Title: Anti-hyperalgesic effects of bupropion, a dopamine and noradrenaline reuptake inhibitor, in a rat model of neuropathic pain

Background: Antidepressants are often used for the treatment of neuropathic pain, and their analgesic effects rely on increased noradrenaline and serotonin levels in the spinal cord. Clinical studies have also shown that bupropion, a dopamine and noradrenaline reuptake inhibitor, has strong efficacy in neuropathic pain; however, the role of spinal cord dopamine in neuropathic pain is unknown. In the present study, we determined the efficacy and underlying mechanisms of intrathecal administration of bupropion in a rat model of neuropathic pain.

Methods: Male Sprague-Dawley rats underwent spinal nerve ligation (SNL), and mechanical hyperalgesia was determined by measuring the withdrawal threshold to paw pressure. In vivo microdialysis was performed in the dorsal horn of the lumbar spinal cord to measure noradrenaline and dopamine concentrations after intrathecal injection of bupropion. We also measured the noradrenaline and dopamine contents in the ipsilateral dorsal lumbar spinal cord in normal rats and in rats 2, 3, and 4 weeks after SNL.

Results: Intrathecal injection of bupropion produced a dose-dependent anti-hyperalgesic effect (3–100 μg). The effect (30 μg) was dose-dependently reversed by intrathecal pretreatment with the α2-adrenoceptor antagonist idazoxan (3–30 μg) and D2-receptor antagonist sulpiride (3–30 μg). Microdialysis revealed that noradrenaline and dopamine concentrations in the spinal dorsal horn were increased after intrathecal injection of bupropion (30 μg). Furthermore, the noradrenaline and dopamine contents in the spinal dorsal horn were increased 2 weeks after SNL and then decreased gradually.

Conclusions: These findings suggest that plasticity of descending inhibition pathways

such as the noradrenaline and dopamine systems contributes to the maintenance of neuropathic pain and that spinal cord noradrenaline and dopamine both play an inhibitory role in neuropathic pain.

Key words: Bupropion, Antidepressants, Neuropathic pain, Noradrenaline, Dopamine

Introduction

Peripheral nerve injury leads to neuropathic pain, which is associated with various changes in sensory processing from the primary afferent neurons to the spinal cord and on to supraspinal and cortical regions. Brainstem-spinal descending inhibitory pathways, including noradrenergic neurons, suppress nociceptive signals from primary afferent neurons to the spinal dorsal horn.[1] Antidepressants such as tricyclic antidepressants and serotonin-noradrenaline reuptake inhibitors are thus recommended as first-line drugs for the treatment of neuropathic pain,[2] as a recent study showed that increased noradrenaline levels in the spinal cord underlie the therapeutic effect of antidepressants in neuropathic pain.[3]

Dopamine also plays a crucial role in nociceptive transmission in the central nervous system. In the brain, animal studies have shown that the dopaminergic system is involved in pain modulation,[4-6] and that dopamine receptor agonists predominantly suppress pain related responses[7] via dopamine D2 receptors.[8] Furthermore, focal electrical stimulation of the A11 area in the brain suppresses the nociceptive responses of spinal dorsal horn neurons.[9] These findings suggest that activation of descending dopaminergic pathways and subsequent release of dopamine in the spinal cord play an important role in the control of nociceptive transmission. However, the role of spinal cord dopamine in neuropathic pain is not clear.

Bupropion is a noradrenaline and dopamine reuptake inhibitor that has been reported to show strong efficacy against neuropathic pain.[10] We hypothesized that bupropion suppresses neuropathic pain by increasing noradrenaline and dopamine levels in the spinal cord. To test this hypothesis, we examined the efficacy and mechanisms of anti-hyperalgesic effects of intrathecally administered bupropion using a rat model of

neuropathic pain produced by spinal nerve ligation (SNL). Because previous studies have suggested that the descending noradrenergic system shows plastic changes after nerve injury,[3,12,13] we also examined the change of noradrenaline and dopamine levels in the lumbar spinal cord over time after SNL.

Materials and Methods

Surgical preparation

Adult male Sprague-Dawley rats (250 g) were used. The animals were housed on soft bedding in a temperature-controlled environment under a 12-h light-dark cycle with free access to food and water. The animals were allowed to habituate to the housing facilities prior to surgery or behavioral testing. The experiments were approved by the Animal Care and Use Committee of the Gunma University Graduate School of Medicine.

SNL was performed as previously described.[14] In brief, animals were anesthetized with isoflurane (2%) in oxygen, and the right L5 spinal nerve was tightly ligated with 5-0 silk and cut just distal to the ligature. The wound was then closed. Ten days after SNL surgery, an intrathecal catheter was inserted for drug administration. A sterilized 32-gauge polyethylene catheter (RecathCo, Allison Park, PA) connected to 8.5 cm of Tygon external tubing (Saint-Gobain Performance Plastics, Akron, OH) was inserted through the cisterna magna while the rat was under isoflurane anesthesia as previously described.[15] The catheter was passed caudally 7.5-8.0 cm from the cisterna magna to the lumbar enlargement. The animals were allowed to recover for 7 days before drug testing.

Behavioral testing

The withdrawal threshold to pressure applied to the hind paw, expressed in grams, was measured using an analgesimeter (Ugo Basile, Comerio, Italy) as previously described.[16] In brief, the analgesimeter device is used to apply increasing pressure to the hind paw. When the animal withdraws its paw, the pressure is immediately released, and

the withdrawal threshold is output in grams. Animal training for this test was performed three times before the drug treatment, and a cut-off of 250 g was used to avoid tissue injury. We tested the animals two or three times at 2- to 3-day intervals. The experimenter performing the behavioral test was blinded to the drug treatment and dose.

Drug administration

The first series of experiments was performed to examine the time course and dose response of the anti-hyperalgesic effects of intrathecally administered bupropion (0, 3, 10, 30, and 100 μg). The withdrawal thresholds were determined before (before SNL surgery) and at 0 (before drug injection), 15, 30, and 60 min after injection, then at 60-min intervals until 480 min after injection. The second series of experiments was performed to determine the effects of intrathecal pre-treatment with the α2-adrenoceptor antagonist, idazoxan, or the dopamine D2-receptor antagonist, sulpiride. Each antagonist (0, 3, 10, and 30 μg) was administered intrathecally 15 min before bupropion (30 μg) injection. For intrathecal injection, bupropion and idazoxan were dissolved in saline, and sulpiride was dissolved in a mixture of 67% dimethylsulfoxide (DMSO) and 33% saline. The drugs were injected intrathecally in a volume of 5 μL, followed by a 15-μL injection of saline to flush the catheter. Bupropion and idazoxan were purchased from Sigma (St. Louis, MO), and sulpiride was purchased from Tocris (Ellisville, MO).

Microdialysis

Microdialysis was performed as previously described.[17] Anesthesia was induced with 3% isoflurane and maintained with 1.5% isoflurane in 100% oxygen through a nose cone. The left femoral vein was cannulated for fluid infusion. The rectal temperature

was maintained at 37°C to 38°C with a heating pad placed beneath the animal. The L3 to L6 level of the right spinal cord was exposed by thoracolumbar laminectomy, and then the rat was placed in a stereotaxic apparatus. After the dura was punctured with a 30 gauge needle, the microdialysis probe (OD = 0.22 mm, ID = 0.20 mm, length = 1 mm; A-I-8-01; Eicom Co., Kyoto, Japan) was inserted from just lateral to the dorsal root and advanced at an angle of 15-30° to a depth of 1 mm using a micromanipulator (model WR-88; Narishige, Tokyo, Japan). The microdialysis probe was perfused with Ringer's solution (147 mmol/L NaCl, 4 mmol/L KCl, and 2.3 mmol/L $CaCl_2$) at a constant flow rate (1 μL/min) using a microsyringe pump (ESP-64; Eicom Co.). After 120 minutes of constant perfusion, two consecutive samples were collected to determine basal noradrenaline and dopamine concentrations in the dialysate. The effective dose of bupropion (30 μg) or saline (5 μL) was administered through the intrathecal catheter, and 15-min perfusate fractions were collected into an auto injector (EAS-20; Eicom Co.). The noradrenaline and dopamine concentrations in the perfusate were analyzed using high-performance liquid chromatography (HPLC) with electrochemical detection using an HTEC-500 analyzing system (Eicom Co.). The chromatographic conditions were as follows: The mobile phase comprised 0.1 mol/L ammonium acetate buffer (pH 6.0), 0.05 mol/L sodium sulfonate in methanol (7:3 vol/vol), and 50 mg/L Na2-EDTA, and the column was an EICOMPAC CAX (2.0 mm × 200 mm; Eicom Co.). The working electrode was glassy carbon (WE-3G, Eicom Co.) with a flow rate of 0.25 ml/min. The detector voltage was set to 0.45 V. The detector temperature was set to 35.0°C. The retention time for noradrenaline and dopamine was 5.4 min and 7.1 min, respectively.

Noradrenaline and dopamine contents in the spinal cord

We also measured the noradrenaline and dopamine contents in the spinal dorsal horn in normal and SNL rats at 2, 3, and 4 weeks after SNL surgery, as previously described.[18] To isolate the dorsal horn of the spinal cord, the portion corresponding to segments L4–L6 was divided into four constituent quadrants: dorsal right, dorsal left, ventral right, and ventral left. The dorsal right (ligation side) portion of the spinal cord was weighed and homogenized in 500 µL of 0.2 mol/L perchloric acid containing 0.1 mmol/L Na_2-EDTA and isoproterenol (0.02 mg/mL) as an internal standard, and centrifuged at 20,000g at 0°C for 15 minutes. The supernatants were adjusted to pH 3.0 by adding 1 mol/L sodium acetate and then filtered through a centrifugal filter with a pore size of 0.45 µm (Millipore, Bedford, MA). Samples (10 µL) were injected into an HTEC-500 analyzing system (Eicom Co.) and the concentrations of noradrenaline and dopamine were analyzed using HPLC with electrochemical detection. The chromatographic conditions were as follows: The mobile phase comprised 0.1 mol/L phosphate buffer (pH 6.0) containing 5 mg/L Na_2-EDTA, 190 mg/L sodium 1-octanesulfate acid, and 17% methanol, and the column was an EICOMPAK SC-5ODS (3.0 × 150 mm, Eicom Co.). The working electrode was glassy carbon (WE-3G, Eicom) with a flow rate of 0.5 mL/min. The detector voltage was set at 0.75 V. The detector temperature was set at 35.0°C. The retention time for noradrenaline and dopamine was 4.43 min and 9.56 min, respectively.

Statistics

We selected a sample size of six, assuming a minimal meaningful difference of 7% and a within-group standard deviation of 4%, based on previous studies. The

statistical analysis was conducted using SigmaPlot 12 (Systat Software Inc., San Jose, CA). The data were normally distributed and are presented as the mean ± SEM. The data were analyzed using a one-way or two-way analysis of variance (ANOVA), followed by Student-Newman-Keuls post hoc tests for multiple comparisons. $P < 0.05$ was defined as statistically significant.

Results

3.1. Anti-hyperalgesic effects of bupropion

Intrathecal injection of bupropion (3–100 μg) produced dose-dependent anti-hyperalgesic effects ($P < 0.001$ by two-way ANOVA); the peak effect was observed at 120 min, although the effect continued to 480 min after injection of the 100 μg dose ($P < 0.05$ by Student-Newman-Keuls post hoc test; Fig. 1), which was the end of the measurement period in the experiment. No adverse behavioral effects, such as motor effects, sedation, or agitation, were observed, and the righting reflex and stepping reflex were normal. Intrathecal pre-treatment with idazoxan, an alpha 2-adrenoceptor antagonist (3–30 μg), dose-dependently reversed the anti-hyperalgesic effect of bupropion (30 μg) ($P < 0.001$ by two-way ANOVA). The maximum dose of idazoxan by itself (30 μg) did not alter withdrawal thresholds compared with the saline group (Fig. 2A). Intrathecal pre-treatment with sulpiride, a dopamine D2-receptor antagonist (3–30 μg), dose-dependently reversed the anti-hyperalgesic effect of bupropion (30 μg) ($P < 0.001$ by two-way ANOVA). The maximum dose of bupropion itself (30 μg) did not alter withdrawal thresholds compared with the saline group (Fig. 2B).

3.2. Increased noradrenaline and dopamine levels in the spinal cord after injection of bupropion revealed by microdialysis

Fig. 3 shows the time course of the change of the noradrenaline and dopamine concentrations in the dorsal horn of the spinal cord in SNL rats after bupropion injection. The baseline noradrenaline and dopamine concentrations before bupropion injection were 2.48 ± 0.38 pg/15 μl and 0.89 ± 0.19 pg/15 μl, respectively ($n = 6$). After intrathecal injection of bupropion (30 μg), the concentrations of both noradrenaline and

dopamine were increased ($P < 0.001$ and $P = 0.001$, respectively, by two-way ANOVA). The noradrenaline concentration increased within 15 min and reached approximately 30% of the baseline value at 45 min, and the increase continued for more than 90 min compared to the saline-treated group. The concentration of dopamine also increased, with a peak at 30 min maintenance of the increase for 45 min after injection.

3.3. Noradrenaline and dopamine contents in the spinal cord of normal and SNL rats

The noradrenaline and dopamine contents in homogenized tissue from the ipsilateral dorsal spinal cord of normal rats and SNL rats were also determined (Fig. 4). The noradrenaline concentration in SNL rats 2 weeks after nerve ligation was higher (1617.5 ± 104.0 pg/g, $n = 6$) than that in normal rats (1196.3 ± 117.6 pg/g, $n = 6$, $P < 0.001$ by Student-Newman-Keuls post hoc test after one way-ANOVA). However, the noradrenaline concentration in SNL rats 3 weeks (925.6 ± 49.9 pg/g) and 4 weeks (1037.5 ± 100.3 pg/g, $n = 6$) after ligation were decreased compared with those in normal rats ($P < 0.001$ and $P = 0.009$, respectively, by Student-Newman-Keuls post hoc test after one way-ANOVA).

At 2 weeks after SNL, an increase in the dopamine concentration in the ipsilateral dorsal spinal cord (220.7 ± 33.8 pg/g, $n = 6$) was observed relative to normal rats (142.9 ± 23.9 pg/g, $n = 6$, $P = 0.044$ by Student-Newman-Keuls post hoc test after one way-ANOVA), and the dopamine concentration subsequently returned to a level similar to that in normal rats.

Discussion

Bupropion is a dopamine-noradrenaline reuptake inhibitor whose acute administration decreases the reuptake of dopamine and noradrenaline into synaptosomes,[19] reduces the firing rate of central noradrenaline- and dopamine-containing neurons,[20] and increases extracellular striatal dopamine levels.[21] We hypothesized that intrathecal administration of bupropion would suppress neuropathic pain symptoms through increased noradrenaline and dopamine levels in the spinal cord. We found that intrathecal administration of bupropion indeed produced dose-dependent anti-hyperalgesia through increased noradrenaline and dopamine levels in the spinal cord, with the effects mediated by spinal α2-adrenoceptors and dopamine D2 receptors. Furthermore, the anti-hyperalgesic effect of the maximum dose of bupropion (100 μg) continued for more than 8 h without any adverse effects.

We recently demonstrated that increased noradrenaline levels in the spinal cord play a critical role in the inhibitory effect of antidepressants on neuropathic pain symptoms.[3] Previous studies demonstrated the potency and efficacy of intrathecal injection of α2-adrenoceptor agonists such as clonidine and dexmedetomidine, which mimic the effects of spinally released noradrenaline, in neuropathic pain states.[22,23] These pharmacological effects in neuropathic pain may be associated with spinal cord plasticity, as animal models of neuropathic pain have shown increased expression of inhibitory α2-adrenoceptors on C-fibers,[24] increased G protein-coupling of spinal α2-adrenoceptors,[25] and increased α2-adrenoceptor-mediated activation of inhibitory cholinergic interneurons.[23,26] Consistent with these reports, intrathecal injection of bupropion in the present study attenuated SNL-induced hyperalgesia, and the effect was dose-dependently reversed by idazoxan, an α2-adrenoceptor antagonist. In the

microdialysis studies, noradrenaline levels were increased in the dorsal horn of the spinal cord after intrathecal bupropion injection. These results indicate that the anti-hyperalgesic effect of bupropion depends on increased noradrenaline levels in the spinal cord.

Dopamine plays an important role in nociceptive transmission and several reports have described direct analgesic actions of dopamine in regions of the brain,[27] such as the striatum,[6,28,29] the basal ganglia,[30] and the nucleus accumbens.[31,32] Dopamine also plays a critical role in nociceptive transmission in the spinal cord through descending inputs from the brain; a previous study reported that no dopaminergic cell bodies are present in the spinal cord.[33] Electrophysiological studies have shown that focal electrical stimulation of the A11 area of the brain suppresses the nociceptive responses of neurons in the dorsal horn of the spinal cord.[9] Furthermore, an in vivo patch clamp analysis revealed that dopamine suppressed a synaptic response to noxious stimuli in substantia gelatinosa neurons in the spinal cord.[34] Behavioral studies have also demonstrated that intrathecal administration of a dopamine agonist has thermal anti-nociceptive effects.[35,36]

Previous studies have shown that dopamine D2 receptors contribute to dopaminergic inhibition of nociceptive transmission in the brain[37,38] and that administration of a D2 receptor antagonist alone in the striatum enhanced pain responses.[6,37] It has also been reported that intrathecal administration of a D2 receptor agonist reduced the thermal hyperalgesia caused by carrageenan-induced chronic inflammation.[39] Therefore, D2 receptors in the spinal dorsal horn may be involved in the attenuation of not only acute nociception but also pathological pain. In the present study, bupropion-induced anti-hyperalgesia was dose-dependently reversed by sulpiride, a D2 receptor antagonist.

Furthermore, in the microdialysis studies, dopamine levels were increased in the dorsal horn of the spinal cord after bupropion injection. These results indicate that increased dopamine level and subsequent activation of D2 receptors in the spinal cord strongly contributed to the anti-hyperalgesic effect of bupropion. To our knowledge, this is the first evidence showing that increased dopamine levels in the spinal cord inhibit neuropathic pain symptoms through spinal D2 receptors.

The change over time of the noradrenaline and dopamine contents in the homogenized tissue from the ipsilateral dorsal lumbar spinal cord after SNL was intriguing. A previous study demonstrated plastic changes in descending noradrenergic neurons after nerve injury[13] where that density of the descending noradrenergic fibers and NA content in the ipsilateral lumbar spinal cord were increased 10 days after SNL in rats. In contrast, Hughes et al.[40] reported a loss of noradrenergic fibers in the ipsilateral lumbar spinal cord 19–21 days after tibial nerve transection in rats. Although the animal models were different, these results suggest that the tone of the descending noradrenergic system dynamically changes over time after nerve injury. Consistent with these findings, the noradrenaline content in the spinal cord in the present study was increased 2 weeks after SNL, followed by subsequent decrease at 3-4 weeks. The dopamine content in the ipsilateral lumbar spinal cord was also increased 2 weeks after SNL but then returned to a level similar to that in normal rats. No previous study have examined the plasticity of the descending dopaminergic system after injury; however, our data indicate that the changes in the descending noradrenaline and dopamine systems over time after nerve injury are similar. Several clinically approved treatments for neuropathic pain, including gabapentin[40] and antidepressants,[3] modulate or mimic the activation of descending noradrenergic pathways to produce analgesia and may

overcome or compensate for decreased function of descending inhibitory pathways. We performed behavioral experiments at approximately 3 weeks after SNL surgery. Although we did not compare the efficacy of bupropion across various time points after nerve injury, the plasticity of descending inhibitory systems over time may contribute to the efficacy of antidepressants for neuropathic pain.

Antidepressants, particularly tricyclic antidepressants and serotonin-noradrenaline reuptake inhibitors, are widely used for the management of neuropathic pain,[2] and it is well known that their analgesic effects are mediated by recruitment of descending inhibitory pathways such as noradrenergic and serotonergic systems.[41] Compared with the large quantity of literature on the noradrenergic and serotonergic descending inhibitory systems, however, little information is available regarding the analgesic effects of dopamine. It has been reported that mechanical allodynia is attenuated by systemic administration of bupropion in animal models of neuropathic pain.[42,43] In a small trial of 41 human patients with neuropathic pain of different etiologies, bupropion showed strong efficacy for pain reduction,[10] and the number needed to treat was calculated as 1.6.[11] Taken together with these previous studies, the present findings provide strong evidence of the inhibitory effect of an NA and DA reuptake inhibitor against neuropathic pain symptoms. Bupropion may thus be useful for treatment of neuropathic pain through a spinal mechanism.

Figure legends

Figure 1. Time course of the anti-hyperalgesic effect of intrathecally injected bupropion (BPR) in rats with SNL. Intrathecally injected bupropion produced a dose-dependent anti-hyperalgesic effect ($P < 0.001$ by two-way ANOVA). All values represent the mean ± SEM for six rats. *$P < 0.05$ compared with the saline (SAL)-treated group by Student-Newman-Keuls post-hoc test at each time point.

Figure 2. Intrathecal pretreatment with an α2-adrenoceptor antagonist and a D2 receptor antagonist dose-dependently reversed the anti-hyperalgesic effect of 30 μg of bupropion ($P < 0.001$ by two-way ANOVA). (A) After the baseline threshold was determined, rats were intrathecally administered saline (SAL) or idazoxan (IDA, 3–30 μg), an α2-adrenoceptor antagonist, followed by bupropion injection 15 min later. (B) After the baseline threshold was determined, rats were intrathecally administered vehicle (VEH) or sulpiride (SUL, 3–30 μg), a D2 receptor antagonist, followed by bupropion injection 15 min later. All values represent the mean ± SEM for six rats. *$P < 0.05$ compared with the control (SAL-SAL or VHE-SAL) group by Student-Newman-Keuls post-hoc test at each time point.

Figure 3. Microdialysis in the dorsal horn of the lumbar spinal cord to determine noradrenaline (A) and dopamine (B) levels after bupropion injection. SNL rats were intrathecally administered saline (SAL) or 30 μg of bupropion (BUP). Levels of both noradrenaline and dopamine were increased after BUP injection ($P < 0.001$ and $P = 0.001$, respectively, by two-way ANOVA). Data are presented over time as a percentage

change relative to the baseline. All values represent the mean ± SEM for six rats. *P < 0.05 compared with the control (SAL or VHE) group by Student-Newman-Keuls post-hoc test at each time point.

Figure 4. The noradrenaline (A) and dopamine (B) content in the ipsilateral dorsal half of the lumbar spinal cord was measured in normal and SNL (2, 3, and 4 weeks after SNL) rats. All values represent the mean ± SEM for six rats. *P < 0.05 compared with normal rats by Student-Newman-Keuls post-hoc test after one-way ANOVA.

Fig. 1

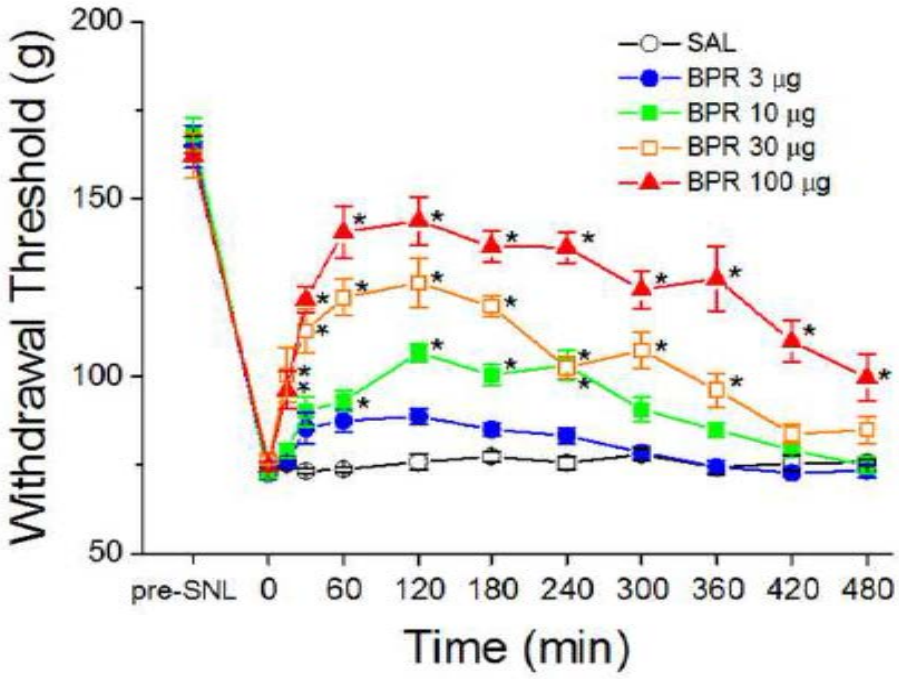
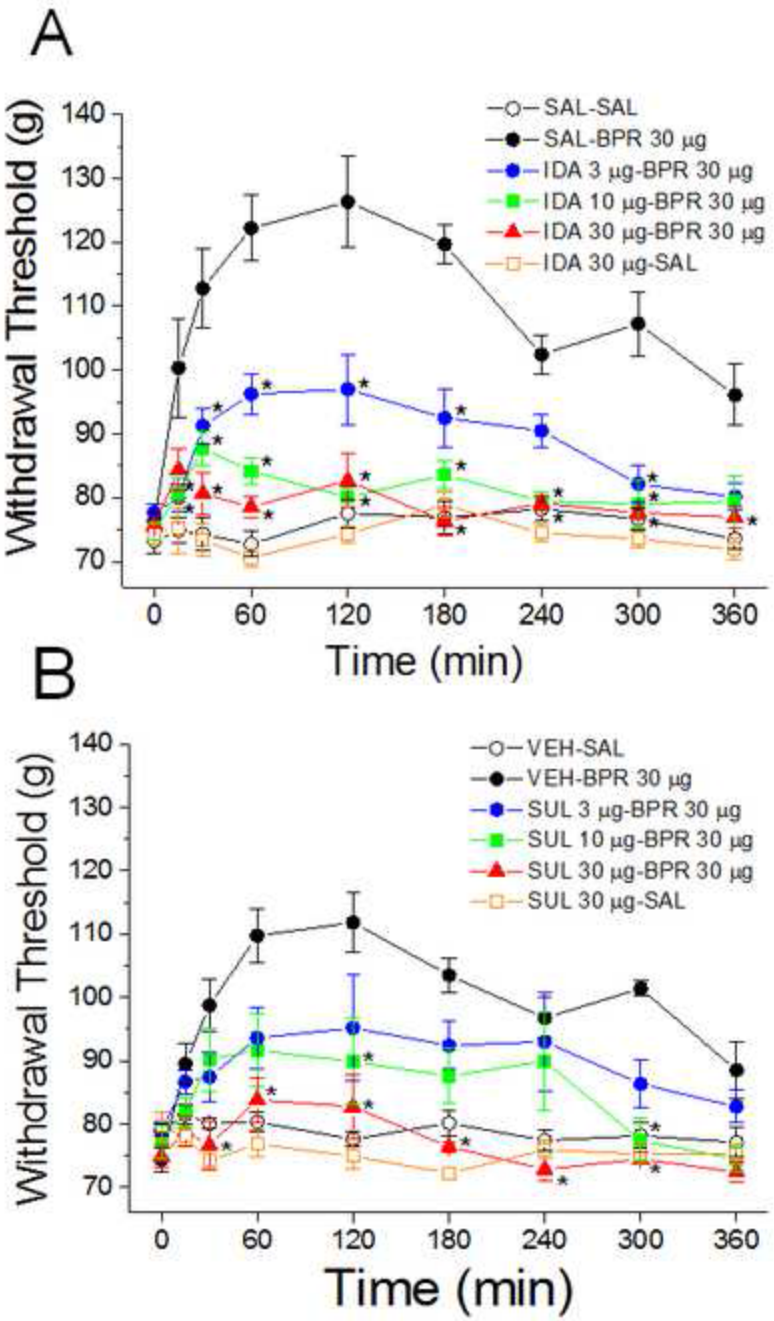


Fig. 2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130
120
110
100
90
80
70
0
60
120
180
240
300
360
Time (min)
SAL-SAL
SAL-BPR 30 µg
IDA 3 µg-BPR 30 µg
IDA 10 µg-BPR 30 µg
IDA 30 µg-BPR 30 µg
IDA 30 µg-SAL
B
Withdrawal Threshold (g)
140
130
120
110
100
90
80
70
0
60
120
180
240
300
360
Time (min)
VEH-SAL
VEH-BPR 30 µg
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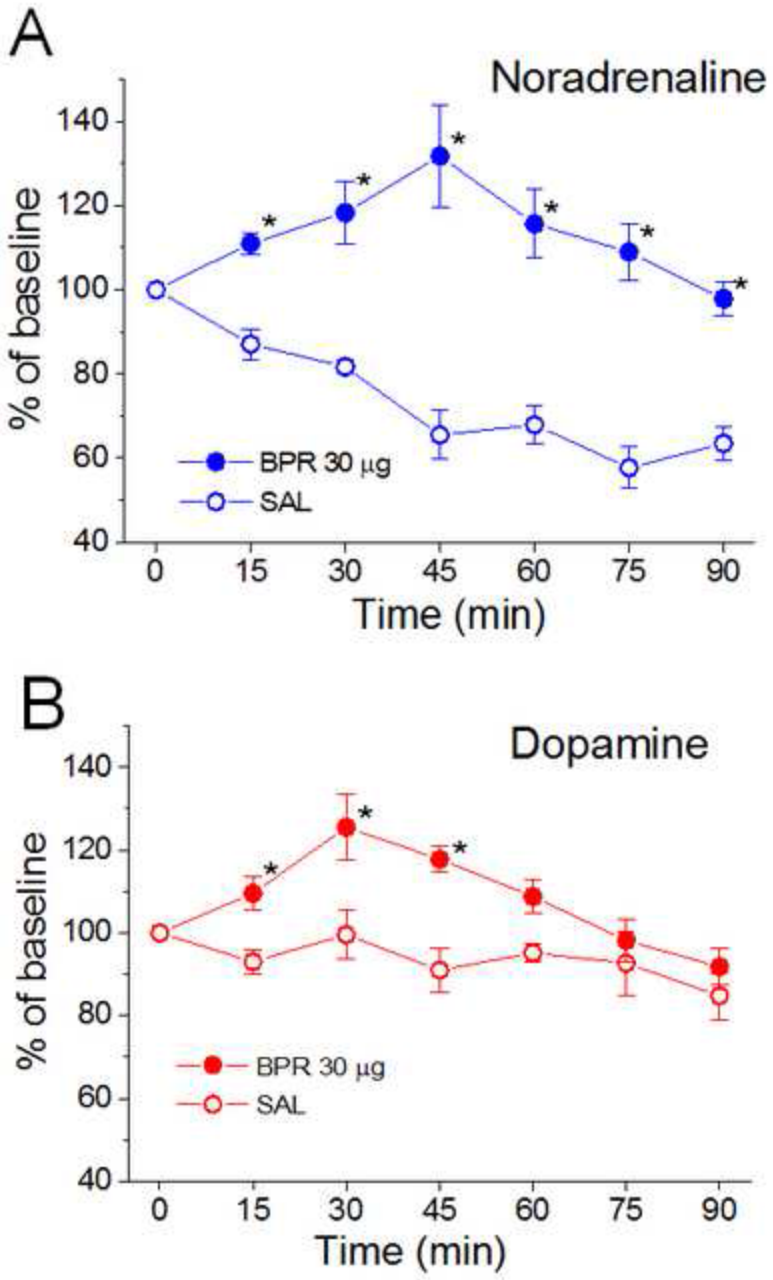
SUL 30 µg-SAL

Fig. 3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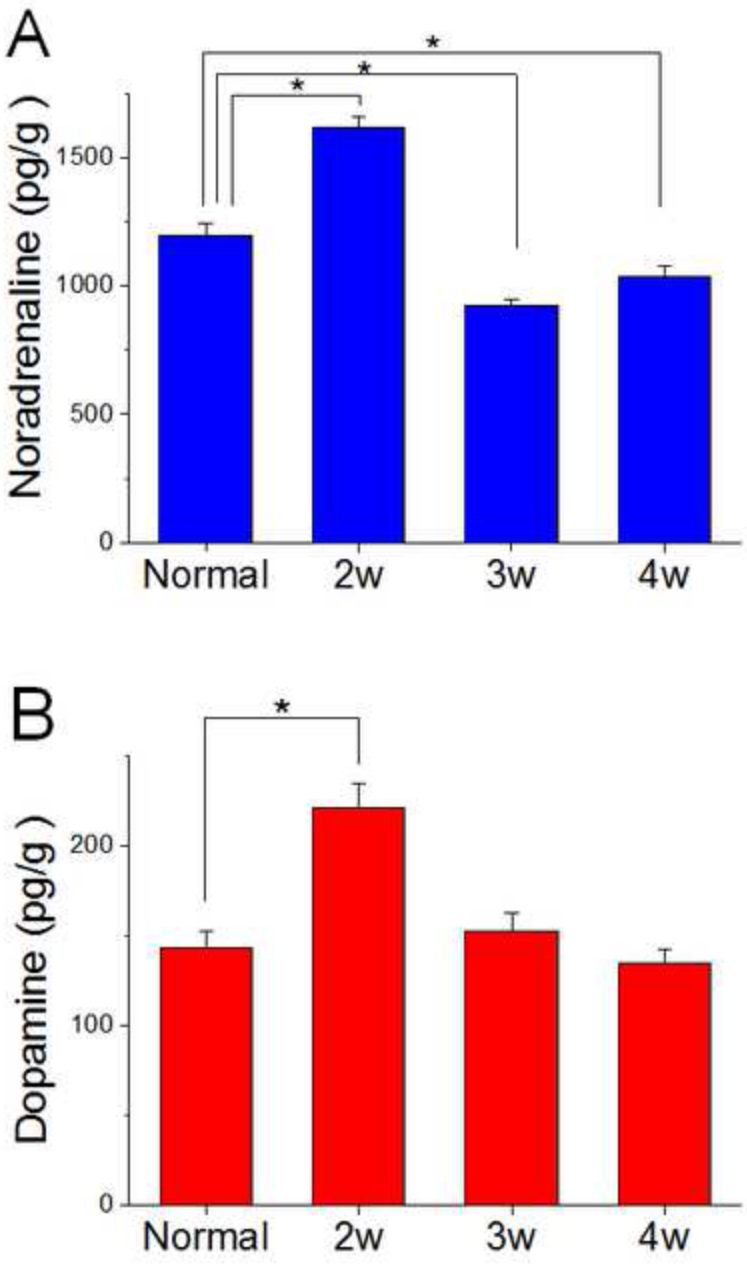
Noradrenaline
% of baseline
140
120
100
80
60
40
0
15
30
45
60
75
90
Time (min)
BPR 30 µg
SAL
B
Dopamine
% of baseline
140
120
100
80
60
40
0
15
30
45
60
75
90
Time (min)
BPR 30 µg
SAL

Fig. 4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1000
500
0
Normal
2w
3w
4w
*
*
*
B
Dopamine (pg/g)
200
100
0
Normal
2w
3w
4w
*

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