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# Does intravenous administration of $GABA_A$ receptor antagonists induce both descending antinociception and touch-evoked allodynia?

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**Abstract**

Effects of intravenous administration of picrotoxin (PTX), a $GABA_A$ receptor antagonist, upon activities of wide dynamic range (WDR) neurons in the lumbar spinal cord were studied in urethane–chloralose anesthetized cats. Intravenous PTX augmented tactile evoked responses of WDR neurons, but reduced nociceptive responses dose-dependently. Spinal transection reversed the suppression of nociceptive responses. In the spinal cat, intravenous PTX enhanced the tactile evoked response. Intravenous PTX enhanced the spontaneous firing of nucleus raphe dorsalis (NRD) and/or ventral periaqueductal gray (PAG) neurons projecting to nucleus raphe magnus. Lidocaine injected into NRD/PAG reversed the antinociceptive action of intravenous PTX. PTX injected into NRD/PAG reduced heat-evoked responses of WDR units. These data suggest that antinociceptive effects of intravenous PTX is primarily due to disinhibitory activation of the descending antinociceptive system originating from NRD and PAG, and that PTX reinforces touch-evoked responses in the spinal cord. 

*Keywords:* $GABA_A$ receptor antagonist; Antinociception; Spinal cord; Wide dynamic range neuron; Periaqueductal gray; Nucleus raphe dorsalis; Allodynia

## 1. Introduction

After an intravenous administration of a subconvulsive dose (0.5 mg/kg) of picrotoxin (PTX), a $GABA_A$ receptor antagonist, the low-threshold mechanoreceptive field of wide dynamic range (WDR) neurons both in the trigeminal subnucleus caudalis and in the spinal dorsal horn expands and the high-threshold surround of receptive field disappears (Yokota and Nishikawa, 1979; Yokota and Nishikawa, 1982). Concomitantly responses of WDR neurons to noxious heat stimulation is abolished (Yokota and Nishikawa, 1982). These results were seemingly inconsistent with the finding that topically applied GAGA receptor agonists inhibit synaptic transmission onto nociceptive neurons in the spinal dorsal horn neurons (Ling et al., 1996). However, GABAergic supraspinal neurons suppress activities of the descending antinociceptive system (Fields et al., 1983, 1988, 1991; Fields and Heinricher, 1985; Moreau and Fields, 1986; Lovick, 1987; Drower and Hammond, 1988; Mason and Fields, 1989; Heinricher et al., 1991; Heinricher and Kaplan, 1991; Heinricher and Tortorici, 1994). Thus, $GABA_A$ receptor antagonists can suppress nociceptive transmission in the spinal cord through supraspinal mechanism, and can enhance nociception through direct spinal action. The question is how supraspinal and spinal mechanisms compete with each other, when administered systemically. The present study was undertaken to clarify this problem.

## 2. Materials and methods

### 2.1. Animals

Experiments were carried out in 44 adult cats. All procedures were consistent with the guidelines of the Animal

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Care and Use Committee of our university and of the Japanese Physiological Society. Anesthesia was induced with ketamine HCl (20 mg/kg; i.m.), and maintained with an intravenous administration of 3.5 ml/kg of a solution of urethane and chloralose (urethane 125 mg/ml, chloralose 10 mg/ml). This was supplemented as required. Blood pressure was continuously monitored via a catheter implanted into the right femoral artery. During single unit recording, animals were paralyzed with pancuronium bromide, and artificially ventilated. End-tidal $CO_2$ was maintained between 3.5% and 4.5% and esophageal temperature was maintained between 37°C and 38°C by a servo-controlled heating pad under the abdomen and by an infrared lamp.

### 2.2. Surgery

With the exception of experiments recording from nucleus raphe dorsalis (NRD) or its adjacent periaqueductal gray (PAG), a laminectomy exposed the lumbar enlargement, which was then covered with a pool of warm mineral oil. Another laminectomy gave an access to either the upper cervical cord or the lower thoracic cord for a transection. In 25 experiments, a craniotomy allowed placement of an injection cannula or a recording microelectrode into PAG/NRD. In 10 experiments, a concentric stimulating electrode was also inserted into nucleus raphe magnus (NRM) of the rostral medulla oblongata so that medullary projecting PAG/NRD output neurons could be identified by antidromic activation. The superficial peroneal (SP) and posterior tibial (PT) nerves were prepared for bipolar electrical stimulation by freeing them from surrounding connective tissue for about 1.5 cm; both were left in continuity with the skin so that receptive fields could be mapped.

### 2.3. Electrophysiological recording

Glass capillary microelectrodes filled with 2% Pontamine sky blue dye in 0.5 M sodium acetate were used to record extracellular single unit activities. During the search for dorsal horn neurons, electrical stimuli at A-fiber strengths (up to 3 V, 0.1 ms duration) were applied to the SP and PT nerves. After the background activity of each dorsal horn neuron was determined, responses to mechanical stimulation of the skin were recorded. Stimuli included gentle brushing of the skin with a soft hair brush (brush), pressure applied to a fold of skin using a pair of broad-tipped forceps (pressure), and pinching with a pair of fine rat-toothed forceps (noxious pinch). Neurons recorded were classified as wide dynamic range (WDR), nociceptive-specific (NS) or low-threshold mechanoreceptive (LTM) neurons. WDR neurons were subsequently tested for responsiveness to C-fiber activation (up to 30 V, 0.5 ms duration) of the SP and/or PT nerve. WDR units with C-fiber input were then tested for responsiveness to noxious heating of the cutaneous receptive field, and subjected to the study of intravenous administration of PTX. Radiant heat stimulation was applied to the center of cutaneous receptive field defined by mechanical stimulation. Precisely controlled skin temperatures were obtained by a servo-controlled thermostimulator (DIA Medical); radiant heating from a quartz halogen lamp was focused with a condenser lens onto the skin. The current to the lamp was controlled by feedback from a thermistor in contact with the skin. The duration of heat stimuli was preset at 20 s. The skin temperature was adapted for 3 min to 35°C before each heat stimulation.

For recording analysis data of tactile responses, gentle stroking across the peripheral receptive field was applied every half second synchronously with auditory signals. The output of the oscilloscope on which the responses of single units were displayed was connected to a window discriminator. The window discriminator output was itself connected to a spike counter. The output from the spike counter consisted of number of spikes occurring in each sequential 1-s bin during a period of background, and both during and after mechanical or heat stimulation of the cutaneous receptive field. The accumulated number of spike discharges during the responses to stimulation after subtraction of the background activity was used as a test of effects studied.

### 2.4. Mapping the receptive fields

The high-threshold surround of receptive field of each WDR unit was mapped with a pair of fine serrated forceps. Moving from outside loci to within the receptive field, we marked with a felt pen the first point at which pinching the skin caused discharges of the unit. The procedure was repeated until a clear outline of receptive field was obtained. The low-threshold center of receptive field was mapped in an analogous fashion, except that a fine soft hair brush was used to stimulate the skin. Both the high-threshold surround and the low-threshold center were transferred on a transparent polyethylene sheet. The sizes of both areas were measured with a planimeter.

### 2.5. Intracerebral injection

In 10 experiments, lidocaine (20 mg/ml) or PTX (3 mg/ml) was delivered into the PAG/NRD in a volume of 5 $\mu$l using a 31-gauge cannula having a beveled tip. This was connected to a 10-$\mu$l Hamilton syringe via a length of polyethylene tubing. The injection cannula, which extended 3 mm beyond the end of the guide cannula, was inserted into the PAG/NRD just prior to the injection and was left in place for a minimum of 5 min after the injection was completed. Progress of the injection was monitored by observing the movement of a small air bubble through the tubing. Only one injection was made per animal except for four experiments in which saline and then lidocaine were injected.

### 2.6. Histological verification

The locations of the units studied were marked by extruding a small amount of Pontamine sky blue from the microelectrode electrophoretically (5 μA DC current, passed for 10 min). At the conclusion of each experiment, the stimulation sites in the brainstem, if available, were lesioned electrolytically, by passing a current of 100 μA DC between the poles of the stimulating electrode for 1 min. Animals were then deeply anesthetized, and perfused through the beating heart with 1 l of a solution of 0.5% potassium ferrocyanide in normal saline, followed by 2 l of 10% formalin. Serial frozen sections (50 μm thick) were cut, stained with Cresyl violet, and the locations of both the stimulation and recording sites checked. The injection sites were also histologically verified on Cresyl violet-stained sections, and plotted on standardized sections.

### 2.7. Statistical analysis

Data are expressed throughout as means ± SE. Statistical significance was tested with *t*-test. In time-course analysis of inhibition after drug administration, paired *t*-test and one-way repeated measures ANOVA were used. *P*-Values $<0.05$ were considered statistically significant.

## 3. Results

Extracellular single unit recordings were made from 32 wide dynamic range (WDR) neurons in the L7 spinal cord. They were located in laminae IV–VI, mostly in lamina V (Expts. 1–3, 5–6) (Rexed, 1952). In addition, nine medullary projecting neurons were recorded in the PAG/NRD (Expt. 4) (Fig. 1).

### 3.1. Experiment 1: antinociceptive action of intravenous PTX on heat-evoked responses of WDR units

Response to noxious heat stimulation applied to the center of cutaneous receptive field was used as an index of nociceptive responsiveness. Peristimulus time histograms of responses of a WDR neuron to noxious heat stimulation are shown in Fig. 2A. Spike discharges increased when the skin temperature of the center of receptive field was raised from 35°C to 48°C. Following an intravenous administration of PTX (0.5 mg/kg), the heat-evoked responses were suppressed tested 20 min after the injection, and resumed after a spinal transection at the level of C3 segment. Mean numbers ± SE of heat-evoked responses in eight units are shown in Fig. 2B. Prior to the PTX administration, the number of spikes/20 s in heat-evoked response was 531.5 ± 113.4. Intravenous PTX significantly reduced the response to 9.6 ± 14.1 ($P < 0.01$, paired *t*-test). After the spinal transection, the heat-evoked response recovered to the control level. Mean number of spikes in heat responses tested 20 min after the transection was 523.5 ± 120.1. Slowly injected PTX did not cause any significant change in blood pressure. Following the spinal transection, the blood pressure temporarily increased and then returned to the control level in about 10 min (Fig. 2C).

In order to determine an adequate dose of intravenous PTX, cumulative effects of intravenous PTX on heat-evoked responses were studied in five WDR neurons (Fig. 3). Ten minutes after the initial administration of PTX (0.15 mg/kg), responses were reduced to 61.2 ± 12.0% of control responses as measured prior to the administration. Thereafter, the responses were maintained around this level. Sixty minutes after the first injection, another 0.15 mg/kg PTX was administered. Ten minutes after the second injection, heat-evoked responses were 44.7 ± 11.8% of the control responses and were maintained around this level thereafter. Sixty minutes after the second injection, 0.2 mg/kg PTX was administered. Ten minutes after the third injection, heat-evoked responses were 4.3 ± 1.3% of the control responses, and were maintained around this level thereafter. A cumulative dose of 0.5 mg/kg PTX was sufficient to almost completely suppress heat-evoked responses.

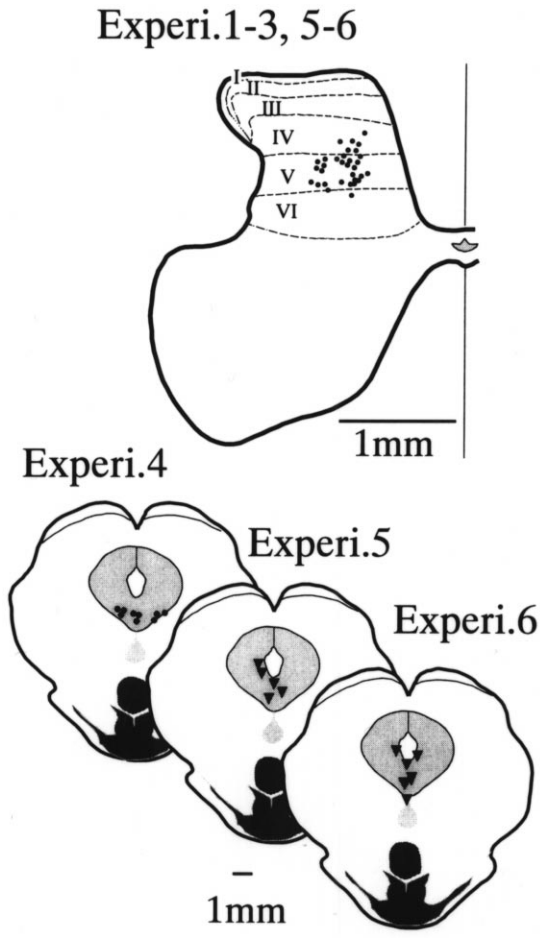


Fig. 1. Locations of recording sites and injection sites. circles: recording sites; triangles: injection sites.

### *3.2. Experiment 2: effects of intravenous PTX on responses of WDR units by mechanical stimulation*

WDR neurons receive not only noxious but also innocuous inputs (Hillman and Wall, 1969). Fig. 4A shows the receptive field of a WDR unit, and Fig. 4B the responses of the unit to mechanical stimulations applied to the center of receptive field. Cutaneous receptive fields of WDR neurons are relatively large, and are divided into three zones according to their responsiveness to mechanical stimulation. Zone 1 is a low-threshold mechanoreceptive center (black area in Fig. 4A), in which neurons have a graded responses to brush, pressure and noxious pinch with increasingly higher frequencies of impulse discharges (Fig. 4B). Zone 2 (cross-hatched area in Fig. 4A) surrounds the low-threshold center, and in this zone pressure and noxious pinch differentially excite the neurons. Zone 3 is a high-threshold surround (shaded area in Fig. 4A), in which only noxious pinch is excitatory.

Effects of intravenous PTX (0.5 mg/kg) and subsequent spinal transection on responses of WDR units to mechanical stimuli were examined (Fig. 4B). The transection was carried out 30 min after the administration at the level of the third cervical cord.

To evaluate effects on the responses to noxious inputs, the total size of cutaneous receptive field was determined by noxious pinch, and the number of spikes evoked by noxious pinch to the center of receptive field was counted. The receptive field shrunk following intravenous PTX, and recovered after subsequent spinal transection (Fig. 5A, right). The size prior to PTX was $8.1 \pm 1.5$ cm$^2$ ($n = 10$). After PTX, the high-threshold surround disappeared and the total receptive field reduced to $1.6 \pm 0.6$ cm$^2$. After the spinal transection, the total receptive field recovered to $7.2 \pm 1.3$ cm$^2$. The difference between the predrug control and the size after PTX was statistically significant ($P < 0.0001$, paired $t$-test), but there was no significant difference between the predrug control and the size after the transection ($P > 0.05$). There were similar changes in number of spikes evoked by noxious pinch (Fig. 5B, right). Spike-counts/10 s prior to, after PTX and after the transection were $650.3 \pm 104.0$, $116.5 \pm 15.9$ and $610.3 \pm 97.6$, respectively. The difference between the predrug control and the count after PTX was statistically significant ($P < 0.0001$), but there was no significant difference between the predrug control and the count after the transection ($P > 0.05$).

In order to evaluate effects on the responses to tactile

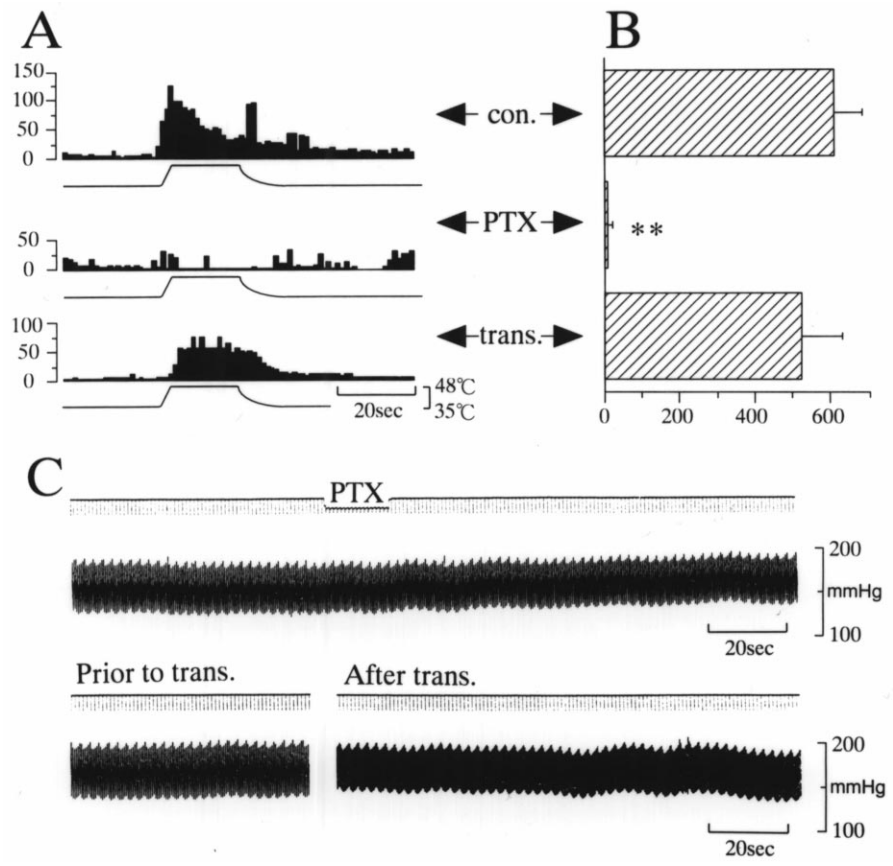


Fig. 2. Effects of intravenous PTX and of subsequent spinal transection on heat-evoked responses. (A) An example of a series of peristimulus histogram (1-s bins). Lower traces indicate local skin temperature changes due to heat irradiation. (B) Summary of effects of intravenous PTX and of subsequent spinal transection on heat-evoked responses ($n = 8$). Data are expressed as percent of the predrug controls. con., control; PTX, after intravenous administration of picrotoxin; trans, after spinal transection. $^{**}P < 0.01$. (C) blood pressure recording.

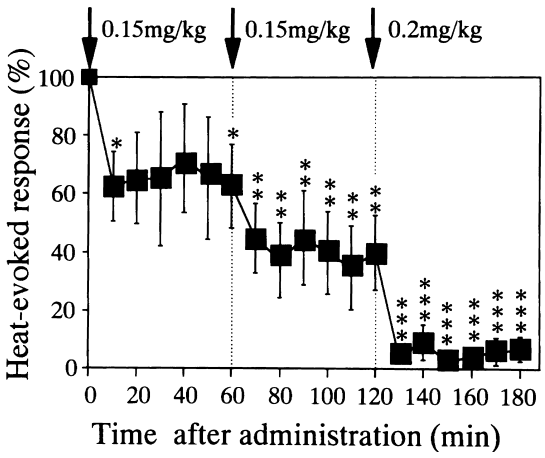


Fig. 3. Mean time course of cumulative effects of intravenous PTX on heat-evoked responses of WDR units. Data are expressed as percent of the predrug controls. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. The time of each injection is indicated by an arrow.

input, the size of low-threshold mechanoreceptive center, and number of spikes evoked by tactile stimulation to this center were measured. The center significantly expanded following intravenous PTX ($P < 0.001$), but did not change after subsequent spinal transection (Fig. 5A, left). The sizes prior to, after the administration and after the transection were $0.8 \pm 0.3$ cm$^2$, $1.6 \pm 0.3$ cm$^2$ and $1.5 \pm 0.6$ cm$^2$, respectively. The number of spikes evoked by brushing increased remarkably following intravenous PTX, and increased further after spinal transection (Fig. 5B, left). Spike counts/10 s prior to, after the administration and after the transection were $229.5 \pm 53.3$, $416.5 \pm 75.4$ and $521.0 \pm 99.8$, respectively. Both the difference between the predrug control and the count after the administration and the difference between the predrug control and the count after the transection were statistically significant ($P < 0.001$ and $P < 0.001$, respectively), but the difference in the count after the administration and the count after the transection was insignificant ($P > 0.05$).

### *3.3. Experiment 3: effects of intravenous PTX on responses of WDR neurons in spinal cats*

Effects of intravenous PTX (0.5 mg/kg) on responses of WDR units were tested in five spinal cats in which the spinal cord was transected at a level of the lower thoracic cord (Fig. 6).

The total size of cutaneous receptive field was $5.8 \pm 0.8$ cm$^2$, and the number of spikes evoked by noxious pinch was $609.2 \pm 215.0$/10 s prior to intravenous PTX. After intravenous PTX, the size of receptive field was $6.0 \pm 0.8$ cm$^2$, and the number of spikes was $595.0 \pm 250.7$ spikes/10 s (Fig. 6A,B, right). There were no significant changes after the administration both in the receptive field size and in the number of spikes ($P > 0.05$, paired $t$-test).

The size of low-threshold center was $1.0 \pm 0.6$ cm$^2$, and the response evoked by brushing the center was $201.0 \pm 65.2$ spikes/10 s prior to intravenous PTX. After intravenous PTX, both increased to $2.2 \pm 1.1$ cm$^2$, and $370.8 \pm 106.3$ spikes/10 s, respectively (Fig. 6A,B, left). The change following the administration in the size of low-threshold center

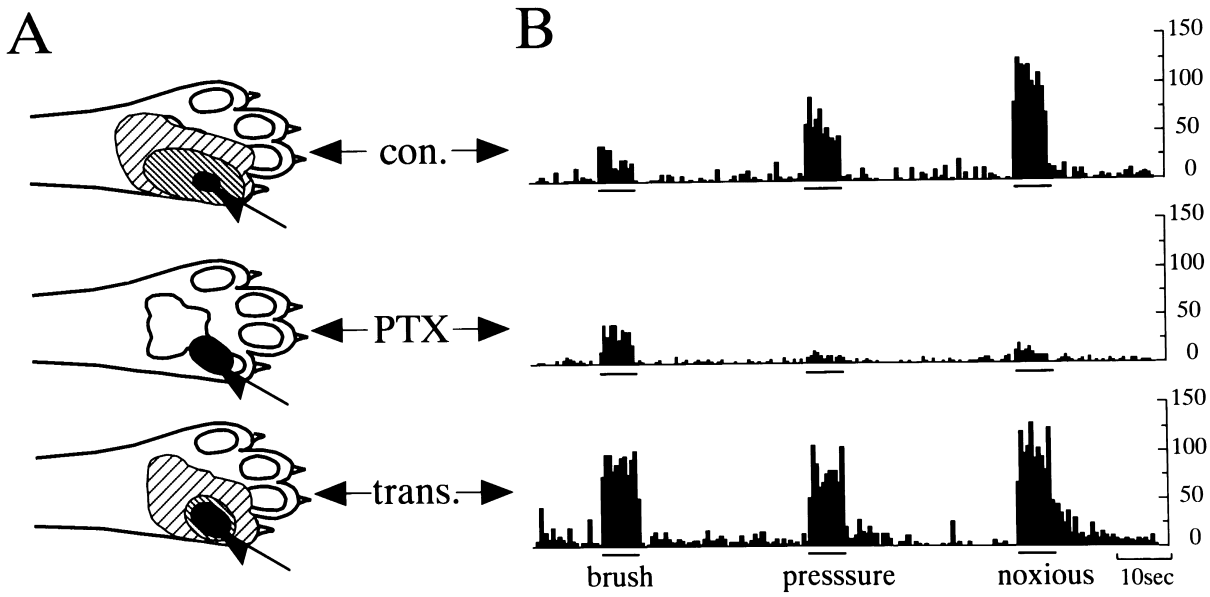


Fig. 4. An example of changes in receptive field organization and in mechanically evoked responses of a WDR unit after intravenous PTX administration and after subsequent spinal transaction. (A) Changes in receptive field. Black area: low-threshold center of receptive field. Cross-hatched area: the area where the unit differentially responded to pressure and pinch. Shaded area: high-threshold surround within which the unit responded only to noxious pinch. (B) Peristimulus histograms (1-s bins) of responses to mechanical stimulation of the center of receptive field (indicated by arrows in A). Bars below histograms show periods of stimulus application. After intravenous PTX (0.5 mg/kg), the high-threshold surround disappeared, and the low-threshold center expanded. Concomitantly, the unit maximally responded to brushing. After a high spinal transection, the high-threshold surround reappeared.

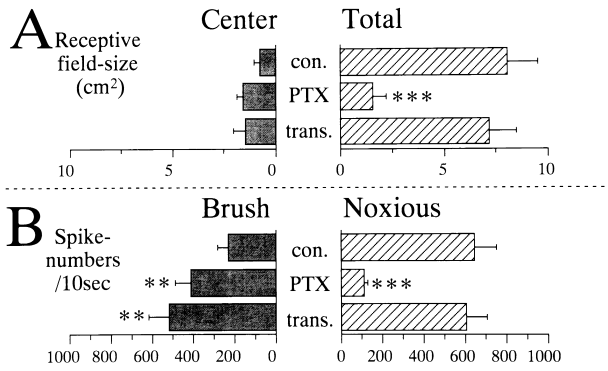


Fig. 5. Effects of intravenous PTX and of subsequent spinal transaction on non-nociceptive and nociceptive responses of WDR neurons ($n = 10$). (A) Changes in receptive field size. (B) Changes in spike numbers evoked by mechanical stimuli to the center of receptive field. $^{**}P < 0.01$, $^{***}P < 0.001$. con., control; PTX, after intravenous administration of picrotoxin; trans, after spinal transection.

was insignificant ($0.1 > P > 0.05$, paired *t*-test), but the increase in the number of spikes was significant ($P < 0.05$).

### *3.4. Experiment 4: responses of medullary projecting PAG/NRD neurons to intravenous picrotoxin*

The above-mentioned experiments suggest that effects of PTX on nociceptive transmission are caused by supraspinal mechanisms. The midbrain periaqueductal gray (PAG) may be one of candidates through which PTX causes descending antinociceptive control. Therefore, effects of intravenous PTX (0.3 mg/kg) on spike discharges of medullary projecting PAG/NRD neurons were studied. The neurons were identified by antidromic stimulation in or near nucleus raphe magnus (NRM). Fig. 7 illustrates an example. The latency of the antidromic responses was 3.2 ms. The unit followed a 100-Hz stimulation (Fig. 7A) and the antidromic spike was collided with spontaneous firing (Fig. 7B). This unit had a low level of spontaneous firing ($0.16 \pm 0.05$ spikes/s) prior to intravenous PTX. The mean number of spikes increased to $3.35 \pm 0.18$/s 10–11 min after PTX and to $10.31 \pm 0.61$/s 30–31 min after PTX (Fig. 7C). The mean time course of changes in spontaneous firing in nine units is plotted in Fig. 8. Spike discharges increased following intravenous PTX in all the units tested. The increase in spike discharges outlasted 60 min. ANOVA revealed that the increase in firing was statistically significant ($F_{8,13} = 2.527$, $P < 0.01$).

### *3.5. Experiment 5: reversal of antinociceptive action of PTX by lidocaine injected into PAG/NRD*

In five cats, 5 $\mu$l of 2% lidocaine was injected into the PAG/NRD 25 min after intravenous PTX (0.5 mg/kg), and changes in heat-evoked responses of spinal WDR units were studied (Fig. 9A). The injection reversed heat-evoked responses which had been suppressed after intravenous PTX. Prior to intravenous PTX, the heat-evoked response was $531.5 \pm 54.7$ spikes/20 s, and 20 min after PTX administration, $9.0 \pm 1.8$ spikes/20 s. The decrease was significant ($P < 0.01$, paired *t*-test). After the lidocaine injection, heat-evoked responses reversed to $523.5 \pm 84.9$ spikes/20 s. The difference in responses between prior to intravenous PTX and after the lidocaine injection was statistically insignificant ($P > 0.05$). Histologically verified midbrain injection sites are summarized in Fig. 1. They were located in the PAG/NRD.

In four cats, normal saline (5 $\mu$l) injected into PAG/NRD. The injection failed to reverse the antinociceptive effects of intravenous PTX (Fig. 9B). Twenty minutes after the saline injection, 5 ml of 20% lidocaine was injected the saline site. The lidocaine injection reversed heat-evoked responses. Prior to intravenous PTX, the heat-evoked response was $374.5 \pm 14.7$ spikes/ 20s, and 20 min after PTX administration, $6.3 \pm 5.3$ spikes/20 s. After the saline injection, the heat-evoked response was $17.7 \pm 5.7$ spikes/20 s. After the lidocaine injection, heat-evoked responses reversed to $235.7 \pm 32.2$ spikes/20 s.

### *3.6. Experiment 6: effects of PTX microinjected into PAG/NRD on the activity of WDR neurons of spinal cord*

In six cats, 5 $\mu$l of 0.15% PTX was injected into PAG/NRD, and effects of the PTX injection on responses of dorsal horn WDR units to heat stimulation were studied. Heat-evoked responses were completely abolished 10 min after an injection of PTX into the ventral PAG, and it partially recovered 60 min after the injection. Mean time-course of effects of PTX injection on heat-evoked responses is plotted in Fig. 10. Prior to the injection, the number of spikes evoked by heat stimulation of the center of receptive field was $499.3 \pm 107.6$. Ten to 30 min after the PTX injection, the number of spikes reduced to $81.7 \pm 9.2$. The mean percentage of response reduced to $20.4 \pm 7.9\%$ of the control response as measured prior to the injection. Fifty minutes after the injection, the number of spikes partially recovered to $180.8 \pm 60.2$. ANOVA revealed that the inhibition of heat-evoked responses was statistically significant ($F_{5,9} = 6.256$, $P < 0.001$). Histologically verified midbrain

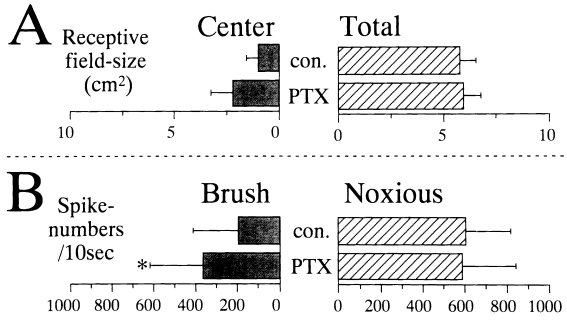


Fig. 6. Effects of intravenous PTX on non-nociceptive and nociceptive responses of WDR units recorded in spinal cats ($n = 5$). (A) Change of receptive field size. (B) Changes in spike numbers evoked by mechanical stimuli to the center of receptive field. $^{**}P < 0.01$, $^{***}P < 0.001$. con., control; PTX, after intravenous administration of picrotoxin.

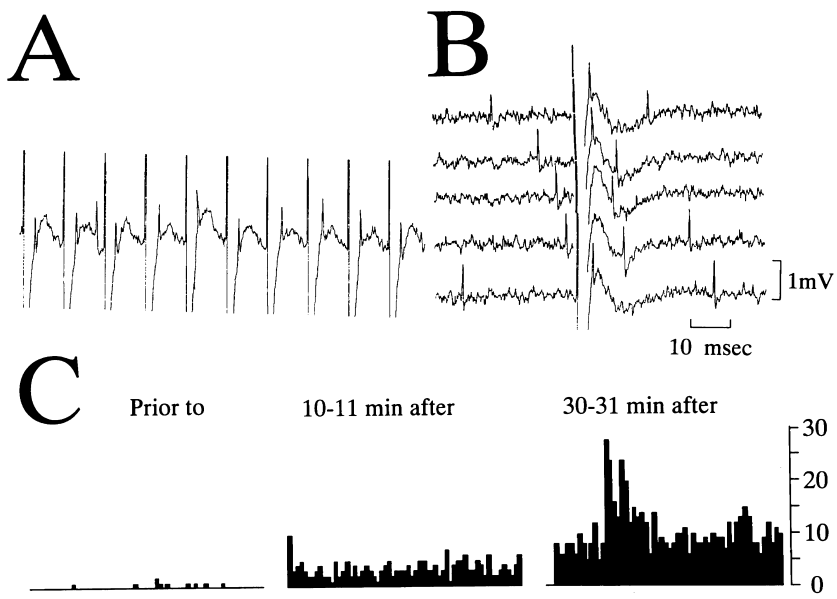


Fig. 7. Responses of a medullary projecting PAG unit to intravenous PTX. (A) Antidromic responses of the unit to NRM stimulation at 100 Hz. (B) Collision test with spontaneous firing. (C) Spike discharges of the unit.

injection sites are summarized in Fig. 1. They were located in the PAG/NRD.

In all six experiments, normal saline (5 $\mu$l) was injected into the ventral PAG. The injection failed to affect the heat-evoked responses.

## 4. Discussion

### *4.1. Effects of intravenous PTX on spinal nociceptive transmission*

In intact cats, intravenous PTX suppressed nociceptive transmission onto WDR neurons, i.e. both high-threshold surround of cutaneous receptive field and nociceptive responses were reduced after intravenous PTX. But intravenous PTX did not suppress nociceptive transmission in spinal cats. The antinociceptive action on spinal WDR neurons induced by intravenous PTX was reversed by a high spinal transection and also by an injection of lidocaine into PAG/NRD. Hence, the site of antinociceptive action of PTX appears to be supraspinal.

$GABA_A$ receptors present $Cl^-$ ionophores that serve to hyperpolarize the cell body by increasing $Cl^-$ conductance (Bowery, 1982; Borman et al., 1987). There are high levels of $GABA_A$ binding sites throughout the PAG (McCabe and Walmley, 1986; Bowery et al., 1987). GABA-Immunoreactive cell bodies were also observed throughout the ventrolateral PAG and in parts of the dorsolateral PAG in the rat (Reiching and Basbaum, 1990). The GABA-synthesizing enzyme glutamate decarboxylase is present in neuronal somata throughout the rostrocaudal extent of the rat, rabbit and opossum PAG (Barbaresi and Manfrini, 1988; Penny et al., 1990). A well-documented descending antinociceptive system is the one arising from the PAG/NRD and relayed through the rostral ventromedial medulla oblongata (RVM) (Basbaum and Fields, 1984; Willis, 1988). It has been reported that microinjection of bicuculline, a competitive $GABA_A$ receptor antagonist, into the PAG inhibits the spinally mediated, nocifensive tail flick reflex (TF) (Moreau and Fields, 1986; Roychowdhury and Fields, 1996), and abolishes responses of spinal dorsal horn neurons to noxious skin heating in the rat (Sandkühler et al., 1989). In the present study, we confirmed that injection of PTX into the PAG/NRD suppresses heat-evoked responses of cat spinal WDR neurons. Taken together, these studies provide convergent evidence that antinociceptive output neurons in the

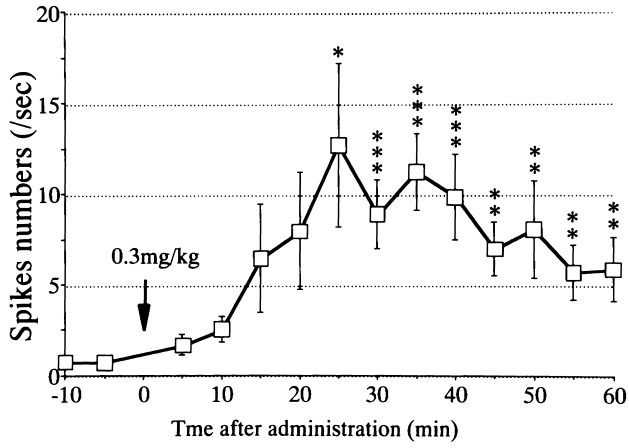


Fig. 8. Mean time course of changes in spike discharges of nine medullary projecting PAG/NRD units following intravenous administration of PTX (0.3 mg/kg). Data are expressed as percent of the predrug controls. $^{**}P < 0.01$, $^{***}P < 0.001$.

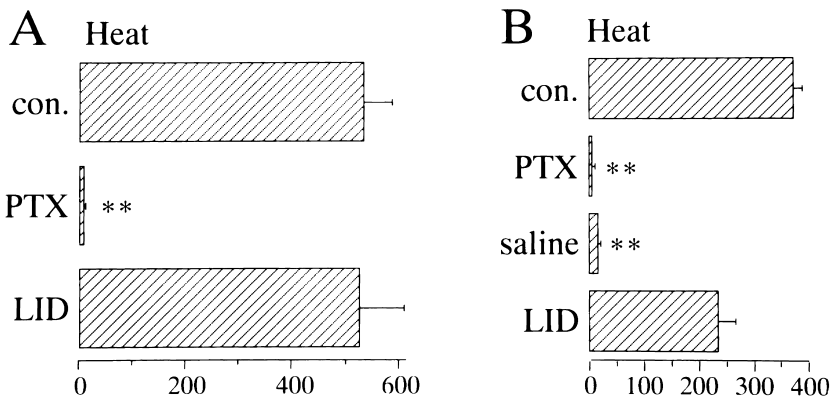


Fig. 9. Effect of saline and lidocaine injection into PAG on intravenous PTX antinociception. $^{**}P < 0.01$. con., before intravenous administration of picrotoxin; PTX, after intravenous administration of picrotoxin; LID, after injection of lidocaine.

PAG/NRD are under a tonic inhibitory control mediated by $GABA_A$ receptors. The observation in the present study that medullary projecting PAG/NRD output neurons were activated by systemic PTX, adds further weight to this inference.

The anatomical link between the PAG and the rostral ventral medulla (RVM) is well known (Abols and Basbaum, 1981; Van Bockstaele et al., 1991). RVM includes nucleus raphe magnus and its adjacent ventral reticular formation. In the cat the adjacent reticular area is roughly coextensive with nucleus raphe magnocellularis (Fields et al., 1991). There are three different classes of neurons in the rat RVM in terms of their physiology and pharmacology (Fields et al., 1983, 1991; Fields and Heinricher, 1985). Neurons of the first class are 'off-cells', which pause their firing prior to execution of nocifensor reflexes such as the tail flick reflex (TF). Neurons of the second class are 'on-cells', which increase their firing prior to the TF. Neurons of the third one are 'neutral cells', which remain unchanged during the TF. Off-cells are proposed to be antinociceptive, while on-cell pro-nociceptive. Bicuculline microinjected into the PAG inhibits the TF and the firing of on-cells, but increases the firing of off-cell (Moreau and Fields, 1986; Pan and Fields, 1996; Roychowdhury and Fields, 1996). Concomitantly the TF-related deceleration of off-cells and acceleration of on-cells in the RVM were reduced. It has been concluded that a GABAergic synapse inhibits neurons in the PAG which modulate nociceptive transmission at the spinal level through actions on neurons in the RVM. Consistent with this conclusion, we found in the present study that intravenous PTX also activates the descending antinociceptive system arising from the PAG/NRD through $GABA_A$ antagonism. Thus, our findings extend previous observations implicating GABA-mediated inhibitory processes in the control of nociceptive modulatory circuitry within PAG/NRD. Morphological demonstration of GABAergic interneurons in the PAG/NRD further supports this interpretation (Wang and Nakai, 1994a,b).

We conclude the antinociceptive effects of intravenous PTX is mainly due to disinhibitory activation of the descending antinociceptive system originating from NRD/PAG, but we cannot exclude the possibility that disinhibitory activation due to PTX also occurs at the RVM and/or at the level of spinal cord. For instance, following local bicuculline microinjection, off-cells enter a prolonged period of continuous firing in the rat (Heinricher and Tortorici, 1994), and GABA-immunoreactive terminals were found presynaptic to enkephalin-immunoreactive cell bodies and dendrites in the cat spinal cord (Liu et al., 1992). There are reports that $GABA_A$ receptor antagonists block the PAG-induced descending inhibition of dorsal horn neuron activ-

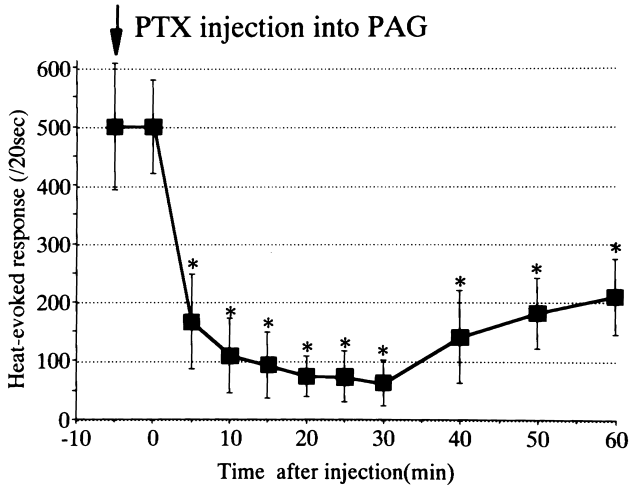


Fig. 10. Mean time course of effects of PTX injection into the PAG/NRD on heat-evoked responses of six WDR units. $^{*}P < 0.05$.

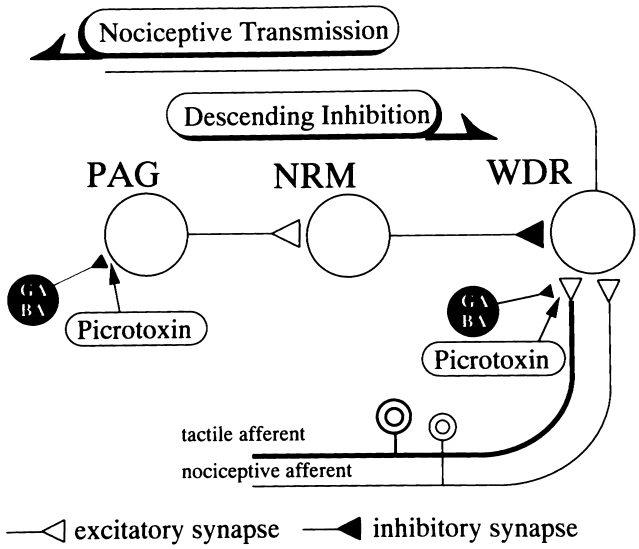


Fig. 11. A schematic diagram showing sites of disinhibitory action of intravenous PTX as revealed in the present experiments.

ity, i.e. they are pronociceptive when directly applied to the spinal cord (Willis, 1988; Peng et al., 1996). Thus the present data suggest that when systemically applied, antinociceptive action of PTX exceeds its direct spinal pronociceptive action.

### *4.2. Effects of intravenous PTX on spinal non-nociceptive transmission*

In the present study, intravenous PTX enhanced spinal non-nociceptive transmission onto WDR neurons in both intact and spinal cats, i.e. the low-threshold center of cutaneous receptive field of spinal WDR neurons expanded and tactile evoked responses were augmented after intravenous PTX. Since these changes after PTX were not reversed by a high spinal transection, the effects were primarily mediated by blockade of GABA receptors in the spinal cord. It has been shown that in the spinal cat that low-threshold mechanoreceptive afferents are excitatory only over a small central area of the receptive field, and are inhibitory over the entire receptive field (Hillman and Wall, 1969). Thus systemic PTX possibly removed the inhibitory action. In contrast, responses of WDR neurons to noxious stimuli were unaffected by systemic PTX in the spinal cat. This implies that PTX removed inhibitory effects of GABA on large afferent inputs exclusively. GABA and glycine are major spinal inhibitory transmitters (Sivilotti and Woolf, 1994). Both are present at high levels in substantia gelatinosa. GABA-containing terminals are frequently presynaptic to the low-threshold myelinated primary afferent terminal complex and form reciprocal synapse (Carlton et al., 1992). This is consistent with our result that intravenous PTX augmented non-nociceptive evoked response exclusively. Consequently, WDR neurons begun to respond to tactile stimuli with high frequency discharges comparable to those evoked by noxious stimuli normally. Whether A$\beta$-fiber input induces pain sensation may be decided by excitation of nociceptive neurons in the cerebral cortex, and pain sensation may occur when tactile stimuli generate a similar number of impulses which noxious stimuli produce normally. Thus phenomenon, similar to allodynia, was generated by intravenous PTX.

Hwang and Yaksh (1997) reported that $GABA_A$ receptor agonists resulted in a dose-dependent antagonism of allodynia by measuring pain-related behavior of neuropathic model rats. Sivilotti and Woolf (1994) demonstrated intrathecal $GABA_A$ receptor antagonists produced facilitation of flexor motoneuron responses to sural nerve stimulation at A$\beta$ strength in neuropathic model rats. The present data are consistent with these previous findings.

## 5. Conclusion

Intravenous PTX activates the descending antinociceptive system through disinhibition at the level of PAG, and augments non-nociceptive evoked responses by removing the effects of GABA which presynaptically inhibits large primary afferent terminals. Sites of disinhibitory action of intravenous PTX in the brainstem and in the spinal cord as revealed in the present study are summarized in Fig. 11.

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