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Buprenorphine Activates μ and Opioid Receptor Like-1 Receptors Simultaneously but Analgesic Effect is Mainly Mediated by μ Receptor Activation in Rat Formalin Test

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ABSTRACT

Buprenorphine is a mixed opioid receptor agonist-antagonist. Recently, buprenorphine has been reported to act as an agonist to opioid receptor like-1 (ORL1) receptor. In the present study, we examined the role of spinal and supraspinal μ receptors and spinal and supraspinal ORL1 receptors in producing an analgesic effect by intrathecally (IT), intracerebroventricularly (ICV) or intraperitoneally (IP) administering buprenorphine in the rat formalin test. Male rats were prepared with IT catheters or ICV injection cannulae. The paw formalin injection (50 μl of 5 % formalin) induces biphasic flinching (phase 1: 0 - 6 min; phase 2: 10 – 60 min) of the injected paw. Buprenorphine, naloxone (μ opioid receptor antagonist) or J113397 (ORL1 receptor selective antagonist) was administered IT, ICV or IP. IT, ICV or IP injection of buprenorphine produces an analgesic effect in a dose dependent manner. The effect of ICV buprenorphine was antagonized by ICV naloxone or ICV J113397 and the effect of IT buprenorphine was antagonized by IT naloxone or IT J113397. The effect of IP buprenorphine was antagonized by IP or IT naloxone, but not ICV naloxone. The analgesic effect of IP buprenorphine was enhanced by IP J113397 or ICV J113397. IP, but not IT or ICV, buprenorphine decreased the number of Fos-like immunoreactivity positive neurons in the L4-5 spinal dorsal horn. These data indicated that buprenorphine affects nociceptive processing by acting at both supraspinal and spinal μ and ORL1 receptors. The analgesic effect of systemically administered buprenorphine was suppressed by the concomitant activation of supraspinal ORL1 receptor.

Buprenorphine is a derivative of the morphine alkaloid thebaine and is used for the treatment of moderate to severe pain (Johnson et al., 2005). Buprenorphine has been reported to have high affinities for μ-, κ-, and δ-opioid receptors with *Ki* values in the nanomolar range. (Huang et al., 2001).

Recently, buprenorphine has been reported to act as an agonist at opioid receptor like-1 (ORL1) receptors (Bloms-Funke et al., 2000). Lutfy et al. (2003) reported that the antinociceptive effect of subcutaneously administered buprenorphine was markedly enhanced, in mice lacking ORL1 receptor, in the tail flick test and that systemic administration of ORL1 receptor antagonist enhanced the analgesic effect of subcutaneously administered buprenorphine. Mice lacking μ-opioid receptor failed to exhibit an antinociceptive effect after subcutaneous administration of buprenorphine (Lutfy et al., 2003). Moreover, buprenorphine did not produce an analgesic effect in μ1-opioid receptor deficient mice (Kamei et al., 1997). These data suggest that the antinociceptive effect of systemically administered buprenorphine is mediated by the activation of μ opioid receptor and that the analgesic effect of systemically administered buprenorphine is suppressed by concomitant activation of ORL1 receptor. On the other hand, spinal ORL1 receptor activation has been reported to produce an analgesic effect (Yamamoto et al., 1997 and 1999). μ opioid receptors and ORL1 receptors are widely located in the nervous system and there is not enough data to determine which μ opioid receptor plays an important role in producing an analgesic effect of buprenorphine and which ORL1 receptor plays an important role in suppressing an analgesic effect of buprenorphine when buprenorphine was administered systemically. Moreover, it is possible that the analgesic effect of systemically administered buprenorphine can be attributed to the activation of spinal ORL1 receptor. In the present study, we investigated the analgesic effect of intrathecal (IT), intracerebroventricular (ICV) or intraperitoneal (IP) administration of buprenorphine and

the effect of naloxone (μ opioid receptor antagonist) or J113397 (ORL1 receptor selective antagonist) on the analgesic effect of IT, ICV or IP administered buprenorphine in the rat formalin test.

Expression of Fos, which is the protein product of the immediate-early protooncogene c-fos, has been widely used to identify populations of neurons that are activated by noxious stimuli (Hunt et al, 1987) and to concomitantly examine the ability of drugs to suppress the expression of Fos-like immunoreactivity (Fos-LI) in the spinal cord in the formalin test (Hammond et al, 1998; Yamamoto et al, 2002). In the present study, the authors also examined the effect of IT, ICV or IP administration of buprenorphine on the expression of Fos-LI induced by paw formalin injection.

METHODS

The following investigations were performed according to a protocol approved by the Institutional Animal Care Committee of Chiba University, Chiba, Japan. Male Sprague-Dawley rats weighing 250-300 g were used.

IT Catheters and ICV Cannulae

Chronic IT catheters were inserted by passing a PE-10 catheter through an incision in the atlanto-occipital membrane to a position 8 cm caudal to the cisterna at the level of lumbar enlargement (Yaksh and Rudy, 1976). The animals were allowed to recover for one week before experimental use.

For ICV injection, a stainless-steel thin-wall injection cannula (24 gauge, 0.64 mm outer diameter, 15 mm long) was stereotaxically placed through a burr hole (0.5 mm caudal to the coronal suture and 1 mm lateral to the sagital suture; 3 mm deep to the dura) into right lateral ventricle. The animals were allowed to recover for three days before experimental use.

Rats showing neurological deficits were not studied.

Formalin Test

To carry out the formalin test, 50 μl of 5 % formalin was injected subcutaneously (SC) into the dorsal surface of the right hind paw with a 27-gauge needle under brief halothane anesthesia. Within 1 min after the formalin injection, spontaneous flinching of the injected paw could be observed. Flinching is readily discriminated and is characterized as a rapid and brief withdrawal or flexion of the injected paw. This pain-related behavior was quantified by counting the number of flinches for 1 min periods at 1 - 2 and at 5 - 6 min, and then for 1 min periods at intervals during the period from 10 to 60 min after the injection. Two phases of spontaneous

flinching behavior (an initial acute phase (phase 1: during the first 6 min after the formalin injection) and a prolonged tonic phase (phase 2: beginning about 10 min after the formalin injection)) were observed. After the observation period, the animals were immediately killed with an overdose of barbiturate.

Immunohistochemistry

Under pentobarbital anesthesia, surgery proceeded with sternotomy, transcardiac aortic needle cannulation, and perfusion with 500 ml of 4 % paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4). Spinal cords were removed and postfixed in the same fixative solution overnight at 4 °C. After storing in 0.01 M phosphate buffered saline (PBS) containing 20 % sucrose for 8 hours at 4 °C, the L 4-5 spinal cord was sectioned to a 40 μm thickness on a cryostat. The sections were processed for Fos immunohistochemistry, by a free-floating ABC technique using rabbit antibody to Fos (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) diluted with PBS containing 5 % normal goat serum and 0.3 % Triton X-100, for 20 hours at 4 °C. The sections were then incubated at room temperature for 90 minutes with a biotinylated goat anti-rabbit immunogloblin G (1:100; Vector Labs, Burlingame, CA) in PBS containing 5 % normal goat serum and 0.3 % Triton X-100. The sections were incubated at room temperature for 1 hour in avidin-biotin complex (1:100; Vector Labs) and visualized with diaminobenzidine and ammonium nickel sulfate. The tissue sections were mounted onto gelatin-coated slides, air-dried, dehydrated in alcohol in a graded manner, cleared in xylene and coverslipped.

Behavioral analysis

Motor functions were evaluated by the performance of two specific behavioral tasks, as follows (Stevens and Yaksh, 1986). 1) The placing/stepping reflex: this

response was evoked by drawing the dorsum of either hind paw over the edge of a tabletop. 2) The righting reflex: an animal placed horizontally with its back on the table will normally show an immediate coordinated twisting of the body around its longitudinal axis to regain its normal position on its feet. To quantify the evaluation of motor functions, both tasks were scored on a scale of 0 to 2 in which 0 = absence of function and 2 = normal motor functions. Animals that were able to perform the motor tasks but did so more slowly than normal animals were assigned a score of 1.

Drugs

The IT administered drugs were delivered in a total volume of 10 μl. The ICV administered drugs were delivered in a total volume of 3 μl. The IP administered drugs were delivered in a total volume of 1 ml. The agents used in this study were buprenorphine (Sigma, St. Louis, MO), J113397 ((1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one, molecular weight = 436, Banyu Pharmaceutical, Tsukuba, Japan) (Ozaki et al., 2000) and naloxone hydrochloride (molecular weight = 364, Sigma).

Experimental Protocol

IT or ICV study

For the dose-response study, buprenorphine was administered IT or ICV 10 min before the formalin injection (IT study: 0.1 ng: n = 5; 1 ng: n = 5; 10 ng: n = 5, ICV study: 1 ng: n = 5; 10 ng: n = 5; 100 ng: n = 5). To obtain control data, vehicle (saline) was injected IT (n = 6) or ICV (n = 5). To verify that the effect of IT or ICV administered buprenorphine on the formalin test was produced by an interaction between buprenorphine and a spinal or a supraspinal naloxone-sensitive μ opioid receptor or a

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spinal or a supraspinal J113397 sensitive ORL1 receptor, respectively, 10 μg of naloxone or 10 μg of J113397 was administered IT (naloxone: n = 5; J113397: n = 5) or ICV (naloxone: n = 5; J113397: n = 5) 10 min before the IT or ICV injection of buprenorphine (IT: 10 ng; ICV: 100 ng). The effect of IT or ICV administration of either 10 μg of naloxone (IT: n = 5; ICV: n = 5) or 10 μg of J113397 (IT: n = 5; ICV: n = 5) on the formalin test was also examined.

IP study

For the dose-response study, buprenorphine was administered IP 10 min before the formalin injection (30 μg/kg: n = 6; 100 μg/kg: n = 5; 300 μg/kg: n = 5). To obtain control data, vehicle (saline, n = 5) was injected IP. To verify that the effect of IP administered buprenorphine in the formalin test was produced by an interaction between buprenorphine and a naloxone sensitive μ opioid receptor, 1 mg/kg of naloxone (n = 5) was administered IP 10 min before the IP injection of 300 μg/kg of buprenorphine. To verify that the effect of IP administered buprenorphine in the formalin test was produced by an interaction between buprenorphine and a J113397 sensitive ORL1 receptor, 1 mg/kg of J113397 (n = 5) was administered IP 10 min before the IP injection of 100 μg/kg of buprenorphine. The effect of IP administration of either 1 mg/kg of naloxone (n = 5) or 1 mg/kg of J113397 (n = 5) on the formalin test was also examined.

To determine which naloxone sensitive μ opioid receptor contributes to the analgesic effect of IP administered buprenorphine, spinal or supraspinal, and which J113397 sensitive ORL1 receptor contributes to the analgesic effect of IP administered buprenorphine, spinal or supraspinal, 10 μg of naloxone or 10 μg of J113397 was administered IT (naloxone: n = 6; J113397: n = 5) or ICV (naloxone: n = 5; J113397: n =

5) 10 min before the IP injection of 300 μg/kg of buprenorphine. To obtain control data, vehicle (saline) was administered IT (n = 8), ICV (n = 5) or IP (n = 5) 10 min before the IP administration of 300 μg/kg or 100 μg/kg buprenorphine.

Immunohistochemical study

10 ng of buprenorphine (IT Study, n = 6), 100 ng of buprenorphine (ICV Study, n = 5) or 300 μg/kg of buprenorphine (IP Study, n = 6) was administered 10 min before the formalin injection, and the expression of Fos-LI was examined 2 hr after the formalin injection. For comparison, vehicle (saline) was administered IT (n = 5), ICV (n = 5) or IP (n = 5).

For the quantitation of Fos-LI, five sections from the L4 and L5 segments of the spinal cord of each rat were randomly selected. The number of Fos-LI positive neurons in the superficial laminae (laminae I and II), the nucleus proprius (laminae III and IV) and the neck of dorsal horn (lamina V) on the side of the spinal cord ipsilateral to the site of formalin injection were counted. Laminae borders were identified by use of anatomical landmarks in gray matter and standard anatomical drawings. The investigator responsible for counting the Fos-LI positive neurons was blind to the drug treatment of each animal. The average of the number of Fos-LI positive neurons in five slices was defined as the number of Fos-LI positive neurons.

Statistical Analysis

Formalin test

For the dose-response analysis, data from phase 1 (0 - 6 min) and phase 2 (10 - 60 min) observations were considered separately. In each case, the cumulative instances of formalin-evoked flinches during the phase 1 and phase 2 were calculated for each rat. Percentage of vehicle control flinches during phase 1 and phase 2 was

calculated in each rat and these individual rat data were then used to construct phase 1 and phase 2 dose-response curves. To evaluate the dose-dependence, one-way analysis of variance (ANOVA) was used. For multiple comparisons, Tukey's test was used. In the antagonist study, the unpaired t-test (two tailed) was used.

Immunohistochemical study

In the comparison of the number of Fos-LI positive neurons between the buprenorphine treated group and the saline treated group in IT, ICV and IP studies, the unpaired t-test (two tailed) was used.

Wherever appropriate, results are expressed as mean ± S.E.M. Critical values that reached a $p<0.05$ level of significance were considered statistically significant.

RESULTS

Behavioral analysis

Two hours after IT injection of 100 ng of buprenorphine, all the rats were dead. After IT injection of 10 ng of buprenorphine, all the animals scored 2 (normal motor function) in the placing/stepping reflex and righting reflex tests 1 hour and 10 min after the drug administration. Thus, 10 ng of buprenorphine is the maximum IT dose used in the present study. After the ICV or IP administration of buprenorphine, all animals scored 2 (normal motor function) in the placing/stepping reflex and righting reflex tests at doses applied in this study. After the IT, ICV or IP administration of naloxone or J113397, all animals scored 2 (normal motor function) in the placing/stepping reflex and righting reflex tests at doses applied in the present study.

IT study

IT injection of buprenorphine decreased the sum of flinches, in both phase 1 and phase 2 flinching behavior, in a dose-dependent manner for doses between 0.1 and 10 ng (Figure 1 and 2, phase 1: $p<0.005$; phase 2: $p<0.001$ by ANOVA). Pre-treatment with 10 μg of naloxone antagonized the analgesic effect of 10 ng of buprenorphine on both phase 1 and phase 2 flinching behavior (Figure 3, Table 1, phase 1: $p<0.01$; phase 2: $p<0.01$, by t-test). Pre-treatment with 10 μg of J113397 antagonized the analgesic effect of 10 ng of buprenorphine on the phase 1 flinching behavior, but not the phase 2 flinching behavior (Figure 3, Table 1, phase 1: $p<0.05$; phase 2: $p>0.7$, by t-test). IT injection of 10 μg of naloxone or 10 μg of J113397 had no effect on the phase 1 and phase 2 flinching behavior as compared with saline treated rats (Figure 3, naloxone: $p>0.2$; J113397: $p>0.3$, by t test).

ICV Study

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ICV injection of buprenorphine decreased the sum of flinches, in both the phase 1 and the phase 2 flinching behavior, in a dose-dependent manner at a dose between 1 and 100 ng (Figure 1 and 2, phase 1: $p<0.005$; phase 2: $p<0.001$ by ANOVA). Pre-treatment with either 10 μg of naloxone or 10 μg of J113397 antagonized the analgesic effect of 100 ng of buprenorphine on both the phase 1 and the phase 2 flinching behavior (Figure 3, Table 1, phase 1: $p<0.01$; phase 2: $p<0.01$, by t-test). ICV injection of 10 μg of naloxone or 10 μg of J113397 had no effect on the phase 1 and the phase 2 flinching behavior as compared with saline treated rats (Figure 3, naloxone: $p>0.1$; J113397: $p>0.1$, by t test).

IP Study

IP administration of buprenorphine decreased the sum of flinches in both the phase 1 and the phase 2 flinching behavior in a dose-dependent manner at a dose between 30 and 300 μg/kg (Figure 1 and 2, phase 1: $p<0.01$; phase 2: $p<0.001$ by ANOVA). Pre-treatment with 1 mg/kg of naloxone (IP) antagonized the effect of 300 μg/kg of buprenorphine on the phase 1 and the phase 2 flinching behavior (Figure 4, Table 1, $p<0.05$ by t test). Pre-treatment with 1 mg/kg of J113397 (IP) enhanced the analgesic effect of 100 μg/kg of buprenorphine on the phase 2, but not on the phase 1, flinching behavior (Figure 4, Table 1, phase 1: $p>0.1$; phase 2: $p<0.005$, by t test). IP injection of 1 mg/kg of naloxone or 1 mg/kg of J113397 had no effect on the phase 1 and the phase 2 flinching behavior as compared with saline treated rats (naloxone study: $p>0.2$; J113397 study: $p>0.05$ by t test, Figure 4).

Pre-treatment with either 10 μg of naloxone (IT) or 10 μg of J113397 (IT) antagonized the effect of 300 μg/kg of buprenorphine on the phase 1 flinching behavior, but not on the phase 2 flinching behavior (Figure 4, Table 1, phase 1: $p<0.05$; phase 2: $p>0.2$, by t test).

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Pre-treatment with 10 μg of naloxone (ICV) had no effect on the analgesic effect of 300 μg/kg of buprenorphine on the phase 1 and the phase 2 flinching behavior (Figure 4, Table 1, phase 1: $p>0.4$; phase 2: $p>0.3$, by t test). Pre-treatment with 10 μg of J113397 (ICV) enhanced the analgesic effect of 300 μg/kg of buprenorphine on the phase 2 flinching behavior, but not on the phase 1 flinching behavior (Figure 4, Table 1, phase 1: $p>0.7$; phase 2: $p<0.05$, by t test).

Immunohistochemical study

Either IT injection of 10 ng of buprenorphine or ICV injection of 100 ng of buprenorphine had no effect on the number of the Fos-LI positive neurons in the laminae I – II, laminae III-IV and lamina V as compared with saline treated rats (laminae I-II: $p>0.2$; laminae III-IV: $p>0.1$; lamina V: $p>0.1$ by t-test, Figure 5). IP injection of 300 μg of buprenorphine decreased the number of the Fos-LI positive neurons in the laminae I – II, but not in the laminae III – IV and lamina V, as compared with saline treated rats (laminae III-IV: $p>0.1$; lamina V: $p>0.1$ by t-test, Figure 5).

DISCUSSION

The formalin test shows two different phases, a first acute phase that is evoked by chemical stimulation to the peripheral nerve and a second prolonged tonic phase that is mediated by the spinal sensitized state induced during the first acute phase (Yamamoto and Yaksh, 1992). When formalin test was used to elucidate drug effects, many mechanistic explanations are available. Moreover, buprenorphine has been reported to produce an analgesic effect in a dose-dependent manner in formalin test, but in some other models, buprenorphine produced bell-shaped dose response curves (Christoph et al, 2005). Thus, we chose the formalin test.

IT study

IT administration of buprenorphine attenuated the flinching behavior at a dose between 0.1 and 10 ng. 100 ng of buprenorphine is the lethal IT dose and we could not examine the effect of IT administration of 100 ng of buprenorphine. IT administered 10 μg of naloxone antagonized the analgesic effect of IT administered 10 ng of buprenorphine on both phase 1 and phase 2 responses. IT administered J113397 antagonized the analgesic effect of IT administered 10 ng of buprenorphine on the phase 1 response, but not on the phase 2 response. These data suggest that, when 10 ng of buprenorphine was administered IT, activation of both naloxone sensitive μ opioid receptor and J113397 sensitive ORL1 receptor is needed to produce an analgesic effect in the phase 1 response and activation of naloxone sensitive μ opioid receptor is enough to produce an analgesic effect in the phase 2 response. It has been reported that IT administered morphine or IT administered nociceptin/orphanin FQ, an agonist of ORL1 receptor, decreased the number of phase 1 and phase 2 flinching behaviors in the rat formalin test (Yamamoto and Yaksh, 1992; Yamamoto et al., 1997). This indicates that activation of either spinal μ receptor or spinal ORL1 receptor is

enough to produce an analgesic effect on the phase 1 and the phase 2 responses evoked by paw formalin injection. This suggests that IT administered 10 ng of buprenorphine activates not enough naloxone sensitive μ receptors alone or not enough J113397 sensitive ORL1 receptors alone to produce an analgesic effect on the phase 1 response. These data also indicated that naloxone sensitive μ opioid receptor activation alone is enough to produce an analgesic effect on the phase 2 response when buprenorphine was administered IT. This suggests that the number of activated naloxone sensitive μ opioid receptor needed to produce an analgesic effect on the phase 2 response is smaller than that needed to produce an analgesic effect on the phase 1 response.

ICV study

ICV administration of buprenorphine attenuated the flinching behavior at a dose between 1 and 100 ng. ICV administration of either 10 μg of naloxone or 10 μg of J113397 antagonized the analgesic effect of ICV administered 100 ng of buprenorphine on both the phase 1 and the phase 2 responses. Thus, when 100 ng of buprenorphine was administered ICV, activation of both μ and ORL1 receptors is needed to produce an analgesic effect and when either μ opioid receptor or ORL1 receptor was blocked by naloxone or J113397, the analgesic effect of ICV administered buprenorphine disappeared. Wang et al. (1999) reported that ICV administration of μ- and κ-opioid receptor agonists produces an analgesic effect on both the phase 1 and the phase 2 responses in the formalin test and that ICV administration of nociceptin/orphanin FQ attenuated the brain μ- and κ-opioid receptor mediated analgesia. These data strongly suggested that activation of brain ORL1 receptor suppressed the brain μ opioid receptor mediated analgesia. The authors do not know

the precise mechanisms that produce an analgesic effect of ICV administered buprenorphine. It has been suggested that the nucleus raphe magnus of the rostral ventromedial medulla (RVM) is a major site of supraspinal nociceptin/orphanin FQ effects on pain processing. In this region both ON cells and OFF cells are located. ON cells fire immediately before a nociceptive reaction, while OFF cells are inhibited by the GABAergic ON cells and, therefore, are silent at the same time. Activation of OFF cells has been reported to induce spinal antinociception via descending antinociceptive tracts (Zeilhofer and Calo, 2003). Inhibition of ON cells by activation of μ opioid receptors causes OFF cell disinhibition and results in spinal antinociception. Nociceptin/orphanin FQ inhibits both ON cells and OFF cells (Zeilhofer and Calo, 2003; Pan et al., 2000). The net effect of nociceptin/orphanin FQ on nociception at supraspinal sites depends on the activation state (resting versus sensitized) of pain controlling neuronal circuits (Zeilhofer and Calo, 2003) and the activation state of opioid receptors other than ORL1 receptor. In the present study, ICV administration of buprenorphine produced an analgesic effect by activation of both μ and ORL1 receptors.

IP study

IP administration of buprenorphine decreased the phase 1 and the phase 2 responses at a dose between 30 and 300 μg/kg.

Effect on phase 1 response

Both IT administration of 10 μg naloxone and IT administration of 10 μg of J113397 antagonized an analgesic effect of IP administered buprenorphine on the phase 1 response. This suggests that activation of both spinal naloxone sensitive μ receptor and spinal J113397 sensitive ORL1 receptor is needed to produce an

analgesic effect on the phase 1 response when buprenorphine was administered IP. Both ICV administration of 10 μg naloxone and ICV administration of 10 μg of J113397 had no effect on an analgesic effect of IP administered buprenorphine in the phase 1 response, and IP administration of 1 mg/kg of naloxone, but not IP administration of 1 mg/kg of J113397, antagonized the analgesic effect of IP administered buprenorphine in the phase 1 response. These data suggest that, when buprenorphine was administered IP, an activation of supraspinal μ and ORL1 receptors had no effect on the phase 1 response and the analgesic effect of IP administered buprenorphine is mediated by the activation of both spinal μ opioid receptors and spinal ORL1 receptors. It is not clear why IP administered J113397 had no effect on the analgesic effect of buprenorphine in the phase 1 response. It is possible that enough J113397 did not reach to the spinal cord to antagonize the effect of IP administered buprenorphine on the phase 1 response.

Effect on phase 2 response

Either IT administration of 10 μg naloxone or ICV administration of 10 μg naloxone had no effect on the analgesic effect of IP administered buprenorphine in the phase 2 response and IP administration of 1 mg/kg of naloxone antagonized the analgesic effect of IP administered buprenorphine in the phase 2 response. These data suggest that the activation of either spinal or supraspinal naloxone sensitive μ opioid receptors is enough to produce an analgesic effect of buprenorphine in the phase 2 response. Either ICV or IP administration of J113397 enhanced the analgesic effect of IP administered buprenorphine in the phase 2 response and IT administration of J113397 had no effect on the analgesic effect of IP administered buprenorphine in the phase 2 response. As noted above, the analgesic effect of ICV administered buprenorphine on phase 1 and phase 2 was antagonized by ICV administration of

J113397. These data suggest that activation of supraspinal ORL1 receptor attenuated the spinal, but not supraspinal, analgesic effect of buprenorphine in the phase 2 response. As mentioned above, the net effect of activation of ORL1 receptor on nociception at supraspinal sites strongly depends on the activation state (resting versus sensitized) of pain controlling neuronal circuits. It has been reported that paw formalin injection induces a sensitized state during phase 2, but not during phase 1 (Yamamoto and Yaksh, 1992). In the rat formalin test, activation of supraspinal ORL1 receptor modulates the spinal analgesic effect of buprenorphine on the phase 2, but not the phase 1, response. These data are consistent with the previous report that a suppressing the ORL1 component by antagonist increases the analgesic effect of systemically administered buprenorphine (Lutfy et al., 2003). It has been reported that systemic administration of buprenorphine produced an analgesic effect with a bell shaped dose-response curve in the rat hot plate test (Bryant et al., 1983) and in the rat neuropathic pain models (Christoph et al., 2005). Although Christoph et al. (2005) suggested that the shape of the dose response curve depends on the nature of the painful stimulus, it is possible that the contribution of ORL1 component of buprenorphine produce a bell shaped dose response curve.

Fos study

IP, but not IT and ICV, administration of buprenorphine significantly decreased the expression of Fos-LI positive neurons in Laminae I-II of the L5 spinal dorsal horn. This indicated that only when buprenorphine activates both supraspinal and spinal μ opioid receptors and supraspinal and spinal ORL1 receptors, buprenorphine suppressed the nociceptive input into the spinal dorsal horn.

In conclusion, 1) buprenorphine activates both μ receptor and ORL1 receptor

and 2) the mechanisms producing the analgesic effect of buprenorphine are very complicated and depend on the site of injection and/or on the activation state (resting versus sensitized) of pain controlling neuronal circuits.

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Footnotes

a) Unnumbered footnote

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Figure Legends

Figure 1

Effects of intrathecal (IT) injection of 10 ng of buprenorphine (BUP; n = 5) and saline (SAL; n = 6) (IT Study), effects of intracerebroventricular (ICV) injection of 100 ng of BUP (n = 5) and SAL (N = 5) (ICV Study) and effects of intraperitoneal (IP) injection of 300 μg/kg (n = 5) and SAL (n = 5) on the time course of the flinches observed after the formalin injection into the dorsal surface of the right rat hind-paw. Drugs were administered 10 min before the formalin injection. The number of flinches/min is plotted vs. time after the formalin injection. Each point represents the mean response and SEM.

Figure 2

Dose-response curves for intrathecal (IT Study), intracerebroventricular (ICV Study) and intraperitoneal (IP Study) injection of buprenorphine. The cumulative instances of formalin evoked flinches in rats, expressed as a percentage of vehicle (saline) evoked flinches during phase 1 and phase 2 of the formalin test are presented. Drugs were administered 10 min before the formalin injection. Each point represents the mean and SEM. The abscissa shows the log dose (ng) in the IT Study and ICV Study and log dose (μg/kg) in the IP Study and the ordinate shows the percentage of vehicle (saline) control flinches during phase 1 or phase 2. * $p<0.05$ as compared with responses at 0.1 ng in the IT Study, $p<0.05$ as compared with responses at 1 ng in the ICV Study and $p<0.05$ as compared with responses at 30 μg/kg in the IP Study. ** $p<0.005$ as compared with responses at 0.1 ng in the IT Study, $p<0.005$ as compared with responses at 1 ng in the ICV Study and $p<0.005$ as compared with responses at 30 μg/kg in the IP Study. # $p<0.05$ as compared with responses as compared with 1 ng in the IT Study and $p<0.005$ as compared with 100 μg/kg in the IP Study.

Figure 3

Effects of 10 μg of naloxone (NAL) or 10 μg of J113397 on the analgesic effect of buprenorphine (BUP). In the intrathecal administration study (IT study), 10 ng of BUP was administered IT. In the intracerebroventricular administration study (ICV study), 100 ng of BUP was administered ICV. For the comparison, 10 μg of NAL or 10 μg of J113397 was administered IT or ICV. Each bar represents the mean and SEM. The ordinate shows the percentage of vehicle (saline) control flinches during phase 1 or phase 2. *$p<0.05$ as compared with responses of BUP treated animals. **$p<0.005$ as compared with responses of BUP treated animals.

Figure 4

Effects of naloxone (NAL) or J113397 on the analgesic effect of intraperitoneal (IP) injection of 100 or 300 μg/kg of buprenorphine (BUP). 1 mg/kg of NAL or 1 mg/kg of J113397 was administered IP 10 min before the BUP IP administration. 10 μg of NAL or 10 μg of J113397 was administered IT or ICV 10 min before the IP administration of BUP. For the comparison, NAL or J113397 was administered IP, IT or ICV. Each bar represents the mean and SEM. The ordinate shows the percentage of vehicle (saline) control flinches during phase 1 or phase 2. * $p<0.05$ as compared with responses of BUP treated animals.

Figure 5

Effect of 10 ng of buprenorphine (BUP) in IT Study, 100 ng of BUP in ICV Study and 300 μg/kg of BUP in IP Study on the number of Fos-LI positive neurons in laminae I-II, laminae III-IV and lamina V on the L4 or L5 segments of the spinal cord ipsilateral to the site of formalin injection. Each bar represents the group mean and SEM. For the

statistical analysis, the unpaired t-test was used.

Table 1

Summary of antagonist study

	IT BUP		ICV BUP		IP BUP					
	IT NAL	IT J	ICV NAL	ICV J	IP NAL	IP J	IT NAL	IT J	ICV NAL	ICV J
Phase 1	Ant	Ant	Ant	Ant	Ant	N/E	Ant	Ant	N/E	N/E
Phase 2	Ant	N/E	Ant	Ant	Ant	Enhance	N/E	N/E	N/E	Enhance

BUP: buprenorphine; NAL: naloxone; J: J113397; Ant: antagonize; N/E: no effect

Figure 1

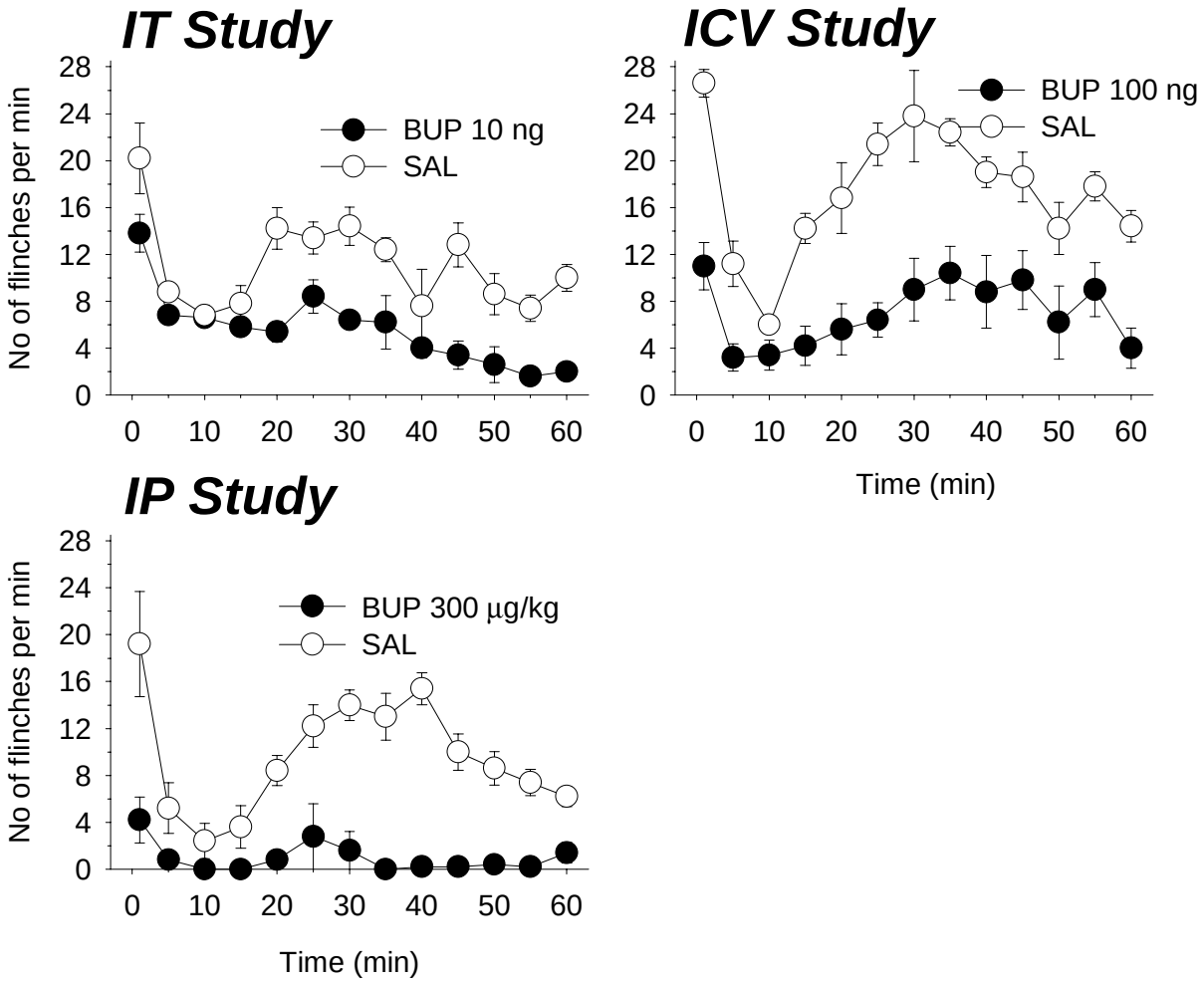

Figure 2

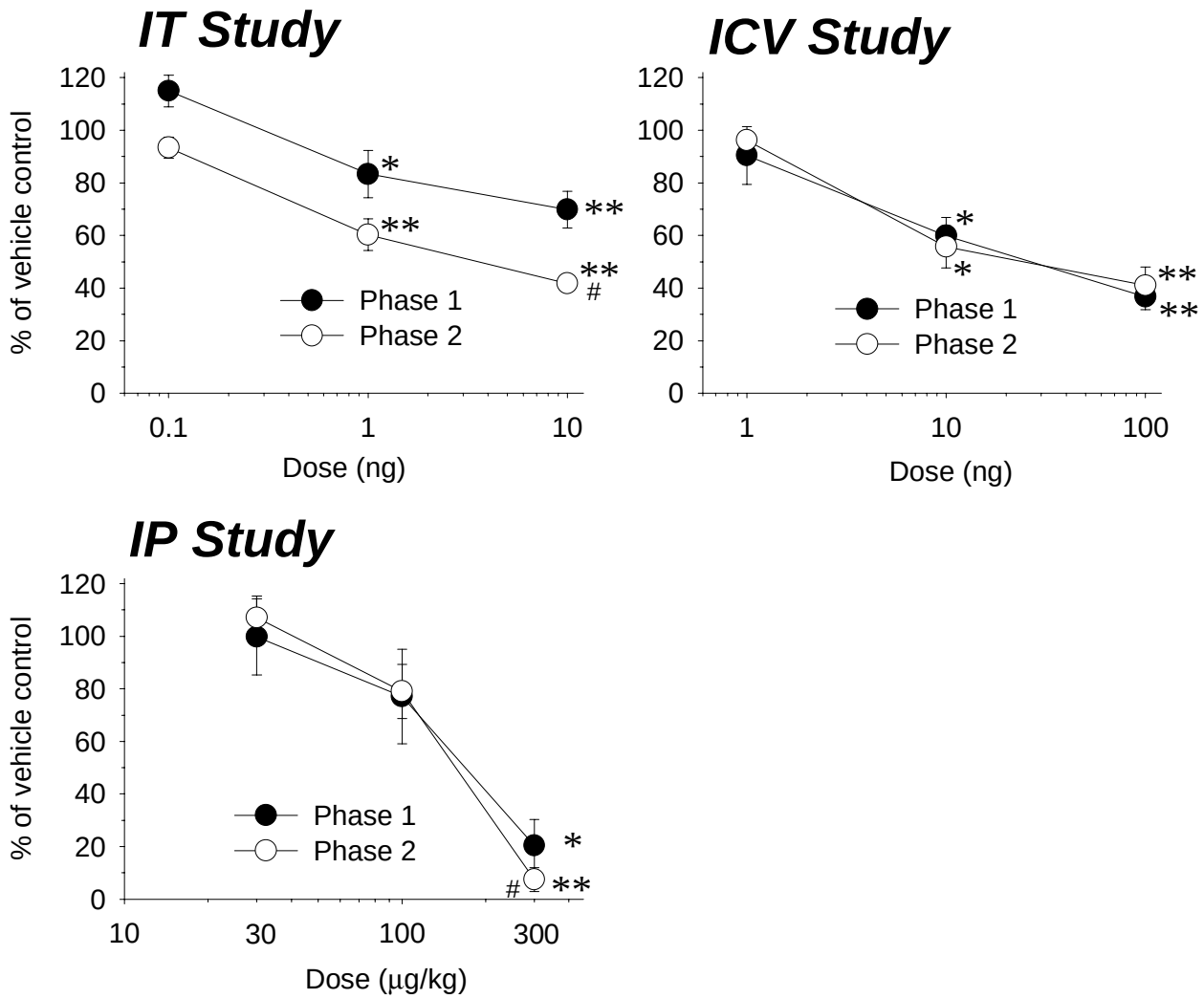

Figure 3

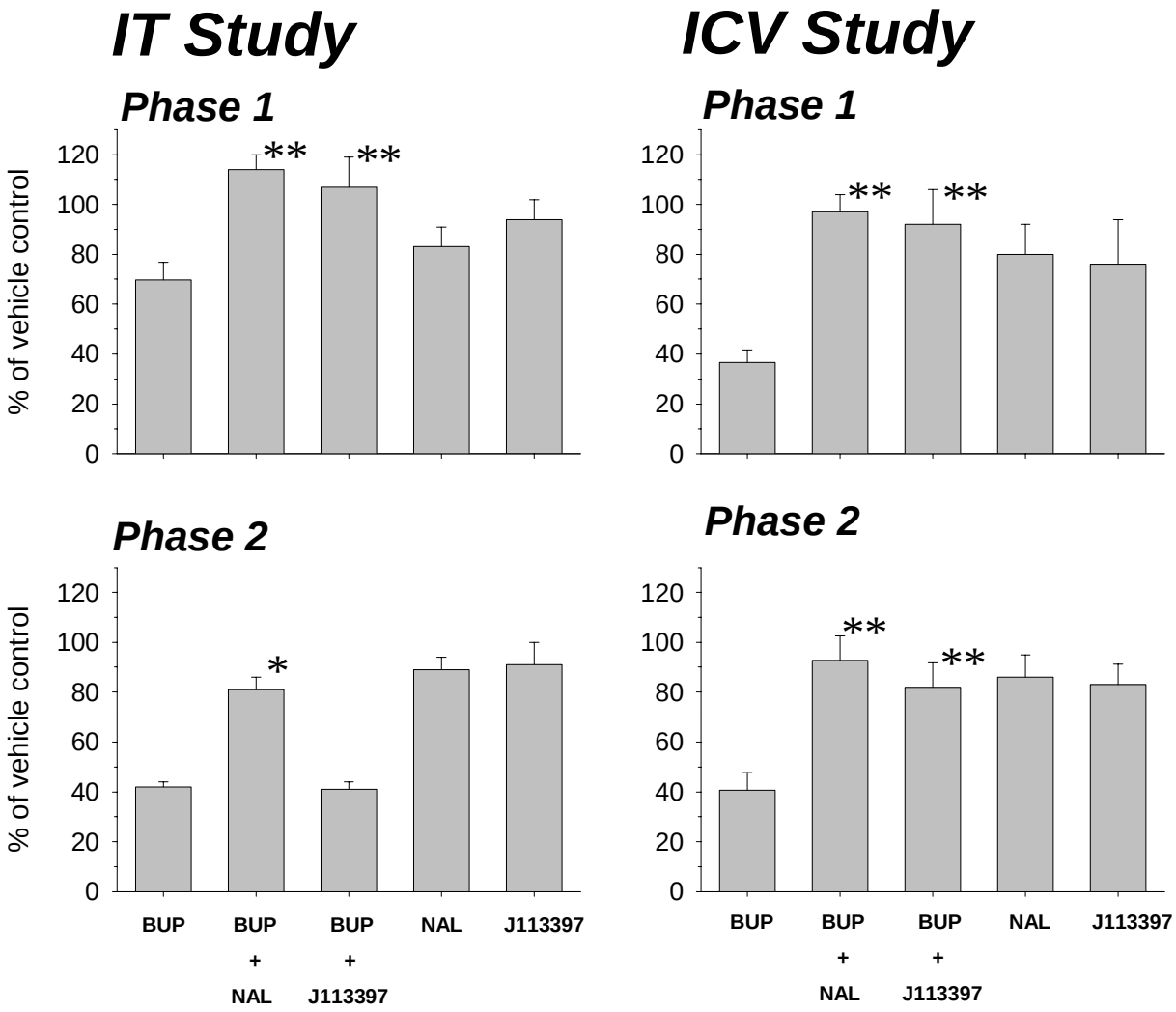

Figure 4

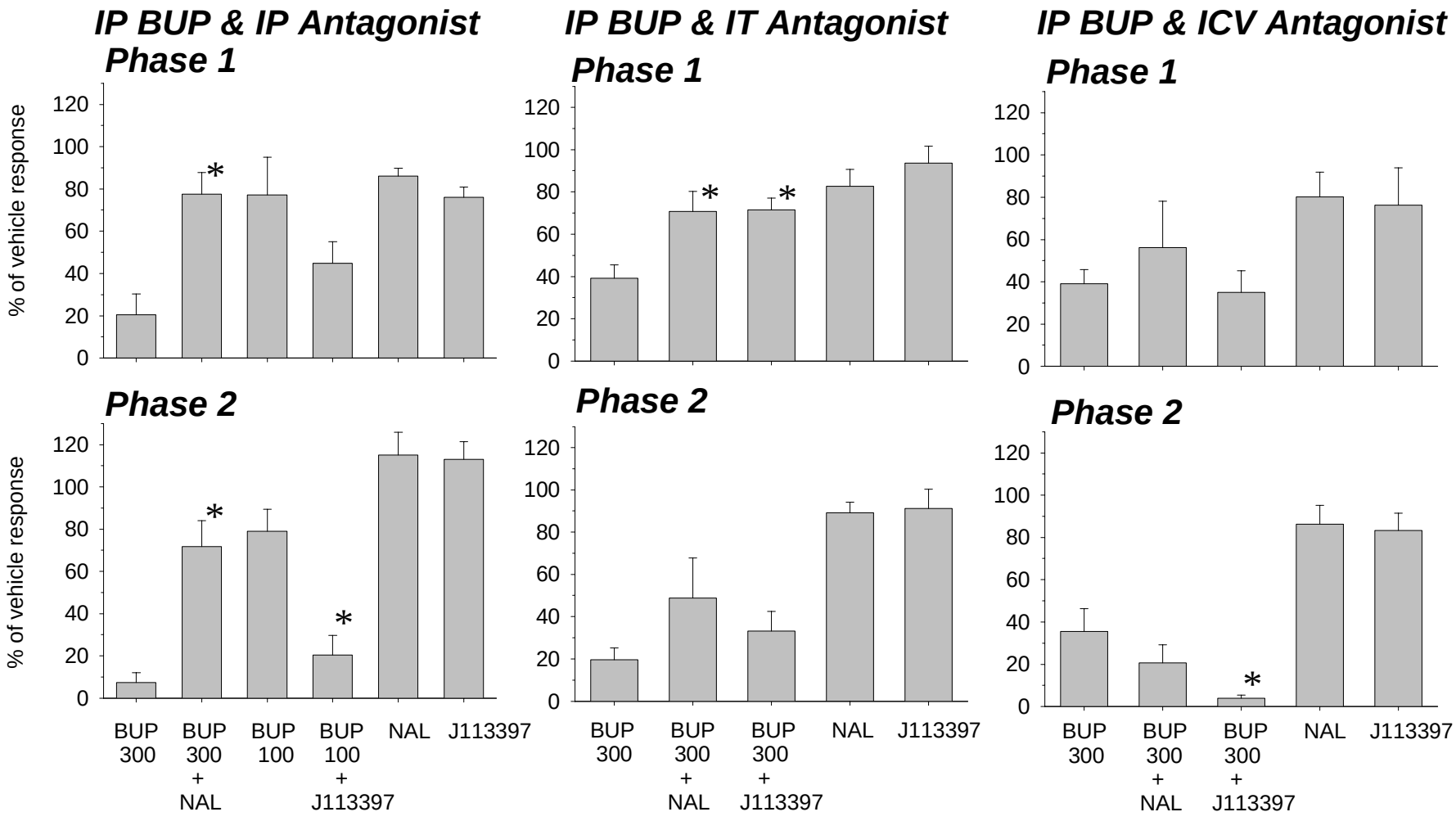

Figure 5