Research Report

Spinal antinociception mediated by a cocaine-sensitive dopaminergic supraspinal mechanism

Judith A. Kiritsy-Roy [a,b], Bai C. Shyu [a,1], Peggy J. Danneman [d], Thomas J. Morrow [a,b,c], Carl Belczynski [a], Kenneth L. Casey [a,b,c,*]

[a] Neurology Service (127), Veterans Affairs Medical Center, 2215, Fuller Road, Ann Arbor, MI 48105, USA,
Departments of [b] Neurology and [c] Physiology and [d] Unit for Laboratory Animal Medicine, The University of Michigan, Ann Arbor, MI 48109, USA

Abstract

The role of dopaminergic descending supraspinal processes in mediating the antinociceptive action of cocaine was studied in the rat using a combination of extracellular neuronal recording and behavioral techniques. Neurons in the superficial laminae (I–II) of the spinal dorsal horn with receptive fields on the tail were recorded in anesthetized rats using insulated metal microelectrodes. Stimulation of the receptive field with either high intensity transcutaneous electrical pulses or with an infrared CO_2 laser beam produced a biphasic increase in dorsal horn unit discharge. Conduction velocity estimates indicated that the early discharge corresponded to activity in Aδ whereas the late response corresponded to activity in C afferent fibers. Cumulative doses of cocaine (0.1–3.1 mg/kg i.v.) inhibited the late response to either electrical or laser stimulation in a dose-related manner. The early response to laser, but not electrical, stimulation was also suppressed by cocaine. Neurons in the spinal dorsal horn with receptive fields on the ipsilateral hindpaw were activated by natural noxious (pinch) or innocuous (tap) somatic stimulation. Cocaine selectively suppressed nociceptively evoked dorsal horn unit discharge. This antinociceptive effect was dose-related (0.3–3.1 mg/kg, i.v.) and antagonized by eticlopride (0.05–0.1 mg/kg, i.v.), a selective D2 dopamine receptor blocker. The same doses of cocaine failed to inhibit the responses of dorsal horn neurons to low threshold innocuous stimulation. Complete thoracic spinal cord transection eliminated the antinociceptive effect of cocaine on dorsal horn neurons and also eliminated the cocaine-induced attenuation of the tail-flick reflex. These data demonstrate that cocaine selectively inhibits nociceptive spinal reflexes and the nociceptive responses of dorsal horn neurons primarily by means of a D2 dopaminergic receptor mechanism. This antinociceptive effect of cocaine is independent of its local anesthetic activity and requires the integrity of the thoracic spinal cord, suggesting that the drug potentiates or activates supraspinal dopaminergic projections to the dorsal horn.

Key words: Cocaine; Dorsal horn neuron; Pain; Tail flick test; Spinal transection; Dopamine

1. Introduction

Previous studies have demonstrated that cocaine produces an antinociceptive effect in animals [15,21,22] and humans [27]. This action of cocaine is independent of its hypertensive [15] or local anesthetic activities [20] and is mediated at least in part by dopaminergic mechanisms [15]. Systemic injections of cocaine at doses that produce a behavioral antinociception inhibit the response to high intensity electrical stimulation of units in the medial thalamus [1,25] and brainstem [2]. However, the precise neuronal mechanisms involved in the antinociceptive action of cocaine remain unclear.

It is possible that cocaine reduces nociceptive input at the level of the spinal cord. This effect could result from a direct inhibition of dorsal horn neurons that receive input from peripheral nociceptors. Alternatively, antinociception could be mediated indirectly via activation or potentiation of descending inhibitory supraspinal processes. Many of the pharmacological effects of cocaine are attributed to inhibition of synaptic neurotransmitter reuptake at monoaminergic nerve endings [10,11], which results in potentiation or enhancement of monoaminergic neurotransmission. Cate-

cholaminergic [12–14,16,24] and serotonergic [7,17,19] cell groups in the brainstem with projections to the dorsal horn are known to have important regulatory influences on spinal transmission of nociceptive input. Cocaine could produce an antinociceptive effect by enhancement of monoaminergic neurotransmission in one or more of these descending monoaminergic systems.

The present experiments were designed to assess spinal antinociceptive activity of cocaine. Because it is a potent local anesthetic, it is not possible to evaluate the effect of intrathecally injected cocaine in behavioral tests such as the hot plate, formalin or tail flick tests. In addition, spontaneous tail twitching results from the psychomotor stimulant effects of cocaine, and this interferes with tail flick testing in fully conscious rats. Therefore, the antinociceptive activity of systemically administered cocaine was studied using the tail flick assay in lightly anesthetized rats. Electrophysiological recording techniques were also used to determine the effects of cocaine on nociceptive and non-nociceptive neuronal responses of dorsal horn neurons. To examine the role of a descending supraspinal mechanism in mediating cocaine antinociception, these pathways were interupted in both the tail flick and unit recording experiments. The results indicate that cocaine, acting by dopaminergic mechanisms, selectively attenuates the nociceptive responses of dorsal horn neurons and the spinally mediated tail flick reflex. Descending supraspinal pathways are required for the full expression of this antinocicptive action.

2. Materials and methods

2.1. Surgical preparation for unit recording

Male Sprague–Dawley rats (300–500 g) were anesthetized with either chloral hydrate (400 mg/kg i.p.) or pentobarbital (Nembutal, 55 mg/kg i.p.). Catheters were implanted in the trachea for mechanical ventilation and in the jugular or tail veins for drug injections. A laminectomy was performed at vertebral segments Th12–L1 for experiments in which the receptive field was on the hindpaw or L2–L3 for experiments with receptive field on the tail. Succinylcholine (1 mg/kg) was administered to produce muscular paralysis; supplemental doses were administered approximately every 1–2 h as determined by the reappearance of flexion reflexes. At these times, supplemental doses of anesthetic were administered. Rectal temperature was monitored and maintained between 37° and 38°C using a thermostatically controlled heating pad. End tidal CO_2 was maintained at 3–4% by adjusting the end tidal volume and rate of the respiratory pump.

2.2. Experimental procedures

Units in the dorsal horn with receptive fields (RFs) on the tail or ipsilateral hindpaw were isolated and recorded using stainless steel or tungsten microelectrodes (impedance = 5–20 MΩ at 100 Hz). Potentials from the dorsal horn units were amplified differentially to

the indifferent electrode, positioned in an adjacent spinous process, and displayed on the oscilloscope. The extracellularly recorded action potentials of single units were converted by an amplitude window discriminator to standard logic pulses and fed to a minicomputer system (Data General, Southboro, MA) for on-line data processing or off-line data analysis.

In some studies, units at spinal segments L2–L3 (vertebral segments Th12–L1) with RFs on the hindpaw were activated using natural stimuli. These units were initially located by their response to non-specific search stimuli (light tapping of the hindpaw). Then, the responses of the units to nociceptive stimuli were tested by pinching the receptive field on the hindpaw with a toothed forceps fitted with an adjustable stop, which controlled the minimum separation of the jaws. Stimuli of reproducible intensity were produced by squeezing a fixed thickness of tissue between the jaws of the forceps until the stop was reached. Similar stimuli applied to the waking rat produce consistent escape responses and frequent attempts to attack the stimulus site. Units that responded exclusively to innocuous somatic stimulation were studied by lightly tapping the receptive field with a blunt instrument. These units were also driven by electrical stimulation, using a pair of stainless steel needle electrodes inserted under the skin of the receptive field. Rectangular constant current pulses (1–10 mA, 0.2–1.0 ms) were delivered using an electrical stimulator (Grass, Model S88).

Units at spinal segments S3–Co1 (vertebral segments L2–L3) with RFs on the tail were located by their response to non-specific search stimuli (light tapping on the tail). These units were activated by noxious stimulation with an infrared CO_2 laser (Directed Energy, Irvine, CA) at pulse intensities that produced a brisk tail flick response in awake animals (10 W, 45 ms). These units were also tested for responses to electrical stimulation using transcutaneous needle electrodes in the RF (10–15 mA, 1 msec).

Spontaneous activity and unit responses to stimulation of the RF were sampled several times during the control period before drug treatments. Drugs were administered as bolus injections in a volume of approximately 0.5 ml per injection through the venous catheter. In some studies, the time course of cocaine effects was monitored by testing the evoked unit responses to stimulation of the RF at 1, 5, 10, 15 and 20 min after administration of cocaine (1–2.5 mg/kg). Only one dose of cocaine was used in these experiments. In other experiments, cumulative dose–response curves for cocaine were obtained by administration of incremental doses of the drug at intervals of 3 min. The evoked unit response was determined at 2 min after each dose. Eticlopride, a D2 dopamine receptor antagonist, was injected (0.05–0.1 mg/kg i.v.) after the final dose of cocaine in some of the experiments.

2.3. Data analysis

The conduction velocity of afferent fibers activated by high intensity electrical stimulation was estimated from the latency to onset of evoked unit discharge and measurements of the distance from the receptive field on the tail to the recording site in the spinal cord.

Peristimulus time histograms were generated and averaged for 5–12 stimuli delivered over a period of 1–2 min before (control) and at predetermined time points after drug injections. In some studies, the unit response to natural noxious or innocuous stimulation was obtained from the histograms as post stimulus firing rate. In other experiments, the areas under the histogram for the early (< 30 msec) and late (> 100 ms) responses to electrical and laser stimulation were obtained using a computerized video digitizing system (Imaging Research Inc., St. Catharine's, Ontario). The unit response to stimulation of the receptive field after cocaine is expressed as percent of the pretreatment control unit response.

Treatments that produced a significant change from control ($P <$ 0.05) were determined using the paired t-test. Differences between

treatment groups were determined using unpaired *t*-tests or analysis of variance. Dose–response data were analyzed by regression analysis.

2.4. Histology

At the end of each experiment, DC current (50 μA, 30 s) was passed through the microelectrode. The animals were then perfused with 10% buffered formalin, and the spinal cords were removed for stioning on a cryostat. Tissues were stained with Cresyl violet and examined for lesions at the recording site in the dorsal horn.

2.5. Tail flick experiments

These studies used an adaptation of the tail flick test of D'Amour and Smith [5]. Rats were lightly anesthetized with chloral hydrate (275 mg/kg i.p.) and were allowed to recover until they exhibited flexion reflexes and had tail-flick latencies of between 4 and 4.5 s. Baseline tail-flick latency (B) was then determined with 5 consecutive readings, each 2 min apart using an automated tail-flick algeometer (IITC, Inc., Woodland Hills, CA). Cocaine (25 mg/kg, i.p.) or saline was administered and tail flick latencies (L) were recorded at 10 min intervals. In some cases, naloxone (1 mg/kg, i.p.; $n = 4$) or saline ($n = 8$) was injected 10 min after cocaine. To prevent tissue damage, the instrument was set to terminate testing automatically whenever the tail flick latency reached 8 s (maximum latency). Tail flick latencies were converted to % Analgesia according to the following formula:

$$((L-B)/(8-B))\times100\%.$$

The effect of cocaine on tail flick latency was also tested in a group of rats with complete transection of the spinal cord. Rats ($n = 8$) were deeply anesthetized with a combination of ketamine (85 mg/kg i.m.) and xylazine (12 mg/kg i.m.) and the spinal cord was transected between thoracic levels 5 and 8. These animals were allowed to recover for 24 h before testing. Because cocaine did not produce hypermotility below the level of the lesion, tail flick testing was conducted without the use of chloral hydrate anesthesia prior to cocaine. Saline was injected 10–20 min before cocaine to control for any effects of the injection procedure alone. Animals were sacrificed immediately after testing and spinal cord lesions were inspected to verify complete transection.

3. Results

3.1. Location of recorded units

In 22 cases in which microelectrode lesions were found, 19 were within the substantia gelatinosa and 3 were at the lateral neck of the dorsal horn. In several experiments in which high impedance tungsten microelectrodes were used, no mark was produced at the recording site. On the basis of micrometer readings from the electrode holders, it was estimated that the units recorded in these experiments were within the superficial laminae of the dorsal horn.

3.2. Synchronous activation of dorsal horn units by high intensity electrical and infrared laser stimulation

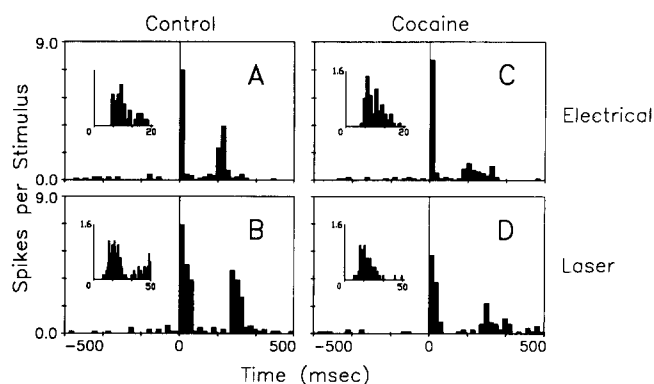Units in the dorsal horn at the level of T3–T4 with receptive fields on the tail were activated initially by

Fig. 1. Effect of cocaine on one dorsal horn unit activated by electrical (10 mA, 1 ms pulses) and CO_2 laser (10 W, 45 ms pulses) stimulation. Multi-trial peristimulus time histograms (bin width = 5 ms) were generated and averaged from 8–12 stimuli delivered to the receptive field on the tail before (A and B) and 5 min after intravenous injection of a total cumulative dose of 3.1 mg/kg cocaine (C and D). Insets in each histogram show, on an expanded time scale, the early (short latency) response to stimulation (bin width = 1 ms). Cocaine suppressed the early and late responses to laser stimulation and the late responses to electrical stimulation.

transcutaneous electrical stimulation (10 mA, 1 ms duration pulses), which produced a biphasic increase in unit discharge. An example of this response for one unit is shown in the peristimulus time histogram of Fig. 1A. Similar results were obtained from a total of 7 units with receptive fields on the tail. The latency to the early discharge (inset) corresponded to afferent fiber conduction velocities of approximately 18.2 ± 1.5 m/s, which is in the range for Aδ afferent fibers. If it is assumed that the late response may be due to direct excitation from afferent fibers, then the average conduction velocity (0.8 ± 0.08 m/s) suggests that the units in these studies also received C fiber input. There was no evidence for a shorter latency unit response to either stimulus, as would be expected if large diameter afferents were stimulated.

Pulses from an infrared CO_2 laser (16 W, 45 ms) directed at the receptive fields of the same 7 units produced a similar biphasic increase in the unit firing rate, with the exception of one unit that responded to laser stimulation with only a long latency response. Fig. 1B shows the laser-evoked response of the same unit shown in Fig. 1A. The latency to both early and late components of the evoked response appears longer after laser stimulation compared to electrical stimulation, probably reflecting the additional time required for activation of tissue nociceptors by the laser compared to the electrical stimulus.

Cumulative doses of cocaine, from 0.1 to 3.1 mg/kg i.v., suppressed selectively the late response of dorsal horn units evoked by electrical stimulation of the tail receptive field. An example of this is shown in Fig. 1C, in which there is no change in the early electrically-

evoked unit response (see inset) after a dose of cocaine that attenuated the late response. The mean effect of cumulative doses of cocaine on both early and late unit responses to electrical stimulation ($n = 7$) is shown in Fig. 2. Although there was no significant change in the early evoked response with increasing doses of the drug ($r = 0.044$, $t = 0.25$, df $= 34$, $P = 0.804$), the late response was reduced by up to 50% after the highest dose of cocaine. Regression analysis of the dose-response data revealed a significant inverse relationship between dose and the late unit response to stimulation ($r = 0.585$, $t = 4.14$, df $= 34$, $P < 0.001$).

To avoid tissue damage that could result from frequent testing with the laser stimulus, the effect of cocaine on laser-evoked activity was determined for these units only after the last cumulative dose of the drug (3.1 mg/kg) rather than after each dose. Under these conditions, cocaine inhibited the late evoked response of all 7 units studied (paired t-test for late response before and after cocaine: $t = 3.553$, df $= 6$, $P = 0.012$). Five of these laser-responsive cells also had a short latency response that was attenuated by the final dose of cocaine (paired t-test for early response before and after cocaine: $t = 4.261$, df $= 4$, $P = 0.013$). An example of the inhibitory effect of cocaine on early (inset) and late laser-evoked unit responses is shown for one of these units in Fig. 1D. The average unit response to laser stimulation for these 5 units before and after cocaine is shown in Fig. 3. Cocaine (3.1

Fig. 3. Comparison of the effect of cocaine on early (initial 30 ms) and late (> 100 ms) responses to electrical versus laser stimulation of the tail. The unit responses to electrical and laser stimulation were determined before (control) and after a cumulative dose of 3.1 mg/kg cocaine. Responses after cocaine are expressed as the mean ($\pm$S.E.M.) percent of the control unit response to each type of stimulus. (*) Indicates a significant change in the unit response to stimulation after cocaine compared to the control unit response ($P < 0.05$, paired t-test). Seven units in the dorsal horn showed late responses to both electrical (10 mA, 1 ms pulse) and CO_2 laser (10 W, 45 ms) stimulation; these were attenuated after the final dose of cocaine. The early response was unaffected by cocaine when elicited by electrical stimulation (7 units), but was attenuated in 5 units that showed an early response to laser stimulation.

mg/kg i.v.) produced a significant decrease in both early and late components of the laser evoked response in these 5 units.

3.3. Effect of cocaine on dorsal horn unit responses to natural somatic stimulation

A total of 22 dorsal horn units with receptive fields on the ipsilateral hindpaw were activated by natural noxious or innocuous stimulation. Six units were activated exclusively by low threshold mechanical stimulation (tap) and adapted rapidly to stimulation in the receptive field. The remaining 16 units were wide dynamic range (WDR) neurons that responded preferentially to noxious mechanical stimulation (pinch).

The effect of natural stimulation on the discharge rate of one WDR and one low threshold unit before and after administration of cocaine is shown in Fig. 4. During the control period before cocaine was administered, the firing rates of the WDR and low threshold units increased with stimulation of the receptive fields by pinching and tapping, respectively. Cocaine (1 mg/kg i.v.) attenuated the response to noxious stimulation (Fig. 4, upper panels) but was without effect on the response to innocuous stimulation (Fig. 4, lower panels). The mean effect of cocaine on 6 WDR and 6 low threshold units is shown in Fig. 5. Cocaine suppressed the WDR unit response to pinch at 5 min after injection, and this inhibitory effect subsided by 10 min after injection. There was no effect of cocaine on the
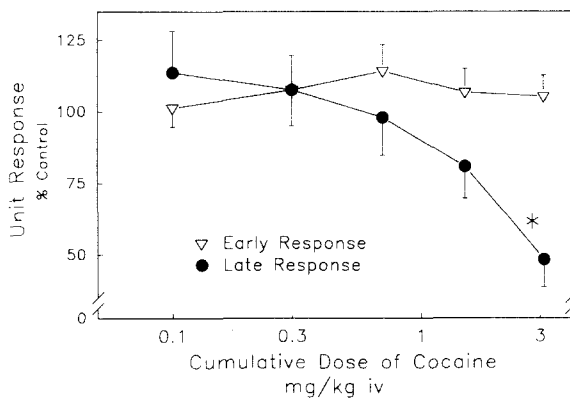

Fig. 2. Effect of cumulative doses of cocaine on dorsal horn unit response to electrical stimulation. Units ($n = 7$) in the dorsal horn with receptive fields on the tail were driven by transcutaneous electrical stimulation (10 mA, 1 ms pulses). The unit response was obtained from peristimulus time histograms, which were averaged from 10–12 stimuli delivered before cocaine (control) and at 2 min after each dose, injected at 3 min intervals. Unit responses after each dose of cocaine are expressed as mean ($\pm$S.E.M.) percent of the control unit response to stimulation. Early responses ($\triangledown$) represent the firing rate of the unit during the initial 20 ms after the stimulus, and late responses ($\bullet$) represent the unit firing rate during the later phase of unit activity. * Indicates a significant linear relationship between log dose of cocaine and unit response to electrical stimulation ($P < 0.001$, regression analysis).
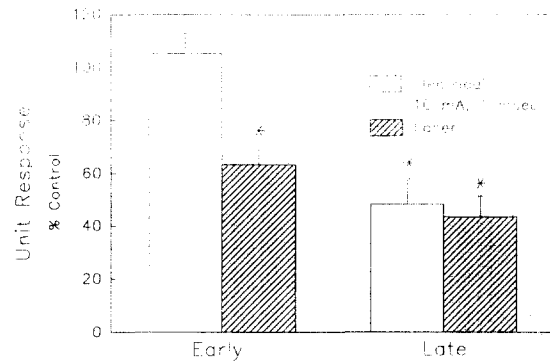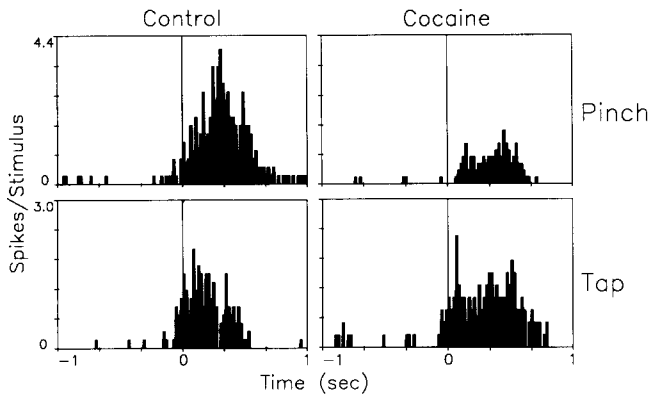
Fig. 4. Effect of cocaine on dorsal horn unit responses to noxious pinch (upper panels) and innocuous tap (lower panels) stimulation. Peristimulus time histograms (bin width = 20 ms) were generated and averaged from 5-6 stimuli delivered to the receptive field on the ipsilateral hindpaw before (left panels) and 5 min after injection of 1 mg/kg cocaine (right panels).

Fig. 6. Effect of cumulative doses of cocaine on dorsal horn unit responses to natural noxious stimulation (pinch) and reversal with eticlopride. Units (n = 7) in the dorsal horn with receptive fields on the ipsilateral hindlimb were stimulated by pinching with toothed forceps. The unit response was obtained from peristimulus time histograms, averaged from 5-6 stimuli before cocaine (control) and at 2 min after each dose of cocaine. Doses were injected at 3-4 min intervals. Unit responses after each dose of cocaine are shown in the filled bars as the mean (±S.E.M.) percent of control. Following the fifth cocaine injection, two doses of eticlopride (50 and 100 μg/kg) were injected at 5 min intervals. The unit response to pinch was determined at 5 min after each dose of eticlopride (hatched bars). * Indicates a significant difference from the response after the highest dose of cocaine (paired t-test, P < 0.05).

response of low threshold units during the 20 min observation period.

To determine if the inhibitory effect of cocaine on nociceptive responses of dorsal horn units required the integrity of descending supraspinal pathways, the spinal cord was transected at the level of T2-T3 in 5 rats. WDR units with receptive fields on the ipsilateral hindpaw were identified at the level of T13-L1 at least 1 h after transection. Cocaine (1 mg/kg i.v.) had no
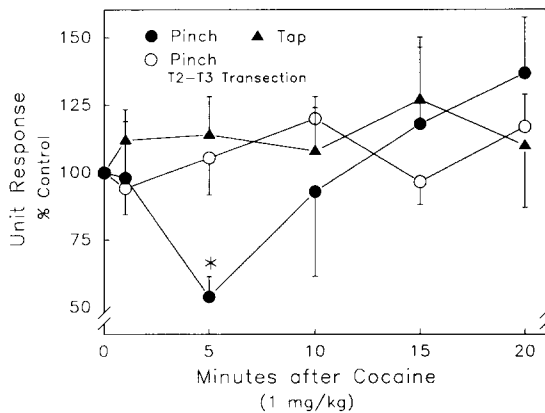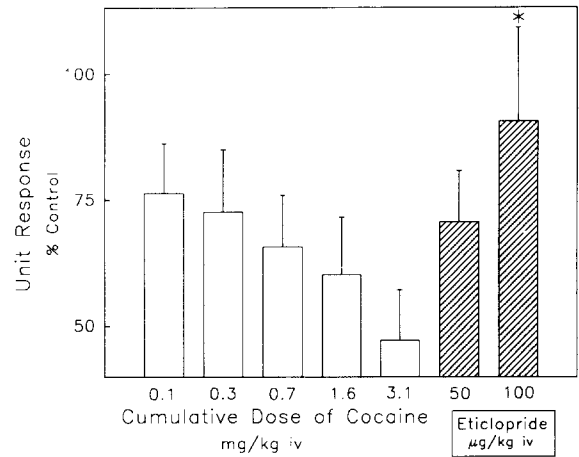

Fig. 5. Effect of single doses of cocaine (1 mg/kg i.v.) on dorsal horn unit responses to natural stimulation in intact rats (filled symbols; n = 6) and on rats with spinal transtions at T2-T3 1-2 h before unit recording (open symbols; n = 5). One unit was studied in each rat. In intact rats, units with receptive fields on the ipsilateral hindlimb were activated by noxious pinch or innocuous tap stimulation. In transected rats, units responded only to noxious pinch with toothed forceps. Control responses to each stimulus were determined before the injection of cocaine from the average peristimulus time histograms generated from 5-6 trials. The same stimuli were delivered at intervals after injection of cocaine, and the mean (±S.E.M.) unit responses are shown at these intervals as percent of the control unit response. * Indicates a significant change in the unit response to stimulation after cocaine compared to the control unit response (P < 0.05, paired t-test).

inhibitory effect on the noxious pinch evoked responses of dorsal horn units in rats with spinal transection (Fig. 5).

Cocaine produced a dose-related inhibition of the unit response to noxious pinch in 7 dorsal horn units (Fig. 6). Cumulative doses of cocaine (0.1 to 3.1 mg/kg i.v.) produced a progressive decrease in the unit response to pinch, with a maximum reduction to about 50% of the control evoked response after a total dose of 3.1 mg/kg (r = 0.359, t = 2.07, df = 30, P = 0.047). Eticlopride (50-100 μg/kg i.v.), a selective D2 dopamine receptor antagonist, administered after the final dose of cocaine, reversed this inhibitory effect within 5 min (paired t-test for cocaine (3.1 mg/kg) vs. eticlopride (100 μg/kg): t = 3.13, df = 5, P = 0.026). This rapid return of responsiveness was not seen at any of the other 5 min intervals between cumulative doses of cocaine, nor was it observed during the 20 min of observation of 3 units following the injection of 2.5 mg/kg cocaine.

3.4. Effect of cocaine on tail flick latency

The effect of cocaine on tail flick latency was determined in 2 groups of rats. One group (n = 26) was intact and lightly anesthetized with chloral hydrate to counteract the excessive movements and motor excita-

tion produced in these restrained animals by cocaine. Tail flick latency was monitored at 10 min intervals after the injection of chloral hydrate (275 mg/kg i.p.) until a robust tail flick reflex with a latency of 4–4.5 s was obtained. At this time, the rats received an injection of saline ($n = 13$) or cocaine ($n = 13$; 25 mg/kg i.p.). This dose and route of administration of cocaine was chosen based on the results of our previous behavioral studies [15]. In these intact rats, tail flick latency increased 10 min after the injection of cocaine. This effect was significant, compared to the response immediately before the injection, for up to 60 min (Fig. 7). Naloxone (1 mg/kg i.p., $n = 4$), administered 10 min after cocaine, had no effect on the response to cocaine (data not shown).

To determine if the antinociceptive effect of cocaine required an intact spinal cord, a group of rats ($n = 8$) underwent a complete thoracic spinal transection (T5–T8) 24–48 h before tail-flick testing. Because of the paralysis of voluntary movements below the transection, the effect of cocaine on the tail-flick reflex of these rats could be determined without the restraining effects of chloral hydrate. Cocaine (25 mg/kg, i.p.) was without effect on tail flick latency in these unanesthetized, spinally transected animals (Fig. 7).
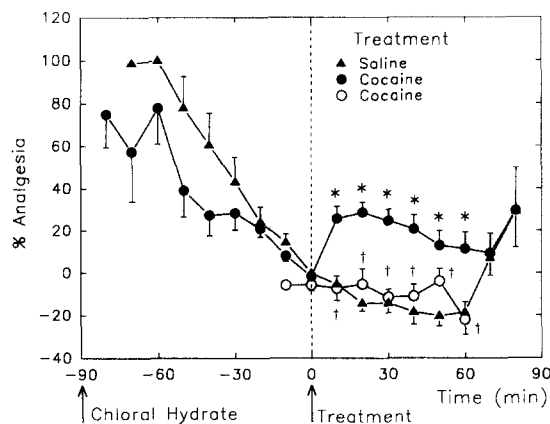

Fig. 7. Effect of cocaine on average (SEM)% analgesia, determined by tail flick latency, in intact and spinally-transected rats. Rats with intact spinal cords ($n = 26$; filled symbols) were anesthetized with chloral hydrate (275 mg/kg i.p.) and allowed to recover for 70–80 min while the tail flick latency was monitored at 10 min intervals. When the reflex latency reached the previously determined normal control level (4–4.5 s), rats received an injection ($t = 0$) of either saline (filled triangles; $n = 13$) or cocaine (filled circles; $n = 13$; 25 mg/kg i.p.) and tail flick latencies were measured for an additional 90 min. A third group of unanesthetized rats ($n = 8$; open circles) received a complete spinal transection 24–48 h before tail flick testing. Cocaine, but not saline, produced a significant increase in tail flick latency in the intact rats compared to the response immediately before injection (*; paired t-test, $P < 0.05$). The same dose of cocaine had no effect on the tail flick latency of the transected rats (†; unpaired t-test between the intact and spinalized groups treated with cocaine; $P < 0.05$).

4. Discussion

The results of these studies provide corroborative behavioral and neurophysiological evidence that cocaine: (1) has a significant antinociceptive effect on spinal nociceptive mechanisms in the rat, and that (2) this effect requires the integrity of supraspinal mechanisms.

Our neurophysiological studies show that cocaine suppresses selectively dorsal horn unit responses to nociceptive stimulation. The responses of dorsal horn WDR units to natural noxious stimulation (pinch), were attenuated in a dose-related manner by cocaine. This effect of cocaine occurred with a short onset and duration of action. In contrast, the same doses of cocaine were without effect on the responses of low threshold units to innocuous stimulation (tapping). It is unlikely that the inhibitory effect of cocaine on nociceptive unit responses resulted from an effect on somatosensory afferents, because Pertovaara et al. [20] reported no local anesthetic effect of systemically-administered cocaine (25 mg/kg) on an electrically evoked C-fiber reflex or on antidromic activation of C-fibers. This suggests that cocaine acts selectively on nociceptive spinal projection neurons or interneurons in the dorsal horn.

Impulses generated by noxious somatosensory stimulation are transmitted to the dorsal horn by small diameter myelinated (Aδ) and unmyelinated (C) afferent fibers [4]. Synchronous activation of somatosensory nociceptors by high intensity electrical stimulation produced a biphasic discharge of dorsal horn units, attributable to Aδ and C afferent input. In these experiments, cocaine suppressed the long latency unit discharge with little effect on the short latency evoked response in each of the 7 units studied, suggesting that cocaine selectively supresses the responses to C-fiber input. Using a similar experimental approach, Pertovaara and Tukeva [23] reported that cocaine inhibited both early and late responses of dorsal horn units to high intensity electrical stimulation, but that this effect was observed in about half of the neurons studied. Differences in results between these studies could be due to differences in sampling or to differences in experimental methods employed, including dosage and route of administration of cocaine, time course of drug effects, and stimulation parameters. Nevertheless, it is apparent from both studies that cocaine has an inhibitory effect on dorsal horn responses to input from C afferent fibers.

High intensity electrical stimulation is a relatively non-selective stimulus that activates a variety of afferent fibers in the receptive field, including low threshold as well as nociceptive afferents. Devor et al. [6] reported that radiant pulses from a CO_2 laser produce

synchronous activation of thermal afferent fibers ($A\delta$ and C) without activation of low threshold mechanoreceptors. They found that units in the dorsal horn that responded to laser stimulation were located in laminae I, II, V and VI. In our study, units in the superficial laminae of the dorsal horn were found to be driven by high intensity CO_2 laser pulses. Laser stimulation of receptive fields on the rat tail produced a biphasic burst of unit discharge, corresponding to activitation of $A\delta$ and C afferents. Both early and late unit responses to laser stimulation were inhibited by cocaine, whereas only the late responses of the same units to high intensity electrical stimulation were suppressed by the drug. This apparent discrepancy could reflect differences in the natural response properties of nociceptors activated by laser versus electrical stimulation or differences in the central temporal summation produced by these different forms of stimulation. Latencies of laser evoked unit discharge were consistently longer for both the early and late responses, reflecting the additional time required for nociceptor activation by the laser stimulus. It is possible that cocaine inhibits both $A\delta$ and C fiber input to the dorsal horn but is more effective when the afferent barrage is less synchronous, as is the case with laser stimulation. There was no evidence that either electrical or laser stimulation on the tail activated $A\beta$ afferents, so it appears that activity in large diameter afferent fibers does not account for the difference in the effect of cocaine on early unit discharge evoked by laser, as compared to electrical, stimulation.

In previous studies, we have reported that cocaine produces a dose-related behavioral antinociceptive effect in the rat hot plate and formalin tests [15]. Both spiperone and eticlopride reversed the behavioral antinociceptive action of cocaine, indicating the involvement of both D1 and D2 dopaminergic mechanisms. Initial attempts to evaluate the possible spinal actions of cocaine using the tail flick test were hindered by cocaine's psychomotor stimulant activity, which resulted in spontaneous tail twitching during the testing procedure in awake rats. This difficulty was circumvented by conducting the tail flick procedure in lightly anesthetized rats [18]. Cocaine prolonged tail flick latency at a dose previously shown to be antinociceptive in the hot plate and formalin tests [15]. The latency to onset and time course for the antinociceptive activity of cocaine in the tail flick test were comparable to those in the hot plate and formalin tests [15]. Since the tail flick reflex is spinally mediated, this finding suggests that the antinociceptive effect of cocaine has a significant spinal component.

Comparison of the time course of cocaine antinociception in the tail flick test and in neurophysiological studies reveals a prolonged action of the drug in the behavioral experiments. This difference in time course probably reflects differences in the dose and route of administration used in these studies. A maximum dose of 3 mg/kg was given intravenously in the unit recording experiments whereas the dose of 25 mg/kg was injected intraperitoneally in the tail flick test.

Inhibition of the tail flick reflex and spinal dorsal horn neuronal response to noxious stimulation could result from a direct postsynaptic action of cocaine on neurons within the dorsal horn or from the activation or potentiation of inhibitory descending projections to the dorsal horn. If cocaine acts directly on intrinsic dorsal horn neurons, then interuption of the descending processes would not be expected to interfere with the antinociceptive activity of cocaine. We examined the effect of upper thoracic spinal transection on antinociceptive effects of cocaine in the tail flick test and in dorsal horn recording experiments. Cocaine was without effect on tail flick response latency or on the response of dorsal horn units to noxious pinch in rats with a complete thoracic spinal transection. These findings rule out the possibility that cocaine has a direct antinociceptive action on neurons within the dorsal horn and suggests that the drug causes activation or potentiation of descending inhibitory projections to the dorsal horn.

The precise neural pathways and mediators of the antinociceptive action of cocaine remain unclear. Cocaine inhibits the reuptake of norepinephrine, dopamine and serotonin [10,11], each of which has been implicated in neuronal mechanisms of pain modulation [8,17,24]. It appears that noradrenergic mechanisms may not be involved in the antinociceptive action of cocaine, since atipamezole, an α_2-adrenoceptor antagonist, failed to reverse the behavioral antinociceptive effect of cocaine in the formalin test [21]. The finding that eticlopride antagonized the antinociceptive action of cocaine in behavioral experiments [15] and in the neurophysiological studies reported here suggests that a dopamine D2 mechanism is involved. The precise location of the dopaminergic pathways involved cannot be ascertained from these experiments. However, the major dopaminergic input to the spinal cord originates from cells in the A11 dopamine cell group and terminates mainly in the dorsal horn and the intermediolateral column, suggesting a role for these descending dopaminergic projections in pain and autonomic regulation [3,26]. Fleetwood-Walker et al. [9] demonstrated that stimulation in the A11 region inhibited nociceptive responses of units in the dorsal horn and that the inhibitory effect was antagonized by sulpiride, a D2 receptor antagonist. It is possible that the antinociceptive action of cocaine is mediated at least in part by the activation or potentiation of this descending A11 dopamine projection, presumably by a presynaptic action within the dorsal horn. Alternatively or additionally, cocaine could activate other supraspinal dopamin-

ergic systems that modulate descending inhibitory pathways to the dorsal horn.

5. Acknowledgements

6. References

[1] Belczynski, C.R.J., Pertovaara, A., Morrow, T.J. and Casey, K.L., The effect of systemic cocaine on spontaneous and nociceptively evoked activity of neurons in the medial and lateral thalamus, *Brain Res.*, 517 (1990) 344–346.

[2] Belczynski, C.R.J., Pertovaara, A., Morrow, T.J. and Casey, K.L., The effect of systemic cocaine on the responses to noxious stimuli and spontaneous activity of medial bulboreticular neurons, *Brain Res.*, 527 (1990) 204–212.

[3] Björklund, A. and Skagerberg, G., Evidence for a major spinal cord projection from the diencephalic A11 dopamine cell group in the rat using transmitter-specific fluorescent retrograde tracing, *Brain Res.*, 177 (1979) 170–175.

[4] Collins, W.F., Nulsen, F.E. and Randt, C.T., Relation of peripheral nerve fiber size and sensation in man, *Arch. Neurol.*, 3 (1960) 381–385.

[5] D'Amour, F.E. and Smith, D.L., A method for determining loss of pain sensation, *J. Pharmacol. Exp. Ther.*, 72 (1941) 74–79.

[6] Devor, M., Carmon, A. and Frostig, R., Primary afferent and spinal sensory neurons that respond to brief pulses of intense infrared laser radiation: a preliminary survey in rats, *Exp. Neurol.*, 76 (1982) 483–494.

[7] Fields, H.L. and Anderson, S.D., Evidence that raphe-spinal neurons mediate opiate and midbrain stimulation-produced analgesia, *Pain*, 349 (1978) 333–349.

[8] Fitzgerald, M., Monoamines and descending control of nociception, *Trends Neurosci.*, 9 (1986) 51–52.

[9] Fleetwood-Walker, S.M., Hope, P.J. and Mitchell, R., Antinociceptive actions of descending dopaminergic tracts on cat and rat dorsal horn somatosensory neurones, *J. Physiol.*, 399 (1988) 335–348.

[10] Friedman, E., Gershon, S. and Rotrosen, J., Effects of acute cocaine treatment on the turnover of 5-hydroxytryptamine in the rat brain, *Br. J. Pharmacol.*, 54 (1975) 61–64.

[11] Hanson, G.R., Matsuda, L.A. and Gibb, J.W., Effects of cocaine on methamphetamine-induced neurochemical changes: charac-

terization of cocaine as a monoamine uptake blocker, *J. Pharmacol. Exp. Ther.*, 242 (1987) 507–513. (Abstract).

[12] Hodge, C.J., Apkarian, A.V., Stevens, R., Vogelsang, G. and Wisnicki, H.J., Locus coeruleus modulation of dorsal horn unit responses to cutaneous stimulation, *Brain Res.*, 204 (1981) 415–420.

[13] Janss, A.J., Jones, S.L. and Gebhart, G.F., Effect of spinal norepinephrine depletion on descending inhibition of the tail-flick reflex from the locus coeruleus and lateral reticular nucleus in the rat, *Brain Res.*, 400 (1987) 40–52.

[14] Jones, S.L. and Gebhart, G.F., Characterization of coeruleospinal inhibition of the nociceptive tail-flick reflex in the rat: mediation by spinal alpha 2-adrenoceptors, *Brain Res.*, 364 (1986) 315–330.

[15] Lin, Y., Morrow, T.J., Kiritsy-Roy, J.A., Terry, L.C. and Casey, K.L., Cocaine: evidence for supraspinal, dopamine-mediated, non-opiate analgesia, *Brain Res.*, 479 (1989) 306–312.

[16] Margalit and Segal, M., Pharmacologic study of analgesia produced by stimulation of the nucleus locus coeruleus, *Psychopharmacology*, 62 (1979) 169–173.

[17] Mokha, S.S., McMillan, J.A. and Iggo, A., Descending control of spinal nociceptive transmission. Actions produced on spinal multireceptive neurones from the nuclei locus coeruleus and raphe magnus, *Exp. Brain Res.*, 58 (1985) 213–226.

[18] Ness, T.J. and Gebhart, G.F., Centrifugal modulation of the rat tail flick reflex evoked by graded noxious heating of the tail, *Brain Res.*, 386 (1986) 41–52.

[19] Oleson, T.D., Twombly, D.A. and Liebeskind, J.C., Effects of pain attenuating brain stimulation and morphine on electrical activity in the raphe nuclei of the awake rat, *Pain*, 4 (1978) 211–230.

[20] Pertovaara, A., Belczynski, C.R., Morrow, T.J. and Casey, K.L., The effect of systemic cocaine on spinal nociceptive reflex activity in the rat, *Brain Res.*, 438 (1988) 286–290.

[21] Pertovaara, A., Kauppila, T. and Tukeva, T., The effect of medetomidine, an a_2-adrenoceptor agonist, in various pain tests, *Eur. J. Pharmacol.*, 179 (1990) 323–328.

[22] Pertovaara, A., Mecke, E. and Carlson, S., Attempted reversal of cocaine-induced antinociceptive effects with naloxone, and opioid antagonist, *Eur. J. Pharmacol.*, 192 (1991) 349–353.

[23] Pertovaara, A. and Tukeva, T., Cocaine: effect on spinal projection neurons in the rat, *Brain Res. Bull.*, 25 (1990) 1–6.

[24] Segal, M. and Sandberg, D., Analgesia produced by electrical stimulation of catecholamine nuclei in the rat brain. *Brain Res.*, 123 (1977) 369–372.

[25] Shyu, B.C., Kiritsy-Roy, J.A., Morrow, T.J. and Casey, K.L., Neurophysiological, pharmacological and behavioral evidence for medial thalamic mediation of cocaine-induced dopaminergic analgesia, *Brain Res.*, 572 (1992) 216–223.

[26] Skagerberg, G. and Lindvall, O., Organization of diencephalic dopamine neurones projecting to the spinal cord in the rat, *Brain Res.*, 342 (1985) 340–351.

[27] Yang, J.C., Clark, W.C., Dooley, J.C. and Mignogna, F.V., Effect of intranasal cocaine on experimental pain in man, *Anesth. Analg.*, 61 (1982) 358–361.