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Antagonistic Effect of Pseudoginsenoside-F11 on the Behavioral Actions of Morphine in Mice

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LI, Z., C. F. WU, G. PEI, Y. Y. GUO AND X. LI. *Antagonistic effect of pseudoginsenoside-F11 on the behavioral actions of morphine in mice.* PHARMACOL BIOCHEM BEHAV **66**(3) 595–601, 2000.—The antagonistic effect of pseudoginsenoside-F11 (PF_{11}) on the various actions of morphine was studied in mice. The results demonstrated that PF_{11}, at the doses of 4 and 8 mg/kg, PO, significantly inhibited morphine (10 mg/kg, SC)-induced memory impairment in the Morris water maze test. PF_{11}, at 4 mg/kg, PO, did not influence conditioned place preference per se, yet markedly blocked the conditioned place preference to morphine. PF_{11} , at the doses of 4 and 8 mg/kg, PO, also significantly antagonized morphine (5 mg/kg, SC)-induced analgesia tested by tail pinch method. PF_{11}, at 4 mg/kg, PO, did not influence locomotor activity per se, yet inhibited the development of the reverse tolerance, as shown by the increase in locomotor activity, to morphine. At the doses of 4 and 8 mg/kg, PO, PF_{11} significantly antagonized the development of analgesia tolerance to morphine in the tail pinch test. Thus, the above results demonstrate for the first time that PF_{11} can antagonize some actions of morphine. However, the mechanism of action of PF_{11} merits further evaluation. © 2000 Elsevier Science Inc.

Pseudoginsenoside-F11 Morphine Memory Analgesia Tolerance Conditioned place preference

MORPHINE, which is clinically prescribed for its analgesic activity, is classified as an addictive drug because it produces a variety of behavioral and physiological changes that are related to its abuse. Acute morphine in animals induces behavioral sensitization and stereotyped behavior (30). Chronic morphine influences behaviors in the conditioned place-preference test and produces physical dependence, characterized by withdrawal symptoms, and tolerance (2,5). In addition, administration of morphine induces memory impairment in several behavioral tasks in animals (14,31,35).

It is well known that the root of *Panax ginseng* C.A. Meyer (Ginseng) has been used for hundreds of years as a treatment for a wide variety of ailments. Many experiments have demonstrated that ginseng extracts antagonized the morphine antinociception and morphine tolerance in rodents (4,16,27). Ginseng saponins and ginsenoside-Rg1, -Rb1 inhibit hyperactivity and conditioned place-preference induced by morphine (18). These findings suggest that ginseng may have potential usefulness for the prevention of adverse actions of morphine abuse.

Pseudoginsenoside-F11 (PF_{11}), an ocotillol-type saponin, is one of the ingredients of *Panax quinquefolium* (American ginseng), previously isolated from leaves of *Panax pseudoginseng* subsp. *himalaicus* H_{ARA} (Himalayan Panax) (33). However, this saponin does not exist in *Panax ginseng* (8). Previous experiments in our laboratory have observed that PF_{11} could improve the scopolamine-induced memory impairments in mice (21), showing a similar property with ginsenosides isolated from *Panax ginseng.* To further evaluate the pharmacological properties of PF_{11}, which has a different structure type from any of the known saponins isolated from roots and leaves of *Panax ginseng* (21), the present study investigated the effects of PF_{11} on the actions of morphine in mice.

METHOD

Animals and Drugs

Male Swiss mice, weighing 18–20 g at the start of experiments, were used. They were housed 10 to a cage under a 12 L:12 D cycle and constant temperature (20 ± 2°C), with water and food freely available.

Pseudoginsenoside-F_{11} was isolated from the aerial parts of *Panax quinquefolium* L. by Department of Chemistry for

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Nature Products of Shenyang Pharmaceutical University. The method for isolation of PF_{11} was the same as that described previously (3). PF_{11} was dissolved in distilled water and administrated intragastrically. Morphine hydrochloride (Shenyang First Pharmaceutical Factory, China) was dissolved in physiological saline and administrated subcutaneously.

Morris Water Maze Test

The task was carried out as previously described (36) in a circular pool (diameter 120 cm, height 40 cm) that was filled to a depth of 20 cm with water at room temperature (20 ± 0.5°C). The water was made opaque by addition of 15 ml India ink. Four equally spaced points around the edge of the pool were designed as four starting positions: east (E), south (S), west (W), and north (N). An escape platform (diameter 6.5 cm) was set 1 cm below the surface of the water and placed in a constant position in the middle of the SW quadrant. The mouse in the pool was trained to find the platform using a variety of extramaze cues, including the desk, wall, window, observer, etc. The observer always sat at the same position.

During the experiment, each mouse was trained four times daily. The mice were placed in the water facing away from the wall from one of four starting sites in a random sequence, and each site was used once everyday. The latency to find the escape platform was measured during each trial. Upon finding and climbing the platform, the mice stayed there 30 s. If the mice failed to find the platform within 60 s, they were placed there by the observer, and a maximum score of 60 s was given. After 30 s rest on the platform, the next trial was initiated.

To investigate the effect of PF_{11} on spatial learning and memory ability in naive mice, the animals were trained daily 60 min after PF_{11} administration, at the doses of 2, 4, and 8 mg/kg, PO, continuously for 5 days. To test the effect of PF_{11} on morphine-treated mice, PF_{11} was orally administered at the doses of 4 and 8 mg/kg consecutively for 5 days (from day 1 to day 5), and the mice were trained 60 min after PF_{11} administration. On days 1 and 2, the mice were trained 30 min after injection of saline. Morphine (10 mg/kg, SC) was administered from day 3 to day 5, and the training started 30 min after morphine administration.

Conditioned Place-Preference Test

Assessment of conditioned place preference: apparatus. The test was conducted as previous described (17) in a chamber consisting of two same-sized compartments (15 × 15 × 13 cm) separated by a guillotine door—one with white walls and the other with black walls. The white compartment had a smooth floor and the black compartment had a grid floor to provide a tactile difference. Mice were allowed free access to both compartments when the guillotine doors were open during the pretesting and final testing phase, and the time spent by the mouse in each compartment was recorded for 15 min.

Procedures for place conditioning. Preliminary data have indicated that naive mice spend more time in the black compartment than in the white compartment when given free access to the entire apparatus for 15 min. Thus, to establish conditioning, we paired morphine with the less favored white compartment. Control mice received a subcutaneous injection of saline immediately before being placed in the black compartment. Mice were placed in the white compartment immediately after administration of morphine (5 mg/kg, SC). PF_{11} was given orally 60 min before morphine or saline administration.

Three Phase Procedure

Pretesting phase. On day 1 and day 2, mice were allowed to move freely between the two compartments. On day 3, baseline preference between the two compartments was established during a 15-min observation period.

Conditioning phase. On days 4, 6, 8, and 10, mice were injected with morphine before confinement in the white compartment for 60 min. On days 5, 7, 9, and 11, mice were injected with saline before confinement in the black compartment for 60 min.

Testing phase. On day 12, the door was opened and the mice were placed in the compartments and allowed to move freely between the two compartments. The time spend by each mouse in the white compartment was recorded during a 15-min observation period.

Analgesia Test

The drug effect was measured by the tail-pinch test. The test was carried out according to the method reported (13,32). Briefly, a paper clip was applied to the tail root of the mouse and the latency of the biting response to the clip was measured. The pressure of the paper clip was adjusted so that the biting response of the naive mice was normally about one second. To prevent tissue damage, a cutoff time of 6 s was selected. The analgesic response was measured every 30 min over a 120-min observation period. PF_{11} was administrated orally consecutively for 5 days. The analgesic effect of morphine was test 60 min after the final administration of PF_{11}, 30 min after morphine (5 mg/kg, SC) administration.

Morphine Tolerance Test

Locomotor activity. The locomotor activity of the mice was measured in a locomotor monitoring cage (15 × 15 × 11 cm, Model XZC-4, Mudanjiang, China) (38). To test the effect of PF_{11} on morphine-induced locomotor sensitization, morphine (10 mg/kg, SC) was injected once a day continuously for 7 days and PF_{11} (4 mg/kg and 8 mg/kg, PO) was administrated 60 min prior to the injection of morphine daily. Every day immediately after the injection of morphine, the mouse was placed in the activity cage for a 5-min adaptation period followed by a 30-min activity recording period.

Analgesia tolerance. The tail-pinch method as described above was used. Morphine (10 mg/kg, SC) was injected once a day consecutively for 9 days, and PF_{11} (4 mg/kg, 8 mg/kg, PO) was administrated 60 min prior to the administration of morphine. The inhibitory effect of PF_{11} on morphine-induced tolerance development was evidenced by the maintaining of analgesic response to a challenge dose of morphine (5 mg/kg, SC) given 24 h after the final injection of morphine. The latency of biting response in the tail-pinch test was estimated at 30, 60, 90, and 120 min after administration of the challenge dose of morphine (5 mg/kg, SC).

Statistics

The results were expressed as the mean ± SEM. Data from the drug effects on morphine-induced locomotor sensitization and development of analgesic tolerance were analyzed by use of unpaired Student's test. Data from the other experiments were analyzed using a one-way analysis of variance (ANOVA) followed by the Dunnett's test for multiple comparison between groups. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Effect of PF_{11} on Morphine-Induced Memory Impairment in Morris Water Maze

The latencies of mice, treated with PF_{11} 2, 4, and 8 mg/kg, to locate the platform were not significantly shortened compared to that of the saline control group, suggesting that PF_{11} per se has no improving effect on spatial memory. The latencies of mice to locate the platform were significantly prolonged after morphine (10 mg/kg, SC) administration on days 3–5 when compared with saline control group. However, the prolonged latencies were markedly shortened after administration of PF_{11} 4 mg/kg [on day 4, $F(2, 23) = 17.01$, $p < 0.001$] and 8 mg/kg [on day 3, $F(2, 23) = 91.79$, $p < 0.001$; on day 4, $F(2, 23) = 12.00$, $p < 0.001$] (Fig. 1).

Effect of PF_{11} on Morphine-Induced Conditioned Place Preference

Treatment with PF_{11} at 4 mg/kg alone did not significantly influence the conditioned place-preference response compared with the saline control group. However, pretreatment with PF_{11} at 4 mg/kg significantly antagonized the conditioned place preference induced by morphine (Fig. 2A), $F(2, 40) = 4.57$, $p < 0.05$. PF_{11} at 8 mg/kg, whether used alone or pretreated with morphine, did not significantly influence the conditioned place-preference response (Fig. 2B).

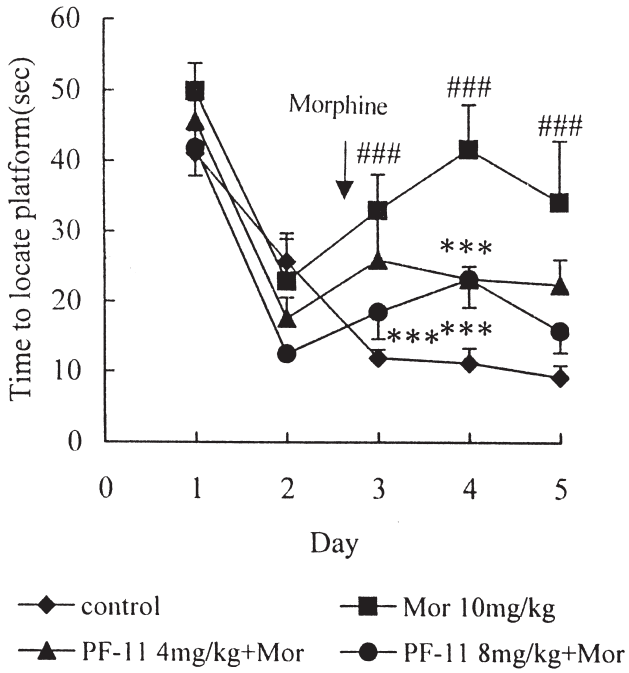

FIG. 1. Effect of pseudoginsenoside-F_{11} on morphine-induced memory impairment in Morris water maze in mice. The mice were injected with saline on days 1–2 and with morphine (10 mg/kg, SC) on days 3–5. Pseudoginsenoside-F_{11}, 4 and 8 mg/kg, was administrated 60 min before saline or morphine administration from days 1 to 5. Each point is the mean ± SEM of the data obtained from eight to nine mice. Mor = morphine. ###$p < 0.001$ vs. saline group. ***$p < 0.001$ vs. morphine group.

Effect of PF_{11} on Morphine-Induced Analgesia

These results are shown graphically in Fig. 3. Morphine (5 mg/kg, SC) exhibited a markedly analgesic effect in the tail-pinch test. PF_{11} per se showed no analgesic effect. However, the morphine-induced analgesic effect was significantly attenuated by administration of PF_{11} at 4 mg/kg (Fig. 3A), $F(2, 24) = 7.25$, $p < 0.01$ at 60 min after morphine administration) and 8 mg/kg (Fig. 3B), $F(2, 24) = 4.53$, $p < 0.05$ at 30 min after morphine administration; $F(2, 24) = 8.60$, $p < 0.01$ at 60 min after morphine administration).

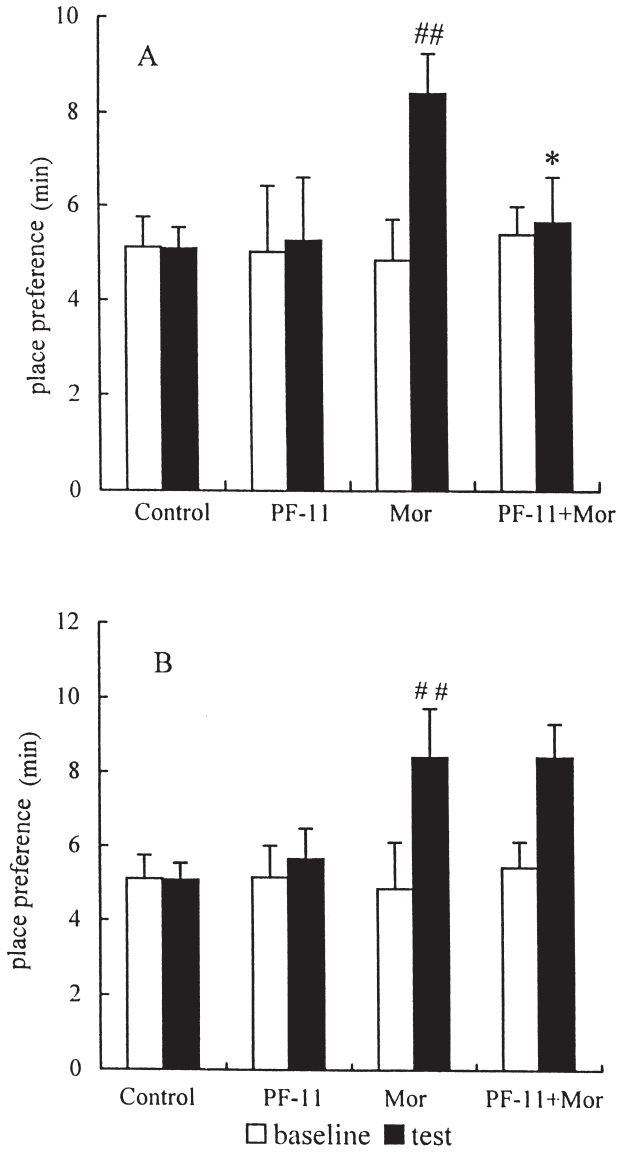

FIG. 2. Effect of pseudoginsenoside-F_{11} on morphine-induced conditioned place preference. Place-preference data are expressed as the time spent in the drug-paired compartment. Pseudoginsenoside-F_{11}, 4 mg/kg (A) and 8 mg/kg (B), was administrated intragastrically to mice 30 min before subcutaneous injection of morphine 5 mg/kg or saline in the conditioned phase. Each column is the mean ± SEM of the data obtained from 13–16 mice. Mor = morphine. ##$p < 0.01$ vs. saline group. *$p < 0.05$ vs. morphine group.

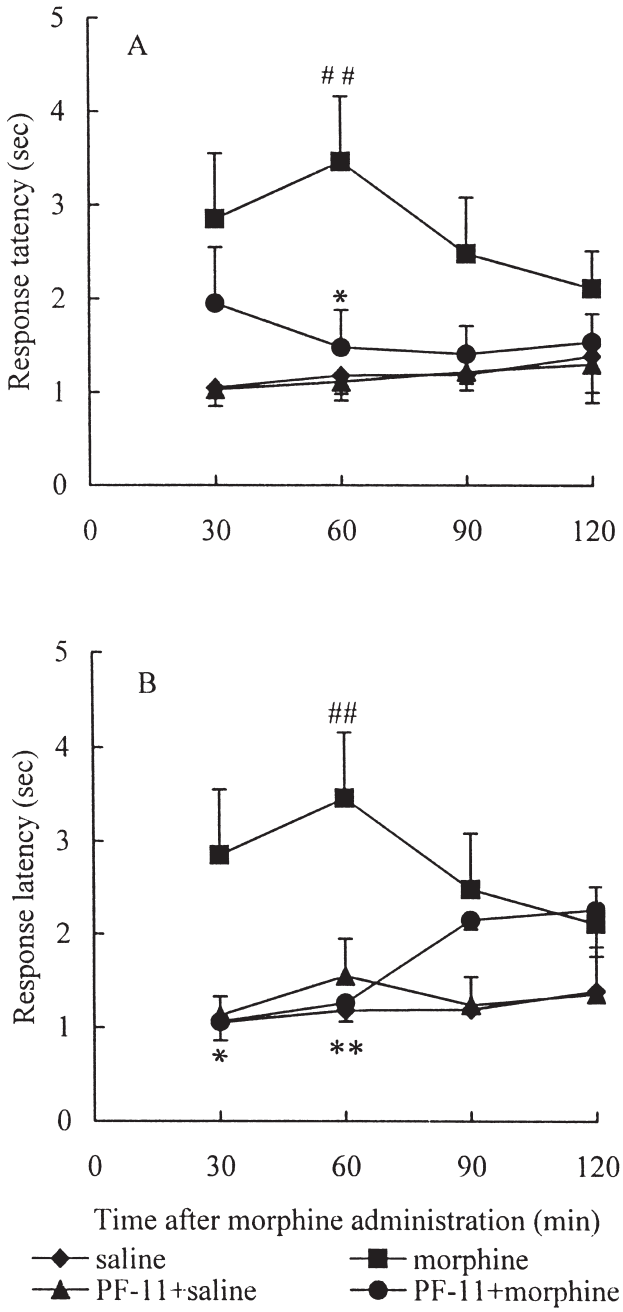

FIG. 3. Effect of pseudoginsenoside-F_{11} on morphine (5 mg/kg, SC)-induced analgesia in tail-pinch test. Pseudoginsenoside-F_{11}, 4 mg/kg (A) and 8 mg/kg (B), was administrated intragastrically for 5 days. Morphine was injected subcutaneously 60 min after the final administration of pseudoginsenoside-F_{11}. The latency was measured every 30 min over 120-min observation period. Each point represents the mean ± SEM of 9–10 mice. #$p < 0.05$, ##$p < 0.01$ vs. saline group. **$p < 0.01$, *$p < 0.05$ vs. morphine group.

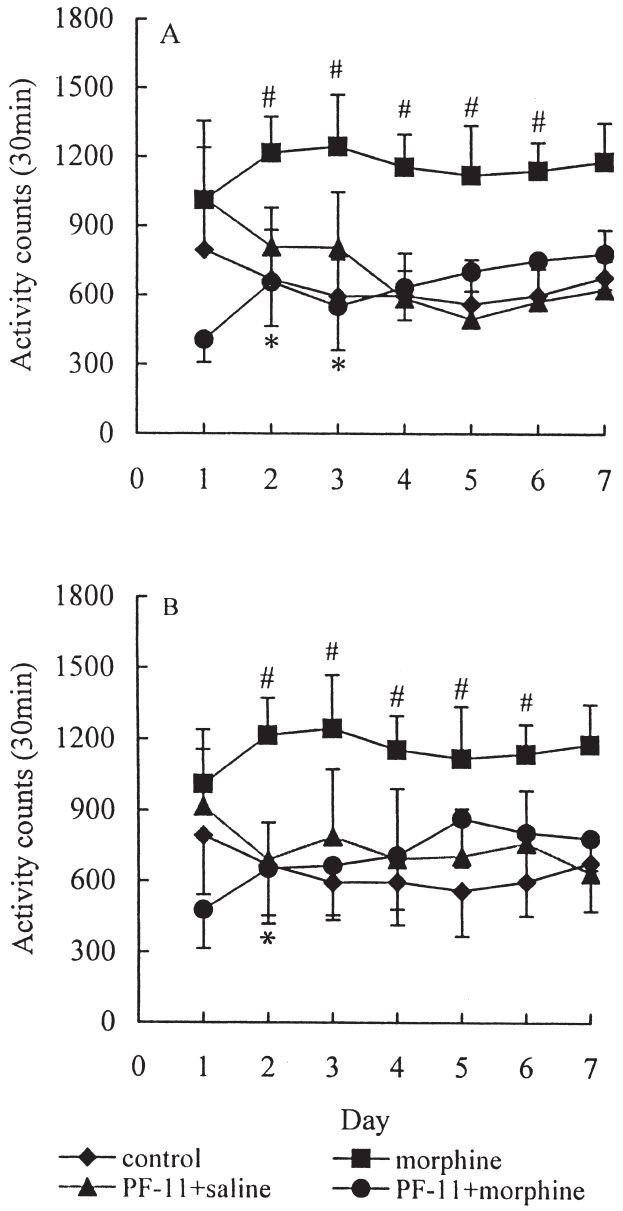

FIG. 4. Effect of pseudoginsenoside-F_{11} on the development of sensitization to the accelerating effect of morphine. Morphine 10 mg/kg was injected subcutaneously seven times at intervals of 24 h. Pseudoginsenoside-F_{11}, 4 mg/kg (A) and 8 mg/kg (B), was given 60 min before morphine injection. Each point represents the mean ± SEM of 11 mice. #$p < 0.05$ vs. saline group. *$p < 0.05$ vs. morphine group.

Effect of PF_{11} on Morphine-Induced Locomotor Sensitization

As seen in Fig. 4, administration of PF_{11} 4 and 8 mg/kg alone had no significant effect on locomotor activity. However, morphine caused a marked increase in activity counts from day 2 to day 6 compared with the saline control group ($p < 0.05$). Morphine-induced hyperactivity was significantly antagonized by pretreatment with PF_{11} (Fig. 4A and B).

Effect of PF_{11} on Morphine-Induced Development of Analgesic Tolerance

After the challenge dose of morphine (5 mg/kg, SC), the analgesic responses between groups were significantly different (Fig. 5). A significant analgesic effect appeared in the

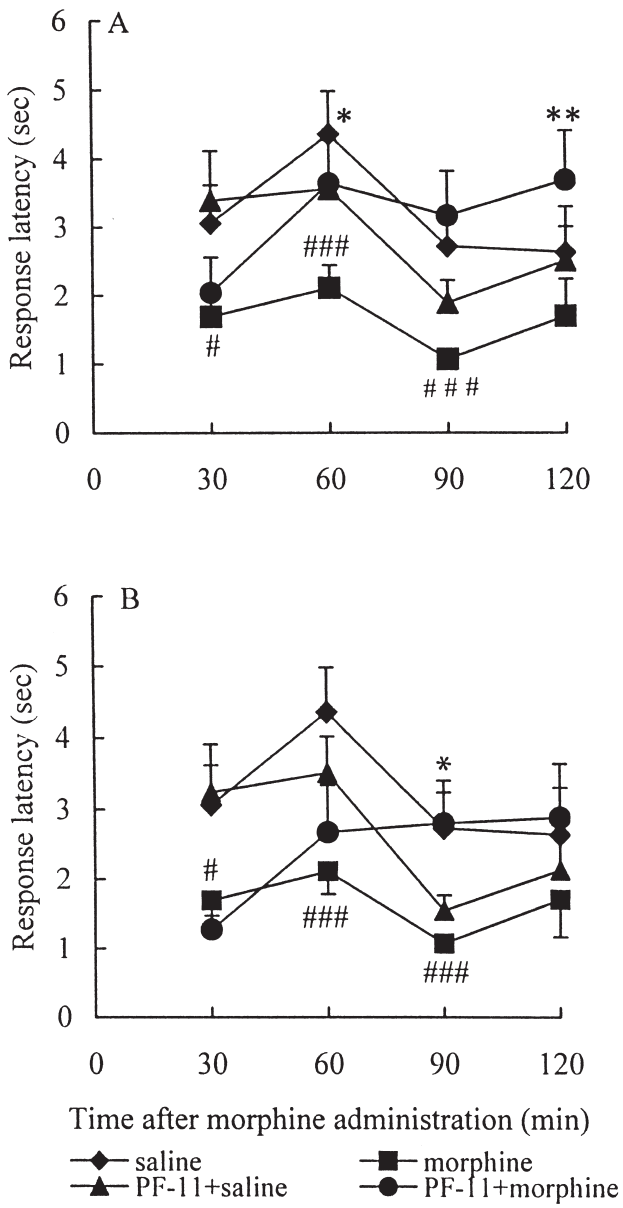

FIG. 5. Effect of pseudoginsenoside-F_{11} on the development of morphine tolerance. Morphine, 10 mg/kg, was injected subcutaneously nine times at the intervals of 24 h, and pseudoginsenoside-F_{11}, 4 mg/kg (A) and 8 mg/kg (B), was given 60 min everyday before morphine injection. Each point represents the mean ± SEM of 11 mice. #$p < 0.05$, ###$p < 0.001$ vs. saline group. **$p < 0.01$, *$p < 0.05$ vs. morphine group.

saline control group. In the morphine (10 mg/kg, SC) control group, the pain threshold decreased significantly, suggesting that morphine tolerance developed. In the PF_{11}, 4 mg/kg + morphine group, the analgesic effect of the challenge dose of morphine (5 mg/kg, SC) was more significant than that of morphine control group ($p < 0.05$, at 60 min and $p < 0.01$, at 120 min after morphine administration, respectively) (Fig. 5A). However, in the PF_{11}, 8 mg/kg + morphine group, no significant increase in analgesic effect was observed after the challenge dose of morphine (Fig. 5B). Moreover, the analgesic effect of morphine was significantly antagonized by PF_{11}, at the dose of 8 mg/kg used alone ($p < 0.05$, at 90 min after morphine administration compared with saline group) (Fig. 5B).

DISCUSSION

The present study has indicated that morphine can induce a memory deficit in Morris water maze performance. These results are consistent with reports that morphine in single doses induces memory deficits in one-trial passive avoidance task (14) and impairs performance in spontaneous alternation task that reflects the spatial working memory (31,35). Moreover, in the present study, PF_{11} per se has no effect on spatial memory, yet can significantly antagonize the morphine-induced memory impairment in Morris water maze task. Our previous experiments have shown that PF_{11} improves scopolamine-induced memory deficits in one-trial passive avoidance test, in two-way active avoidance response test and in water maze test (21). These observations strongly suggest that PF_{11} is a substance that can antagonize memory deficits induced by morphine and scopolamine.

Conditioned place-preference paradigm is considered as a model for the reinforcing properties of drugs, such as morphine (2,5,28), with dependence liability (7). In the present study chronic morphine produced a conditioned placed-preference response, and this response was significantly inhibited by pretreatment with PF_{11} at 4 mg/kg, which per se did not induce the place preference. It is reported that dopaminergic neurons of the ventral tegmental system were involved in reinforcement processes, in which morphine facilitates dopaminergic transmission, either by stimulating the release of dopamine or by inhibiting dopamine uptake (6,10,19,37). Thus, it is possible that the influence of dopamine transmission by PF_{11} is involved in its inhibitory effect on morphine-induced conditioned place preference. It is reported that ginsenosides from *Panax ginseng* influence monoamine transmission in the brain (34).

The present data indicate that PF_{11} significantly attenuated morphine-induced hyperactivity, which is regarded as a sensitization (or reverse tolerance) to morphine (12,30), and antagonized the development of tolerance to morphine analgesia in the tail-pinch test in mice. Although several neuronal systems are involved in morphine tolerance (15,22,24,29,39), the mechanism underlying the development of morphine tolerance remains unclear at the present time. It is suggested that morphine inhibited the activation of adenylyl cyclase, and consequently, the activation of neurons (9). Chronic morphine results in a compensatory upregulation of adenylyl cyclase activity followed by decreased effect of morphine and thus tolerance results. To date, no study has been done to investigate whether PF_{11} affects adenylyl cyclase activity. However, it is reported that ginsenosides, which antagonize the development of tolerance to morphine (4), increase intracellular cAMP levels, stimulate the activity of adenylyl cyclase, and inhibit phosphodiesterase activity (23,26). Moreover, ginseng root saponins can normalize the stress-induced increase in intracellular cAMP levels (11). Because there are many common properties shared by both *Panax ginseng* and *Panax quinquefolium* in traditional Chinese medicine, it is worthwhile to elucidate whether PF_{11} is able to affect the nucleotide catabolism.

The differential effects of ginsenosides may come from the differences of their structure types. PF_{11} has a structure of octillol type that exists in *Panax quinquefolium* (33) and *Panax*

vietnamensis (Vietnamese ginseng) (25). It is found that an octillol type saponin majonside-R2, a compound isolated from *Panax vietnamensis*, dose dependently attenuated opioid-induced antinociception (13). In the present study we have also observed PF_{11} antagonized morphine-induced analgesia. These observations may strongly suggest the potential role of octillol type saponin from the *Panax genus* in regulation of opioids activities, and the mechanism of the action of these substances merits further evaluation.

In conclusion, the present study has given the first evidence that PF_{11} possesses a significant inhibitory property on various morphine-induced actions, such as memory impairment, conditioned place preference, analgesia and the development of tolerance. Although the mechanism of action of PF_{11} is not clear at present, two explanations could be speculated, according to the present results and above discussions. One is that PF_{11} acts, respectively, on the different neuronal systems to antagonize the actions of morphine, such as by activating cholinergic transmission to antagonize morphine-induced memory deficits (1,20,21); by activating dopaminergic transmission to inhibit morphine-induced conditioned place preference (19,37); and by regulating adenylyl cyclase activity to eliminate morphine-induced tolerance development. However, in theory, it seems unlikely that PF_{11}, as a pure substance, could directly affect so many neuronal systems. Another possible explanation is that PF_{11} may act as an antagonist on opioid receptors, so that it can antagonize all actions of morphine observed in the present study. Binding experiments carried out by our laboratory have shown that PF_{11} significantly inhibited the displacement of morphine to [^{3}H]-diprenorphine at μ receptors (manuscript in preparation). Collectively, the present results suggest that PF_{11} may be an interesting candidate for use in the treatment of some adverse effects induced by morphine.

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