Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 3, pp. 675-682, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)00197-9

[D-Pen^2-D-Pen^5]enkephalin, a Delta Opioid Agonist, Given Intracerebroventricularly in the Mouse Produces Antinociception Through Mediation of Spinal GABA Receptors

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Received 20 December 1993

HOLMES, B. B. AND J. M. FUJIMOTO. *[D-Pen^2-D-Pen^5]enkephalin, a delta opioid agonist, given intracerebroventricularly in the mouse produces antinociception through mediation of spinal GABA receptors.* PHARMACOL BIOCHEM BEHAV 49(3) 675-682, 1994.—Intracerebroventricular (ICV) administration of [D-Pen^2-D-Pen^5]enkephalin (DPDPE), a δ opioid receptor agonist, activates a descending antinociceptive pathway that inhibits the tail-flick response in mice. Involvement of spinal GABA receptors in this response was studied by giving GABA antagonists intrathecally. First, antinociception produced by intrathecally administered isoguvacine, a $GABA_A$ agonist, was inhibited by intrathecal bicuculline (GABA receptor antagonist) or picrotoxin (chloride channel antagonist). Then, antinociception induced by ICV DPDPE was antagonized by intrathecal picrotoxin and bicuculline in a dose-and time-dependent manner. Second, intrathecal administration of 2-hydroxysaclofen, a $GABA_B$ antagonist (which inhibited antinociception induced by a $GABA_B$ agonist, baclofen, given IT), produced a shift of the dose-response curve for ICV DPDPE to the right. $GABA_A$ and $_B$ antagonists given together intrathecally produced a greater than additive antagonistic effect against ICV DPDPE-induced antinociception. Thus, the δ agonist action of DPDPE in the brain leads to activation of descending spinal pathways which involve mediation by spinal $GABA_A$ and $GABA_B$ receptors in the antinociceptive response.

DPDPE Delta opioid receptors Spinal GABA Antinociception Descending pathway

IN PRODUCING analgesia, one of the important modes of action of the opioids is the activation of descending noradrenergic and serotonergic pathways from the brain to the spinal cord to supress nociceptive responses (7,20). For μ agonists, such as morphine, one approach to demonstrating the involvement of descending noradrenergic and serotonergic pathways is to administer adrenergic and serotonergic antagonists intrathecally, IT, in animals to inhibit the action of μ agonists given into the brain (64,66). The serotonergic system is also involved in the antinociceptive action produced by the kappa opioid agonists (21,61). Antinociception produced by epsilon receptor activation by β-endorphin is mediated by the spinal release of Met-enkephalin and is inhibited by IT naloxone administration (60). The descending system involved in the antinociceptive action of δ agonists given supraspinally remains unknown even though endogenous δ agonists (such as Met- and Leu-enkephalin) have been known for some time. Knowledge of such pathways is desirable because the cloning of brain δ receptors (48) heightens interest in δ receptor mediated action. Thus, the purpose of the present study was to demonstrate that a highly selective δ agonist [D-Pen^2-D-Pen^5]enkephalin, DPDPE (40,43), when given intracerebroventricularly, ICV, into the brain of mice, activates descending pathways that are mediated by GABA receptors in the spinal cord to produce antinociception in the tail-flick test [preliminary results were reported earlier, (16)]. DPDPE was chosen because administration of endogenously occurring peptides, Met- and Leu-enkephalin, has evanescent action and β-endorphin has predominant epsilon over δ agonist action (60).

The rationale for the present study was based on the recent

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reports of descending GABA mediated antinociceptive systems (35,36). Reichling and Basbaum (47) implicate descending bulbo-spinal GABA neurons that project to the dorsal horn of the spinal cord in producing antinociception in the rat. These GABA immunoreactive neurons originate in the nucleus reticularis paragigantocellularis and nucleus reticularis paragigantocellularis pars alpha as determined by retrograde transport of wheat germ agglutinin horseradish peroxidase in neurons. Others have demonstrated GABAergic neurons projecting from medullary areas to the spinal cord (8,27). In addition, GABA agonists given intrathecally, IT, produce analgesia (1,29,50,52,53,55,65). In a systematic series of work, Hammond and colleagues (35,36) demonstrated that intracerebral microinjections of glutamate into specific brain sites produce antinociception that is mediated by spinal GABA receptors. Site vs. receptor selectivity exists in that intrathecal administration of $GABA_A$ antagonists inhibits the antinociceptive action elicited from the nucleus gigantocellularis pars alpha, whereas that elicited from more medial sites (periaqueductal gray, nucleus raphe magnus) is antagonized by $GABA_B$ antagonists (35,36).

In the present study, a δ receptor selective agonist, DPDPE, was given ICV to produce inhibition of the tail-flick response in mice. The $GABA_A$ receptor antagonist, bicuculline, and the chloride channel antagonist, picrotoxin, given IT, inhibited the DPDPE-induced antinociceptive response. Also, the $GABA_B$ antagonist, 2-hydroxysaclofen, given IT inhibited the antinociceptive response to ICV DPDPE. These results support the concept that DPDPE given into the brain produced antinociception by activating descending systems that were mediated by spinal $GABA_A$ and $GABA_B$ receptors.

METHOD

Animals and Measurement of Antinociceptive Response

Male ICR mice (25–35 g) from Sasco Laboratories (Omaha, NE) were used in the radiant heat tail-flick test (13). The lamp intensity was set to provide a predrug time of 2–4 s, with a cutoff time of 10 s. The percent maximum possible effect (%MPE) is calculated according to the formula (15):

$$\%\text{MPE} = \frac{(\text{Postdrug time} - \text{Predrug time}) \times 100}{(10 - \text{Predrug time})}.$$

Protocol and Drug Administration

The basic protocol involved the ICV administration of the δ opioid agonist, DPDPE, to inhibit the tail-flick response. Involvement of spinal GABA receptors in this δ agonist-induced antinociception was evaluated by administration of either the $GABA_A$ receptor complex antagonists (bicuculline and picrotoxin) or $GABA_B$ antagonist 2-hydroxysaclofen, IT, 5 min before the tail-flick test. The effective doses of the $GABA_A$ or $_B$ antagonists were determined against IT isoguvacine (a $GABA_A$ agonist)- or baclofen (a $GABA_B$ agonist)-induced antinociception, respectively. Dose-response studies were performed for DPDPE given ICV in the presence and absence of $GABA_A$ antagonists, bicuculline, and picrotoxin, or $GABA_B$ receptor antagonist, 2-hydroxysaclofen, given IT.

Solutions containing DPDPE were administered ICV in 4 μl volumes (18). Isoguvacine, baclofen, picrotoxin, bicuculline and 2-hydroxysaclofen solutions were injected IT in 5 μl volumes (25). Drugs were dissolved in a 0.9% NaCl solution or in a 0.9% NaCl solution containing a 0.01% Triton X-100 for DPDPE. The solutions were heated to dissolve bicuculline and 2-hydroxysaclofen. Doses and times of administration of the drugs are given with each experiment in the Results section. Appropriate drug vehicle solutions were given at each site in control groups in place of the drug solutions. All studies were done in compliance with the Institutional Animal Care and Use Committee (Animal Studies Subcommittee).

Statistical Analysis

Student's *t*-test (two groups compared), Dunnett's test (one group compared to all other groups), and ANOVA followed by Newman-Keuls' test (multiple group comparisons) were used for determining significant differences between group means as indicated by a $p \leq 0.05$ (56). The dose-response curves for DPDPE were analyzed by the method of Litchfield and Wilcoxon, as adapted by Dewey et al. (15).

Source of Drugs

DPDPE (Peninsula, Belmont, CA); morphine sulfate $5H_20$ (Mallinckrodt Chemical Works, St. Louis, MO); isoguvacine hydrochloride, (+)-bicuculline, picrotoxin, 2-hydroxysaclofen and (L)-baclofen (Sigma Chemical Co., St. Louis, MO). The doses of the drugs were for the forms stated above.

RESULTS

Determination of the Doses of IT Picrotoxin and Bicuculline Needed to Inhibit the Antinociceptive Action of IT Isoguvacine

In the experiments depicted in Fig. 1A and B, isoguvacine, a $GABA_A$ agonist, given IT (10 μg, 5 min before the tail-flick test) prolonged the tail-flick latency to increase the %MPE. The peak response occurred at 10 min (data not given). This antinociceptive response was antagonized in a dose-dependent fashion by administration of increasing doses of bicuculline and picrotoxin along with the isoguvacine. Isoguvacine action was antagonized by picrotoxin in a dose range of 0.25 to 0.5 μg and bicuculline at 0.25 to 1 μg. Doses of picrotoxin higher than 1 μg produced seizures, biting, and scratching. Picrotoxin tested at 0.25 μg and bicuculline at 0.5 μg had no effect on the tail-flick response by themselves (legend to Fig. 1).

Effect of Different Doses of IT Picrotoxin and Bicuculline on Antinociception Produced by a Fixed Dose of ICV DPDPE

Figure 2A shows that doses of 0.25 to 1 μg picrotoxin, IT, decreased the antinociceptive effect of ICV DPDPE, 10 μg. Similarly, the antinociceptive effect of ICV DPDPE was reduced at the 0.5 and 1 μg dose of bicuculline, IT (Fig. 2B). These doses of bicuculline and picrotoxin were similar to those that inhibited IT isoguvacine induced-antinociception (Fig. 1). The 10 μg dose of DPDPE, ICV, was chosen so as to supress the tail-flick response by about the same amount as did the 10 μg IT dose of isoguvacine. The response to this 10 μg dose of DPDPE reached a peak at 10 min and decreased by about one-half at 30 min (data not given). The results indicated that ICV DPDPE-induced antinociception was inhibited by $GABA_A$ antagonists given IT.

Duration of the Antagonistic Action of IT Picrotoxin and Bicuculline on the Antinociceptive Action of ICV DPDPE

The results presented in Fig. 3A and B indicated that when DPDPE was administered ICV at 10 min before the tail-flick test along with saline IT at 5, 15, 30, and 60 min before the tail-flick test, DPDPE produced consistent antinociceptive responses. Comparison of these groups with those treated with

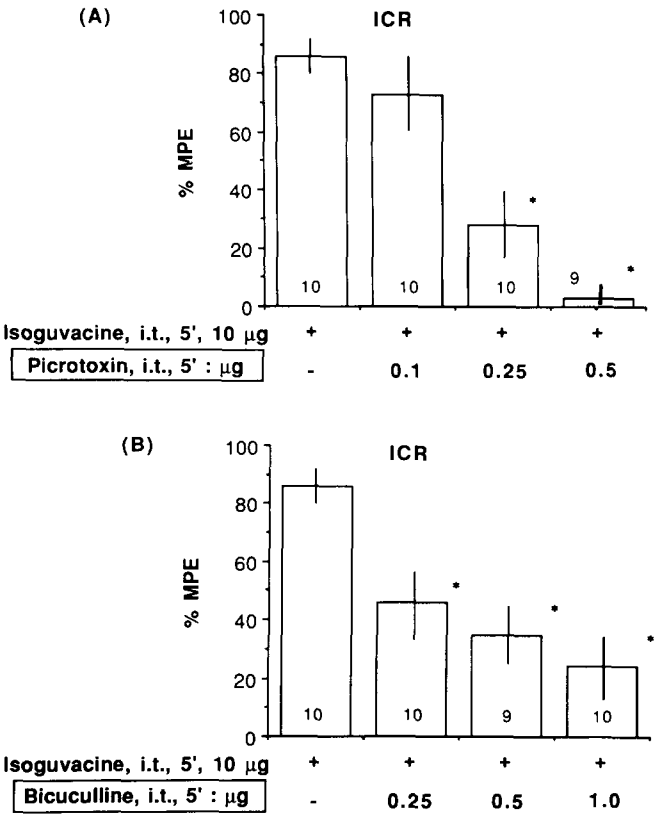

FIG. 1. Dose-response studies for the antagonism of IT isoguvacine-induced antinociception by bicuculline and picrotoxin in ICR mice. (A) Intrathecal picrotoxin in increasing doses (0.1 μg to 0.5 μg) administered together with isoguvacine (10 μg, 5 min before the tail-flick test) produced a dose-dependent antagonism. (B) Intrathecal bicuculline administered together with isoguvacine also produced a dose-dependent antagonism. The * indicates significant difference according to Dunnett's test, $p \leq 0.05$. Hereafter the numbers in the bars represent the number of mice used per group. %MPE is the percent maximum possible effect. The vertical line is the SEM (standard error of the mean). A + indicates that the drug was administered and a − indicates that vehicle (solution) was administered.

DPDPE along with IT picrotoxin (0.25 μg) at the corresponding times, showed that the antinociceptive action of DPDPE was inhibited by picrotoxin given at 5 min (Fig. 3A). At 15, 30, and 60 min after administration of picrotoxin, antagonism was not evident. Similarly, the antinociceptive action of ICV DPDPE was inhibited by the IT administration of 0.5 μg of bicuculline only at 5 min (Fig. 3B). These results suggested that picrotoxin and bicuculline had short durations of action.

Dose-Response Relationship for ICV DPDPE-Induced Analgesia at Fixed Doses of IT Picrotoxin and Bicuculline

Figure 4 shows the dose-response curve for ICV DPDPE-induced antinociception in IT saline treated mice. In additional experiments at a fixed dose of picrotoxin and bicuculline given IT, the dose-response curve for ICV DPDPE was determined. The DPDPE curves were shifted to the right by both bicuculline, 1.0 μg, IT and picrotoxin, 0.25 μg, IT. Picrotoxin shifted the DPDPE curve to the right in a parallel fashion. However, with bicuculline, the slope of the DPDPE curve was significantly different from the slope of the curve for DPDPE alone.

Involvement of Spinal $GABA_B$ Receptors in the Antinociceptive Action of ICV DPDPE

Baclofen, a $GABA_B$ agonist, 0.5 μg, IT, inhibited the tail-flick response at 5 min (Fig. 5A). Administration of a $GABA_B$ receptor antagonist, 2-hydroxysaclofen, at the same time inhibited the baclofen action in a dose-dependent manner.

Figure 5B shows the dose-response curve for ICV DPDPE in the presence of 2-hydroxysaclofen. The ICV DPDPE curve was shifted to the right in the presence of 7.5 μg of 2-hydroxysaclofen, given IT, at 5 min before the TFT. The dose-response curve for ICV DPDPE and IT saline is shown as a dotted line because these data are from Fig. 4. These results indicated that $GABA_B$ receptors were also involved in mediating the antinociceptive response to ICV DPDPE.

A duration of action study was performed for 2-hydroxysaclofen (Fig. 5C). DPDPE was given ICV at a con-

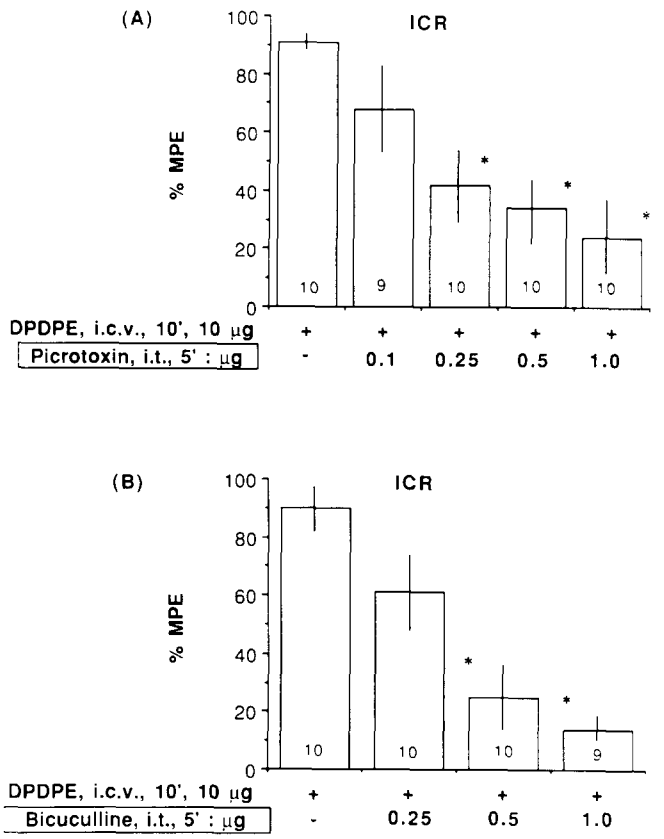

FIG. 2. Antagonism of ICV DPDPE-induced antinociception by IT picrotoxin and bicuculline. (A) Picrotoxin, IT, given 5 min before the tail-flick test antagonized ICV DPDPE (10 μg, 10 min) in a dose-dependent fashion. (B) Intracerebroventricular DPDPE-induced antinociception was also antagonized by bicuculline, IT, (5 min). An * indicates significant difference from control ICV DPDPE according to Dunnett's test $p \leq 0.05$. After each of the following treatments with groups of 10 mice each, the %MPE ± SEM were: ICV vehicle, 4 μl, 10 min, plus picrotoxin, IT, 0.25 μg, 5 min: 0.7 ± 2.5; ICV, vehicle, 4 μl, 10 min, plus bicuculline, IT, 0.5 μg, 5 min: 5.2 ± 2.0, which indicated bicuculline and picrotoxin had no effect on the tail flick by themselves.

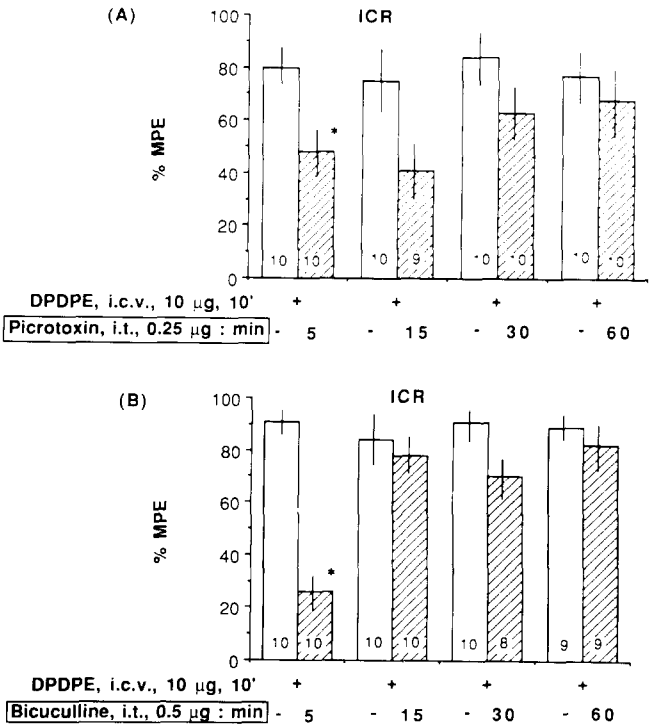

FIG. 3. Time course for antagonism of ICV DPDPE-induced antinociception by IT bicuculline and picrotoxin. The dose (10 μg) and time of administration (10 min before the tail-flick test) was kept constant for the ICV DPDPE given to all groups. (A) Picrotoxin antagonism of DPDPE-induced antinociception was significant only at 5 min. (B). Bicuculline, IT, only antagonized ICV DPDPE at 5 min and was back to control by 15 min before the tail-flick test. An * indicates significant difference according to Student's t-test, $p \leq 0.05$.

stant dose and time (10 μg at 10 min) before the TFT. The 7.5 μg dose of 2-hydroxysaclofen was given at various time from 5 min to 60 min before the TFT. The maximal antagonism was at 5 min and the antagonism was over within 30 min.

Because the results indicated that both $GABA_A$ and $GABA_B$ receptors were involved, the possibility was evaluated that blocking both receptors at the same time would have a greater effect than blocking each receptor separately. The results in Fig. 6 supported this possibility. An IT dose of 2.5 μg of 2-hydroxysaclofen, 0.1 μg of picrotoxin and 0.1 μg bicuculline were found to produce no effect on ICV DPDPE-induced antinociception. One-half of this dose of each antagonist was given in combinations of two: picrotoxin/2-hydroxysaclofen, bicuculline/2-hydroxysaclofen or picrotoxin/bicuculline. Note that the 0.05 μg dose of picrotoxin, given together with 1.25 μg 2-hydroxysaclofen, produced a significantly greater inhibition of DPDPE-induced antinociception than the 0.1 μg dose of picrotoxin or the 2.5 μg dose of 2-hydroxysaclofen. Similarly, the 0.05 μg dose of bicuculline given together with 1.25 μg 2-hydroxysaclofen produced a greater effect than either the 0.1 μg bicuculline or the 2.5 μg 2-hydroxysaclofen treatments separately. If the two drugs (given at one-half doses) in each combination were having strictly an additive effect, then the response should have been no different than to either drug given at the full dose. The latter occurred when 0.05 μg of picrotoxin was combined with 0.05 μg bicuculline, that is, when two $GABA_A$ antagonists were given together. When the combination (picrotoxin/2-hydroxysaclofen, bicuculline/2-hydroxysaclofen) contained antagonists for both $GABA_A$ and $GABA_B$ receptors, the effect was more than additive.

DISCUSSION

It is well established that DPDPE given ICV in mice produces antinociception through stimulation of δ receptors (40,43). It is likely that the antinociceptive action of DPDPE given supraspinally is modulated by a descending pathway to the spinal cord to supress the tail-flick response. The tail-flick response elicited by radiant heat is a spinal response that remains intact in spinally transected mice (63). In mice, agonists can be given intracerebroventricularly along with various antagonists given intrathecally to characterize antinociceptive descending systems. As an example, Wigdor and Wilcox (64), through the use of selective antagonists IT, found that morphine given ICV in the mouse activates both descending noradrenergic and serotonergic spinal systems (with the noradrenergic component being the stronger) to produce antinociception. As mentioned in the introduction, others have since then used this approach to study the descending pathways of antinociception for μ, κ, and epsilon agonists acting in the brain. For δ agonists, noradrenergic and serotonergic pathways are not involved in the descending pathway (46).

The present findings indicated that both spinal $GABA_A$ and $_B$ receptors were involved in ICV DPDPE-induced antinociception. Involvement of $GABA_A$ receptors was shown by the ability of IT bicuculline and picrotoxin to inhibit the ICV DPDPE-induced inhibition of the tail-flick response. The duration of this antagonism by picrotoxin and bicuculline was very short (5 min). The reason for this short action is not known, but the present protocol was similar to that used by Alhaider et al. (2) in mice. These antagonists were effective at doses that were similiar to those that antagonized the antinociception produced by IT isoguvacine, the $GABA_A$ agonist. Bicuculline, given IT at a fixed dose of 1 μg, produced a right-

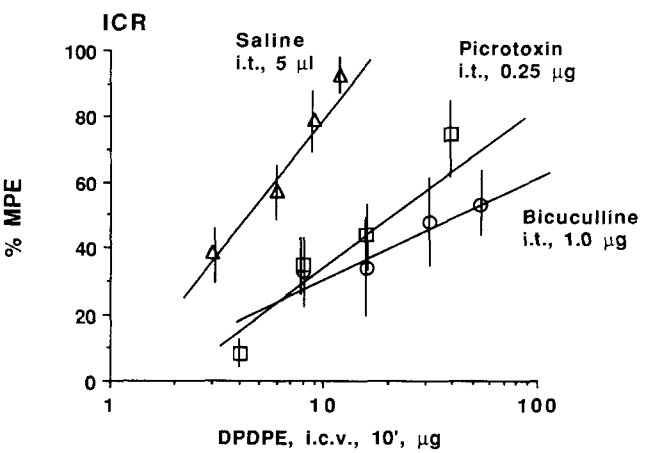

FIG. 4. Dose-response studies for ICV DPDPE-induced antinociception as modified by IT picrotoxin and bicuculline. The control group received DPDPE and saline, IT (triangles). A fixed dose of picrotoxin (0.25 μg) produced a parallel shift to the right (squares). Bicuculline (1.0 μg) also produced a rightward shift but a significant difference in the slope from the control group occurred (circles). The ED_{50} with 95% confidence interval for DPDPE, ICV, 10 min, with IT saline, 5 min: 4.2 (2.0–8.7) μg; with IT picrotoxin, 18.5 (11.2–30.3) μg; with IT bicuculline, 38.0 (9.6–150.9) μg.

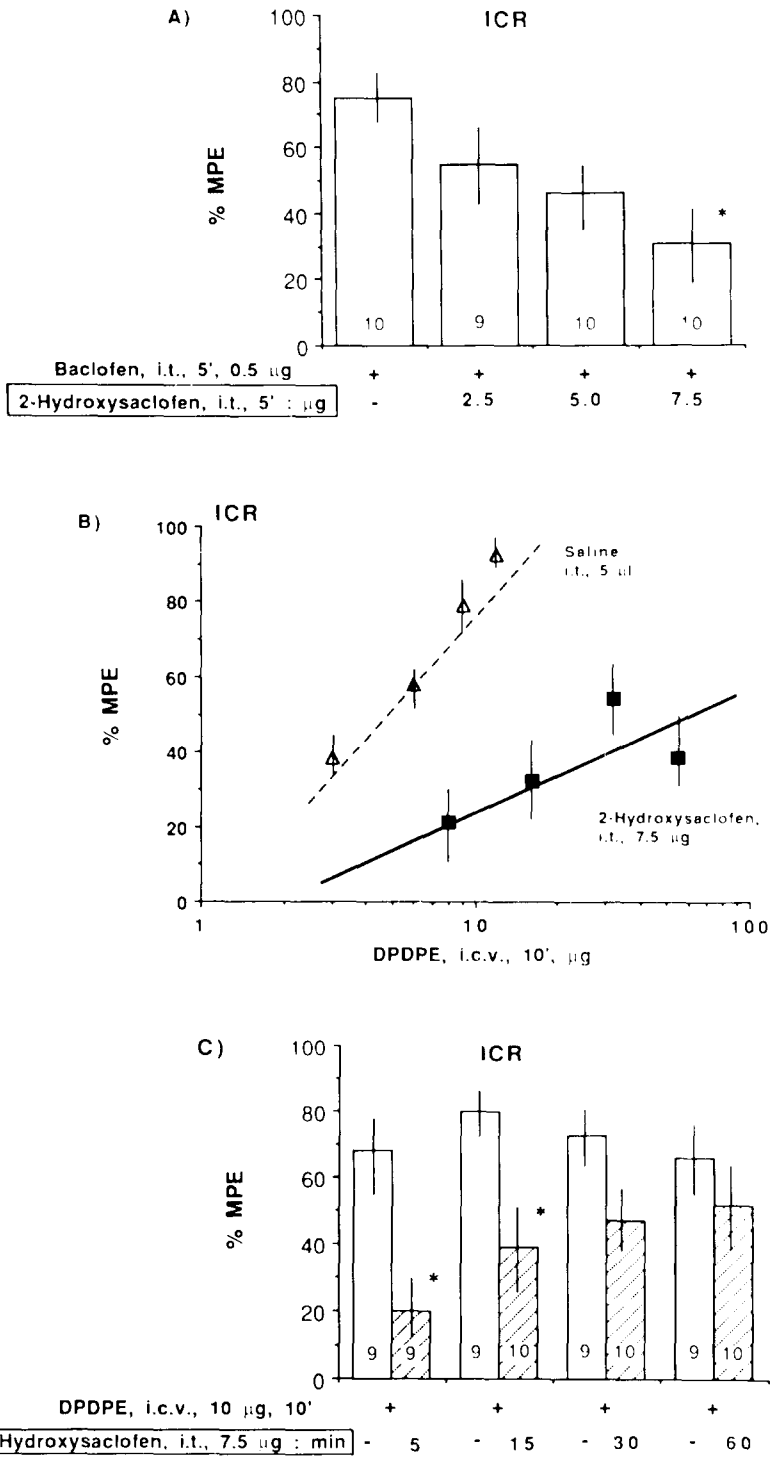

FIG. 5. (A) Intrathecal baclofen, a $GABA_B$ agonist, inhibited the TFT at a dose of 0.5 µg (5 min). Combinations of various doses of 2-hydroxysaclofen with baclofen, IT, resulted in a dose-dependent decrease in baclofen-induced antinociception. The 7.5 µg dose of IT 2-hydroxysaclofen was the most effective. An * indicates significant differences according to Dunnett's test $p \leq 0.05$. (B) Figure 5B is the dose response study for ICV DPDPE-induced antinociception as modified by the 7.5 µg dose of 2-hydroxysaclofen. The dotted line for DPDPE indicates the same data as presented in Fig. 4. 2-Hydroxysaclofen, given intrathecally, produced a parallel shift of the DPDPE curve to the right. (C) The time course of antagonism by 2-hydroxysaclofen on DPDPE analgesia was shown to peak at 5 min. The * indicates that significant antagonism occured at the 5- and 15-min time period according to Student's *t*-test. The plain bars represent DPDPE alone while the striped bars represent the addition of IT 2-hydroxysaclofen.

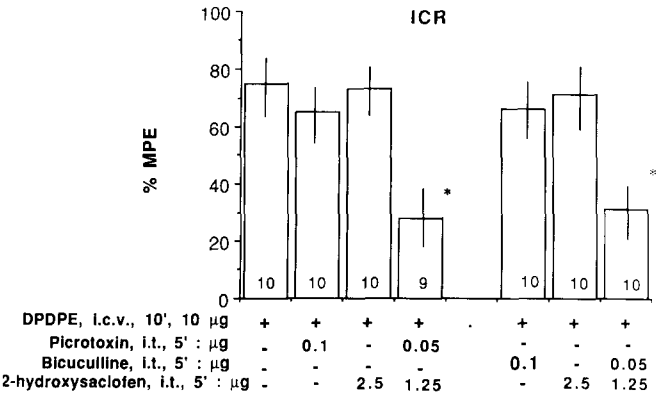

FIG. 6. The greater than additive interaction between $GABA_A$ and $GABA_B$ antagonists given together against ICV DPDPE-induced antinociception. The designated, seperate (whole) dose of each antagonist (picrotoxin, bicuculline, or 2-hydroxysaclofen) was ineffective against ICV DPDPE-induced antinociception. One-half of the dose of the given antagonist was given in the combination of picrotoxin/2-hydroxysaclofen or bicuculline/2-hydroxysaclofen. These combinations gave a greater than additive antagonistic effect. The * indicates significant difference from all other groups according to ANOVA followed by Newman-Keuls test. A study done by combining one-half the dose of picrotoxin and bicuculline gave 53 ± 8% (MPE ± SEM). This mean value was no different from that for the DPDPE group or the groups given DPDPE with the whole dose of either bicuculline or picrotoxin.

ward shift in the dose-response curve for ICV DPDPE. The slope of this DPDPE curve was significantly different from the slope of the control DPDPE curve, indicating a nonparallel shift. Picrotoxin (0.25 µg, IT) produced a rightward parallel shift in the dose-response curve for ICV DPDPE.

Isoguvacine given IT in the rat produces antinociception (29,35) which is antagonized by coadministration of picrotoxin or bicuculline. Bicuculline is a selective antagonist at the $GABA_A$ receptor (26,32). The $GABA_A$ action can also be inhibited by picrotoxin, which blocks the chloride ion channel that the GABA/benzodiazepine receptor complex modulates (17,32,41,54,57).

$GABA_A$ receptor activation increases chloride conductance (51,57). Opening of chloride channels results an influx of chloride. This influx stabilizes the membrane potential, blocks the action potential, and results in decreased neurotransmitter release. This stabilization can be accomplished through hyperpolarization or depolarization of the membrane (51). Picrotoxin blocks the chloride ion passage, therefore allowing neurotransmitter release. Bicuculline competitively binds to the GABA binding site to cause a hyperpolarization of the primary afferents (6,14,30,32,57). Activation of receptors postsynaptically on spinal nociceptive sensory projection neurons may be involved in producing analgesia (2).

Spinal $GABA_B$ receptors were implicated in the mediation of ICV DPDPE-induced antinociception (Fig. 5). Antinociception induced by IT baclofen, a $GABA_B$ agonist, was antagonized by 2-hydroxysaclofen. The dose-response curve for ICV DPDPE-induced antinociception was shifted to the right in a nonparallel fashion. Doses of DPDPE above 55 µg caused overt motor stimulation in the mice treated with 2-hydroxysaclofen; higher doses of DPDPE were not tested. Thus, the dose response curve stopped at a mean MPE of 51.2%. A presumptive ED_{50} value would be about 55 µg to yield a shift of approximately 12-fold. The 2-hydroxysaclofen had a short duration of action (15 min).

Baclofen, the $GABA_B$ agonist (9), has been shown to be antinociceptive in a variety of situations. Baclofen, given either IP or SC, produces antinociception in the tail-flick, hot plate, and abdominal stretching tests (31,45,52). Baclofen given intrathecally is antinociceptive in a series of behavioral tests (19,24,55,65). High doses of baclofen produce flaccidity (45,65); however, at the dose used here (0.5 µg, IT), no overt signs such as muscle weakness and ataxia were seen. 2-Hydroxysaclofen is a selective antagonist at $GABA_B$ receptors (9). It is a competitive antagonist at $GABA_B$ receptors in the guinea pig ileum and mouse vas deferens and is 10-fold more potent against baclofen than phaclofen (9). 2-Hydroxysaclofen, IT, inhibits the antinociceptive action of IT baclofen in the hot plate and tail-flick test in rats (4).

$GABA_B$ receptor action appears to work through a second-messenger system (9,10). Both baclofen and $GABA_B$ can bind postsynaptically to the $GABA_B$ receptor to increase potassium conductance causing hyperpolarization of the primary afferent A δ and C fibers. Baclofen and $GABA_B$ can also act presynaptically to block Ca channels to produce shortening the Ca component of the action potential (3,4,9). The $GABA_B$ receptor antagonist, 2-hydroxysaclofen, binds competitively to $GABA_B$ receptors to inhibit either the hyperpolarization or block the inhibition of Ca flow through the channel (12).

Our results indicated that $GABA_A$ and $GABA_B$ receptor responses were involved simultaneously in the mediation of the descending analgesic pathway for ICV DPDPE-induced antinociception. The two paired combinations of picrotoxin or bicuculline with 2-hydroxysaclofen, all given at one-half of already ineffective doses, produced greater inhibition of ICV DPDPE-induced antinociception than would be expected from an additive effect. That is, additive effects from the one-half dose combinations should have been the same as for the responses to the whole dose of a given antagonist (no effect). The combination of half the dose of bicuculline with half the dose of picrotoxin gave a response, which was not different from either the whole dose of bicuculline or picrotoxin separately. This kind of one-half plus one-half dose protocol for paired combinations has been used previously to demonstrate an additive antinociceptive interaction (IT serotonin and morphine) and an antagonistic interaction (IT serotonin and D-Ala^2-N-$MePhe^4$-gly ol^5, a selective μ agonist (5)). The present results indicated that ICV DPDPE simultaneously activates spinal $GABA_A$ and $GABA_B$ receptors to produce antinociception.

Our results complement in several respects reports in the literature. First, intracerebral microinjections of L-glutamate into sites in the rat brain produce antinociception that is blocked by GABA antagonists given IT (35,36). Responses elicited from the more lateral reticular sites (such as the nucleus gigantocellularis pars alpha) involve spinal $GABA_A$ receptor mediation, although more medial sites involve $GABA_B$ receptor-mediated antinociception (35,36). It is not known whether DPDPE activates the same system as that activated by the microinjection of L-glutamate. Second, there are bulbospinal GABAergic neurons that project to the dorsal horn (28,38,47) at least in animals other than the mouse. The GABAergic link affected by IT administration of GABA antagonists might reside in these long projection neurons or more likely interneurons in the dorsal horn. Neurons in the superficial lamina of the dorsal horn contain L-glutamic acid decarboxylase, the enzyme responsible for GABA synthesis (23,37). Immunoperoxidase procedures demonstrate localization of L-

glutamic acid decarboxylase and probable GABAergic axon terminals in lamina II and III of the dorsal horn of the rat spinal cord which are ascribed to substantia gelatinosa interneurons (6). Light and electron microscopic studies also show GABAergic neurons in the dorsal horn (44,58,59). GABA receptors are found in the dorsal horn as well (23,33,38,42,49). $GABA_A$ and $GABA_B$ receptors are found on C fiber and Aδ fiber afferent terminals (14). Localization of GABA neurons and receptors in the areas associated with nociception suggests a modulatory role for GABA on the nociceptive system.

In the future, it would be of interest to determine whether it is possible to show that activation of different sites in the brain with δ agonists will activate selectively spinal $GABA_A$ or $GABA_B$ receptors as was possible with the microinjection of L-glutamate (35,36). Because DPDPE is a δ_1 receptor agonist, a possible difference in selectivity of GABA receptors activated by δ_2 receptor agonists might exist. Whether the supraspinal antinociceptive action of endogenous δ agonist peptides, Met- and Leu-enkephalin involve modulation by spinal GABA receptors should be examined.

In conclusion, DPDPE (the prototypic δ agonist), given ICV, produced antinociception. The IT adminstration of either $GABA_A$ or $_B$ antagonists inhibited this antinociceptive response. Because more than an additive effect was obtained by giving both types of antagonists together, it appears that both $GABA_A$ and $_B$ receptors simultaneously modulate the spinal antinociceptive action.

ACKNOWLEDGEMENTS

We wish to thank Dr. Donna Hammond of the Department of Anesthesia and Critical Care, University of Chicago, for preprints of publications and Dr. Lawerence Isaac of the Department of Pharmacology, University of Illinois, for stimulating our thinking.

This work was supported by a grant from the National Institute on Drug Abuse (DA00451) and Research Career Scientist Award from the Veterans Administration (JMF). A preliminary report of part of this work was presented earlier (16).

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