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Inhibition of the Antianalgesic Action of Dynorphin A in Mice by Cholera Toxin

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ARTS, K. S., J. M. FUJIMOTO AND S. M. CRAIN. *Inhibition of the antianalgesic action of dynorphin A in mice by cholera toxin.* PHARMACOL BIOCHEM BEHAV 46(3) 623-629, 1993.—Dynorphin A-(1-17) (Dyn A) administered intrathecally (IT) or released spinally in the mouse produces an antianalgesic action. The present experiments indicate that IT administration of cholera toxin inhibited the antianalgesic action of Dyn A. When clonidine was administered intracerebroventricularly (ICV) to release spinal Dyn A, IT cholera toxin inhibited the antianalgesic action of Dyn A so that the analgesic component of action of clonidine became evident. Dyn A given IT inhibited the analgesic action of morphine given ICV. Cholera toxin given IT eliminated the antagonistic action of Dyn A. These results, in addition to others, indicate that IT cholera toxin antagonized the action of Dyn A. When the antianalgesic action of Dyn A was attenuated by pretreatment with dynorphin antiserum or by pretreatment that produced desensitization to Dyn A, cholera toxin had no effect. These results suggested that the antianalgesic action of Dyn A is mediated by activation of opioid receptors that are positively coupled to adenylate cyclase through a G_s regulatory protein.

Dynorphin Antianalgesia Cholera toxin G_s Morphine Clonidine

CERTAIN drugs, such as physostigmine, clonidine, midazolam, and naloxone, administered intracerebroventricularly (ICV) in mice release dynorphin A-(1-17) (Dyn A) in the spinal cord to produce an antianalgesic action (11-14,20,26). The antianalgesic action of the endogenously released Dyn A can be demonstrated in the tail-flick test by antagonism of the analgesic action of morphine given intrathecally (IT). Furthermore, the antianalgesic action of Dyn A is abolished by IT administration of the opioid antagonists, naloxone and norbinaltorphimine, at doses that, respectively, do not affect the analgesic action (tail-flick test in mice) of DAMGO (Tyr-D-Al^2-Gly-NMePhe^4-Gly-ol^5, a mu agonist, or U50,488H {*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate hyrdrate}, a kappa agonist, given IT. Dyn A action is attenuated by IT pretreatment with dynorphin antiserum and by production of desensitization to Dyn A by pretreatment with agents that release Dyn A (1, 13,14).

Dynorphins, as well as mu and delta opioid agonists, have inhibitory and excitatory effects on the duration of the action potential of sensory neurons in cultured mouse dorsal root ganglion (DRG)-spinal cord explants and dissociated DRG neurons [see review (7)]. The inhibitory action that shortens the duration of the action potential occurs through a decrease in calcium or increase in potassium conductance (16,17,35, 36). Dynorphin-(1-13) has an excitatory action to prolong the action potential duration of DRG neurons by decreasing potassium conductance (31). Furthermore, other mu, delta, and kappa opioid agonists given at low (ca. nM) concentrations prolong the action potential duration, whereas at higher (mM) concentrations, these opioids shorten the action potential duration (4,6,7,10,28,31). The opioid excitatory effect on DRG neurons is blocked by cholera toxin (CTX), indicating that this opioid effect is mediated by receptors coupled through a G_s protein to adenylate cyclase and cAMP-dependent, voltage-sensitive ionic conductances (29). CTX decreases the efficacy of the ligand to activate G_s-coupled opioid receptors. In contrast, pertussis toxin (PTX) blocks opioid-induced shortening of the DRG action potential, which is mediated by activation of PTX-sensitive G_i/G_o-coupled inhibitory opioid receptors (7,17,24,28). CTX also inhibits the excitatory action of opiates in certain other preparations. Opioid enhancement

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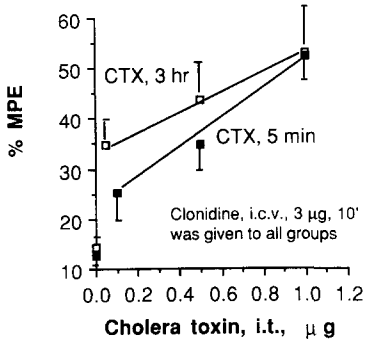

FIG. 1. The IT administration of CTX uncovered the analgesic action of ICV clonidine. Clonidine (3 μg) given ICV 10 min before the tail-flick test (TFT) along with IT saline at 3 h or 5 min before the TFT had very little analgesic effect (two points at zero cholera toxin dose). In the remaining groups, CTX was given IT 3 h or 5 min before the TFT along with ICV clonidine at 10 min. The effect of ICV clonidine was enhanced by the treatment with CTX (at 1 μg, $p < 0.05$ by Dunnett's test, for 5 min and 3 h CTX vs. saline controls). Eight to 10 mice were used for each point.

of electrically stimulated release of enkephalins from myenteric neurons of the isolated guinea pig ileum preparation is inhibited by CTX (and not by PTX) without an effect on the basal release of enkephalins induced by electrical stimulation (15). CTX inhibits the contracture induced by naloxone in the isolated ileum preparation from morphine-dependent guinea pigs without any effect on the electrically induced muscle twitch response; only the naloxone-induced muscle contracture response is affected by CTX (23).

The purpose of the present study was to determine if the antianalgesic action of Dyn A as found in mice is mediated by opioid receptors that are coupled to a G_s protein system. Because CTX inhibits the excitatory effects of opioids on peripheral neurons as discussed above, we used CTX as a tool in the present study to determine if the antianalgesic action of Dyn A is inhibited by CTX. The first protocol involved ICV administration of clonidine to release endogenous Dyn A in the spinal cord. Clonidine given ICV not only releases Dyn A but has an antinociceptive component of action that can be uncovered if the Dyn A component of action is attenuated (12,13). CTX, given IT, enhanced the analgesic action of ICV clonidine, indicating that CTX inhibited the Dyn A component of action of clonidine. Another protocol examined the ability of Dyn A administered IT (11,14) or released in the spinal cord by IT administration of capsaicin (2) to inhibit the antinociceptive action of morphine given ICV. The action of Dyn A to inhibit morphine-induced antinociception was eliminated by IT administration of CTX.

METHOD

Animals and Antinociceptive Test

Male ICR mice, Sasco Laboratories (Omaha, NE), weighing 20 to 30 g, were used in all experiments. Each animal was used only once. The antinociceptive response (for brevity referred to also as analgesia) to morphine was evaluated by the radiant heat tail-flick test (TFT) (8) using a lamp intensity that provided a predrug latency time of 2–4 s and a 10-s cutoff time. The percent maximum possible effect (% MPE) for each mouse and mean for the group was calculated using the following formula (9):

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

Intracerebroventricular and Intrathecal Administration of Agents

A 4-μl volumeof saline or drug solution was administered ICV (18). A 5-μl volume of saline or solution of agents was administered IT (21). Drugs administered IT were cholera toxin (CTX), Dyn A, capsaicin, and dynorphin antiserum (Dyn AS). The usual times for the ICV and IT injections were at 10 and 5 min before the TFT, respectively. CTX was given IT at various times stated with each experiment. Dynorphin antiserum was administered as a pretreatment 1 h before the TFT. All times, as indicated, refer to times before the TFT.

Experimental Protocols to Assess the Effect of CTX

The first experimental protocol involved ICV administration of clonidine (3 μg, 10 min) and CTX (IT, 5 min or 3 h) before the tail-flick test to see if CTX treatment inhibited the

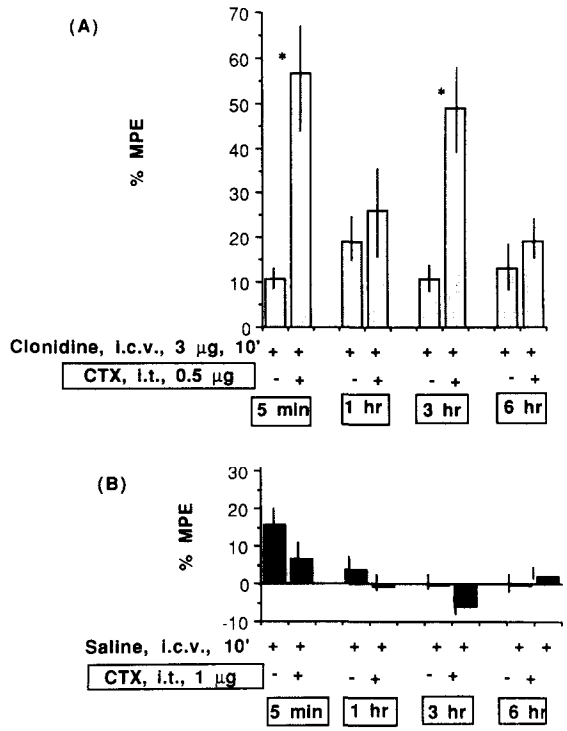

FIG. 2. The time course of the enhancement of ICV clonidine analgesia by CTX (IT, 1 μg) was determined by varying the time of administration of CTX while keeping the time and dose of ICV clonidine constant. At 5 min, IT CTX enhanced ICV clonidine-induced analgesia as before. (A) At 1 h, IT CTX had no effect on the action of clonidine even though at 3 h IT CTX effectively enhanced clonidine-induced analgesia. The biphasic action of CTX was also found in other experiments to be described later. Asterisk indicates significant differences from control (ICV clonidine + IT saline) using Student's *t*-test. (B) A control experiment showed that IT CTX given at the designated times produced no significant difference from groups given IT saline at the same times. Saline was given ICV at 10 min before the TFT to all groups in place of clonidine. Eight to 10 mice were used for each group. A + indicates treatment stated at left; − indicates drug vehicle treatment.

antianalgesic component of clonidine. Clonidine has a latent analgesic component that can be uncovered by inhibiting the spinal Dyn A-mediated antianalgesic component (12,13). If CTX inhibited the action of spinally released Dyn A, clonidine would manifest an increased analgesic response. Dose-response and time-response relationships for the effect of CTX on the clonidine response were determined. A way to attenuate the Dyn A component is to administer capsaicin as a pretreatment so as to produce desensitization to the subsequent action of Dyn A (2). Thus, in one experiment, capsaicin (10 mg/kg, 0.1 ml/10 g body weight) was given SC as a 6-h pretreatment and the combination of clonidine and CTX was tested as above. If CTX were to inhibit Dyn A action, CTX should have no effect in the capsaicin-pretreated mice. In one experiment, CTX was heated to 56°C in a hot water bath for 20 min to inactivate its action.

In a second protocol, morphine (4 μg) was given ICV 10 min before the TFT and Dyn A was given IT to inhibit this morphine-induced analgesia. CTX was administered IT at 5 min or 3 h to evaluate its effect on this action of Dyn A. Dyn A was given IT in a dose of 10 pg (5 fmol).

In a third protocol, ICV morphine was used to induce analgesia as above. Capsaicin (0.1 μg) was given IT to antagonize morphine-induced analgesia through presumptive release of Dyn A (2). CTX was given IT at 5 min or 3 h to see if it modified the action of endogenously released Dyn A in a system different from the clonidine system above.

Source of Drugs

Materials were obtained from the following sources: morphine sulfate·$5H_2O$ (Mallinckrodt Chemical Co., St. Louis, MO); Dyn A (Bachem, Inc., Torrance, CA); capsaicin and cholera toxin (Sigma Chemical Co., St. Louis, MO). Dynorphin antiserum was the same as used in a previous publication (13). Doses of the drugs refer to the form of the drugs stated here. Drugs were dissolved in 0.9% sodium chloride solution or in the case of Dyn A in 0.01% Triton X-100 in 0.9% sodium chloride solution. Warming and stirring was required to dissolve the capsaicin. The times, doses, and routes of drug administration and number of mice used per group are stated in each experiment.

RESULTS

CTX-Induced Enhancement of ICV Clonidine Antinociception

A 3-μg dose of clonidine given ICV 10 min before the tail-flick test had little effect on the latency of the tail-flick response in mice that had been treated 5 min or 3 h before the tail-flick test with IT saline (Fig. 1, two points on the ordinate at zero cholera toxin dose). When CTX was administered IT either 5 min or 3 h before the tail-flick test, increases in the antinociceptive action of clonidine were obtained that appeared to be linearly related to the dose of cholera toxin. The 5-min and 3-h times were derived from an experiment where the time of administration of 3 μg of clonidine was fixed at 10 min, while the time of administration of CTX (IT) was changed (Fig. 2A). The administration of CTX at 5 min and 3 h increased the tail-flick latency induced by clonidine. CTX had no effect at 1 h. Thus, a biphasic effect was produced by CTX with a nadir at 1 h. The 5-min and 3-h times were used for CTX in other experiments. Control groups given IT CTX, 1 μg, had tail-flick latencies that were not different from control groups treated with ICV and IT saline at each of these times (Fig. 2B).

Inactivation of CTX by Heating

The ability of CTX to increase clonidine analgesia was demonstrated again (group 1 vs. 2, at 5 min and 3 h, Fig. 3). This CTX effect no longer occurred when the CTX was heated for 20 min at 56°C (group 3 at 5 min and 3 h). Heating inactivates CTX (29).

Evaluation of CTX Action on Clonidine-Induced Antinociception Under Conditions Where the Dyn A Component of Action of Clonidine Has Been Attenuated

Pretreatment 1 h before the TFT with dynorphin antiserum enhanced the antinociceptive action of ICV clonidine (Fig.

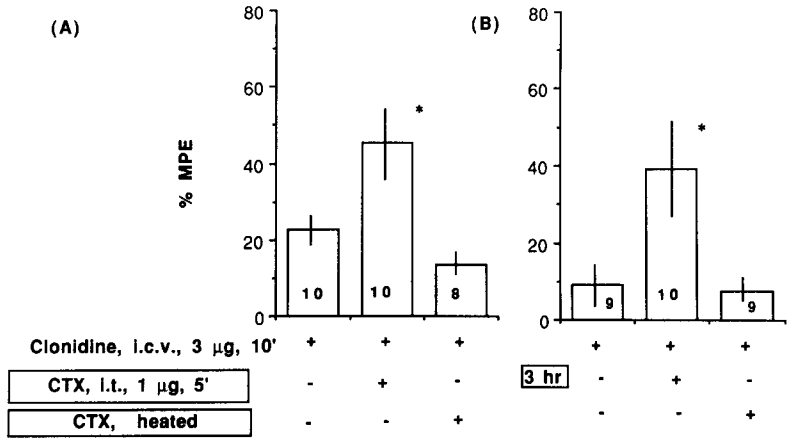

FIG. 3. Heating the cholera toxin for 20 min at 56°C (CTX heated) destroyed the ability of cholera toxin to enhance ICV clonidine action. CTX or heated CTX was given either (A) 5 min or (B) 3 h before the TFT. Asterisk indicats significant difference from other groups using ANOVA followed by Newman-Keuls' test ($p < 0.05$). Numbers within the bar were the number of mice used in each group.

4A, group 1 vs. 3). CTX enhanced the antinociceptive action of clonidine (group 1 vs. 2). Giving both the Dyn AS and the CTX did not result in an effect significantly greater than that obtained for either the CTX or Dyn AS groups. Thus, the response was less than an additive and the effect of the two appeared not to be independent.

Six-hour pretreatment with 10 mg/kg (SC) of capsaicin produces desensitization to the subsequent effect of Dyn A (2). An increase in the analgesic action of ICV clonidine was produced by the capsaicin pretreatment (group 4 vs. 1, Fig. 4B). When both CTX and capsaicin treatments were given (group 5), the effect was no greater than the effect of either CTX (group 2) or capsaicin (group 4) treatments separately.

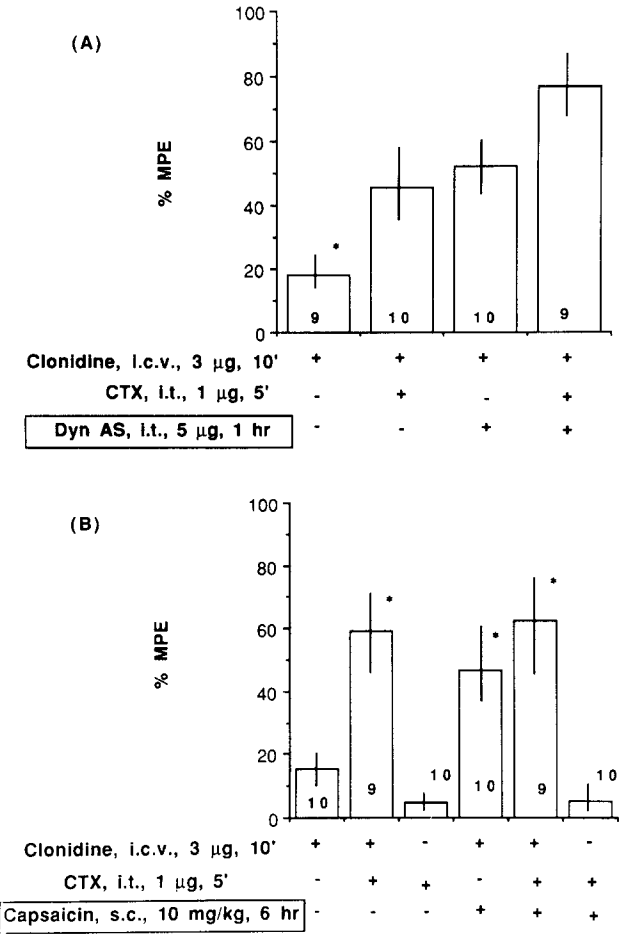

FIG. 4. Pretreatment with dynorphin antiserum (Dyn AS) or capsaicin pretreatment eliminated the ability of IT CTX to enhance ICV clonidine-induced analgesia. (A) Both IT CTX and dynorphin antiserum increased the analgesic action of ICV clonidine. When the two agents were combined, the response was no different than when each agent was given alone. (B) Capsaicin (10 mg/kg, SC) 6-h pretreatment, which produces desensitization to the subsequent action of Dyn A-enhanced, clonidine-induced analgesia (group 4 vs. 1). In the group pretreated with capsaicin, cholera toxin (IT) did not produce a greater effect on clonidine-induced analgesia (group 5) than either the group pretreated with capsaicin (group 4) or CTX IT (group 2). Groups with asterisk were not significantly different from each other but were significantly different from all groups without an asterisk, as determined by ANOVA followed by Newman-Keuls' test (in both A and B).

This meant that the CTX effect and capsaicin effects were not additive. As above, the effect of the two treatments appeared not to be independent. Thus, the results (Fig. 4A, B) indicated that when the Dyn A component of action of clonidine was attenuated by either Dyn AS pretreatment or desensitization to Dyn A by capsaicin pretreatment, CTX did not produce any additional enhancement of clonidine analgesia.

Evaluation of the Ability of CTX to Affect the Antianalgesic Action of Dyn A Administered IT Against ICV Morphine-Induced Antinociception

Morphine given ICV 10 min before the tail-flick test produced an antinociceptive effect (Fig. 5, group 1 in each set of time). This antinociceptive effect was antagonized by the IT administration at 5 min of Dyn A, 10 pg (group 1 vs. 2 in each set), as expected (11,14). This antianalgesic action of IT Dyn A was eliminated by IT administration of CTX at 5 min and 3 h, but not at 1 and 18 h. The nadir at 1 h created a biphasic action as in the previous experiments (Fig. 2). Also, CTX treatment did not alter the response to ICV morphine (group 3 vs. 1 in each set).

Capsaicin given IT releases spinal Dyn A to antagonize ICV morphine-induced analgesia (2). Capsaicin (IT) decreased the analgesic action of ICV morphine as expected (Fig. 6, group 1 vs. 2 for each set). This action of IT capsaicin was reversed by CTX given IT at 5 min and 3 h, but not at 1 h. This time frame of action of CTX against the effect of capsaicin was like that seen earlier for CTX on the Dyn A action (Fig. 5).

DISCUSSION

CTX given IT inhibited the antianalgesic action of Dyn A. This conclusion was based on the results from three experi-

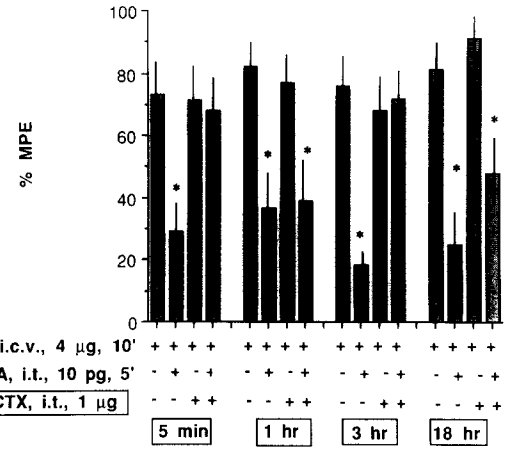

FIG. 5. The effect of IT CTX was evaluated on the antagonistic action of IT Dyn A against ICV morphine-induced analgesia. The analgesia induced by ICV morphine (10 min) was antagonized by IT Dyn A (5 min) (group 1 vs. 2) at each time: 5-min, 1-, 3-, and 18-h treatment with IT saline. At 5 min and 3 h, IT CTX eliminated the effect of Dyn A (group 4 vs. 2 in respective set). At 1 h, CTX had no effect on the action of IT Dyn A to antagonize morphine-induced analgesia (group 2 vs. 4). By 18 h, CTX IT had no effect on the antagonism of morphine-induced antinociception by IT Dyn A. The biphasic effect of CTX corresponded in time to the biphasic effect seen in Fig. 3. Significance, indicated by an asterisk, was determined by ANOVA followed by Newman-Keuls' test within each set of experiments at the given time. Eight to 10 mice were used in each group.

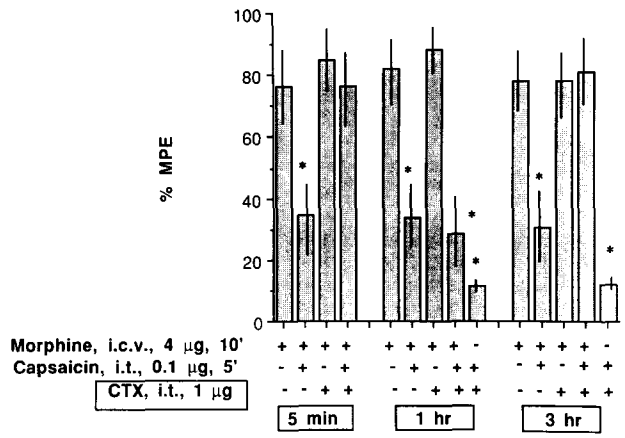

FIG. 6. The effect of IT CTX was evaluated on the antagonistic action of IT capsaicin against ICV morphine-induced analgesia. The analgesia induced by ICV morphine was antagonized by IT capsaicin (group 1 vs. 2, at 5 min, 1 h, and 3 h after IT saline). At 5 min and 3 h, IT CTX eliminated the effect of capsaicin (group 4 vs. 2 in respective set). At 1 h, CTX had no effect on the action of IT capsaicin to antagonize morphine-induced analgesia (group 2 vs. 4). The biphasic effect of CTX corresponded in time to the biphasic effect seen in Fig. 5. Asterisk indicates significant differences, as in Fig. 5. Eight to 10 mice were used in each group.

mental protocols where CTX given IT: a) enhanced the analgesic action of ICV clonidine, b) antagonized the effect of IT Dyn A on ICV morphine-induced analgesia, and c) eliminated the antagonistic action of IT capsaicin on ICV morphine-induced analgesia. Clonidine given ICV has two components to its action in the TFT (12,13). First, ICV clonidine releases spinal Dyn A, which has an antianalgesic action. Second, ICV clonidine has an analgesic action that remains latent until the spinal Dyn A antianalgesic component is attenuated. In the present study, when CTX was given IT, it increased the analgesic response to ICV clonidine. This result was compatible with IT CTX inhibiting the Dyn A component of clonidine action to uncover the analgesic component. Also, dynorphin antiserum given IT 1 h before the tail-flick test enhances the antinociceptive effect of ICV clonidine by attenuating the action of endogenously released Dyn A (13). In the presence of dynorphin antiserum, IT CTX did not further enhance the antinociceptive of action of clonidine (Fig. 4A). Another way to attenuate spinal Dyn A action is to produce desensitization to Dyn A (14). Capsaicin releases spinal Dyn A and, like pretreatment with Dyn A itself, attenuates the response to Dyn A (2). Capsaicin, given SC as a 6-h pretreatment, produced desensitization to the antianalgesic action of Dyn A (Fig. 4B). In the capsaicin-pretreated group, ICV clonidine produced an antinociceptive effect. CTX did not increase this analgesic action of clonidine in the capsaicin-pretreated mice. The latter result indicated that when the mice were desensitized to the action of Dyn A, CTX had no effect because CTX works by antagonizing the action of Dyn A. If IT CTX were directly synergizing with the analgesic component of action of clonidine, CTX should have continued to synergize even when the Dyn A component was attenuated.

In the experiments where morphine was given ICV, Dyn A administered IT decreased morphine-induced analgesia (Fig. 5) as expected (11,14). This action of Dyn A was blocked by the IT administration of 1 μg of CTX; IT CTX itself did not affect morphine-induced analgesia. Because the Dyn A was administered IT rather than endogenously released, the results indicated more directly that CTX inhibited the action of Dyn A.

As a variation to the two protocols (ICV clonidine and ICV morphine plus IT Dyn A), the third protocol involved ICV morphine-induced analgesia antagonized by IT administration of capsaicin. Capsaicin given IT antagonizes ICV morphine analgesia through release of spinal Dyn A (2). CTX given together with IT capsaicin eliminated the action of capsaicin, suggesting that CTX was inhibiting the action of Dyn A.

CTX inhibits the excitatory action of opioids on DRG neurons through inactivation of G_s protein to reduce the efficiency of coupling between the opioid ligand receptor and G_s (23). The present results indicate that CTX inhibited the antianalgesic action of Dyn A, which appeared to be a stimulatory action through G_s on the adenylate cyclase system. CTX given IT increases the analgesic potency of SC morphine in the tail withdrawal response of rats to hot water, while pertussis toxin decreases the potency of morphine (37). This action of CTX might be explained by our present finding that IT CTX appears to block the Dyn A antianalgesic component of spinal morphine action, thereby enhancing analgesia. Recently, we have demonstrated that the analgesic action of IT morphine in mice can be enhanced by attenuating the spinal Dyn A component by IT administration of opioid antagonists or dynorphin antiserum (Holmes and Fujimoto, submitted). CTX given IT should also enhance the analgesic action of IT morphine, an experiment that we will perform in the future. It is of interest that the obverse effect of inhibition of analgesia by IT administration of PTX has been reported (19,22). The effect of PTX correlates with the finding that in dorsal root ganglion-spinal cord explants in vitro, PTX blocks the inhibitory effects of opioids on sensory-evoked, dorsal horn synaptic network responses [(5); see (24)].

Even though the results in the present study with CTX given at 5 min and 3 h before the tail-flick test gave similar consistent results with the three protocols, it must be mentioned that at the 1-h pretreatment time, CTX did not produce an inhibitory effect. We do not know the reason for this biphasic action of CTX. It might, for example, be due to excitatory effects of CTX resulting from its anti-GTPase action, which could elicit a transient period of activation of the adenylate cyclase/cyclic AMP system in these neurons, in addition to the direct attenuation by CTX of the efficacy of G_s-coupled excitatory opioid receptor functions. Such dual effects of CTX have been observed in electrophysiologic studies of excitatory opioid receptor functions in sensory neurons in culture (29) and in many biochemical studies, e.g., G_s-coupled beta-adrenergic receptor functions in erythrocyte membranes (32).

It should be noted that in two of the protocols, clonidine and morphine were given ICV remote from the site where CTX was given IT This approach had the advantage that the CTX was most likely not acting at the receptor site at which clonidine and morphine were acting in the brain. A caveat is that Sanchez-Blazquez and Garzon (27) found that when CTX is given ICV in mice it enhances ICV morphine- and clonidine-induced analgesia. We have no direct evidence that when CTX was given IT, it was not getting up into the brain. If CTX given IT were producing effects by reaching the brain, it would seem that the responses at 5 min after administration would be different from those at 3 h. We would not expect CTX to reach the brain in high amounts at 5 min. Because the effects of spinally released and IT administered Dyn A were

at the spinal level, we believe that the CTX administered IT was acting in the spinal cord. Sachez-Blazquez and Garzon (27) propose that ICV morphine- and clonidine-induced analgesia are enhanced by CTX inhibition of G_s protein. Thus, it appears possible that the excitatory action of Dyn A on the spinal cord is mediated by activation of similar G_s-coupled opioid receptors as in the brain. These results suggest that morphine and clonidine may have excitatory effects in the brain just as Dyn A has in the spinal cord. To make matters more complex, microinjection of both CTX and PTX into the locus coeruleus and periaqueductal gray area of rats inhibits the antinociceptive action of morphine microinjected into the locus coeruleus and the periaqueductal gray area (3). CTX inhibition of morphine-induced analgesia in these cases may have been due to interference with G_s-coupled opioid receptor-mediated stimulation of the release of inhibitory modulators, e.g., adenosine (34) or serotonin (25) in these CNS tissues, thereby attenuating this indirect mode of morphine-induced analgesia (7,30).

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REFERENCES

1. Aksu, F.; Holmes, B. B.; Fujimoto, J. M. Opioid antagonists: Indirect antagonism of morphine analgesia by spinal dynorphin A. Pharmacol. Biochem. Behav. 45:409–418; 1993.
2. Arts, K. S.; Fujimoto, J. M.; Tseng, L. F. Involvement of dynorphin A and not substance P in the spinal antianalgesic action of capsaicin against morphine-induced antinociception in mice. J. Pharmacol. Exp. Ther. 261:643–651; 1992.
3. Bodnar, R. J.; Paul, D.; Rosenblum, M.; Liu, L.; Pasternak, G. W. Blockade of morphine analgesia by both pertussis and cholera toxins in the periaqueductal gray and locus coeruleus. Brain Res. 529:324–328; 1990.
4. Chen, G. G.; Chalazonitis, A.; Shen, K. F.; Crain, S. M. Inhibitor of cyclic AMP-dependent protein kinase blocks opioid-induced prolongation of the action potential of mouse sensory ganglion neurons in dissociated cell cultures. Brain Res. 462:372–377; 1988.
5. Crain, S. M.; Crain, B.; Makman, M. H. Pertussis toxin blocks depressant effects of opioid, monoaminergic and muscarinic agonists on dorsal-horn network responses in spinal cord-ganglion cultures. Brain Res. 400:185–190; 1987.
6. Crain, S. M.; Shen, K. F.; Chalazonitis, A. Opioids excite rather than inhibit sensory neurons after chronic opioid exposure of spinal cord-ganglion cultures. Brain Res. 455:99–109; 1988.
7. Crain, S. M.; Shen, K.-F. Opioids can evoke direct receptor-mediated excitatory effects on sensory neurons. Trends Pharmacol. Sci. 11:77–81; 1990.
8. D'Amour, F. E.; Smith, D. L. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72:74–79; 1941.
9. Dewey, W. L.; Harris, L. S.; Howes, J. P.; Nuite, J. A. The effect of various neurohormonal modulators on the activity of morphine and the narcotic antagonists in the tail flick and phenylquinone test. J. Pharmacol. Exp. Ther. 175:435–442; 1970.
10. Fan, S.-F.; Shen, K.-F.; Crain, S. M. Opioids at low concentrations decrease opening of K^+ channels in sensory ganglion neurons. Brain Res. 558:166–170; 1991.
11. Fujimoto, J. M.; Rady, J. J. Intracerebroventricular physostigmine-induced analgesia: Enhancement by naloxone, β-funaltrexamine and nor-binaltorphimine and antagonism by dynorphin A (1–17). J. Pharmacol. Exp. Ther. 251:1045–1052; 1989.
12. Fujimoto, J. M.; Arts, K. S. Clonidine administered intracerebroventricularly in mice produces antianalgesic effect which may be mediated spinally by dynorphin A (1–17). Neuropharmacology 29:351–358; 1990.
13. Fujimoto, J. M.; Arts, K. S.; Rady, J. J.; Tseng, L.-F. Spinal dynorphin A (1–17): Possible mediator of antianalgesic action in mice. Neuropharmacology 29:609–617; 1990.
14. Fujimoto, J. M.; Holmes, B. B. Systemic single dose morphine pretreatment desensitizes mice to the spinal antianalgesic action of dynorphin A (1–17). J. Pharmacol. Exp. Ther. 254:1–7; 1990.
15. Gintzler, A. R.; Xu, H. Different G proteins mediate the opioid inhibition or enhancement of evoked (5-methionine)-enkephalin release. Proc. Natl. Acad. Sci. USA 88:4741–4745; 1991.
16. Gross, R. A.; Macdonald, R. L. Dynorphin A selectively reduces a large transient (N-type) calcium current of mouse dorsal root ganglion neurons in cell culture. Proc. Natl. Acad. Sci. USA 84: 5469–5473; 1987.
17. Gross, R. A.; Moises, H. C.; Uhler, M. D.; Macdonald, R. L. Dynorphin A and cAMP-dependent protein kinase independently regulate neuronal calcium currents. Proc. Natl. Acad. Sci. USA 87:7025–7029; 1990.
18. Haley, T. J.; McCormick, W. G. Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br. J. Pharmacol. 12:12–15; 1957.
19. Hoehn, K.; Reid, A.; Sawynok, J. Pertussis toxin inhibits antinociception produced by intrathecal injection of morphine, noradrenaline and baclofen. Eur. J. Pharmacol. 146:65–72; 1988.
20. Holmes, B. B.; Fujimoto, J. M. Naloxone and norbinaltorphimine administered intracerebroventricularly antagonize spinal morphine-induced antinociception in mice through the antianalgesic action of spinal dynorphin A (1–17). J. Pharmacol. Exp. Ther. 261:146–153; 1992.
21. Hylden, J. L. K.; Wilcox, G. L. Intrathecal morphine in mice: A new technique. Eur. J. Pharmacol. 67:313–316; 1980.
22. Lutfy, K.; Chang, S.-C. J. L.; Candido, J.; Jang, Y.; Sierra, V.; Yoburn, B. C. Modification of morphine-induced analgesia and toxicity by pertussis toxin. Brain Res. 544:191–195; 1991.
23. Lux, B.; Schulz, R. Effect of cholera toxin and pertussis toxin on opioid tolerance and dependence in the guinea pig-myenteric plexus. J. Pharmacol. Exp. Ther. 237:995–1000; 1986.
24. Makman, M. H.; Dvorkin, B.; Crain, S. M. Modulation of adenylate cyclase activity of mouse spinal cord-ganglion explants by opioids, serotonin and pertussis toxin. Brain Res; 445:303–313; 1988.
25. Pycock, C. J.; Burns, S.; Morris, R. In vitro release of 5-hydroxytryptamine and gamma-aminobutyric acid from rat periaqueductal grey and raphe dorsalis region produced by morphine or an enkephalin analogue. Neurosci. Lett. 22:313–317; 1981.
26. Rady, J. J.; Fujimoto, J. M. Dynorphin A (1–17) mediates midazolam antagonism of morphine antinociception in mice. Pharmacol. Biochem. Behav.; in press.
27. Sanchez-Blazquez, P.; Garzon, J. Cholera toxin and pertussis toxin on opioid- and α_2-mediated supraspinal analgesia in mice. Life Sci. 48:1721–1727; 1991.
28. Shen, K. F.; Crain, S. M. Dual opioid modulation of the action potential duration of mouse dorsal root ganglion neurons in culture. Brain Res. 491:227–242; 1989.
29. Shen, K.-F.; Crain, S. M. Cholera toxin-A subunit blocks opioid excitatory effects on sensory neuron action potentials indicating mediation by Gs-linked opioid receptors. Brain Res. 525:225–231; 1990.
30. Shen, K.-F.; Crain, S. M. Cholera toxin-B subunit blocks excitatory effects of opioids on sensory neuron action potential indicating that GM1 ganglioside may regulate Gs-linked opioid receptor functions. Brain Res. 531:1–7; 1990.

31. Shen, K.-F.; Crain, S. M. Dynorphin prolongs the action potential of mouse sensory ganglion neurons by decreasing a potassium conductance whereas another specific kappa opioid does so by increasing a calcium conductance. Neuropharmacology 29:343–349; 1990.
32. Stadel, J. M.; Lefkowitz, R. J. Differential effects of cholera toxin on guanine nucleotide regulation of beta-adrenergic agonist high affinity binding and adenylate cyclase action in frog erythrocyte membranes. J. Cyclic Nucl. Res. 7:363–374; 1981.
33. Steel, R. G. D.; Torrie, J. H. Principles and procedures of statistics with special reference to the biological sciences. New York: McGraw-Hill Book Company, Inc., 1960:99–111.
34. Sweeney, M. J.; White, T. D.; Sawynok, J. Morphine, capsaicin and K^+ release purines from capsaicin-sensitive primary afferent nerve terminals in the spinal cord. J. Pharmacol. Exp. Ther. 248: 447–454; 1989.
35. Werz, M. A.; Macdonald, R. L. Opioid peptides selective for mu- and delta-opiate receptors reduce calcium-dependent action potential duration by increasing potassium conductance. Neurosci. Lett. 42:173–178; 1983.
36. Werz, M. A.; Macdonald, R. L. Dynorphin and neoendorphin peptides decrease dorsal root ganglion neuron calcium-dependent action potential duration. J. Pharmacol. Exp. Ther. 234:49–56; 1985.
37. Wheeler-Aceto, H.; Cowan, A. Studies with phasic and tonic noxious stimuli suggest that both cholera and pertussis toxin sensitive mechanisms mediate morphine antinociception in the rat spinal cord. Soc. Neurosci. Abstr. 17:727 (A291.6); 1991.