学位論文

Effects of an intrathecal TRPV1 antagonist, SB366791, on morphine-induced itch, body temperature, and antinociception in mice

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榊原賢司

Abstract

Purpose: Transient receptor potential vanilloid 1 (TRPV1) not only is activated by multiple stimuli but also is involved with histamine-induced itch. The effects of TRPV1 on morphine-induced itch are unknown. We examined the effects of intrathecal administration of TRPV1 antagonist on morphine-induced itch, body temperature, and antinociception for mice.

Methods: Each C57/BL6j mouse was intrathecally administered with one of the following solutions: morphine, SB366791 (as the TRPV1 antagonist), morphine + SB366791, saline, or vehicle. For each mouse, each instance of observed scratching behavior was counted, the body temperature was measured, and the nociceptive threshold was determined using the tail-immersion test.

Results: SB366791 dose-dependently reduced the scratching behavior induced by the administration of morphine. SB366791 and the morphine + SB366791 groups did not manifest an increase in body temperature. Antinociceptive effects were observed to occur dose-dependently for morphine but not for SB366791. Compared with morphine alone, the administration of morphine + SB366791 did not reduce significant antinociceptive effects.

Conclusion: We propose that an intrathecal TRPV1 antagonist, SB366791, reduced morphine-induced itch without causing hyperthermia and did not suppress morphine-induced antinociception for mice.

Keywords: pruritus, opioid, TRPV1, nociception, hyperthermia

Introduction

Although morphine is indispensable and widely used for pain management, it presents some adverse effects, including nausea, vomiting, and respiratory depression, with itch being a major effect. The frequency and severity of morphine-induced itch vary with the dose and route of administration.[1] The incidence of neuraxial morphine-induced itch is sometimes severe, ranging from 30% to 90% in patients receiving morphine.[1] Previously, histamine antagonists have failed to suppress morphine-induced itch.[2,3] Conversely, opioid receptor antagonists can block morphine-induced itch but are not clinically available because they inhibit opioids' antinociceptive effects. Therefore, there is no standard treatment for morphine-induced itch.

Transient receptor potential vanilloid 1 (TRPV1) is the first cloned TRP family member channel.[4] Recently, some TRP channels were considered as the molecular sensors of chemical, thermal, and mechanical noxious stimuli that evoke pain and itch.[5] TRPV1 is activated by multiple stimuli, including capsaicin, heat, pH, endocannabinoids, and endogenous lipids.[6] TRPV1 antagonists have shown antinociceptive effects in several pain models including inflammatory, cancer, and postoperative pain model.[7-9] We previously demonstrated the important role of TRPV1 on histamine-induced itch.[10] However, hyperthermia is a major side effect of the systemic administration of TRPV1 antagonists.[11-13]

The effects of an intrathecal TRPV1 antagonist on morphine-induced itch without causing hyperthermia remain unknown. Herein, we examined the effects of an intrathecal TRPV1 antagonist on morphine-induced itch, body temperature, and antinociception for mice.

Materials and methods

Animals

All experiments were approved by the Animal Care and Use Committee of Shimane university (No. IZ 27 – 117, 139) and conducted according to the regulations for animal experiment at Shimane university. The studies were conducted on male C57/BL6j mice (21 – 27 g), which were housed in light (lit from 8:00 to 20:00) and temperature controlled (23 – 25°C) environment. Food and water were freely available.

Drugs

Morphine hydrochloride (Takeda Pharmaceutical, Tokyo, Japan) was dissolved in physiologic saline. Morphine hydrochloride was dissolved in vehicle consisting of ethanol and saline at a 1:9 dilution when we studied the effect of intrathecal SB366791 on morphine-induced itch. The dosage of morphine was determined based on the method described in a previous study.[14] SB366791 (Wako Pure Chemical Industries, Osaka, Japan), the TRPV1 antagonist to be used in this study, was dissolved in a vehicle, and its dosage was determined as previously described.[15] SB366791 cannot be dissolved in physiologic saline. Therefore, we used the vehicle.

Intrathecal Injection Method

Lumbar punctures were performed as previously described.[16] The experiments were performed only after each administrator had achieved a success rate of > 90% in intrathecal injection training sessions, which involved the administration of 5 μl lidocaine (2%). The volume of each drug was 5 μl.

Scratching Behavior

This experiment was conducted from 9:00 to 16:00. Scratching behavior was counted as previously described.[17] Two days before starting this study, the mice were habituated each day under the same conditions of observation. After acclimation for 30 min, each mouse was intrathecally administered with one of the following solutions: morphine (0.1, 0.3, or 1.0 nmol) dissolved in saline, 0.3-nmol morphine dissolved in vehicle, SB366791 0.1 nmol, 0.3-nmol morphine + SB366791 (0.01, 0.03, or 0.1 nmol), saline, or vehicle. After intrathecal solution administration, the scratching behavior of each mouse was videotaped for 60 min under unmanned conditions. The temporal and total numbers of scratches performed by the individual mouse's hind paws during the first 60 min after intrathecal injection were counted. This test was performed in a blinded manner.

Observation of Body Temperature

This experiment was performed from 9:00 to 16:00. The mice were habituated every day for 2 days under the same conditions of observation. Each mouse received an intrathecal injection of one of the following agents: 0.3-nmol morphine dissolved in saline, 0.1-nmol SB, 0.3-nmol morphine + 0.1-nmol SB, saline, or vehicle. The body temperature was measured using an infrared thermometer (Ubi-x, CISE 99TS, Tokyo, Japan) on the

back of each mouse (the area shaved for intrathecal injection)[18] at 10, 20, 30, 40, 50, and 60 min after the performance of the intrathecal injection. This test was performed in a blinded manner.

Tail-Immersion Test

This experiment was performed from 9:00 to 17:00. The mice were habituated every day for 2 days under the same conditions of observation. The nociceptive threshold was determined as previously described.[14] The tail of each mouse was submerged in water at 48.0°C ± 0.5°C, and the time to tail withdrawal was observed. On the testing day, each mouse was gently held in a soft cloth, and its tail was immersed in the heated water before and after 5, 15, 30, 60, 90, 120, and 150 min of the performance of the intrathecal injection of one of the following solutions: morphine (0.1, 0.3, or 1.0 nmol) dissolved in saline, SB366791 (0.01, or 0.1 nmol), 0.3-nmol morphine + 0.1-nmol SB366791, saline, or vehicle. If a mouse did not remove its tail from the heated water, a 20 s cut-off was used to prevent tissue damage and an upper limit of latency of 20 s was recorded. This test was performed in a blinded manner.

Statistical Analysis

The number of scratches, body temperature, and latencies of the tail-immersion test are presented as the mean ± standard error of the mean (SEM). The total number of scratches was analyzed using one-way analysis of variance (ANOVA), followed by Scheffe's test. Changes in the number of scratches, body temperature, and tail-withdrawal latencies were analyzed using two-way repeated-measures ANOVA, followed by Scheffe's test. Statistical analyses were performed by using Stat-View 5.0 (Abacus Concepts, Inc. Berkley, CA, USA). Differences were considered significant at $P < 0.05$.

Results

The total number of mice used in all of the experiments was 156; the number of mice observed was 62 for scratching behavior, 40 for body temperature, and 54 for the tail-immersion test. We used 8 mice for vehicle group of scratching behavior and all groups of body temperature. We used 6 mice for the other groups of scratching behavior and all groups of the tail-immersion test. No mouse showed any neurologic deficits resulting from intrathecal injection. No mouse was excluded from this study.

Scratching Behavior

In the saline, 0.1-nmol morphine, and 1.0-nmol morphine groups, the total numbers of scratches were 12.7 ± 1.7, 33.8 ± 13.2, and 66.2 ± 19.5, respectively. The number of scratches was significantly higher in the 0.3-nmol morphine group than in the saline group (127.5 ± 23.2; $P = 0.001$; $F_{3, 20} = 9.2$) (Fig. 1A). The peak of scratching behavior was seen at 10 – 20 min after undergoing an intrathecal administration in the mice of the 0.3-nmol morphine group and at 0 – 10 min after undergoing an intrathecal administration in the mice of the 1.0-nmol morphine group; the number of scratches decreased after these times in all of the groups (Fig. 1B).

The scratching behavior of the 0.1-nmol SB366791 group (10.2 ± 1.0) was not significantly different from that of the vehicle group (9.8 ± 2.2; $P > 0.99$) (Fig. 2). Scratching behavior was significantly increased for the 0.3-nmol morphine dissolved in vehicle (0.3-nmol morphine + vehicle) group (122.7 ± 26.7) in comparison to that of the vehicle group ($P = 0.002$). In contrast, compared with the vehicle group, the 0.3-nmol morphine + 0.01-nmol, 0.03-nmol, and 0.1-nmol SB366791 groups did not exhibit significant increases in the number of scratches (68.3 ± 12.5; $P = 0.12$, 42.5 ± 18.3; $P = 0.71$, 29.2 ± 10.7; $P = 0.95$, respectively; $F_{5, 32} = 9.3$). SB366791 dose-dependently reduced the scratching behavior that was induced by morphine at 0.3 nmol. In addition, the total numbers of scratches for the groups receiving 0.3-nmol morphine + 0.03-nmol or 0.1-nmol SB366791 were significantly decreased compared with that of scratches of the 0.3-nmol morphine + vehicle group ($P = 0.02$ and $P = 0.004$, respectively) (Fig. 2).

Body Temperature

The body temperature of the mice ranged from 35.8°C to 36.2°C, among all of the groups for 60 min after the intrathecal injection ($P = 0.087$). Compared with the body-temperature measurements for the vehicle group, those of the SB366791 group and the morphine + SB366791 group did not manifest an increase in body temperature (Fig. 3).

Tail-Immersion Test

Intrathecal morphine dose-dependently produced antinociceptive effects. The latency of withdrawal of the tail following tail immersion in heated water was significantly prolonged from 5 min to 15 min after administration for the 0.3-nmol morphine group ($P = 0.007$ and $P = 0.0423$, respectively) and from 5 min to 90 min and to 150 min after administration for the 1.0-nmol morphine group, compared with the latency observed for

the saline group (P = 0.0001 – 0.044) (Fig. 4A). Intrathecally administered SB366791 did not produce thermal antinociceptive effects, in comparison with the effects observed for the vehicle group (P = 0.95) (Fig. 4B). The latency was significantly prolonged from 5 min to 120 min after administration for the 0.3-nmol morphine group, compared with that for the saline group (P = 0.001 – 0.015) (Fig. 4C). Morphine at 0.3 nmol + SB366791 at 0.1 nmol produced antinociceptive effects corresponding to a latency increase from 5 min to 120 min, compared with the effects observed for the vehicle group (P < 0.0001 – 0.025). Morphine at 0.3 nmol + SB366791 at 0.1 nmol did not produce significant thermal antinociceptive effects, compared with the effects observed for the 0.3-nmol morphine group (P = 0.21 – 0.99) (Fig. 4C).

Discussion

Three main findings were observed in this study. First, intrathecally administered SB366791, which is a TRPV1 antagonist, dose-dependently inhibited morphine-induced itch following an intrathecal administration in mice. Second, intrathecal SB366791 did not raise body temperature. Third, intrathecal SB366791 did not suppress morphine-induced antinociception of a thermal stimulus. Therefore, an intrathecal TRPV1 antagonist, SB366791, produced potent antipruritic effects for intrathecal morphine-induced itch, without serious adverse effects such as hyperthermia.

In clinical studies, several drugs have been used to treat morphine-induced itch.[14,19-23] 5-HT-receptor antagonists may relieve morphine-induced itch, although the results of clinical trials are debatable.[19,20] Some reports have indicated that a subanesthetic dose of propofol resulted in antipruritic effects on morphine-induced itch; however, the efficacy of propofol for morphine-induced itch remains controversial.[21,22] In both basic research and clinical studies, the administration of kappa opioid receptor agonists was found to have antipruritic effects on morphine-induced itch,[14,23] although the mice retained some scratching behavior;[14] additionally, 50% of patients continued to suffer from opioids-induced itch.[23] There is no standard therapy for morphine-induced itch. It is well known that the systemic administration of morphine has a sedative effect.[24] We showed that the intrathecal administration of high-dose morphine also causes sedation with a decrease in the scratching behavior in mice.[14] Therefore, intrathecal morphine administration did not produce scratching behavior dose-dependently. Furthermore, 0.3-nmol morphine was selected as a combination dose with SB366791. To the best of our knowledge, no studies

have reported the ability of intrathecal TRPV1 antagonists to attenuate morphine-induced itch. This study demonstrated that an intrathecal TRPV1 antagonist inhibited intrathecal morphine-induced itch.

The molecular mechanisms of morphine-induced itch are not completely understood, but the mu opioid receptor (MOR) isoform MOR1D and the gastrin-releasing peptide receptor (GRPR) are known to play a critical role in these mechanisms. MOR1D and GRPR are colocalized in the dorsal horn of the spinal cord.[25] Morphine induces heterodimerization and co-internalization of MOR1D and GRPR. GRPR activates the phospholipase C (PLC)/inositol 1, 4, 5 triphosphate (IP3)/calcium signaling pathway. This PLC/ IP3/calcium signaling pathway evokes morphine induced itch.[25]

Although the mechanisms of antipruritic effects of TRPV1 antagonists on morphine-induced itch are unknown, possible mechanism may include as followed. TRPV1 is mainly expressed in the central and peripheral terminals of primary sensory neurons.[26] The central terminal of the primary sensory neurons lies in the dorsal horn of the spinal cord and is concentrated in the superficial laminae. MOR and TRPV1 are colocalized in the superficial laminae of dorsal horn.[26] Furthermore, TRPV1-expressing neurons release gastrin-releasing peptide (GRP) in the dorsal horn of the spinal cord, resulting in the evocation of the GRP–GRPR signaling pathway.[27] These studies and our results indicate the possibility that TRPV1 interact between MOR1D and GRPR in the spinal cord.

Although SB366791 is not a pure TRPV1 antagonist, SB366791 is a potent and high selectivity TRPV1 antagonist which has little or no effect on the activity against a wide range of receptors, including opioids.[28] In our data, SB366791 did not inhibit morphine-induced antinociception. Therefore, we suggest that SB366791 produces antipruritic effects through TRPV1 and not MOR.

It has been reported that a TRPV1 antagonist produces little or no antinociceptive effects in naïve models.[29] Consistent with this report,[29] an intrathecal TRPV1 antagonist did not produce antinociceptive effects in naïve mice in our study. However, TRPV1 antagonists have shown antinociceptive effects in several pain models.[7-9] TRPV1 expression was increased in bone cancer pain mice model.[30] In addition, TRPV1 is functionally upregulated in postoperative pain mice model.[31] This difference between naïve models and pain models may depend on the activation of TRPV1, including the increase of TRPV1 expression or its up-regulation in the pain models. [30,31] The antinociceptive effects of morphine vary depending on the animal models. Morphine is

effective against postoperative and inflammatory pain compared with neuropathic pain.[32,33] Bone cancer pain is resistant to morphine compared with inflammatory pain.[34] Down regulation of MOR expression is thought to attenuate sensitivity of bone cancer pain to morphine.[33] It has been reported that the combination of morphine and TRPV1 antagonists has potent analgesic effect on bone cancer model.[35]

Although TRPV1 antagonists have been widely accepted as next-generation pain therapies, many clinical studies of TRPV1 antagonists have been put on hold, mainly because of adverse events.[36,37] In fact, the systemic use of TRPV1 antagonists in basic research studies has been shown to cause hyperthermia. [11-13] The present study showed that an intrathecal TRPV1 antagonist did not affect the body temperature of mice.

There are some limitations to our study. First, the mechanisms of the antipruritic effects observed after the intrathecal administration of SB366791 combined with morphine were not clear. Further studies are needed to address these issues. Second, although no mice exhibited any side effects, such as motor dysfunction, after the intrathecal administration of SB366791, the neurotoxicity of SB366791 at the spinal level was not clarified.

Conclusion

This study demonstrated that intrathecal SB366791 reduced intrathecal morphine-induced itch without causing hyperthermia and did not suppress morphine-induced antinociception for mice.

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Author Contributions

S.S. and N.I. designed the project. S.S., M.S., Y.K. and M.H. performed the experiments. S.S. and N.I. wrote the manuscript. All authors discussed the results, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no competing interests.

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Figure 1: Scratching behavior induced by the intrathecal administration of morphine.

Notes: (A) The total number of scratches was significantly higher for the 0.3-nmol morphine group than for the saline group ($P = 0.001$). (B) The time course of scratching behavior after the administration of saline or morphine (0.1, 0.3, or 1.0 nmol). The peak of scratching behavior was at 10 – 20 min after undergoing an intrathecal administration for the mice of the 0.3-nmol morphine group and at 0 – 10 min after undergoing an intrathecal administration for the mice of the 1.0-nmol morphine group. Data are presented as the mean ± standard error of the mean (SEM) for the 6 - 8 mice in each group. $^{*}P < 0.05$ compared with the saline group. One-way analysis of variance (ANOVA), followed by Scheffe's test in (A), two-way repeated-measures ANOVA, followed by Scheffe's test in (B).

Figure 2: Scratching behavior induced by the intrathecal administration of morphine or/and SB366791.

Notes: The total numbers of scratches for the groups receiving 0.3-nmol morphine + 0.03-nmol or 0.1-nmol SB366791 were significantly decreased compared with the total number of scratches of the 0.3-nmol morphine group ($P = 0.02$ and $P = 0.004$, respectively). Data are presented as the mean ± SEM for the 6 mice in each group. $^{*}P < 0.05$ compared with the vehicle group. $^{\dagger}P < 0.05$ compared with the 0.3-nmol morphine dissolved in vehicle group. One-way analysis of variance (ANOVA), followed by Scheffe's test.

Abbreviation: SB = SB366791.

Figure 3: Time course of body temperature over the first 60 min following the administration of saline, vehicle, 0.3-nmol morphine, 0.1-nmol SB366791, or 0.3-nmol morphine + 0.1-nmol SB366791.

Notes: The body temperature of the mice ranged from 35.8°C to 36.2°C in all groups for 60 min after intrathecal injection ($P = 0.087$). Data are presented as the mean ± SEM for the 6 mice in each group. Two-way repeated-measures analysis of variance.

Abbreviations: SB = SB366791, ns = not significant.

Figure 4: Antinociceptive effects measured by the tail-immersion test. Antinociceptive effects after the administration of (A) saline or morphine (0.1, 0.3, or 1.0 nmol); (B) vehicle or SB (0.01 nmol, or 0.1 nmol) and (C) saline, vehicle, 0.3-nmol morphine, or 0.3-nmol morphine + 0.1-nmol SB.

Notes: Antinociceptive effects were observed to occur dose-dependently for morphine but not for SB366791 ($P = 0.95$). Compared with morphine alone, the administration of morphine + SB366791 did not reduce significant antinociceptive effects ($P = 0.21 – 0.99$). Data are presented as the mean ± SEM for the 8 mice in each group. $^{*}P < 0.05$ compared with the saline group. $^{\dagger}P < 0.05$ compared with the vehicle group. Two-way repeated-measures analysis of variance followed by Scheffe's test.

Abbreviations: SB = SB366791, ns = not significant.

Figure 1A

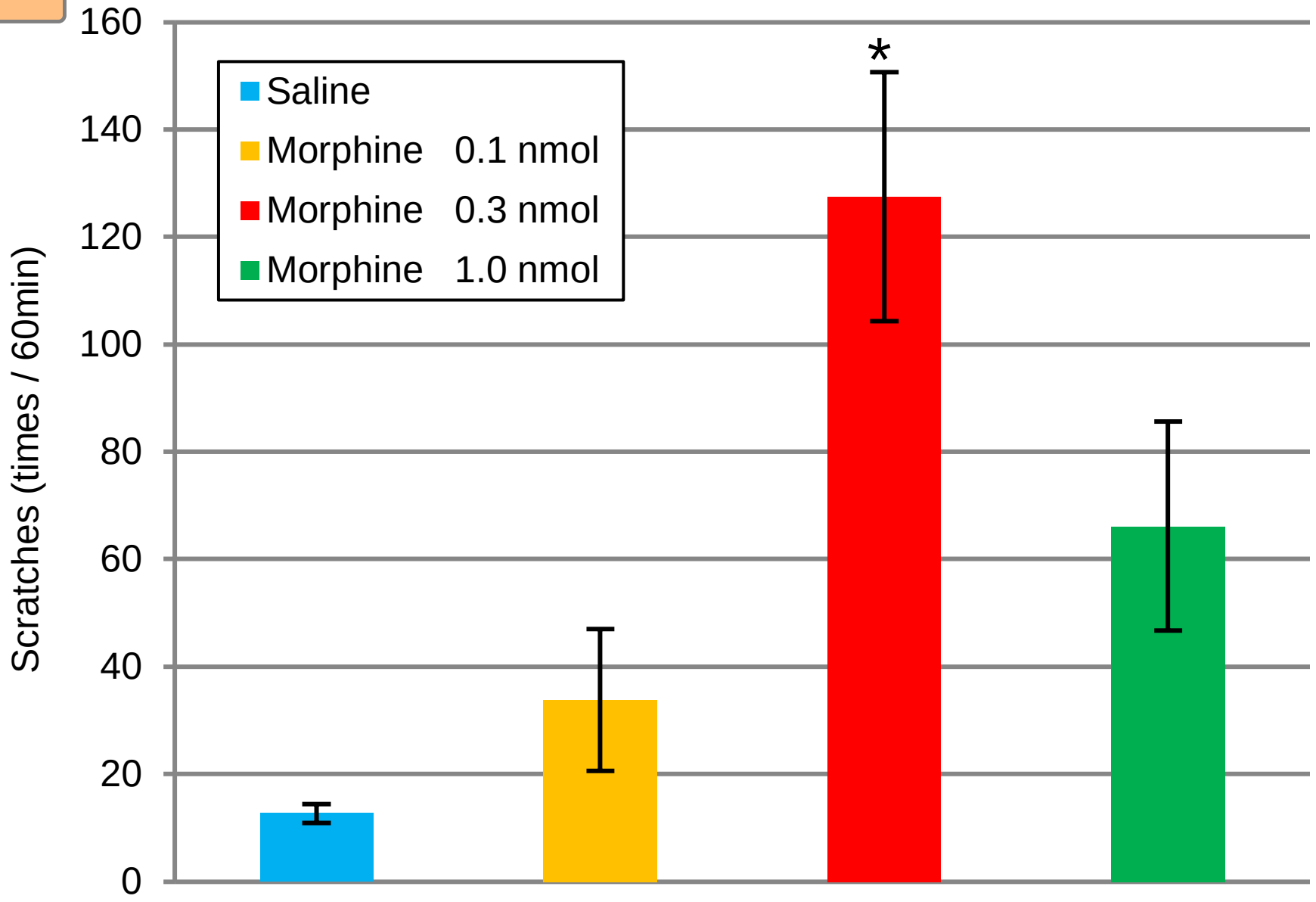
*P < 0.05 VS. Saline
Saline
Morphine 0.1 nmol
Morphine 0.3 nmol
Morphine 1.0 nmol
Scratches (times / 60min)
160
140
120
100
80
60
40
20
0
*

Figure 1B

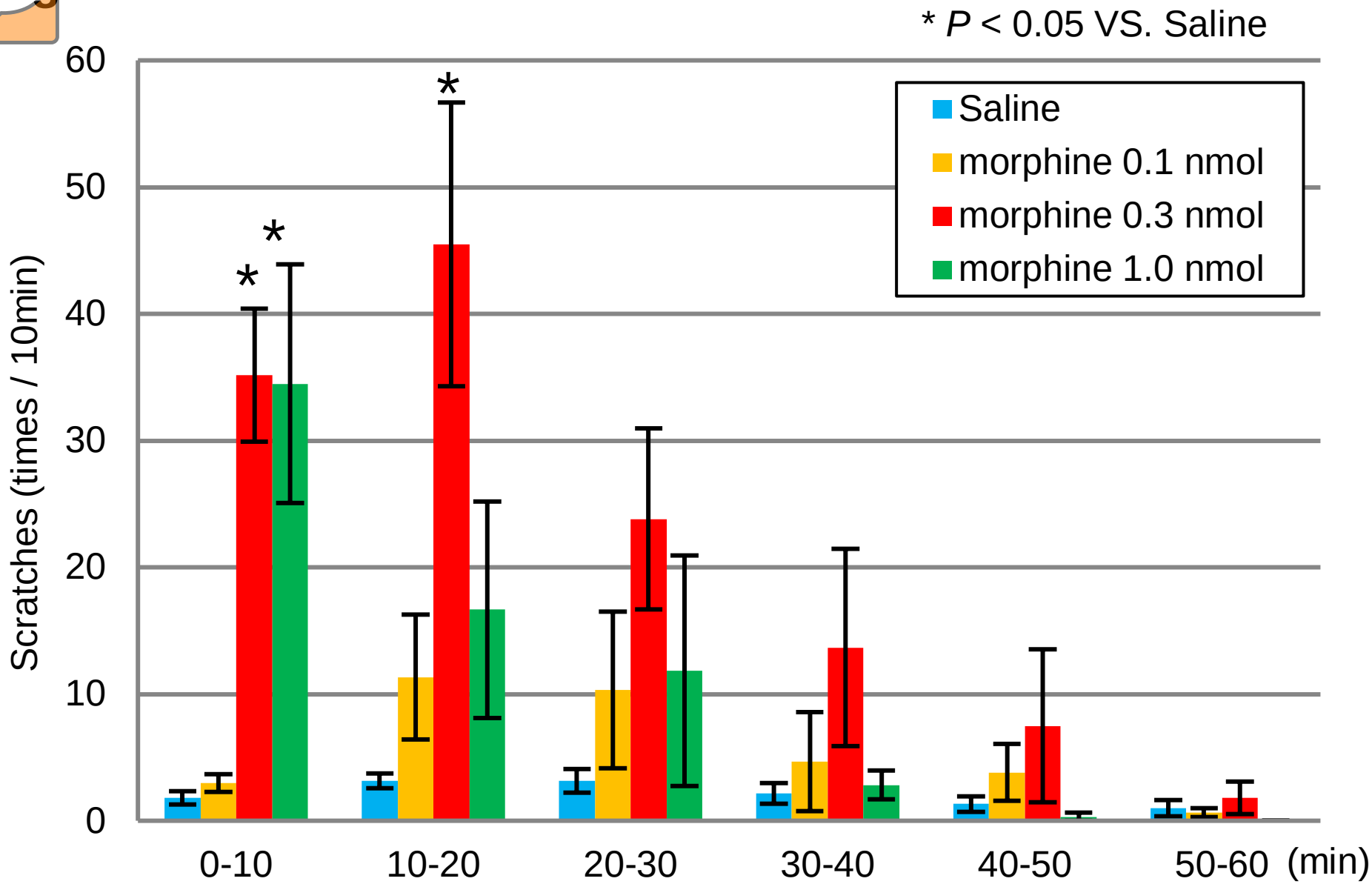
* P < 0.05 VS. Saline
Saline
morphine 0.1 nmol
morphine 0.3 nmol
morphine 1.0 nmol
Scratches (times / 10min)
60
50
40
30
20
10
0
0-10
10-20
20-30
30-40
40-50
50-60
(min)
*
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Figure 2

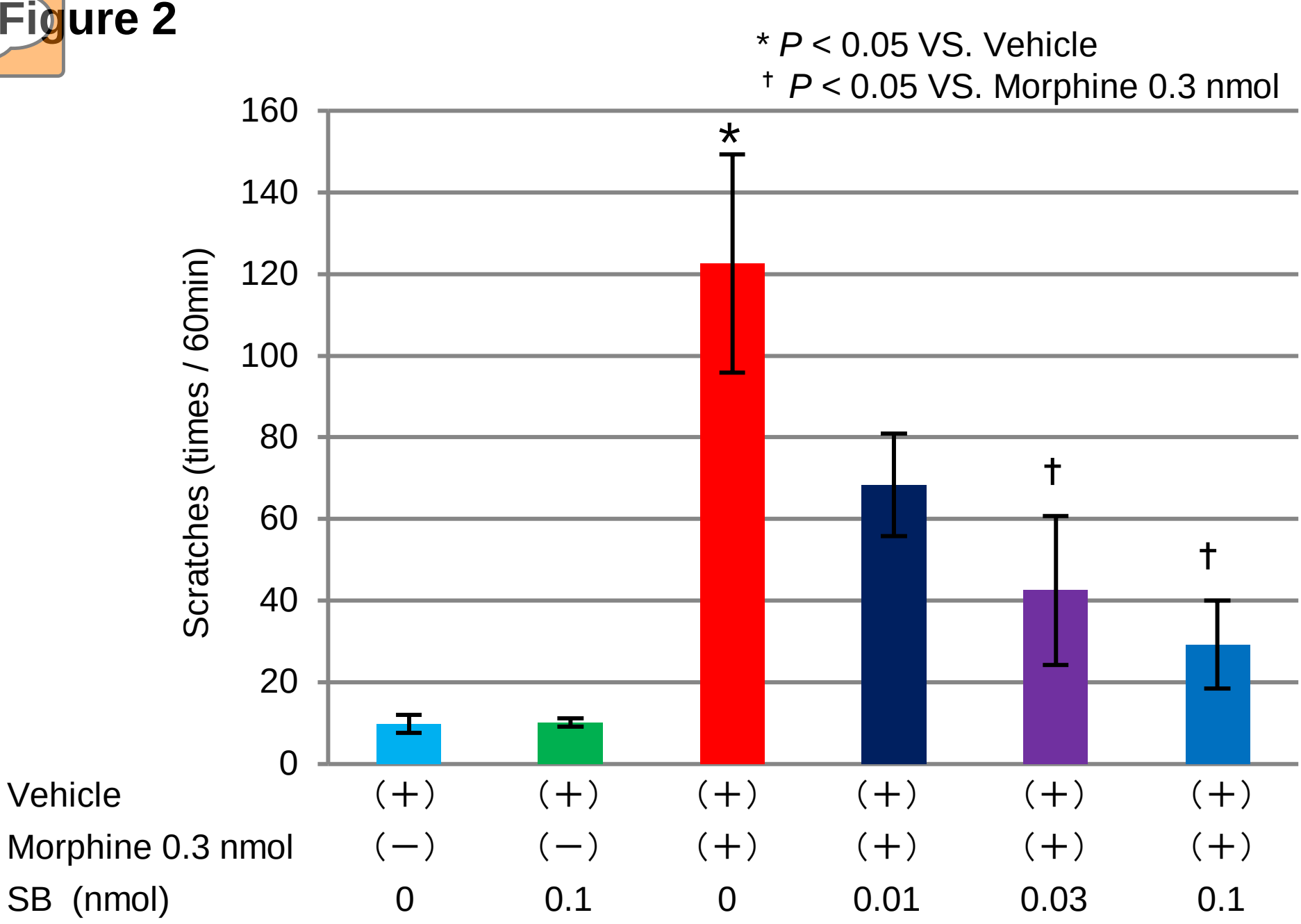

* P < 0.05 VS. Vehicle
† P < 0.05 VS. Morphine 0.3 nmol
Scratches (times / 60min)
160
140
120
100
80
60
40
20
0
*
†
†
Vehicle (+) (+) (+) (+) (+) (+)
Morphine 0.3 nmol (−) (−) (+) (+) (+) (+)
SB (nmol) 0 0.1 0 0.01 0.03 0.1

Figure 3

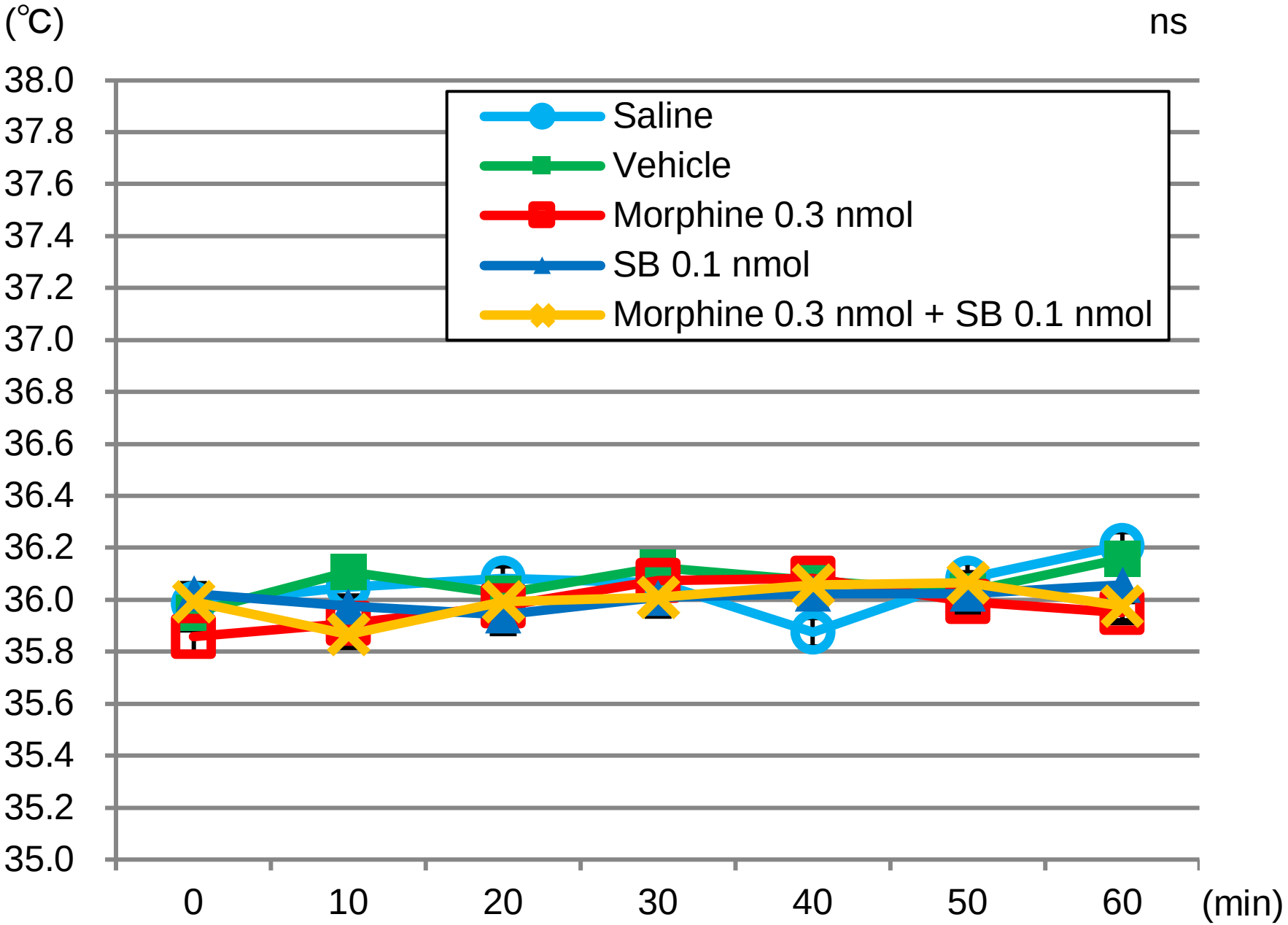

(℃)
ns
38.0
37.8
37.6
37.4
37.2
37.0
36.8
36.6
36.4
36.2
36.0
35.8
35.6
35.4
35.2
35.0
Saline
Vehicle
Morphine 0.3 nmol
SB 0.1 nmol
Morphine 0.3 nmol + SB 0.1 nmol
0
10
20
30
40
50
60
(min)

Figure 4A

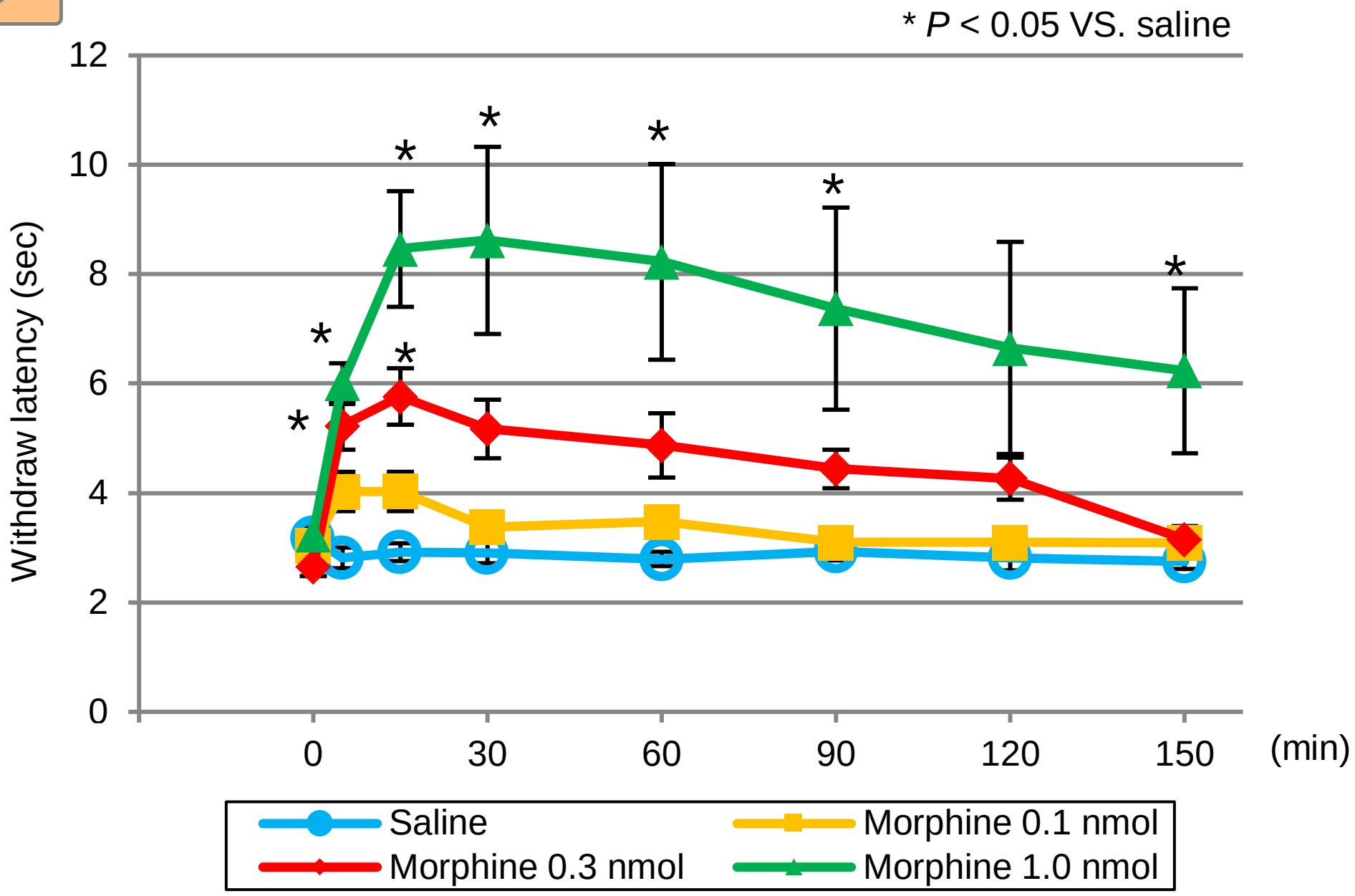

* P < 0.05 VS. saline
Withdraw latency (sec)
12
10
8
6
4
2
0
0
30
60
90
120
150
(min)
Saline
Morphine 0.1 nmol
Morphine 0.3 nmol
Morphine 1.0 nmol

Figure 4B

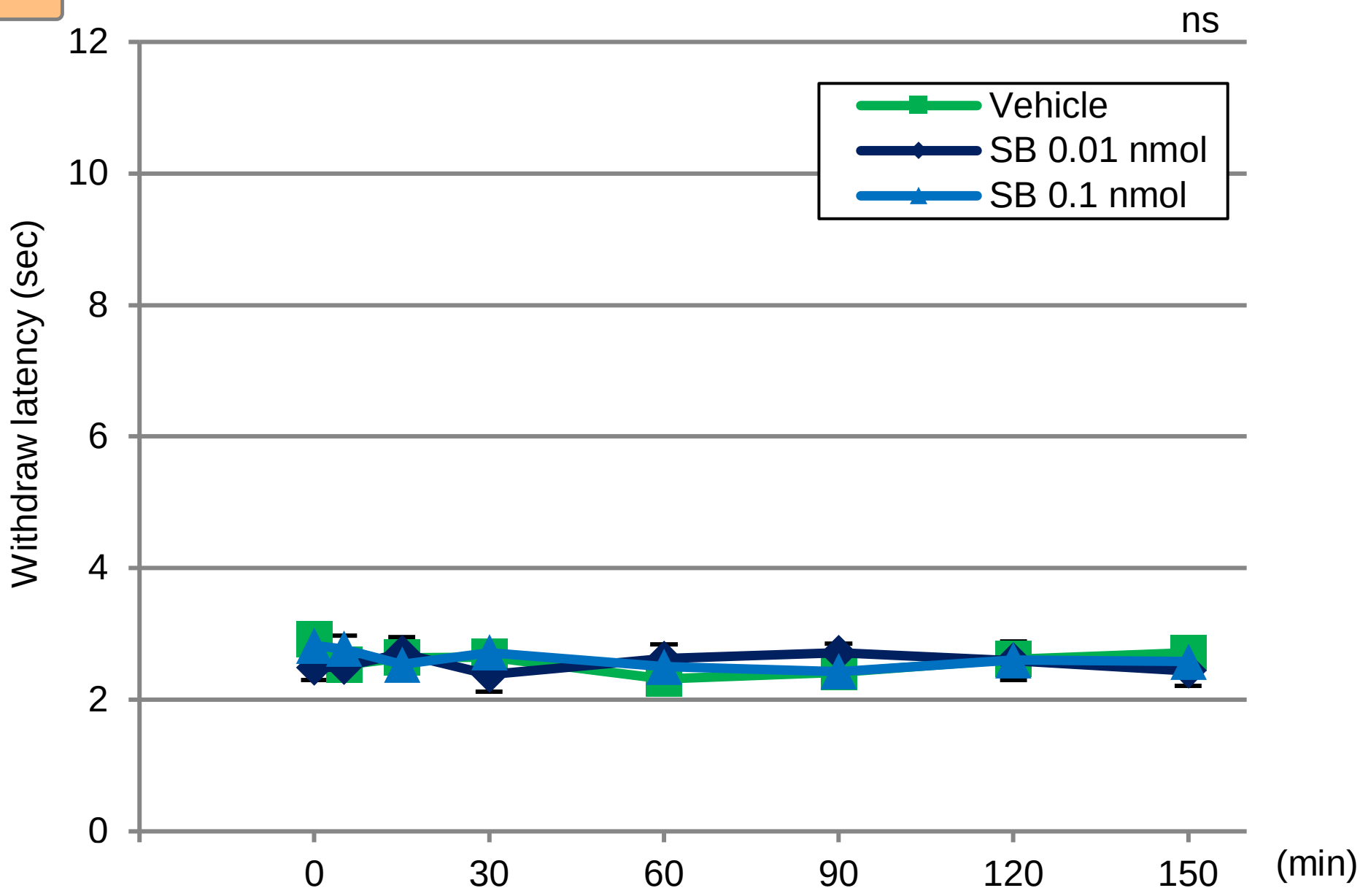
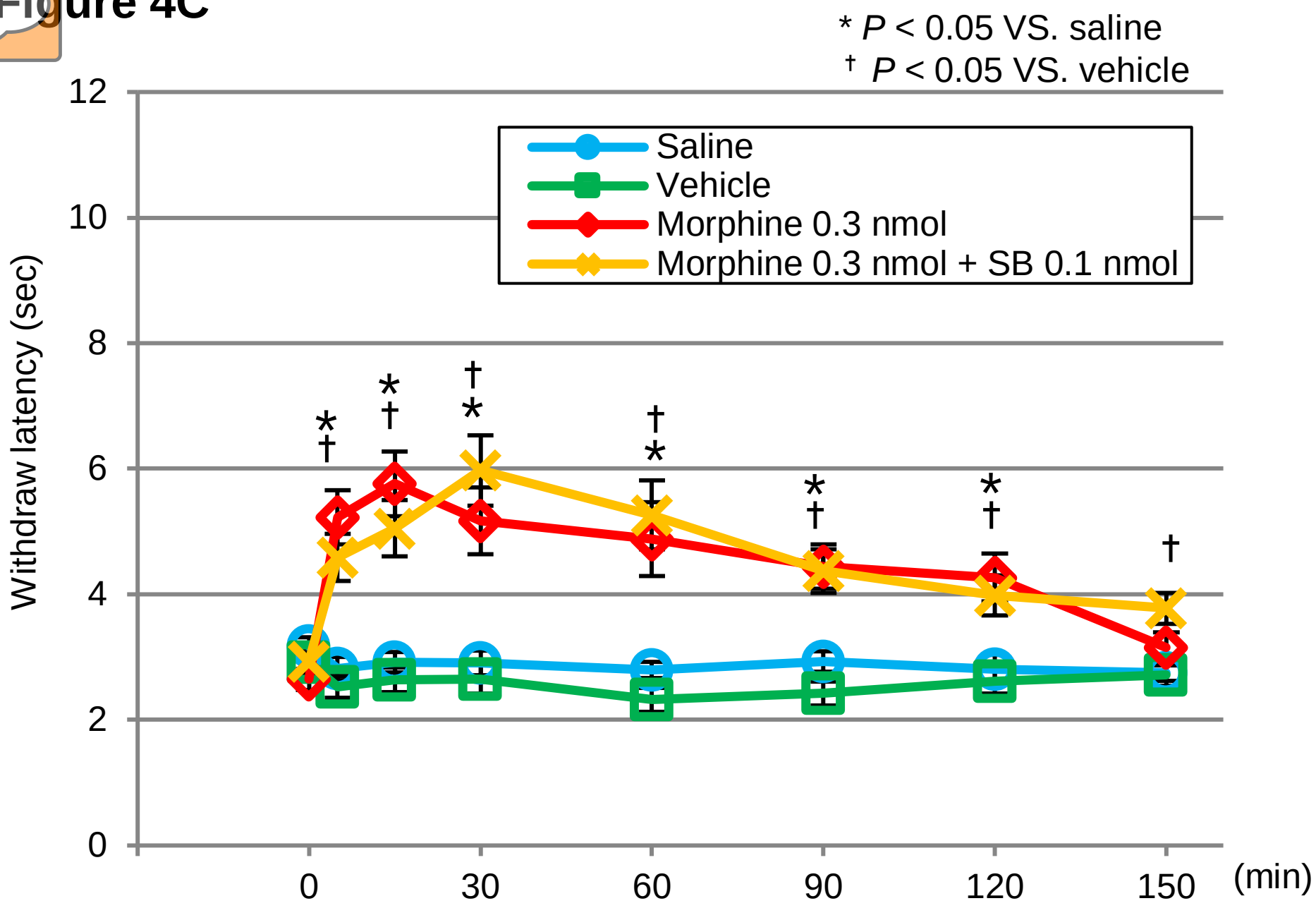

ns
12
10
8
6
4
2
0
Withdraw latency (sec)
Vehicle
SB 0.01 nmol
SB 0.1 nmol
0
30
60
90
120
150
(min)

Figure 4C


* P < 0.05 VS. saline
† P < 0.05 VS. vehicle
Saline
Vehicle
Morphine 0.3 nmol
Morphine 0.3 nmol + SB 0.1 nmol
Withdraw latency (sec)
12
10
8
6
4
2
0
0
30
60
90
120
150
(min)