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Title Page

Effects of the NMDA receptor antagonist perzinfotel (EAA-090) on chemically-induced thermal hypersensitivity

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Abbreviations: perzinfotel (EAA-090; [2-(8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)-ethyl]phosphonic acid, selfotel (CGS-19755; *cis*-4-(phosphonomethyl) piperidinge-2-carboxylic acid), dizocilpine [(MK-801; (+)-10,11-dihydro-5-methyl-5H-dibenzo [a,d]cyclohepten-5, 10 imine (Z)-2-butenedioate (1:1)]), CGP-39653; D,L-(E)-2-amino-4-propyl-5-phosphono-3-pentenoic acid

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Abstract

Perzinfotel (EAA-090; [2-(8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)-ethyl]phosphonic acid) is a selective, competitive NMDA receptor antagonist with high affinity for the glutamate site. The current study evaluated whether perzinfotel would have antinociceptive effects or block thermal hypersensitivity associated with the administration of chemical irritants in rats. Perzinfotel lacked antinociceptive effects but dose- and time-dependently blocked prostaglandin E_2 (PGE_2)- and capsaicin-induced thermal hypersensitivity in a warm-water tail withdrawal assay in rats. Doses of 10 mg/kg intraperitoneal (IP) or 100 mg/kg oral (PO) blocked PGE_2-induced hypersensitivity by 60-80%. The magnitude of reversal was greater than other negative modulators of the NMDA receptor studied, such as un-competitive channel blockers (e.g., memantine, dizocilpine and ketamine), a NR2B selective antagonist (e.g., ifenprodil), and other glutamate antagonists (e.g., selfotel, CPP, CGP-39653), up to doses that suppressed operant rates of responding. In contrast to other negative modulators of the NMDA receptor studied, which typically decreased operant rates of responding at doses that lacked antinociceptive effects, perzinfotel did not modify response rates at doses that blocked irritant-induced thermal hypersensitivity. Collectively, these studies demonstrate that perzinfotel has therapeutic ratios for effectiveness versus adverse effects superior to those seen with other competitive and un-competitive NMDA receptor antagonists studied.

Introduction

Excitatory amino acids (EAAs) acting at NMDA receptors play a role in both acute and chronic pain. Increases in afferent input and glutamate release within the spinal cord have been observed after peripheral injection of an irritant (e.g., carrageenan) or tissue injury (Sluka and Westlund, 1993; Kawamata and Omote, 1996; Dickenson et al., 1997). Repeated stimulation of primary afferent fibers can progressively increase the magnitude and duration of action potentials in spinal cord (often termed 'windup'), which can lead to central sensitization (Ma and Woolf, 1995). This neuronal hyperexcitability manifests itself in a lowered threshold to evoked activity (i.e., hyperalgesia), an expansion of receptive fields (i.e., secondary hypersensitivity), and an ability of non-noxious input to evoke neuronal activity (i.e., allodynia). In preclinical studies, NMDA receptor antagonists reverse neuronal hyperexcitability and reverse hypersensitivity in several inflammatory and neuropathic animal pain models associated with various pathophysiologic mechanisms (Mao et al., 1993; Chaplan et al., 1997; Suzuki et al., 2001). In clinical studies, NMDA receptor antagonists, such as ketamine and dextromethorphan, reduce windup pain, spontaneous pain, hyperalgesia and allodynia in patients with post-herpetic neuralgia pain, phantom limb pain, and diabetic neuropathy pain (Stubhaug and Breivik, 1997; Rabben et al., 1999; Sang, 2000). However, the narrow separation between effectiveness and adverse effects, including sedation and psychotomimetic effects, of clinically available NMDA receptor antagonists has severely hampered their utility for the treatment of neuropathic pain.

Perzinfotel is a selective, competitive small molecule antagonist that blocks the actions of glutamate at the NMDA receptor (Kinney et al., 1998; Childers et al., 2002;

Sun et al., 2004). Previous studies have demonstrated its effectiveness in several animal stroke models and anticonvulsant models (Childers et al., 2002). Importantly, perzinfotel has an adverse effect profile superior to many other reported competitive (e.g., CGS-19755) and un-competitive (e.g., dizocilpine) antagonists (Kinney et al., 1998; Childers et al., 2002). Given the apparent involvement of NMDA receptors in pain conditions, perzinfotel was evaluated in preclinical pain assays to determine whether the compound would have antinociceptive, antiallodynic or antihyperalgesic effects.

Materials and Methods

Animal maintenance and research were conducted in accordance with the National Research Council's policies and guidelines for the handling and use of laboratory animals outlined in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The laboratory facility was licensed by the United States Department of Agriculture and accredited by the American Association for Accreditation of Laboratory Animal Care. Research protocols were approved by the Wyeth Institutional Animal Care and Use Committee in accordance with the guidelines of the Committee for Research and Ethical Issues of IASP (Zimmermann, 1983).

Subjects. Male Sprague-Dawley rats (Charles River, Kingston/Stoneridge, NY) weighing 200-250g at time of arrival were individually housed in wire cages in a climate-controlled room. A 12-hour light/dark cycle (lights on at 06:30) was in effect for all animals and water was available ad libitum. In the operant responding study, rats were food restricted to 10-15 g food post-session and food pellets earned during sessions. For all oral dosing studies, rats were fasted for approximately 16 hr before drug administration. With these exceptions, all other rats were feed ad libitum.

Thermal sensitivity assessed by warm-water tail withdrawal. To asses baseline thermal sensitivity, the terminal 10 cm of the tail was placed into water warmed to 34, 38, 42, 46, 50, 54 or 58 °C. The latency in seconds for the animal to remove the tail from the water was used as a measure of nociception. If the animal did not remove the tail within 20 sec, the experimenter removed the tail and a maximum latency of 20

sec was recorded. The antinociceptive effects of morphine or perzinfotel were evaluated in a cumulative dosing paradigm. Under this procedure, doses of morphine or perzinfotel, increasing in 0.5 log unit increments, were administered intraperitoneal (IP) every 30 min. Tail-withdrawal latencies were assessed during the 5-min period at the end of each 30 min period (i.e., 25-30 min after drug administration).

PGE_2- and capsaicin-induced thermal hypersensitivity assessed by warm-water tail withdrawal. Following the assessment of baseline thermal sensitivity, thermal hypersensitivity was produced by a intradermal (ID) 50 μL injection of prostaglandin E_2 (PGE_2; Sigma, St. Louis, MO) or capsaicin (Sigma, St. Louis, MO) into the distal 1 cm of the tail. Temperature-effect curves were generated before (baseline) and after (15, 30, 60, 90, and 120 min) PGE_2 (0.01 – 0.1 mg) or capsaicin (0.001 – 0.1 mg) injection. To evaluate whether baseline thermal sensitivity or the magnitude of thermal hypersensitivity changed after repeated PGE_2 administration, eight rats were administered PGE_2 weekly for three weeks and thermal sensitivities were assessed before and 30 min after the administration of 0.1 mg PGE_2. Based on results from the current study, as well as results of PGE_2 and capsaicin in other species, such as rhesus monkeys (Brandt et al., 2001), subjects were tested a maximum of three times with a minimum of five days between tests (one baseline 0.1 mg PGE_2 or 0.01 mg capsaicin assessment and one or two compound tests in the presence of 0.1 mg PGE_2 or 0.01 mg capsaicin).

To assess the chronic effects of perzinfotel, 10 mg/kg perzinfotel was administered and the duration of PGE_2-induced thermal hypersensitivity was assessed 30 min later for 2 hr. Subjects were then dosed daily with 10 mg/kg perzinfotel for two

weeks. The ability of perzinfotel to block thermal hypersensitivity was re-assessed 1 week and again 2 weeks after the beginning of chronic daily treatment under conditions identical to the first determination.

The ability of compounds to block 0.1 mg PGE_2- or 0.01 mg capsaicin-induced thermal hypersensitivity was assessed using a single dose procedure. Under this procedure, a single dose of compound was administered IP or PO 30 min before the injection of PGE_2 or capsaicin and thermal sensitivities were assessed 30 min after PGE_2 or capsaicin injection (i.e., drug effects were evaluated 60 min after administration). This pretreatment time was chosen based on preliminary rodent pharmacokinetic studies, which indicated that the time to peak concentration (t_{max}) of perzinfotel was between 0.3 and 1 hr after PO administration (20 and 100 mg/kg) and 0.3 hr after IP administration (10 mg/kg). Apparent terminal half-lives ($t_{1/2}$) were 1.3 to 7.8 hr after PO administration and 0.5 hr after IP administration. In addition, preliminary time course studies indicated that the behavioral effects of perzinfotel were maximal when administered 30 min before PGE_2 by the oral route of administration. For IT administration of perzinfotel, rats were anesthetized with isoflurane and an incision was made along the dorsal midline from approximately L3-S2. Perzinfotel was administered into the intrathecal space at the level of the lumbar enlargement in a volume of 20 ul using a 50 ul Hamilton syringe. PGE_2 was administered at the same time as IT perzinfotel and thermal hypersensitivity was evaluated 30 min later.

Effects of perzinfotel on schedule-controlled responding. To evaluate the potential for drugs to modify tail-withdrawal latencies by mechanisms unrelated to pain

(i.e., sedation), compounds were also evaluated for their ability to suppress operant rates of responding. Experimental sessions were conducted in operant conditioning chambers located inside ventilated sound-attenuating chambers that were equipped with white noise to mask extraneous sounds (Med Associates Inc., Georgia, VT). A response lever and a food trough were located on the front panel of the operant chamber. The operant chambers were controlled and monitored by computers with hardware and software from Med Associates Inc.

Rats were trained to respond on one lever under a fixed ratio-30 (FR30) schedule of food presentation (BioServ 45mg pellets; Frenchtown, NJ). Daily experimental sessions consisted of 3 components. Each component consisted of a 10-min timeout period followed by a 10-min response period; thus, daily sessions totaled 60 min. During the timeout period, the chamber was dark, and there were no programmed consequences. During the response component, the house light was illuminated, and lever pressing was associated with an audible feedback click. Experimental sessions were conducted daily (Mon-Fri). Test sessions assessing the effects of compounds were typically conducted two days per week (Tue and Fri) provided response rates were within 20% of the previous 5 training day mean on the day preceding the test. Compounds were administered IP or PO at the start of the first cycle. To equate rate effects with irritant-induced thermal hypersensitivity, which assessed the effects of drugs 60 min after administration, response rates for only the last cycle (i.e., 50-60 min after drug administration) were used for comparison.

Data Analysis. Temperature-effect curves were generated for each experimental condition for individual rats. The temperature that produced a half-maximal increase in the tail-withdrawal latency (i.e., T_{10}) was calculated from each temperature-effect curve. The T_{10} was determined by interpolation from a line drawn between the point above and the point below 10 sec on the temperature-effect curve. For these studies, thermal hypersensitivity was defined as a leftward shift in the temperature-effect curve and a decrease in the T_{10} value. Statistical analysis was done using a within subjects repeated measures analysis of variance on T_{10} values. The criterion for significant reversal of the T_{10} value from the chemical irritant alone was $p < 0.05$.

Reversal of thermal hypersensitivity was defined as a return to baseline of the temperature-effect curve and the T_{10} value. Blockade of irritant-induced thermal hypersensitivity was quantified as the percentage return to baseline values (% Reversal) according to the following equation:

$$\% \text{ Reversal} = \frac{(T_{10}^{\text{drug+irritant}}) - (T_{10}^{\text{irritant}})}{(T_{10}^{\text{baseline}}) - (T_{10}^{\text{irritant}})} \times 100$$

in which $T_{10}^{\text{drug+irritant}}$ is the T_{10} after a drug in combination with PGE_2 or capsaicin, T_{10}^{irritant} is the T_{10} after PGE_2 or capsaicin alone, and T_{10}^{baseline} is the T_{10} under control conditions.

Operant response rates for the last cycle were converted to percentage of vehicle control by using the average rate from the previous training day as the control value (i.e., average of 3 cycles). ED_{50} values and 95% confidence limits for both decreases in

operant responding and reversal of thermal hypersensitivity were calculated by linear regression when at least three data points were available on the linear portion of the dose-effect curve or by interpolation when two data points (one above and one below 50%) were available. ED_{50} values were typically not calculated when effects did not reach a magnitude of at least 50%.

Drugs. Memantine and dizocilpine (MK-801) were purchased from RBI (Natick, MA). Amitriptyline, prostaglandin E_2 and ifenprodil were purchased from Sigma (St. Louis, MO). Selfotel (CGS-19755), L-701324 and (R,S) CPP were purchased from Tocris (Ellisville, MO). Morphine was purchased from Mallinckrodt, Inc. (St. Louis, MO) and ketamine was purchased from J. A. Webster (Sterling, MA). Ifenprodil, and L-701324 were dissolved in 2% tween80 / 0.5% methylcellulose and sterile water. Low concentrations of perzinfotel were dissolved in sterile water, and concentrations for oral dosing higher than 10 mg/ml were dissolved in 2% tween80 / 0.5% methylcellulose and sterile water. All other compounds were dissolved in sterile water. Drug concentration doses were calculated using the molecular weight of the base form and were administered in a volume of 1 ml/kg with the dose administered calculated as mg/kg.

Results

Baseline thermal nociception and irritant-induced thermal hypersensitivity in the warm-water tail withdrawal assay. Throughout the course of these studies, rats typically left the tail in the water for the full 20 sec at low temperatures (42 and 46 °C), removed the tail after approximately 8 sec at intermediate temperatures (50 °C), and rapidly (within 4-5 sec) removed the tail at high temperatures (54 and 58 °C). Average baseline T_{10} values throughout these studies were generally between 49 and 51 °C (Figure 1; points above '0').

PGE_2 and capsaicin produced thermal hypersensitivities that were dose-dependent and transient. After the administration of either PGE_2 or capsaicin, maximal changes in the T_{10} were observed 15 min after injection (Figure 1). Thermal sensitivities returned to near control values by 120 min. To determine if weekly administration of PGE_2 modified baseline sensitivities or thermal hypersensitive to subsequent PGE_2 administration, a dose of 0.1 mg PGE_2 was administered weekly to eight rats. Over the course of this study, neither baseline thermal sensitivity (wk 1 = 49.5 ± 0.2; wk 2 = 49.4 ± 0.3; wk 3 = 49.7 ± 0.2) nor PGE_2-induced thermal hypersensitivity (wk 1 = 44.3 ± 0.3; wk 2 = 44.6 ± 0.1; wk 3 = 44.0 ± 0.3) evaluated 30 min after PGE_2 administration were significantly altered.

Based on these results, drugs were assessed 30 min after the administration of 0.1 mg PGE_2 or 0.01 mg capsaicin. Figure 2 shows the baseline temperature effect curve at this time (the dotted line at the 10 sec latency represents the T_{10} value). The ID administration 0.1 mg PGE_2 or 0.01 mg capsaicin into the distal end of the tail shifted the temperature effect curve to the left and produced a decrease in the T_{10}. Thirty min after administration, rats removed the tail from temperatures of water that were previously

innocuous (34 – 46 °C) and rapidly removed the tail from temperatures of water that had previously been partially noxious (50 °C). A dose of 0.1 mg PGE_2 produced a 4.5 °C decrease in the T_{10}, whereas a dose of 0.01 mg capsaicin produced a larger 8.4 °C decrease in the T_{10}.

Effects of perzinfotel on baseline thermal sensitivity and chemically-induced thermal hypersensitivity. The antinociceptive effects of perzinfotel and morphine were assessed in a warm-water tail withdrawal assay in rats. The *mu*-opioid receptor agonist morphine administered IP dose-dependently and significantly increased tail-withdrawal latencies and increased the T_{10} at doses greater than 1.0 mg/kg (Figure 3). In contrast, perzinfotel did not modify tail-withdrawal latencies or increase the T_{10} value up to a dose of 30 mg/kg IP.

To determine if perzinfotel had effects under conditions of hypersensitivity, such as those that might be produced under inflammatory conditions, perzinfotel was tested after the administration of PGE_2. A dose of 10 mg/kg IP perzinfotel administered 30 min before PGE_2 significantly blocked PGE_2-induced thermal hypersensitivity over the 120 min test (Figure 4). The potency of perzinfotel was not modified during daily IP treatment. Following 1 week of daily administration of 10 mg/kg, perzinfotel was slightly more effective for blocking thermal hypersensitivity. This effect was sustained after a second week of daily dosing.

Morphine dose-dependently blocked PGE_2-induced thermal hypersensitivity. Doses higher than 0.3 mg significantly blocked thermal hypersensitivity and a dose of 3 mg/kg fully blocked hypersensitivity (Figure 5; left panel). The ED_{50} for morphine in this assay is shown in Table 1. Similar to morphine, perzinfotel dose-dependently

blocked PGE_2-induced thermal hypersensitivity. A dose of 3 mg/kg perzinfotel IP administered 30 min before PGE_2 significantly blocked PGE_2-induced hypersensitivity. A higher dose of 10 mg/kg IP blocked PGE_2-induced thermal hypersensitivity by 79%. Perzinfotel was also effective after oral administration with significant blockade observed after 30 mg/kg and a 62% reversal observed after 100 mg/kg. Based on ED_{50} values, perzinfotel was 22-fold less potent after oral administration than after IP administration (Table 1). Perzinfotel was also effective after IT administration. Concentrations of 0.3, 1 and 3 nM reversed PGE_2-induced thermal hypersensitivity by 34.8 ± 4.4, 56.3 ± 6.9 and 61.1 ± 5.5 percent, respectively. The calculated ED_{50} for IT perzinfotel was 0.90 (95% CL 0.49 – 1.65) nM

Similar to their effects in the PGE_2 assay, morphine and perzinfotel dose-dependently blocked capsaicin-induced thermal hypersensitivity. The potency of morphine in the capsaicin assay (ED_{50} = 1.43; 95% CL 1.14 – 1.79) was similar to its potency in the PGE_2 assay. Doses of perzinfotel higher than 1 mg/kg IP significantly blocked capsaicin-induced hypersensitivity and a dose of 10 mg/kg produced a 77% reversal. After PO administration, all doses of perzinfotel (10-100 mg/kg) significantly blocked capsaicin-induced thermal hypersensitivity. Perzinfotel was 6.6-fold less potent after PO administration (ED_{50} = 31.0; 95% CL 23.3 – 41.2) compared to IP administration (ED_{50} = 4.7; 95% CL 3.67 – 6.1).

Effects of other NMDA receptor antagonists on PGE_2-induced thermal hypersensitivity. Few other glutamate site NMDA receptor antagonists blocked PGE_2-induced thermal hypersensitivity to a similar magnitude as perzinfotel. Doses of 1 and 3 mg/kg CPP significantly blocked thermal hypersensitivity (Figure 6). A dose of 10

mg/kg CPP produced a 56% reversal, however, a higher dose of 30 mg/kg did not substantially block hypersensitivity further (57%). Selfotel significantly blocked PGE_2-induced hypersensitivity at doses of 10 and 30 mg/kg, however, this effect was not greater than a 25% reversal. Similarly, a dose of 10 mg/kg CGP-39653 only produced a 23% reversal.

The ability to block PGE_2-induced thermal hypersensitivity was not common to all negative modulators of the NMDA receptor. For example, none of the NMDA receptor channel blockers, including memantine, ketamine or dizocilpine, produced greater than a 20% reversal of PGE_2-induced thermal hypersensitivity (Figure 7) up to doses that produced observable signs of sedation and ataxia. Similarly, the NR2B selective NMDA receptor subtype selective antagonist ifenprodil produced a maximal 16% reversal up to doses that produced observable signs of sedation and ataxia. In contrast, the glycine site antagonist L-701324 did significantly block PGE_2-induced thermal hypersensitivity at doses of 3 and 10 mg/kg IP doses. However, the highest dose of 10 mg/kg L-701324 was also associated with observable signs of sedation and ataxia.

Effects of NMDA receptor antagonists on schedule-controlled responding. To quantify drug-induced behavioral disruptions, compounds were also evaluated for their ability to suppress operant rates of responding. In rats responding under a FR30 schedule of food reinforcement, doses of morphine greater than 1 mg/kg significantly decreased response rates (Figure 8; left panel). An IP dose of 3 mg/kg perzinfotel did not modify rates of responding. Larger IP doses of 10 and 30 mg/kg significantly decreased response rates. When administered PO, doses of perzinfotel up to 178 mg/kg did not modify rates of responding. A larger dose of 300 mg/kg decreased rates of responding by

53% of normal control response rates. Rate-decreasing effects of high doses of perzinfotel produced similar levels of rate suppression over the duration of the 1 hr study. For example, 300 mg/kg PO perzinfotel decrease response rates to 55.6 ± 17.3, 50.3 ± 17.2 and 46.7 ± 17.0 percent of control during the first, second and third cycle, respectively. Similarly, a dose of 30 mg/kg IP perzinfotel decreased response rates to 48.2 ± 17.2, 46.8 ± 20.6 and 47.4 ± 20.6 percent of control during the first, second and third cycle, respectively.

All of the NMDA receptor antagonists evaluated dose-dependently decreased response rates (Figure 8; right panel). Dizocilpine was the most potent NMDA receptor antagonist for decreasing rates of responding. Other NMDA receptor antagonists decreased response rates over a similar dose range (3-30 mg/kg) and had similar ED_{50} values (between 5.8 and 23.5 mg/kg). Table 1 shows the ED_{50} values of compounds for reversing PGE_2-induced thermal hypersensitivity, the ED_{50} values of compounds for decreasing operant rates of responding and the respective dose ratio for these two effects. Perzinfotel had the largest dose ratios by both IP and PO routes of administration compared to all other compounds evaluated. Morphine had the next largest ratio followed by L-701324 and (R,S,) CPP. All other NMDA receptor antagonists evaluated suppressed rates of responding at doses that were ineffective for reversing PGE_2-induced thermal hypersensitivity. Thus, dose ratios for these compounds were less than one.

Discussion

The current study examined the ability of NMDA receptor antagonists to block irritant-induced thermal hypersensitivity. Consistent with its mechanism of action, perzinfotel lacked antinociceptive effects but did prevent chemical irritant-induced thermal hypersensitivity. The ratio to prevent PGE_2-induced thermal hypersensitivity verses decreases in response rates was larger for perzinfotel than for the other compounds evaluated. In comparison, other NMDA receptor antagonists evaluated significantly suppressed response rates at doses lower than those that modified PGE_2-induced thermal hypersensitivity. This study demonstrates that perzinfotel has a therapeutic ratio for effectiveness versus nonspecific effects superior to that observed with the NMDA receptor antagonists evaluated.

Thermal hypersensitivity produced by chemical irritants. PGE_2 is a metabolite of the arachidonic acid cascade and its effects are mediated by endoperoxide receptors located on primary afferent nociceptors. Agonist stimulation of these receptors activates cAMP-dependent protein kinase, which produces a number of secondary events including enhancement of Ca^{2+} and Na_V currents (Bley et al., 1998). Capsaicin sensitizes primary afferent nociceptors through agonst actions at TRPVR1 (Szallasi and Blumberg, 1999). In the current study, both of these irritants produced hyperalgesia (increased sensitivity to noxious temperatures) and allodynia (increased sensitivity to non-noxious temperatures). These effects of PGE_2 and capsaicin in rodents extends previous findings in rhesus monkeys (Negus et al., 1993; Brandt et al., 2001) and in humans (Sciberras et al., 1987; LaMotte et al., 1991). Given the similarities between the dose range and temperature responses in preclinical and clinical studies, these results suggest that

capsaicin- and PGE_2-induced thermal hypersensitivity could be useful surrogate endpoints when assessing novel compounds having potential clinical utility.

Effects of perzinfotel and other NMDA receptor antagonists on chemically-induced thermal hypersensitivity. In the present study, perzinfotel lacked antinociceptive effects under conditions where morphine increased tail-withdrawal latencies in normal animals. These results are consistent with the lack of antinociceptive effects observed with other compounds of this class (Dickenson et al., 1997; Lutfy et al., 1997), and indicate that perzinfotel does not modify normal pain sensitivity.

Perzinfotel dose-dependently blocked PGE_2-induced thermal hypersensitivity after IP and PO administration. However, the ability to block PGE_2-induced thermal hypersensitivity was not common to all NMDA receptor antagonists. For example, the un-competitive NMDA receptor antagonists memantine, ketamine and dizocilpine (MK-801), the NR2B-subunit selective antagonist ifenprodil, and even other competitive glutamate site antagonists (selfotel and CGP-39653) were only minimally effective for preventing PGE_2-induced thermal hypersensitivity. It is noteworthy that at doses that produced sedation, ataxia and impairment of operant responding, animals still were able to remove their tails from warm water indicating that animals could still perceive and respond to nociceptive stimuli. Although, some studies have demonstrated that ketamine or dizocilpine can decrease inflammatory pain after intrathecal administered (Ren et al., 1992; Klimscha et al., 1998), the current results indicate that dose-limiting adverse effects associated with systemic administration of these compounds impedes observations of therapeutic efficacy, results consistent with other studies in both rodents and humans (Boyce et al., 1999; Sang, 2000).

Effects of perzinfotel and other NMDA receptor antagonists on operant responding. Doses of perzinfotel that fully blocked PGE_2-induced thermal hypersensitivity were not associated with disruptions in other behaviors. For example, doses of perzinfotel that decreased operant responding by 50% were 5- to 10-fold higher than doses that produced a 50% blockage of PGE_2-induced thermal hypersensitivity. In contrast, other negative modulators of the NMDA receptor evaluated decreased rates of responding and elicited observable signs of sedation and ataxia at doses that lacked any antihyperalgesic effects. L-701324 and (R,S) CPP were the exceptions and did produce greater than a 50% reversal of PGE_2 at doses lower than those that decreased rates of responding; however, the separation between alleviating thermal hypersensitivity and decreasing response rates was small. Phamacokinetic studies with perzinfotel demonstrate rapid absorption and elimination after both PO and IP routes of administration. Consistent with peak concentrations, the antihyperalgesic effects of perzinfotel in the PGE_2 assay are maximal 1 hr after PO administration and wane by 5 hr after administration (unpublished observation). In the current study, rate-decreasing effects of high doses of perzinfotel produced similar levels of rate suppression over the duration of the 1 hr study. These results suggest that the behavioral effects of perzinfotel were not fluctuating at the time of testing and that the behavioral assessments were close to the peak plasma concentrations.

Pharmacology of perzinfotel. Perzinfotel is a selective, competitive NMDA receptor antagonist with high affinity (30 nM) for the glutamate site (Kinney et al., 1998). Perzinfotel lacks activity at more than 60 other receptors, ion channels or uptake sites (Childers et al., 2002; Sun et al., 2004). In vitro, perzinfotel blocks NMDA-induced

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currents with an IC_{50} of 0.48 μM and glutamate-induced neurotoxicity with an IC_{50} of 1.6 μM. In contrast, perzinfotel does not have appreciable affinity (up to a concentration of 100 μM) for kainate, AMPA and strychnine-insensitive glycine receptors and does not block AMPA- or kainate-induced neurotoxicity (up to a concentration of 1mM). To date, the activity of perzinfotel is consistent with actions at the NMDA receptor.

The mechanisms for the unique profile of perzinfotel in the current study are being investigated. One possibility is that perzinfotel does not readily cross the blood brain barrier (BBB), thereby precluding centrally mediated adverse effects typical of other NMDA receptor antagonists. Previous studies have demonstrated that peripheral NMDA receptors are involved in heat hypersensitivity associated with inflammation and that this hypersensitivity can be blocked by the local administration of a NMDA receptor antagonist (Davidson et al., 1997; Davidson and Carlton, 1998; Du et al., 2003). Thus, perzinfotel might be blocking peripheral nociceptor input and subsequent central sensitization. However, other data is not fully consistent with this notion. In vivo, systemic administration of perzinfotel blocks lethality induced by an ICV ED_{90} dose of NMDA (ED_{50} = 2.1 mg/kg; IP) and blocks maximal electroshock convulsions (ED_{50} = 4.8 mg/kg; IP) in mice (Kinney et al., 1998; Childers et al., 2002). Perzinfotel also reverses established tactile hypersensitivity produced by L5/L6 spinal nerve ligation and chronic constriction injury of the sciatic nerve (unpublished results); both neuropathic pain models thought to be strongly mediated by central mechanisms (Kawamata and Omote, 1996). Moreover, pharmacokinetic studies have shown that perzinfotel does cross the BBB, albeit weakly (unpublished results). Together with the IT efficacy in the

current study, a purely peripheral action of perzinfotel is not fully consistent with its known activity.

A second possibility for the unique activity of perzinfotel could be related to its actions at subpopulations of NMDA receptors. NMDA receptor complexes are comprised of NR1 subunits (of which there are 8 splice variants) and NR2 subunits (of which there are 4 subtypes, NR2A-D). In addition to having discrete localization, subunit composition imparts unique biophysical and pharmacologic properties (Sirinathsinghji and Hill, 2002). Compounds used in the current study have been shown to have different NMDA receptor subtypes selectivity. For example, L-701,324, ketamine, dizocilpine and memantine show little selectivity among the NR1/NR2 subunit combinations (Yamakura et al., 1993; Sucher et al., 1996; Parsons et al., 1999). Selfotel, CGP-39653 and CPP have slightly higher (2- to 3- fold) selectivity for NR1/NR2A subunits than for other NR2 subunits (Laurie and Seeburg, 1994; Christie et al., 2000). In oocytes expressing different NR1/NR2 subunits, perzinfotel was 8- to 13-fold more selective for the NR1/NR2A than either NR1/NR2B or NR1/NR2C (Sun et al., 2004). Thus, among the compounds evaluated in the current study, only perzinfotel was selective for the NR1/NR2A subunit.

Studies suggest that compounds having NR1/NR2B selectivity are more effective for alleviating pain and lack adverse effects compared to non-selective antagonists (Boyce et al., 1999; Chazot, 2004). For example, ifenprodil has 400-fold selectivity for NR1/NR2B over NR1/NR2A (Williams, 1993) and blocks both mechanical hypersensitivity associated with sciatic nerve ligation and carrageenan administration (Boyce et al., 1999). However, like the current study, these effects occurred over a

similar dose range as those that impaired rotarod performance (Boyce et al., 1999). Although ifenprodil has selectivity for NR1/NR2B, it also has activity at other non-NMDA receptors such as α_1-adrenoceptors (Chenard et al., 1995) and Ca^{2+} channels (Bath et al., 1996) that might contribute to the impairment of other behaviors. More selective NR1/NR2B compounds (e.g., CP-101606) might reverse PGE_2-induced thermal hypersensitivity at doses that are not associated with side effects. However, there are accumulating data suggesting that NR2A plays a significant role in inflammatory pain. For example, mRNA and protein levels of the NR2A subunit are upregulated to a greater extent than NR2B mRNA in the rostral ventromedial medulla (RVM) after carrageenan injection in the hindpaw of rats (Miki et al., 2002). In another study, formalin injected into the paw increased NR2A mRNA expression in the spinal cord (Gaunitz et al., 2002). However, upregulation of NR2A mRNA might be specific for inflammatory pain states since nerve injury decreased NR2A in the spinal cord (Karlsson et al., 2002). Taken together, the current study indicates that NR2B selectivity is not the only avenue for improving the adverse effect profile of NMDA antagonists; selectivity for other NMDA receptor subunits might also be important for identifying compounds with therapeutic potential.

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Figure legends

Figure 1. Thermal hypersensitivity produced by chemical irritants. Abscissa: time in minutes after PGE_2 (left panel) or capsaicin (right panel) injection. Ordinate: temperature of water in degrees Celsius that produced 10-second tail-withdrawal latency. All points show mean (± 1 SEM) data from 8 rats. Asterisk indicates significant ($p<0.05$) differences in the chemical irritant treated T_{10} value from the baseline "0" T_{10} value.

Figure 2. Temperature effect curves before and after chemical irritants in the warm-water tail withdrawal assay. Abscissa: Temperature in degrees Celsius. Ordinate: Latency in sec for rat to remove its tail from water. All points show mean (± 1 SEM) data from 8 rats except 'Baseline,' which is the mean (± 1 SEM) data from the 16 rats.

Figure 3. Effects of perzinfotel and morphine in the warm-water tail withdrawal assay. Abscissa: Dose of drug in milligrams per kilogram administered IP. Ordinate: temperature of water in degrees Celsius that produced 10-second tail-withdrawal latency. All points show mean (± 1 SEM) data from 8 rats. Asterisk indicates significant ($p<0.05$) differences from the 'Sal' T_{10} value.

Figure 4. Thermal hypersensitivity produced by PGE_2 and reversal by perzinfotel. Abscissa: time in minutes following 0.1 mg PGE_2 injection. Ordinate: temperature of water in degrees Celsius that produced 10-second tail-withdrawal latency. One week following baseline evaluation of hypersensitivity produced by PGE_2, 10 mg/kg

perzinfotel was administered IP ('Perzinfotel'). Animals were then treated daily with 10 mg/kg perzinfotel with redeterminations of the effects of PGE_2 determined after 1wk of daily dosing ('Perzinfotel 1wk') and again after 2 wk of daily dosing (Perzinfotel 2wk'). Perzinfotel was administered 30 min before PGE_2. All points show mean (± 1 SEM) data from 8 rats. Asterisk indicates significant ($p<0.05$) differences in the drug treated T_{10} value from the baseline capsaicin alone T_{10} value.

Figure 5. Effects of perzinfotel or morphine administered under conditions of chemically induced thermal hypersensitivity. Abscissa: Dose of drug in milligrams per kilogram administered IP or PO. Ordinate: Percent reversal of thermal hypersensitivity. Perzinfotel or morphine was administered 30 min before 0.1 mg PGE_2 (left panel) or 0.1 mg capsaicin (right panel), which was administered 30 min before the test. Each point shows mean (± 1 SEM) data from 8 rats. Asterisk indicates significant ($p<0.05$) differences in the drug treated T_{10} value from the baseline PGE_2 or capsaicin alone T_{10} value.

Figure 6. Effects of other competitive glutamate site NMDA receptor antagonists under conditions of thermal hypersensitivity produced by PGE_2. Abscissa: dose of drug in milligrams per kilogram. Ordinate: Percent reversal of thermal hypersensitivity. PGE_2 (0.1 mg) was injected 30 minutes following drug administration, and the temperature-effect curves were determined 30 minutes later. Each point shows mean (± 1 SEM) data from 8 rats. Asterisk indicates significant ($p<0.05$) differences in the drug treated T_{10} value from the baseline PGE_2 alone T_{10} value.

Figure 7. Effects of other inhibitors of the NMDA receptor complex under conditions of thermal hypersensitivity produced by PGE_2. Abscissa: dose of drug in milligrams per kilogram. Ordinate: Percent reversal of thermal hypersensitivity. PGE_2 (0.1 mg) was injected 30 minutes following drug administration, and the temperature-effect curves were determined 30 minutes later. Each point shows mean (± 1 SEM) data from 8 rats. Asterisk indicates significant ($p<0.05$) differences in the drug treated T_{10} value from the baseline PGE_2 alone T_{10} value.

Figure 8. Effects of drugs on operant rates of responding. Abscissa: dose of drug in milligrams per kilogram. Ordinate: mean rates of responding on the third cycle presented as a percentage of control response rates. Each point shows mean (± 1 SEM) data from 4-10 rats. Asterisk indicates significant ($p<0.05$) differences in the drug treated rate of responding value from the control rate of responding value.

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Table 1

Mean ED_{50} values in milligrams per kilogram (± 95% confidence limits) of test drugs to block PGE_2-induced thermal hypersensitivity, to decrease operant rates of responding and the dose ratio of these effects.

Test Drug	PGE_2[a]	Response Rates[b]	Dose Ratio
Perzinfotel (IP)	2.5 (1.9 – 3.4)	26.8 (13.7 – 52.5)	10.7
Perzinfotel (PO)	55.5 (32.58 – 94.5)	296.9 (215.5 – 409.0)	5.3
Morphine (IP)	1.00 (0.98 – 1.02)	3.8 (1.7 – 8.3)	3.8
L-701324 (IP)	3.7 (2.8 – 4.8)	8.8 (4.4 – 17.7)	2.4
(R,S) CPP (IP)	10.5 (5.8 – 18.9)	22.8 (11.8 – 43.7)	2.2
Ketamine (IP)	N.A. (>17.8)	14.1 (10.1 – 19.7)	< 0.8
Memantine (IP)	N.A. (>10.0)	6.8 (6.3 – 7.5)	< 0.7
CGP-39653 (IP)	N.A. (>10)	6.1 (4.4 – 8.6)	< 0.6
Dizocilpine (IP)	N.A. (>0.3)	0.12 (0.09 – 0.16)	< 0.4
Ifenprodil (IP)	N.A. (>30.0)	12.4 (10.4 – 14.7)	< 0.4
CGS-19755 (IP)	N.A. (>30.0)	5.8 (4.5 – 7.3)	< 0.2

[a] PGE_2 values are mean data from 7-8 rats.

[b] Response Rates values are mean data from 4 – 10 rats.

N.A., not active up to highest dose tested (> highest dose tested in mg/kg)

Figure 1

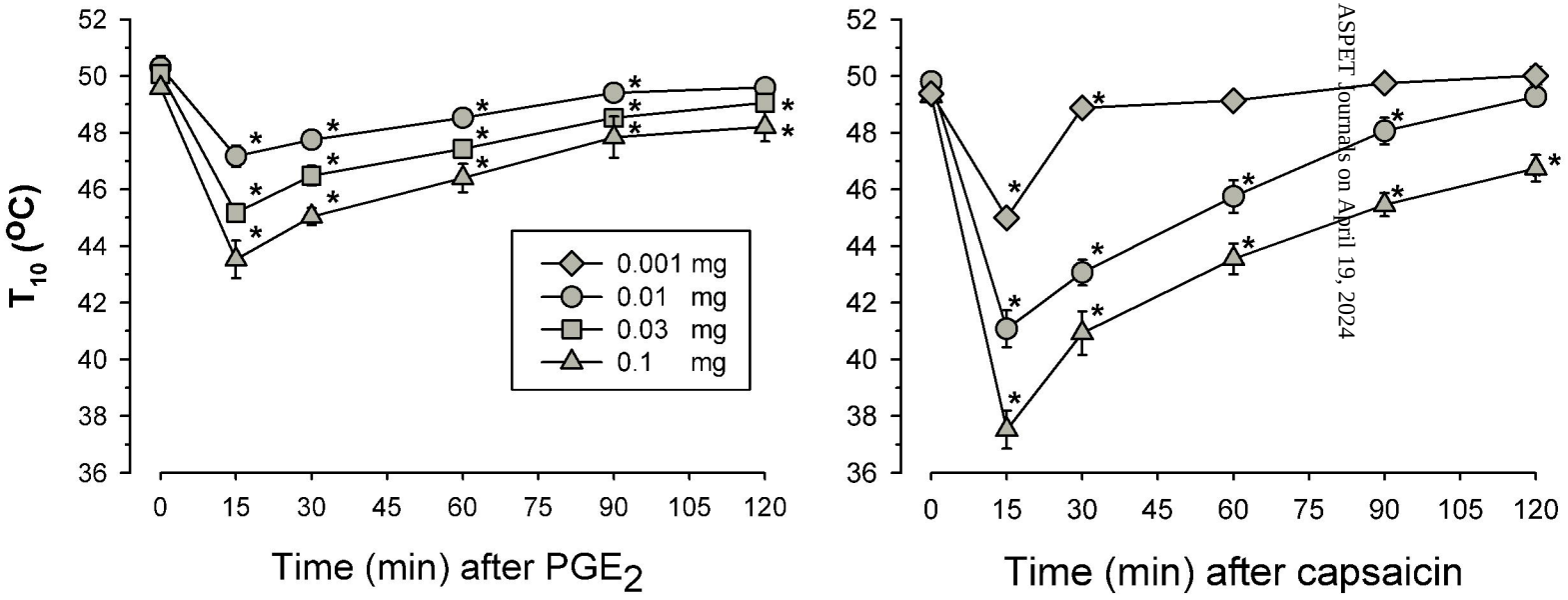

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Figure 2

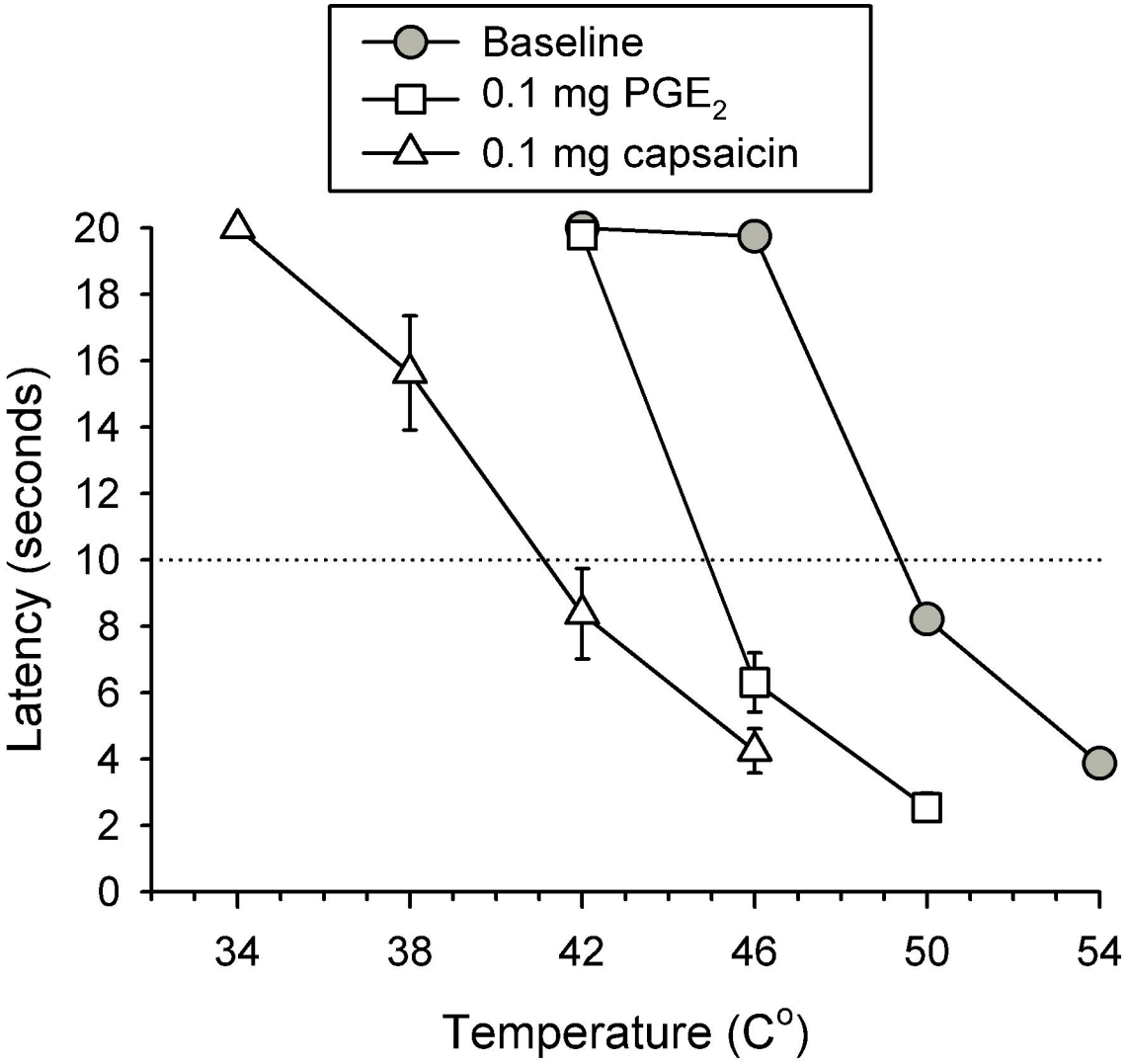

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Figure 3

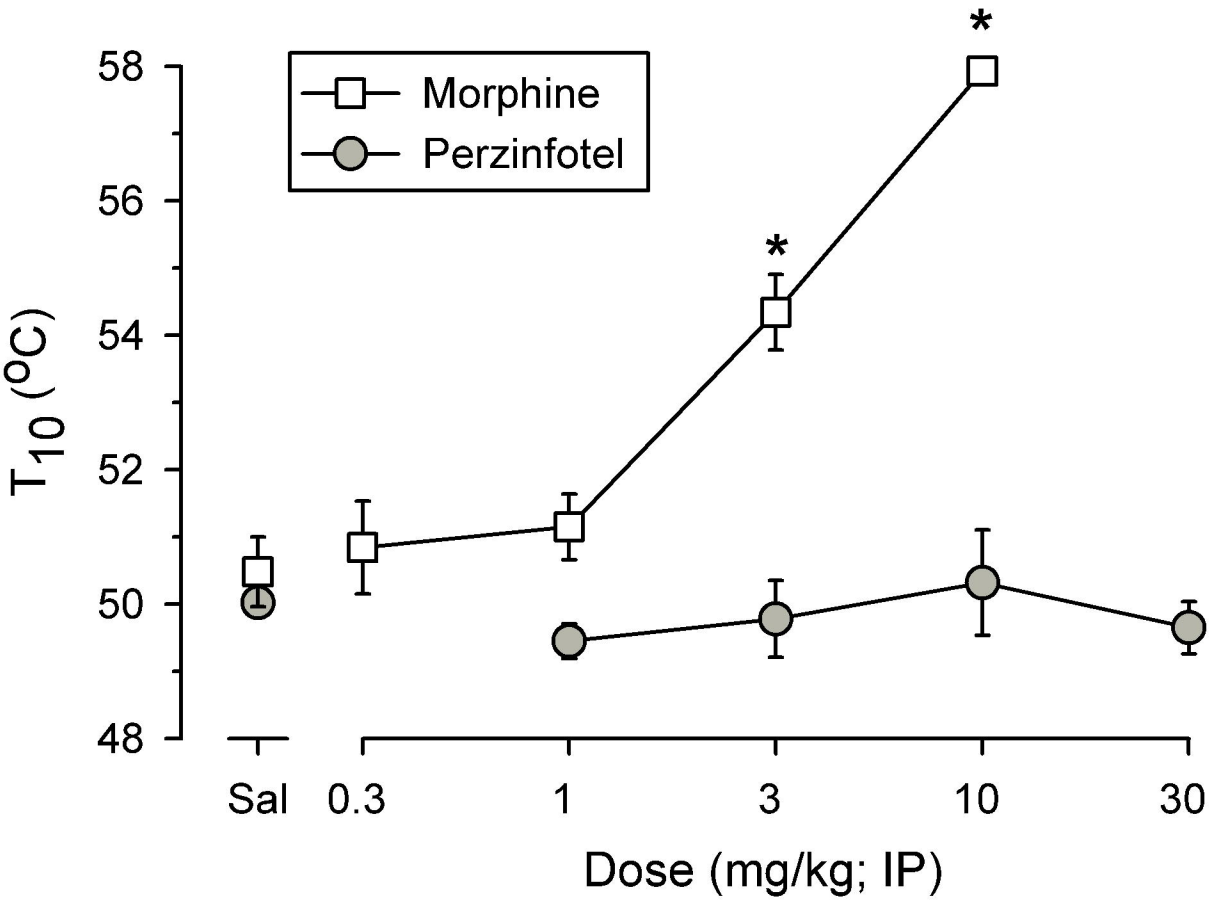

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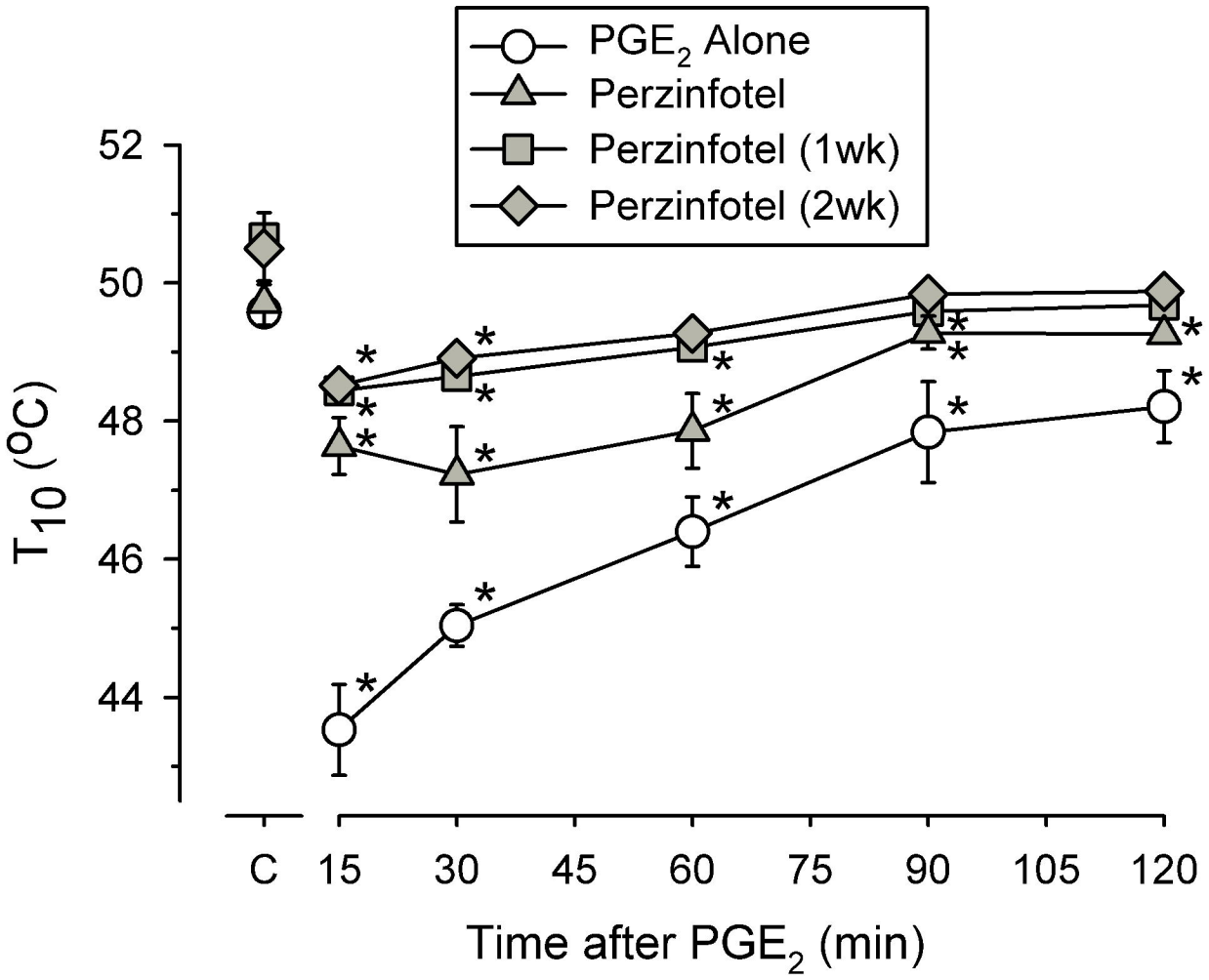
Figure 4



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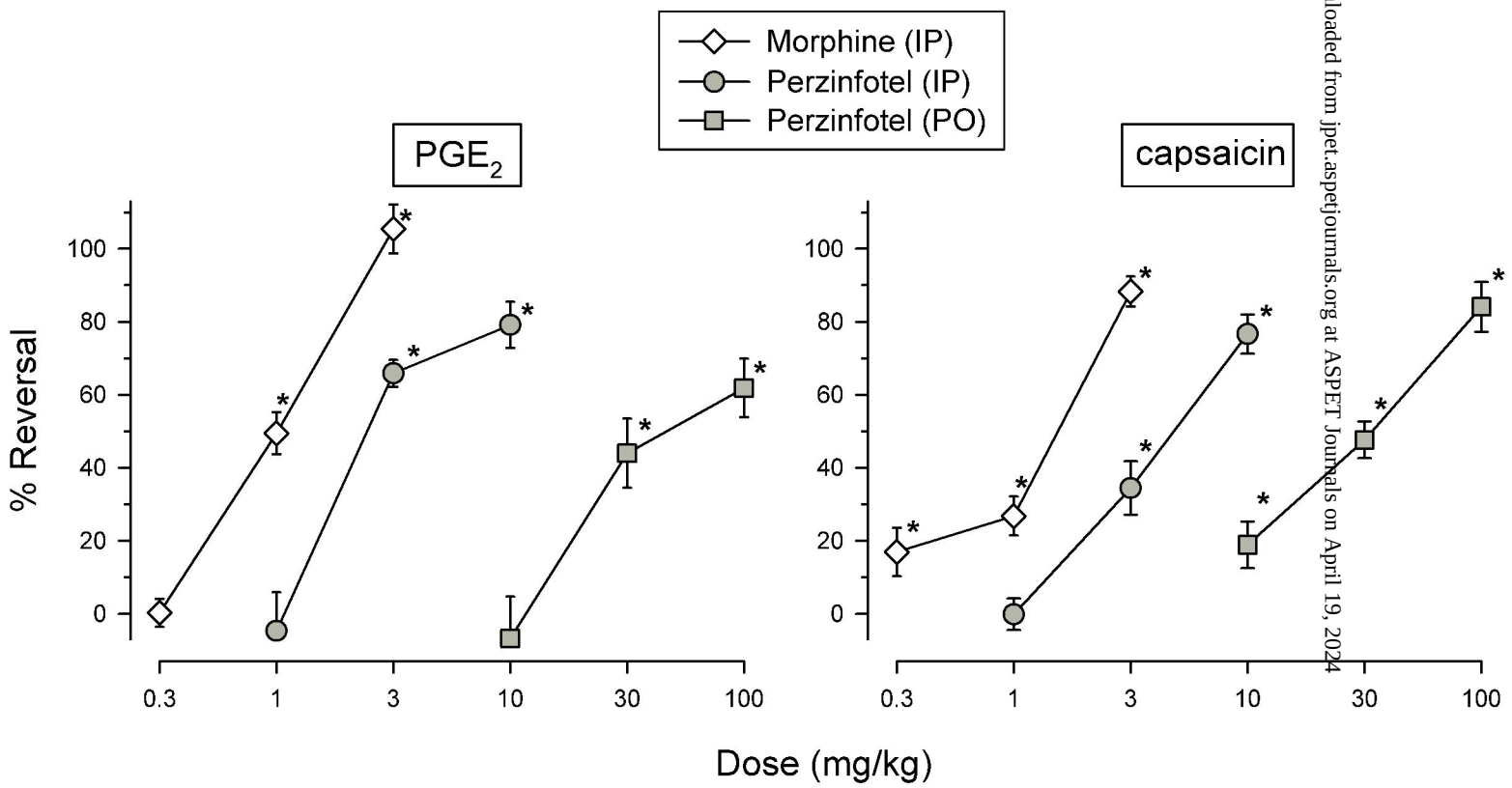

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Figure 6

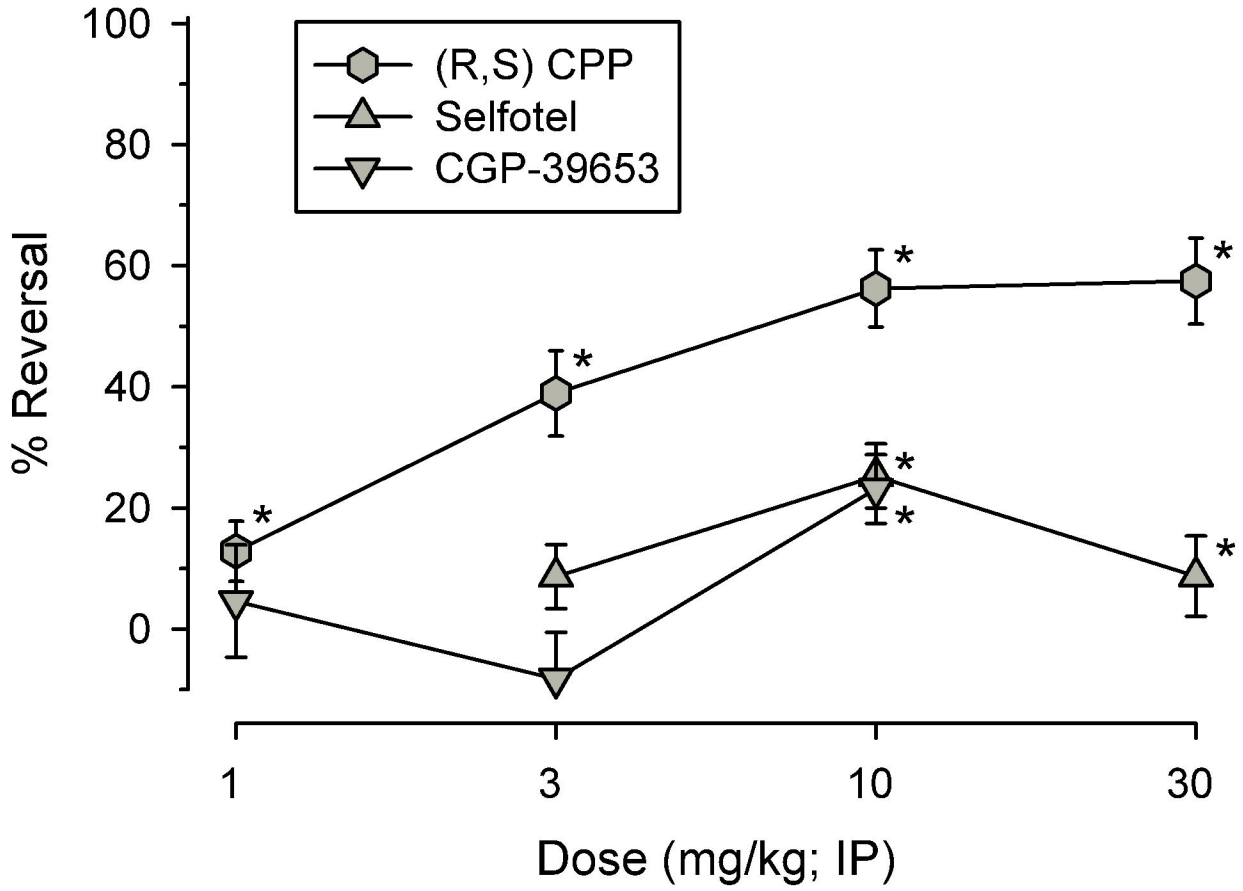

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Figure 7

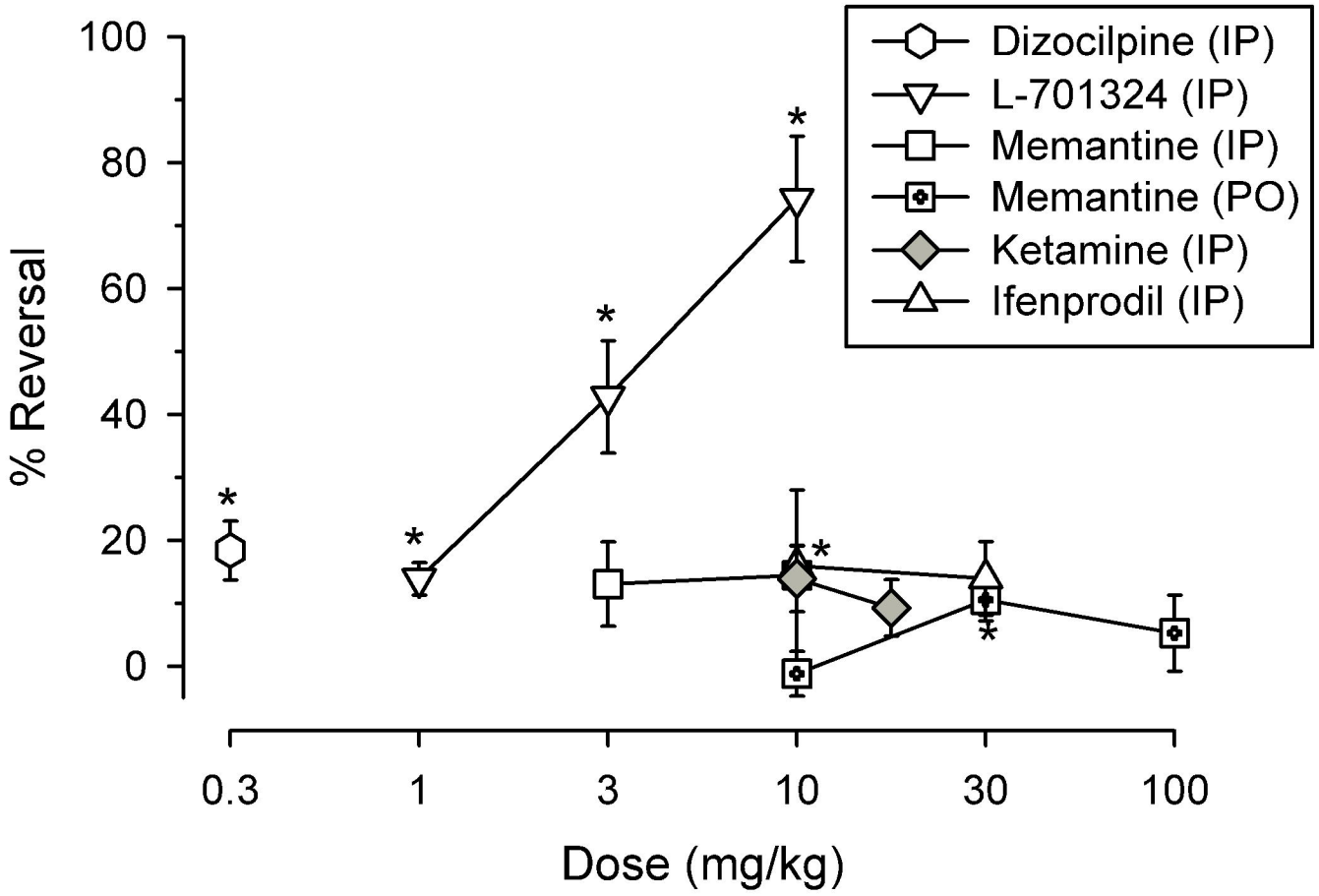

Figure 8