The Neurochemical Systems that Mediate Antinociception in Spinalized Rats

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ABSTRACT

We have previously shown that exposure to three brief (2 sec) 3.0 mA shocks elicits an opioid hypoalgesia, and that exposure to longer (75 sec) tailshocks elicits a nonopioid hypoalgesia in spinalized rats. The present study explores whether cholinergic and noradrenergic systems play a role in the production of these antinociceptive effects. Experiment 1, showed that the cholinergic antagonist scopolamine elicits hyperalgesia in spinalized rats, but does not affect the magnitude of antinociception observed after either brief or long tailshocks. Experiment 2 showed that alpha-2-noradrenergic antagonist yohimbine does not affect baseline levels of pain reactivity. Yohimbine did, however, attenuate the antinociception observed after both shock schedules. Implications of the results are discussed.

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INTRODUCTION

Considerable evidence exists that exposure to a variety of aversive stimuli can elicit a decrease in pain reactivity, or hypoalgesia. For example, exposure to electric shock, cold water, biting, or restraint can elicit a hypoalgesia that lasts up to 2 hours (Akil, Madden, Patrick, & Barchas, 1976; Bodnar, Kelly, Brutus, & Glusman, 1980; Miczek, Thompson, & Shuster, 1982). This basic effect, known as environmentally-induced hypoalgesia, has been demonstrated in a variety of species and across a range of pain reactivity tests (Bodnar et al., 1980; Chesher & Chan, 1977; Jackson, Maier, & Coon, 1979; Moskowitz, Terman , & Liebeskind, 1985; Willer, Dehen, & Chambier, 1981). In many situations the hypoalgesia appears to be mediated by endogenous opiates since the hypoalgesia is blocked by opiate antagonists (e.g. naltrexone and naloxone) and morphine tolerance (Akil et al., 1976; Drugan, Grau, Maier, Madden, & Barchas, 1981; Grau, Hyson, Maier, Madden, & Barchas, 1981; Lewis, Cannon, & Liebeskind, 1980; Maier, Davies, Grau, Jackson, Morrison, Moye, Madden, & Barchas, 1980; Watkins & Mayer, 1982). However, under other conditions, the hypoalgesia is not affected by these manipulations, which suggests nonopioid mechanisms are also involved (Grau et al., 1981; Lewis et al., 1980; Lewis,

Sherman, & Liebeskind, 1982; Watkins & Mayer, 1982).

The mechanisms which elicit environmentally-induced hypoalgesia appear to decrease pain, at least in part, by attenuating the flow of nociceptive information at the level of the spinal cord (for a review, see Basbaum & Fields, 1984; Basbaum, Marley, O'Keefe, & Clanton, 1977; Terman, Shavit, Lewis, Cannon, & Liebeskind, 1984; Watkins & Mayer, 1982). Supporting this, it has been shown that manipulations which elicit hypoalgesia inhibit spinally mediated measures of pain reactivity (e.g., tail withdrawal from radiant heat [the "tail-flick test"] (D'Amour & Smith, 1941; Grau, 1987a; Irwin, Houde, Bennett, Hendershot, & Seevers, 1951; Meagher, Chen, Salinas, & Grau, submitted).

Recent work from our laboratory suggests that these spinal antinociceptive systems can be activated in multiple ways, and that the mode of activation employed depends on the severity of the aversive stimulus used to elicit hypoalgesia (Meagher, Grau, & King, 1989, 1990; Meagher et al., submitted). For example, exposure to relatively mild tail-shock (3, 0.75 sec, 1.0 mA) elicits both a transient nonopioid and long-lasting opioid hypoalgesia on the tail-flick test (Grau, 1987a, 1987b). Under these conditions, forebrain systems appear to play a critical role in activating the spinal antinociceptive system since the hypoalgesia is eliminated by manipulations which disrupt forebrain functioning (e.g., decerebration, lesioning the frontal cortex

or administration of a high dose of pentobarbital [Grau, 1987a; Meagher, 1989, 1990]). In contrast, when an organism is exposed to more severe shock schedules, the activation of spinal antinociception mechanisms is governed by lower-level neural systems. For example, exposure to three, long (25 sec), 1.0 mA tail-shocks elicits a strong nonopioid hypoalgesia which is eliminated by spinal transection but is unaffected by manipulations which disrupt forebrain processing (Meagher et al., 1989, 1990). This suggests that under these conditions, the activation of spinal antinociceptive mechanisms is controlled by neural systems within the brainstem. Finally, exposure to relatively severe tail-shock (e.g., 2 sec to 75 sec of 3.0 mA) elicits an antinociception which survives a spinal transection at the second thoracic vertebrae (T2). Thus, under these conditions, the antinociceptive systems appear to be directly activated, within the spinal cord, by afferent nociceptive inputs. Interestingly, the form of the antinociception observed in spinalized rats depends on shock severity. For example, exposure to three brief (2 sec), 3.0 mA tail-shocks elicits a strong naltrexone reversible (opioid) antinociception on the tail-flick test; whereas three long (75 sec), 3.0 mA tail-shocks elicits a strong naltrexone insensitive (nonopioid) antinociception (Meagher et al., submitted).

In recent years, considerable progress has been made towards elucidating the neurochemical systems that mediate the

hypoalgesia observed after exposure to relatively mild aversive stimuli. This research has revealed that both noradrenergic and cholinergic systems play an important role in the production of forebrain and brainstem mediated antinociceptive effects (Grau, Illich, Chen, & Meagher, 1991). For example, we have shown the cholinergic antagonist scopolamine blocks both the opioid and nonopioid antinociception observed after very mild shocks but potentiates the brainstem mediated nonopioid antinociception observed after longer shocks (Grau et al., 1991). In contrast, both forebrain and brainstem mediated antinociceptive effects appear to be blocked by the alpha-2-noradrenergic antagonist, yohimbine (Danysz, Minor, Jonsson, Post, & Archer 1986; Jones & Gebhart, 1986; Lichtman & Fanselow, 1989).

The present paper looks at whether cholinergic or noradrenergic systems play a role in the production of the spinally mediated antinociception observed after exposure to either brief (2 sec) or long (75 sec) tailshock. Experiment 1 tests impact of the cholinergic antagonist scopolamine, and Experiment 2 tests the effects of the alpha-2-noradrenergic antagonist yohimbine.

GENERAL METHODS

Subjects. The subjects were male Sprague-Dawley rats obtained form Harlan, Houston, Texas. The rats were ordered at an age of 90 days and were given 10 days to acclimate after they arrived. They were maintained on a 12:12-hr light:dark cycle. The subjects were individually housed and maintained on ad libitum food and water.

Surgery and Histology. Rats were anesthetized with 40 mg/kg thiopental i.p., a short-acting anesthetic. To stabilize and position the rat's body for surgery, its head was held in a stereotaxic instrument and a small "pillow" was placed under its chest. The rats received laminectomies at T2 with a heat-cauterizing electrode according to the following procedure: (a) after T2 was localized tactilely, an 1" anterior-posterior incision was made; (b) the muscle tissue was cleared around T2, and a tissue retractor was used to isolate the cord; (c) a small rongeur was used to expose the T2 segment of the cord; and (d) the exposed cord was transected by heat cauterization. The exposed spinal cord was covered with Oxycel (Parke-Davis), and the wound was closed with autoclips. All rats were given 8-10 hr recovery before testing. The transections were verified by: 1) inspecting the cord during the operation; and 2) observing the behavior of the subjects after they recovered to ensure they exhibited paralysis behind the level of the forepaws. In past studies

(e.g., Meagher et al. 1990, submitted), post mortem inspection of the cord has verified that, without exception, when these criteria are met, the cord is completely transected

Apparatus. During behavioral testing, the rats were restrained in Plexiglas tubes (22 cm length, 6,8 cm internal diameter). The front of each tube was covered by a Plexiglas sheet. Inside each tube was a flat rectangular platform on which the rat could lie (5.5 cm wide and 5.3 cm from the top of the tube). The exterior surfaces of the tubes were covered with duct tape. Ventilation holes were drilled into the top, middle portion of the tubes. The rats were confined in the tubes by a band of adhesive tape that spanned the rear opening just above the rat's tail. This allowed the tail, which projected between the band of adhesive band and the base of the tube, to move freely. The tube was placed in a sound attenuating chamber. During shock presentation, the door to each chamber was closed.

Tail-shock was provided by a 660-V transformer that produced a constant-current 3.0 mA shock. The shock electrode, constructed from a modified fuse clip, was lightly coated with electrode paste. The electrode was taped to the rat's tail, approximately 15 cm behind the rear of the tubes.

A radiant heat tail-flick device was used to assess changes in nociception. The radiant heat source was provided by a 375-W movie light (Sylvania, type EBR) that was located 18 cm above the base of the apparatus. To focus the radiant

heat onto the tail, a condenser lens was positioned 8 cm below the light. The aluminum base of the device had a triangular groove cut into it (0.8 cm wide, 0.4 cm deep), in which the rat's tail was positioned. Test trials were automatically terminated by a lateral movement of the tail (minimum 0.5 cm) that was detected by a photocell positioned below the groove. A timer automatically recorded the duration of the trial to the nearest 0.01 second.

The apparatus was located in an isolated room. A space heater maintained the room temperature at approximately 26.5 degree Celsius and provided a background noise level of about 60 dB.

Procedure. Subjects were tested eight to ten hours after surgery (between 7:00 p.m. and 11:00 p.m.). After the subjects received either the test drug or its vehicle, they were placed in the restraining tubes and given 15 min to acclimate. Each rat then received four tail-flick tests at 2-min intervals. To prevent tissue damage, an 8-sec cut-off was used. The last three tail-flick latencies were averaged to provide a measure of the rat's baseline levels of nociception. Immediately after baseline testing, the shock electrode was attached to the rat's tail with adhesive tape. One third of the subjects in each drug condition then received three, 2-sec, 3.0 mA shocks at 20-sec intervals (Brief Shock). Another third received three, 75-sec, 3.0 mA shocks at 20-sec intervals (Long Shock). The remaining third were treated the

same except that shock was withheld (Unshocked). After the last shock, or an equal period of restraint for the unshocked groups, the shock electrodes were removed, and five tail-flick trials were administered at 2-min intervals.

Experiment 1: Role of Cholinergic Systems

As mentioned above, prior research has revealed that the cholinergic antagonist, scopolamine, attenuates forebrain mediated antinociceptive effects but potentiates brainstem mediated antinociception (Grau et al., 1991). In addition, scopolamine per se often elicits hyperalgesia in intact rats (Feigley, Beakey, & Saynisch, 1976; Grau et al., 1991; Watkins, Katayama, Kinscheck, Mayer, & Hayes, 1984). In my first experiment, I test whether scopolamine affects either baseline levels of pain reactivity or environmentally-induced hypoalgesia in spinalized rats. Two shock paradigms were used to elicit hypoalgesia. A brief shock procedure (3, 2 sec, 3.0 mA) that has been previously shown to elicit a strong naltrexone reversible hypoalgesia (opioid) in spinalized rats, and a long shock procedure (3, 75 sec, 3.0 mA) that elicits a naltrexone-insensitive hypoalgesia in spinalized rats (Meagher et al., submitted).

Methods

Subjects. Forty-eight (n = 8) rats served as subjects.

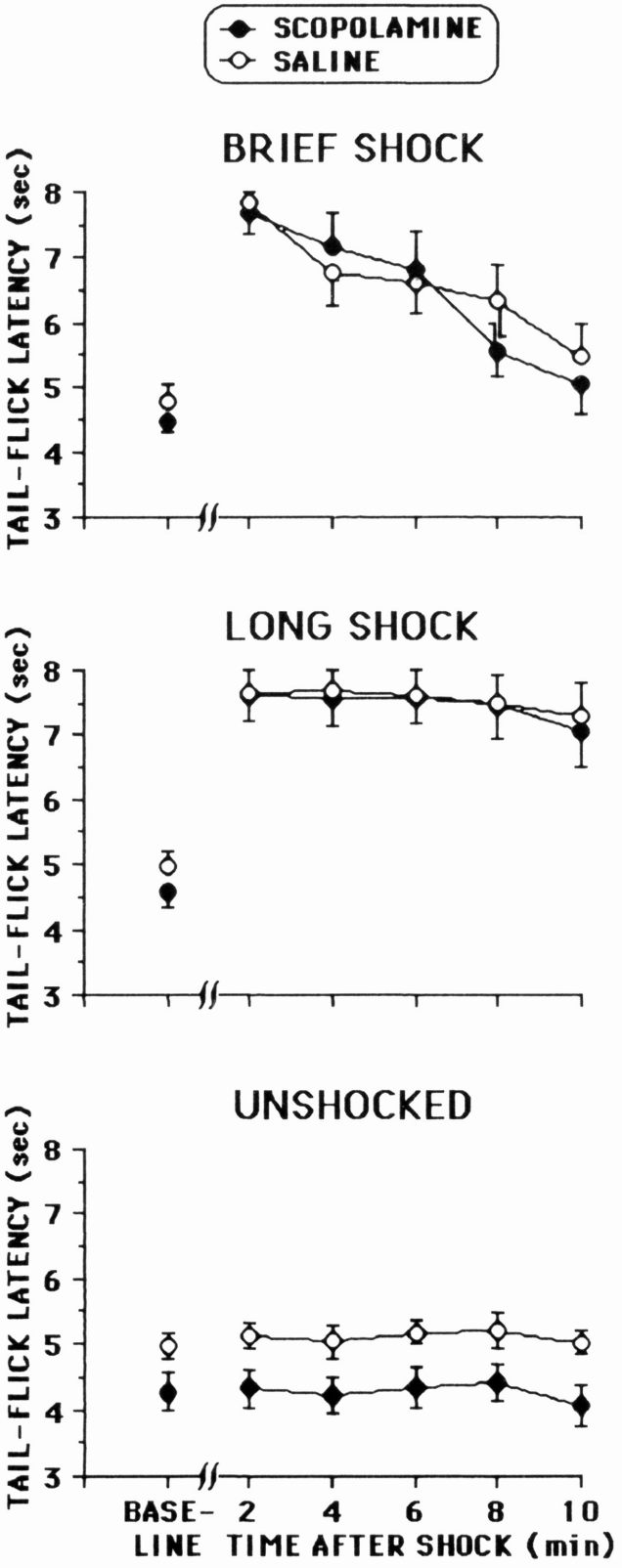
Procedure. Prior to testing, half the subjects received an intraperitoneal (i.p.) injection of 1 mg/kg of scopolamine (The dose of scopolamine employed was based on past research which has shown that it elicits a strong hyperalgesia and blocks forebrain mediated hypoalgesic effects in intact rats [Grau, et al., 1991]). The other half received its vehicle,

saline. The subjects were then placed in the restraining tubes and tested as described previously.

Results

The results are depicted in Figure 1. The mean baseline tail-flick latencies are shown at the left of each graph. It appears that scopolamine produced a slight decrease in tail-flick latencies (hyperalgesia). An analysis of variance (ANOVA) confirmed that the drug had a significant impact on baseline tail-flick latencies, $F(2, 42) = 6.77$, $p < .05$. As one would expect, neither the magnitude of this effect, nor the overall means, varied depending upon whether subjects were assigned to the brief shock, long shock or unshocked conditions, both Fs $< .47$, $p > .05$.

Figure 1. Tail-flick latencies observed in spinalized rats treated with either scopolamine (filled circles) or saline (open circles). (The baseline scores are depicted on the left side of each graph. The levels of pain reactivity observed after Brief Shock, Long Shock, or in subjects that remained Unshocked, are depicted in the top, middle and bottom panels, respectively.)


SCOPOLAMINE
SALINE
BRIEF SHOCK
LONG SHOCK
UNSHOCKED
TAIL-FLICK LATENCY (sec)
8
7
6
5
4
3
BASE-LINE
2
4
6
8
10
TIME AFTER SHOCK (min)

The mean tail-flick latencies observed after shocks, or an equivalent period of restraint, are depicted to the right of the baseline scores. It is clear that both shock schedules elicited a strong antinociception which was not affected by scopolamine. In addition, it appears the long shock schedule elicited a stronger antinociception than the brief shock schedule. An ANOVA confirmed that the main effect of shock treatment was significant, $F(2, 42) = 34.32$, $p < .001$. There was also a significant trials effect and trials by shock treatment interaction, both Fs > 12.17, $p < .001$. Neither the main effect of drug treatment, nor any of its higher order interactions, approached statistical significance, all Fs < 1.87, $p > .05$. Newman-Keuls test ($p < .05$) was then used to compare the overall post shock means collapsed across drug treatment. This test revealed that both shock schedules elicited antinociception relative to the unshocked control and that the long shock procedure elicited a significantly greater antinociception.

Elsewhere we found that when one controls for the impact of scopolamine on baseline levels of antinociception, the drug appears to potentiate the nonopioid, brainstem mediated, antinociception observed after three, 25 sec, 1.0 mA shocks (Grau et al., 1991). To elucidate whether such an effect might be observed in the present experiment, an analysis of covariance (ANCOVA) was performed to statistically control for the impact of the drug on baseline levels of pain reactivity.

I again found a significant effect of shock treatment, $F(2, 41) = 42.75$, $p < .001$. More importantly, neither the main effect of drug treatment, nor its interaction with shock treatment, approached statistical significance, both Fs $< .38$, $p > .05$. Thus, even when the impact of scopolamine on baseline scores is controlled for, the drug has no effect on the magnitude of shock-induced antinociception.

Discussion

Prior work had shown that scopolamine, per se, elicits hyperalgesia on the tail-flick test (Feigley et al, 1976; Grau et al., 1991; Watkins et al., 1984). This effect appears to be centrally mediated since methyl-scopolamine, which does not cross the blood-brain barrier, has no impact on baseline levels of pain reactivity (Grau et al., 1991). The present experiment revealed that a similar effect is observed in spinalized rats, which suggests the critical cholinergic synapse lies within the spinal cord. In contrast, scopolamine had no impact on the magnitude of shock-induced antinociception, which suggests a cholinergic synapse does not play a critical role in the production of either the opioid or nonopioid antinociception observed in spinalized rats.

Experiment 2: Role of Noradrenergic Systems

Experiment 2 tests whether the alpha-2-noradrenergic antagonist yohimbine affects either baseline levels of pain reactivity or environmentally-induced antinociception in spinalized rats.

Methods

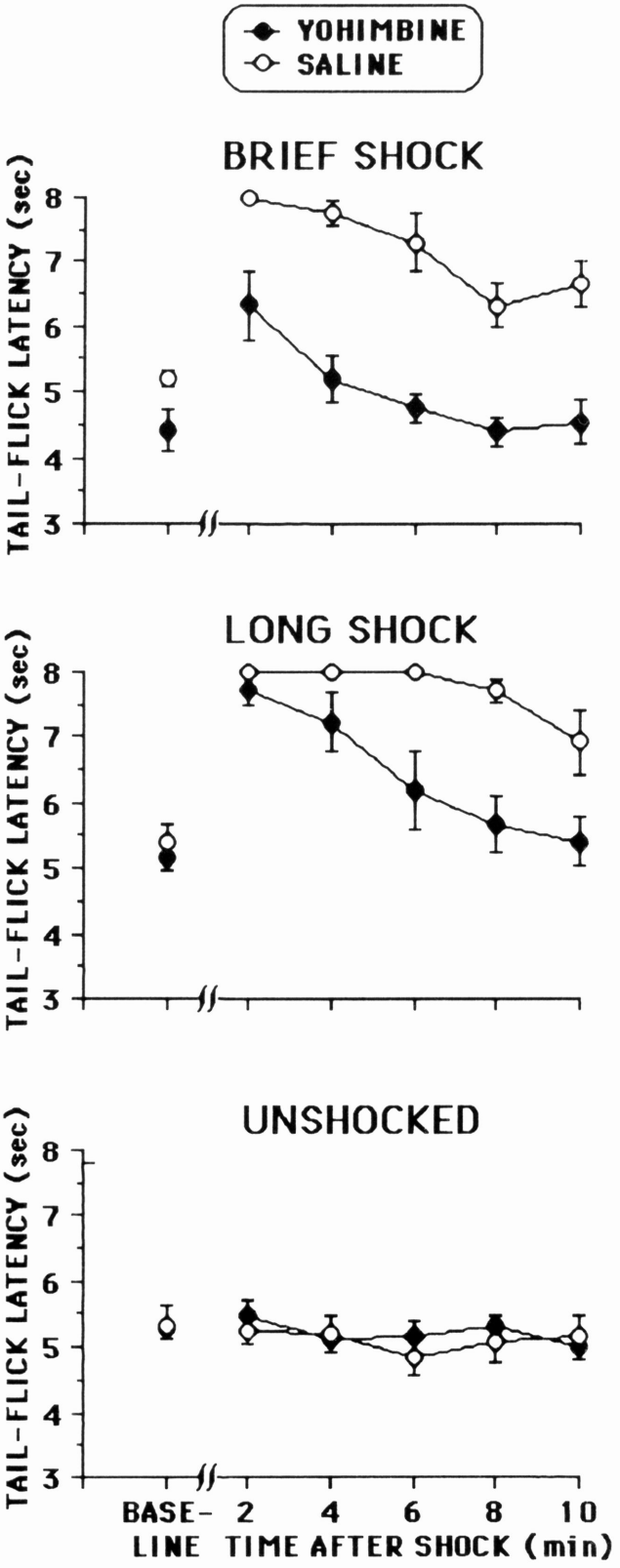
Subjects. Thirty-six subjects (n =6) served as subjects in this experiment.

Procedure. Half of the subjects were given 10 mg/kg of yohimbine i.p. The remaining subjects received its vehicle, saline. The subjects were then placed in the restraining tube and tested as described previously.

Results

The mean baseline scores are depicted on the left of each graph in Figure 2. It is apparent that yohimbine had little impact on baseline levels of pain reactivity and that the groups did not differ prior to shock treatment, all F's = 3.07, $p > .05$.

Figure 2. Tail-flick latencies observed in spinalized rats treated with either yohimbine (filled circles) or saline (open circles). (The baseline scores are depicted on the left side of each graph. The levels of pain reactivity observed after Brief Shock, Long Shock, or in subjects that remained Unshocked, are depicted in the top, middle and bottom panels, respectively.)


YOHIMBINE
SALINE
BRIEF SHOCK
LONG SHOCK
UNSHOCKED
TAIL-FLICK LATENCY (sec)
8
7
6
5
4
3
BASE-
LINE
2
4
6
8
10
TIME AFTER SHOCK (min)

The tail-flick latencies observed after each shock treatment are depicted to the right of the baseline scores. As in Experiment 1, both shock schedules elicited a strong antinociception relative to the saline controls. Yohimbine appears to have eliminated the opioid antinociception observed after brief shock and attenuated the nonopioid antinociception observed after long shock. An ANOVA confirmed that the main effects of shock and drug treatment were significant, both Fs > 29.50, $p < .001$. In addition, the drug by shock treatment interaction showed that the magnitude of the antinociception observed depended on drug treatment, $F(2, 30) = 10.27$, $p < .001$. The within subjects terms revealed that both the trials effect, $F(4, 120) = 23.62$, $p < .001$, and its interaction with shock treatment, $F(8, 120) = 5.04$, $p < .001$, were significant. The interaction between trials and drug treatment was also significant, $F(4, 120) = 2.45$, $p < .05$. The three-way interaction between trials, shock treatment and drug condition was not significant, $F(8, 120) = 1.92$, $p > .05$. The Newman-Keuls test ($p < .01$) was then used to compare the post shock means. This test revealed that both of the long-shocked groups, and the saline-treated brief-shocked group were hypoalgesic relative to both unshocked groups and the yohimbine-treated group that received brief shock. In addition, both of the saline-treated shocked groups were hypoalgesic relative to the yohimbine-treated brief-shocked groups. No other differences were significant.

Discussion

In contrast to scopolamine, yohimbine did not affect baseline levels of pain reactivity. The drug did, however, attenuate both the opioid antinociception observed after brief (2-sec 3.0 mA) shocks and the nonopioid antinociception observed after long (75-sec 3.0 mA) shocks. This suggests that noradrenergic systems play a critical role in producing these two forms of antinociception.

Although yohimbine eliminated the opioid antinociception observed after brief shock, it only attenuated the nonopioid antinociception observed after long shocks. There are a number of potential explanations as to why yohimbine failed to eliminate long shock-induced antinociception in spinalized rats. One possibility is that the dose employed was insufficient to completely block the alpha-2-noradrenergic receptors. Alternatively, the nonopioid antinociception observed after long shocks may be mediated by two independent pathways, only one of which depends on a noradrenergic synapse. Further research is needed to distinguish these two alternatives.

GENERAL DISCUSSION

The present set of experiments was designed to elucidate whether cholinergic or noradrenergic systems play a role in the production of spinally mediated opioid or nonopioid antinociception. As has been repeatedly observed in intact rats (Feigley et al., 1976; Grau et al., 1991; Watkins et al., 1984), Experiment 1 showed that scopolamine per se elicited hyperalgesia. This, in conjunction with past work which suggests that the hyperalgesia is centrally mediated, implies scopolamine induces hyperalgesia by disrupting cholinergic transmission within the spinal cord. In contrast, scopolamine did not affect the magnitude of either the opioid or nonopioid mediated antinociception observed in spinalized rats. Thus, these antinociceptive effects do not depend on a cholinergic synapse.

Experiment 2 assessed the impact of the alpha-2-noradrenergic antagonist yohimbine. Yohimbine did not affect baseline levels of pain reactivity. It did, however, attenuate both the opioid antinociception observed after brief shock as well as the nonopioid antinociception observed after long shock. Thus, a noradrenergic synapse appears to play a crucial role in the production of both of these antinociceptive effects. It remains unclear, however, whether the critical noradrenergic synapse lies within the spinal cord. The problem here is that yohimbine, when administered

systemically, can affect peripheral as well as central noradrenergic sites. To determine whether a peripheral or central site is critical, we need to test whether intrathecal yohimbine attenuates shock-induced antinociception in spinalized rats. This study is currently in progress.

The present set of experiments explored the neurochemical systems which mediate antinociception in spinalized rats. We chose to begin our studies on the neurochemical basis of antinociception at the level of the spinal cord for a number of reasons. First, relative to higher brain regions, we know much more about the neural circuitry that underlies pain modulation at the level of the spinal cord. In addition, spinal mechanisms may provide the foundation and basic architecture upon which supraspinal pain modulating systems are built. Further research is needed to determine what other neurochemical systems are involved in the generation of antinociception at the level of the spinal cord. For example, a survey of recent literature suggests GABAergic and serotonergic systems may also play a role. The role of these systems is currently being investigated.

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