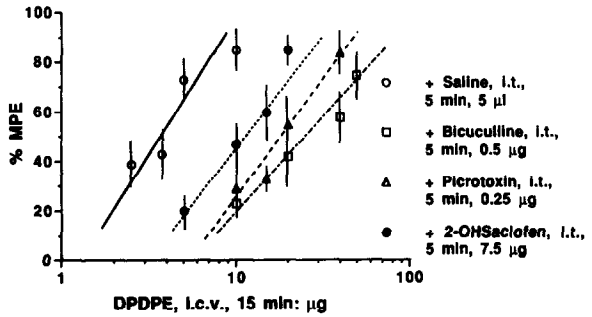

FIG. 2. Dose-response relationships for ICV DPDPE were determined with saline or the GABA antagonists administered IT. DPDPE given ICV 15 min before the tail flick test produced a dose-dependent antinociceptive response when given with control saline injection, IT, at 5 min (open circles). The 15-min time for DPDPE was taken from a previous publication (14). This dose-response curve was shifted to the right in a parallel fashion by administration of bicuculline IT (squares). A similar result was obtained when picrotoxin (triangles) was administered IT; a parallel, rightward shift in the DPDPE dose-response curve occurred. 2-Hydroxysaclofen administered IT also produced a parallel, rightward shift of the DPDPE dose-response curve (filled cirlcles). Thus, both $GABA_A$ and $GABA_B$ receptors in the spinal cord were involved in the antinociceptive activity of DPDPE given ICV in Swiss Webster mice. Each point represents the mean % MPE for each dose administered to 8-10 mice and the lines through the points indicate the SEM.

Baclofen, a $GABA_B$ agonist, given IT (0.5 µg) produced antinociception that was inhibited by coadministration of 2-hydroxysaclofen (Fig. 1C). Thus, activation of $GABA_B$ receptors also produced antinociception. These results in Swiss Webster mice involving spinal $GABA_A$ and $GABA_B$ receptors were similar to those obtained previously in ICR mice (6).

Spinal GABA Receptor Involvement in ICV DPDPE-Induced Antinociception

Administration of DPDPE into the brain of Swiss Webster mice produced dose-dependent antinociception (Fig. 2, circles). Administration of bicuculline (0.5 µg) IT produced a parallel, rightward shift in the dose-response curve for DPDPE-induced antinociception (Fig. 2, squares). The ED_{50} value (95% confidence interval) changed from 3.75 µg (2.44-5.78 µg) to 24.2 µg (14.67-39.93 µg), which represented a sixfold shift in the dose-response curve. A similar shift was observed following picrotoxin (0.25 µg) administration IT (Fig. 2, triangles); the ED_{50} value for ICV DPDPE in the presence of IT picrotoxin was 17.1 µg (10.49-27.87 µg) (a fivefold shift). Thus, $GABA_A$ receptors in the spinal cord mediated the antinociceptive action of ICV DPDPE.

Administration of 2-hydroxysaclofen (7.5 µg) IT also produced a parallel, rightward shift in the dose-response curve for DPDPE (Fig. 2, filled circles). The ED_{50} value was shifted threefold to 11.2 µg (7.27-17.25 µg). Therefore, spinal $GABA_B$ receptors were also involved in DPDPE-induced antinociception. These results indicated that DPDPE acted the same in Swiss Webster as in ICR mice (6) and suggested that both $GABA_A$ and $GABA_B$ receptors were involved in the antinociception produced by ICV DPDPE. None of the antagonists given IT along with ICV saline (in place of DPDPE) produced antinociception compared to the IT saline group (Table 1). The % MPE value for bicuculline was significantly different from the IT saline-matched control (Table 1) and appeared to be due to the somewhat higher % MPE value than usually obtained for saline treatment.

TABLE 1

DETERMINATION OF THE ANTINOCICEPTIVE ACTIVITY OF GABA ANTAGONISTS GIVEN BEFORE THE TAIL FLICK TEST

Treatment, IT, 5 min Before Tail Flick Test	*n*	% MPE (SEM)
Saline given ICV 15 min before TFT		
Saline (5 µl)	10	17.7 (4.0)
Bicuculline(0.5 µg)	10	0.3 (2.7)*
Picrotoxin (0.25 µg)	10	11.0 (3.4)
2-Hydroxysaclofen (7.5 µg)	8	6.0 (5.6)
Saline given ICV 10 min before TFT		
Saline (5 µl)	10	8.4 (6.2)
Bicuculline (0.5 µg)	10	8.0 (1.3)
Picrotoxin (0.25 µg)	10	6.5 (2.9)
2-Hydroxysaclofen (7.5 µg)	8	13.1 (2.4)

*Significantly different from saline group using Dunnett's test, $p < 0.05$.

Spinal GABA Receptors Involved in the Antinociceptive Action of ICV Heroin

Heroin, administered ICV in Swiss Webster mice, produced a dose-dependent antinociceptive response (Fig. 3, open circles). Administration of bicuculline (0.5 µg) IT produced a ninefold rightward shift in the heroin dose-response curve (Fig. 3, squares), changing the ED_{50} value from 0.93 µg (0.58-1.49 µg) to 8.3 µg (6.15-11.21 µg). Likewise, ICV heroin-induced antinociception was inhibited by administration of picrotoxin (0.25 µg) IT (Fig. 3, triangles). The ED_{50} value for heroin in the presence of picrotoxin was 6.9 µg (3.56-13.39 µg), indicating a sevenfold shift in the dose-response curve. Thus, $GABA_A$ receptors in the spinal cord were involved in the antinociceptive activity of ICV heroin.

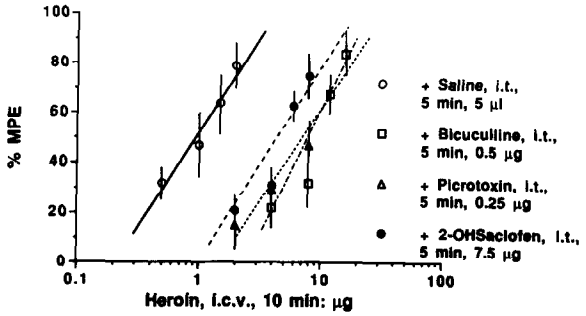

FIG. 3. Dose-response relationships for ICV heroin were determined with saline or the GABA antagonists administered IT. Heroin administered ICV 10 min before the tail flick test produced dose-dependent antinociception in the presence of IT saline treatment given at 5 min (open circles). The 10-min time for heroin was obtained previously (14). Administration of bicuculline IT at 5 min produced a parallel, rightward shift in the heroin dose-response curve (squares). Similarly, the dose-response curve for heroin was shifted to the right in a parallel manner when picrotoxin was administered IT (triangles). Treatment with the $GABA_B$ receptor antagonist, 2-hydroxysaclofen, also produced a rightward shift in the heroin dose-response curve (filled circles). Therefore, ICV heroin, like DPDPE, produced antinociception that involved $GABA_A$ and $GABA_B$ receptors in the spinal cord.

TABLE 2

DETERMINATION OF THE ANTINOCICEPTIVE ACTIVITY OF MORPHINE (2 μg) GIVEN ICV AT 10 MIN ALONG WITH TREATMENT (IT) WITH SALINE OR GABA ANTAGONIST AT 5 MIN BEFORE THE TAIL FLICK TEST

Treatment, IT, 5 min Before TFT	*n*	% MPE (SEM)
Saline (5 μl)	8	76.0 (9.0)
Bicuculline (0.5 μg)	10	62.4 (7.3)
Picrotoxin (0.25 μg)	10	68.7 (11.8)
Saline (5 μg)	9	80.8 (7.2)
2-Hydroxysaclofen (7.5 μg)	9	77.0 (9.5)

$GABA_B$ receptor involvement in heroin-induced antinociception was examined next. Administration of 2-hydroxysaclofen (7.5 μg) IT along with ICV heroin produced a parallel, rightward shift in the heroin dose-response curve. The ED_{50} value for ICV heroin-induced antinociception when 2-hydroxysaclofen was given IT was 4.85 μg (2.9-8.1 μg), which represented a fivefold shift in the dose-response curve. Thus, spinal $GABA_A$ and $GABA_B$ receptors were involved in the antinociception produced by ICV heroin. The antagonists administered IT after ICV saline (in place of heroin at 10 min) produced no alteration in the tail flick response (Table 1).

Lack of Involvement of Spinal GABA Receptors in ICV Morphine-Induced Antinociception

Morphine (2 μg) given ICV in Swiss Webster mice produced an antinociceptive response (Table 2). Administration of bicuculline (0.5 μg) and picrotoxin (0.25 μg) IT did not alter ICV morphine-induced antinociception (Table 2). These results suggested that spinal $GABA_A$ receptors were not involved in ICV morphine-induced antinociception. Administration of the $GABA_B$ antagonist, 2-hydroxysaclofen (7.5 μg), IT also had no effect on the antinociceptive activity of ICV morphine (Table 2). Therefore, the antinociception produced by ICV morphine acting on μ receptors (14) did not involve spinal GABA receptors.

DISCUSSION

The first aim of this study was to show that the δ agonist action of DPDPE in the brain of Swiss Webster mice produced antinociception that was mediated by spinal $GABA_A$ and $GABA_B$ receptors. Initial experiments showed that isoguvacine, a $GABA_A$ agonist, and baclofen, a $GABA_B$ agonist, produced antinociception in the tail flick test. These actions were inhibited by $GABA_A$ (bicuculline, picrotoxin) and $GABA_B$ (2-hydroxysaclofen) antagonists, respectively. Next, the antinociceptive action produced by ICV administration of DPDPE was inhibited by IT administration of bicuculline, picrotoxin, and 2-hydroxysaclofen. Thus, spinal $GABA_A$ and $GABA_B$ receptors were involved in ICV DPDPE-induced antinociception. These results in Swiss Webster mice were similar to those previously described in ICR mice (6). Because of this similarity, the discussion covered in the previous publication is omitted here. The present discussion focuses on the results with heroin.

The second purpose of the study was to show that the antinociception produced by the δ agonist action of ICV heroin was mediated by spinal GABA receptors. As indicated in the Introduction, this purpose arose from a special feature of heroin. Heroin acts on δ receptors in Swiss Webster mice, but on μ receptors in ICR mice, whereas DPDPE acts on δ receptors in both mice (14). When heroin acts on μ receptors in ICR mice, descending serotonergic and noradrenergic systems are activated. Antinociception is inhibited by methysergide, a nonselective serotonin receptor antagonist, and yohimbine, an α_2-adrenergic antagonist, given IT. Present results showed that ICV heroin-induced antinociception, in Swiss Webster mice, was inhibited by IT administration of bicuculline, picrotoxin, and 2-hydroxysaclofen. Thus, both spinal $GABA_A$ and $GABA_B$ receptors played a role in ICV heroin-induced antinociception. This finding was consistent with the recent finding that heroin acts like DPDPE to activate δ_1 receptors in the brain of Swiss Webster mice (15). Furthermore, when the receptor selectivity of heroin changed from μ in the ICR mice to δ_1 in Swiss Webster mice, a corresponding change from the serotonergic and noradrenergic receptor mediation to $GABA_A$ and $GABA_B$ receptors occurred. These findings indicate that Swiss Webster mice might serve as a convenient in vivo model in screening for potential antinociceptive δ agonists. Determination of δ agonist activity by simultaneous ICV administration of nonselective δ receptor antagonists, like naltrindole (16), or selective δ_1 antagonists, like [D-Ala^2,Leu^5,Cys^6]enkephalin and 7-benzylidenenaltrexone (8,12), or δ_2 antagonists, like naltrindole-5′-isothiocyanate (8) and naltriben (16), along with evaluation of involvement of spinal GABA receptors would provide useful differentiation. Present findings, though simply stated, give no insight into the factors that control whether heroin will act on μ or δ receptors in the brain.

One further consideration is of interest. Antinociception elicited from L-glutamate-sensitive sites located in medial medullary loci such as the nucleus raphe magnus activate spinal $GABA_B$ receptors whereas loci in lateral sites such as the nucleus gigantocellularis pars alpha selectively activate spinal $GABA_A$ receptors (9,10). If such analogous selectivity of loci exists in mice as in rats, then the present results suggest that δ_1 agonists may act on both loci in the brain because ICV DPDPE- and heroin-induced antinociception involves both $GABA_A$ and $GABA_B$ spinal receptors. Studies are in progress to evaluate whether the antinociception produced by δ_2 agonists given ICV would involve spinal GABA receptors.

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