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Research report

Dual effects of the lichen glucan PB-2, extracted from *Flavoparmelia baltimorensis*, on the induction of long-term potentiation in the dentate gyrus of the anesthetized rat: possible mediation via adrenaline β- and interleukin-1 receptors

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Abstract

We have previously found that oral or intravenous (i.v.) administration of the polysaccharide fraction PB-2, extracted from the lichen *Flavoparmelia baltimorensis*, facilitated the induction of long-term potentiation (LTP) in the dentate gyrus (DG) in vivo. In this study, the mechanism underlying the effect of PB-2 on the induction of LTP was investigated in the DG of anesthetized rat focusing on the contribution of the interleukin-1 (IL-1) receptor and the adrenaline β-receptor. An i.v. injection of IL-1ra (10^{-9} g/kg), an antagonist of the IL-1 receptor, had no effect on the basal response in the DG; however, this treatment augmented the enhancement of LTP induced by a single i.v. injection of PB-2 (10^{-3} g/kg). This potentiating effect was also observed following intracerebroventricular (i.c.v.) injection of IL-1ra (10^{-15}–10^{-11} g). An i.v. injection of IL-1β (3.5×10^{-15}–3.5×10^{-9} g/kg) inhibited the induction of LTP, which was diminished by the previous application of IL-1ra. These results suggest that the activation of the IL-1 receptor induces the suppression of LTP in PB-2-treated rats, and that endogenous IL-1β contributes to the IL-1 receptor activation. An i.c.v. infusion of metoprolol (7.5×10^{-6} g), an antagonist of the adrenaline β_1-receptor, attenuated the enhancement of LTP induced by an i.v. injection of PB-2. These results suggest that PB-2 has two different effects on the LTP, an enhancing effect and an inhibiting one, and that it exhibited the significant enhancing effect on the LTP as a total balance of these effects.

Theme: Excitable membranes and synaptic transmission
Topic: Long-term potentiation: pharmacology

Keywords: Interleukin-1β; Adrenaline β-receptor; Lichen; Polysaccharide; Long-term potentiation; Hippocampus

1. Introduction

Long-term potentiation (LTP) is a form of synaptic plasticity that is observed in some brain regions, such as the hippocampal formation [1,6] and cerebral cortices [10,19,22]. Indeed, LTP in the hippocampal formation is considered to be the primary cellular model of learning and memory [5]. We have demonstrated that some extracts of thallophytic plants are candidate materials for retrieving from the memory impairments associated with senile dementia because they have the ability to increase the level of LTP that occurs in the hippocampus [11,30]. Recently, we have demonstrated that oral administration or intravenous (i.v.) injection of PB-2, a water-soluble polysaccharide

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fraction extracted from the lichen *Flavoparmelia baltimorensis*, facilitates the induction of LTP in the dentate gyrus (DG) of the anesthetized rat [15]. PB-2 had no effect on LTP if administered intracerebroventricularly (i.c.v.), suggesting that the primary site of action of PB-2 was not in the central nervous system (CNS) but in the periphery. However, the mechanism underlying the LTP-enhancing-effect of PB-2 has yet to be elucidated.

Interleukins (ILs) are known to act as mediators for host defense responses in the periphery [9]. Recent evidence has determined various roles of ILs in the CNS. Among the ILs, the proinflammatory cytokine, interleukin-1β (IL-1β), has been reported to have a dominant effect on CNS functions such as the control of appetite and sleep [23,25]. It has also been suggested that IL-1β stimulates the hypothalamo–pituitary–adrenal axis [27], implicating a role in the relationship between the peripheral and the central relationship. There are several lines of evidence to suggest that the expression of functional IL-1β is high not only in the hypothalamus but also in the hippocampus [26], and that a high density of IL-1 receptors exists in the hippocampus [12,24,27], suggesting the involvement of IL-1β in memory processes and LTP. While evidence for significant roles of IL-1β in the CNS is accumulating, very little is known about its role in the periphery with regard to its influence on central synaptic function.

The adrenaline β-receptor has been shown to relate the hippocampal synaptic function such as the basal synaptic transmission and the induction of LTP [33]. It is reported that the activation of β-receptor induced an increase of the synaptic potential in the hippocampal formation, which was parallel with the activation of the locus ceruleus [20,21]. Furthermore, the possibility has risen that the peripheral IL-1β controls the central norepinephrine, as an endogenous ligand of the β-receptor [29]. Therefore, in the present study, we investigated the role of the IL-1 receptor and the β-receptor in the PB-2-induced enhancement of LTP in anesthetized rats. We proposed the dual effect of PB-2 on the induction of LTP because of the simultaneous activation of the enhancing effect and the inhibiting one due to the activation of the adrenaline β-receptor and the IL-1 receptor, respectively.

2. Materials and methods

2.1. Isolation of PB-2

PB-2 was isolated from the lichen *Flavoparmelia baltimorensis* as described previously [31,32]. Briefly, *Flavoparmelia baltimorensis* (6.22 g) was first processed with ethanol, and the residue was extracted with water. The aqueous extract (0.758 g) was suspended in 150 ml of a mixture of ethanol and water (2:1), and then centrifuged at 10,000 rpm for 30 min at 4 °C. The precipitate (PB-1, 0.475 g) was resuspended in 50 ml of water and centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant yielded 144.1 mg of PB-2. The structure of the main constituent of PB-2 was analyzed by ^{1}H-nuclear magnetic resonance spectrometry, and revealed to be a linear α-glucan with α(1–3) and α(1–4) linkages.

2.2. Animals

Male Wistar rats (5–8 weeks old; Nihon SLC, Shizuoka, Japan) were housed in a temperature- and humidity-controlled environment (23 ± 1 °C, approximately 50% humidity) on a 12:12 h light/dark cycle initiated at 8:00 a.m., and with free access to food and water. The care and handling of the animals conformed to the Guidelines for Animal Experiment of College of Pharmacy, Nihon University.

2.3. Electrophysiology

Electrophysiological recording of field potentials in the DG of the anesthetized rat was performed as described previously [15]. Briefly, rats were deeply anesthetized with an intraperitoneal injection of a combination of urethane (1.0 g/kg) and α-chloralose (25 mg/kg). Rats were judged to be sufficiently anesthetized when their hindlimb withdrawal reflex in response to a light pinprick had disappeared. They were then fixed into a stereotaxic frame equipped with a head holder.

A bipolar electrode was placed in the entorhinal cortex (8.1 mm posterior to bregma, 4.4 mm lateral to the midline, approximately 3.0 mm ventral to the dura) to stimulate the bundle of the perforant path. A monopolar recording electrode was placed in the granule cell layer of the ipsilateral dentate gyrus (3.5 mm posterior to bregma, 2.0 mm lateral to the midline, approximately 3.0 mm ventral to the dura). A field synaptic potential superimposed with a population spike (PS) was elicited by a single-pulse test stimulation. The stimulus intensity was set to evoke the half-maximal amplitude of a PS. We recruited the optimum amplitude of the evoked PS as described in the previous report [15]. Several brief high-frequency stimulations were used to induce LTP. This usually took the form of a weak tetanic stimulation consisting of 30 pulses at 60 Hz. In the IL-1β experiment, however, strong tetanic stimulations consisting of 100 pulses at 100 Hz or 100 pulses at 250 Hz were used. The effects of the drugs were assessed by analyzing the time course of changes in the PS amplitude after applying tetanic stimulation. LTP magnitude was measured by averaging the data recorded 30–60 min after the tetanic stimulation.

2.4. Drug injection

For the i.c.v. injection, 5.0 μl of the desired concentration of a drug was injected into the contralateral ventricle (0.8 mm posterior to bregma, 1.5 mm lateral to the midline, 3.5 mm ventral to the dura) with the aid of a microsyringe after

obtaining the stable postsynaptic response. The responses were observed for a further 25 min to allow the drug to diffuse systemically and to ensure constancy of the baseline responses. For the i.v. injection, 1.0 ml/kg of the desired concentration of a drug was injected into a venous cannula that had been inserted into the femoral vein.

2.5. Chemicals

IL-1ra was purchased from Genzyme/Techne. All other chemicals were purchased from Sigma.

3. Results

3.1. PB-2-induced enhancement of LTP in the DG

We have reported previously that a single i.v. injection of PB-2 facilitates the induction of LTP in the DG in a bell-shaped manner, where a significant increase was observed at doses of 1.0×10^{-3} and 5.0×10^{-3} g/kg [15]. Thus, in this study, a PB-2 dose of 1.0×10^{-3} g/kg was used to enhance LTP. We determined the optimal timing of an injection of PB-2 by administering single i.v. infusions of PB-2 (1.0×10^{-3} g/kg) 60, 30, or 15 min before, and immediately after tetanic stimulation consisting of 30 pulses at 60 Hz (Fig. 1). The enhancement of LTP was observed when PB-2 was injected before the stimulation; however, a significant increase in LTP was observed only when the injection was administered 15 min before the tetanic stimulation (control, 112.97 ± 2.96, $n = 5$; PB-2 (−15 min), 154.90 ± 7.59, $n = 6$; $P < 0.05$). Thus, in subsequent experiments, PB-2 was injected 15 min before the tetanic stimulation, as was the case in our previous report.

3.2. Effect of IL-1ra on the induction and PB-2-induced enhancement of LTP

In order to examine the role of endogenous IL-1 in the PB-2-induced modulation of LTP, a soluble IL-1 receptor (IL-1ra), an antagonist of the IL-1 receptor, was infused solely to the femoral vein so as to antagonize the IL-1 receptors (Fig. 2). An i.v. infusion of IL-1ra (10^{-9} g/kg) alone did not induce any significant changes in the evoked basal response. Although LTP tended to be enhanced by pretreatment with IL-1ra, this effect was not significant as compared with the control group. An i.v. infusion of PB-2 under the previous IL-1ra-infused condition did not affect the basal responses (Fig. 2A). The same treatment with IL-1ra (10^{-9} g/kg) increased the PB-2-induced enhancement of LTP (PB-2 alone, 154.03 ± 6.00, $n = 4$; PB-2 + 10^{-9} g/kg IL-1ra, 193.46 ± 6.73, $n = 5$; $P < 0.05$), although lower doses of IL-1ra did not affect that enhancement. Next, we investigated the contribution of the central IL-1 receptor on the PB-2-induced enhancement of LTP (Fig. 3). Pretreatment with IL-1ra (10^{-15}–10^{-11} g) by i.c.v. injection augmented the

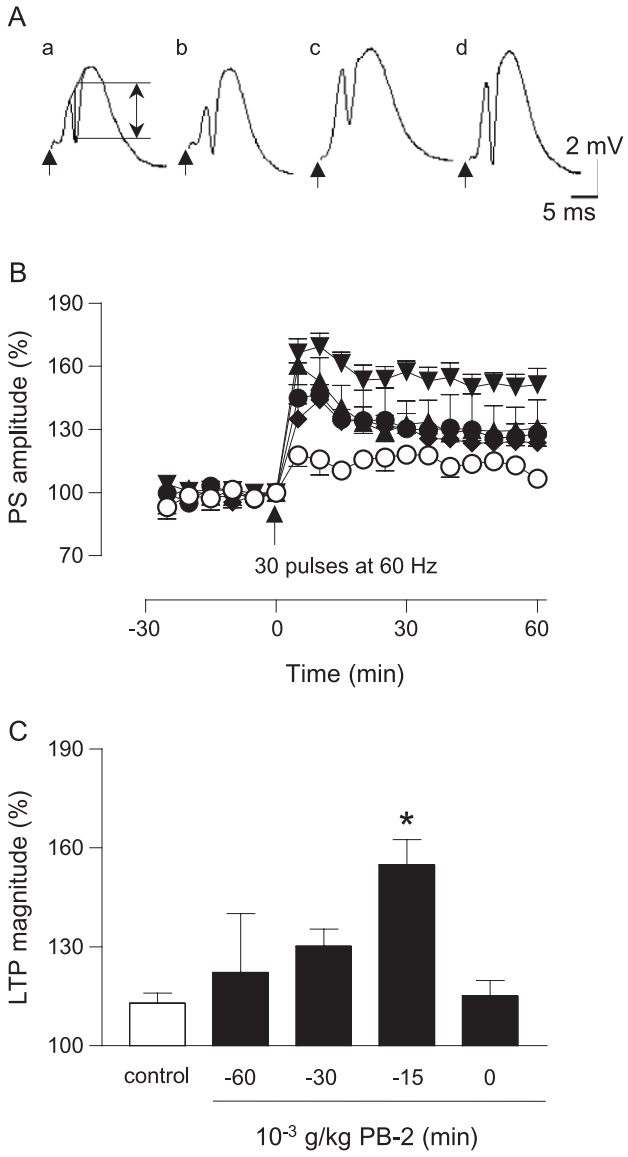

Fig. 1. Optimal timing of injection of PB-2 to enhance LTP. (A) Sample traces of evoked synaptic potentials recorded from the dentate granule cell layer in control and PB-2-treated conditions. Left two traces indicate sample recordings in control condition at immediately before (a) and 60 min after (b) tetanic stimulation. Right two samples are from the PB-2-treated (15 min before tetanic stimulation) condition at immediately before (c) and 60 min after (d) tetanic stimulation. Low-frequent test stimulation was delivered at indicated by an arrow. PS amplitude was monitored by measure of depth of negative-going component that indicated by double-directed arrow in a. (B) Time-course changes in the PS amplitude of control (○), PB-2-injected animals at 60 (●), 30 (▲) or 15 (▼) min before and immediate after (0 min: ◆) tetanic stimulation. The effect of PB-2 on the induction of LTP induced by tetanic stimulation consisting of 30 pulses at 60 Hz was examined by i.v. injection of PB-2. Results are expressed as percent difference from the average value 0–5 min before tetanic stimulation. Data are expressed as means ± SEM. (C) Effect of the timing for PB-2 injection on LTP magnitude. LTP magnitude is expressed by the average data 30–60 min after tetanic stimulation. Data are expressed as means ± SEM (control, $n = 5$; PB-2 groups, $n = 6$ each) *$P < 0.05$ vs. control (Tukey's test following analysis of variance).

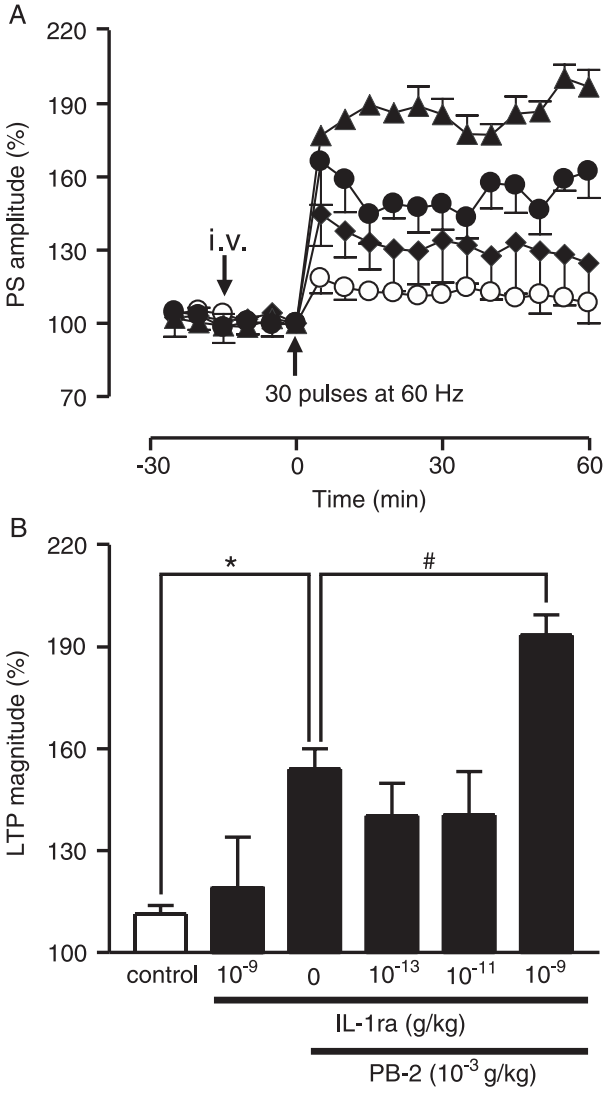

Fig. 2. Peripheral IL-1 receptor-mediation to the PB-2-induced enhancement of LTP. (A) Time-course changes in the PS amplitude of control (○), 10^{-3} g/kg PB-2- (●), 10^{-9} g/kg IL-1ra + 10^{-3} g/kg PB-2- (▲) or 10^{-9} g/kg IL-1ra- (◆) injected animals. Drugs were delivered by a single i.v. injection 15 min before tetanic stimulation. Results are expressed as percent difference from the average value 0–5 min before tetanic stimulation. Data are expressed as means ± SEM. (B) Comparison of the effect of IL-1ra on PB-2-induced enhancement of LTP. Data are expressed as means ± SEM (control, $n = 5$; IL-1ra alone, $n = 4$; PB-2 alone, $n = 4$; 10^{-3} g/kg PB-2 + 10^{-13} g/kg IL-1ra, $n = 3$; 10^{-3} g/kg PB-2 + 10^{-11} g/kg IL-1ra, $n = 3$; 10^{-3} g/kg PB-2 + 10^{-9} g/kg IL-1ra, $n = 5$) $^{*}P < 0.05$, $^{\#}P < 0.05$ (Tukey's test following analysis of variance).

enhancing effect of PB-2 without affecting the evoked basal responses. A significant augmentation was observed at a dose of 10^{-11} g (PB-2 alone, 146.85 ± 2.71, $n = 5$; PB-2 + 10^{-11} g IL-1ra, 185.47 ± 8.47, $n = 4$; $P < 0.05$).

3.3. Effect of IL-1β on the induction of LTP

Since it has been suggested that the IL-1 receptor contributes to the activation of the PB-2 accelerating mechanism, we next examined the involvement of IL-1β in the activation of IL-1 receptors. To this end, we investigated the effect of IL-1β on the induction of LTP. A single infusion of various doses of IL-1β (3.5×10^{-15}–3.5×10^{-9} g/kg) given via the femoral vein reduced the LTP induced by electrical stimulation consisting of 30 pulses at 60 Hz or 100 pulses at 100 Hz. However, none of the inhibitory effects reached statistical significance (Fig. 4). We investigated further the effect of IL-1β on the LTP induced by applying a stronger electrical stimulation. The stronger tetanic stimulation consisting of 100 pulses at 250 Hz induced a greater degree of LTP that was

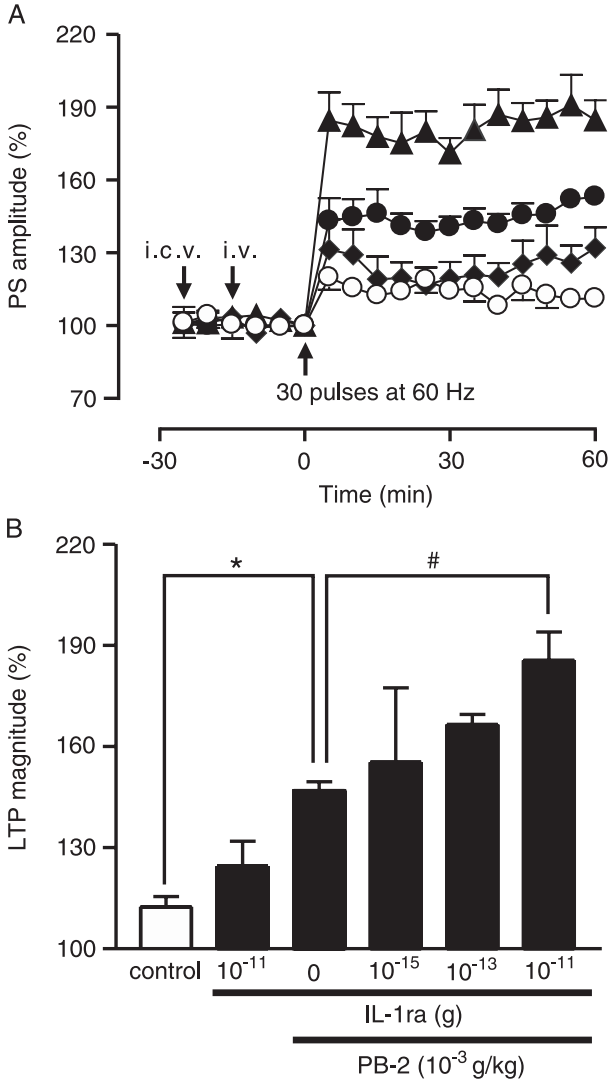

Fig. 3. Central IL-1 receptor-mediation to the PB-2-induced enhancement of LTP. (A) Time-course changes in the PS amplitude of control (○), 10^{-3} g/kg PB-2- (●), 10^{-11} g IL-1ra+10^{-3} g/kg PB-2- (▲) or 10^{-11} g IL-1ra- (◆) injected animals. IL-1ra was delivered by an i.c.v. injection 25 min and PB-2 by an i.v. injection 15 min before tetanic stimulation. Results are expressed as percent difference from the average value 0–5 min before tetanic stimulation. Data are expressed as means ± SEM. (B) Comparison of the effect of IL-1ra on PB-2-induced enhancement of LTP. Data are expressed as means ± SEM (control, $n = 5$; IL-1ra alone, $n = 3$; PB-2 alone, $n = 5$; 10^{-3} g/kg PB-2 + 10^{-15} g/kg IL-1ra, $n = 3$; 10^{-3} g/kg PB-2 + 10^{-13} g/kg IL-1ra, $n = 3$; 10^{-3} g/kg PB-2 + 10^{-11} g/kg IL-1ra, $n = 4$) $^{*}P < 0.05$, $^{\#}P < 0.05$ (Tukey's test following analysis of variance).

similar magnitude of LTP induced by 60 Hz-stimulation under PB-2- and IL-1ra-injected condition (Fig. 5). An injection of IL-1β (3.5×10^{-15}–3.5×10^{-9} g/kg) inhibited the induction of LTP that was induced by strong tetanic stimulation. The doses that resulted in significant changes lay in the range 3.5×10^{-12}–3.5×10^{-9} g/kg (control, 170.85 ± 13.69, $n = 5$; 3.5×10^{-12} g/kg IL-1β, 121.39 ± 11.44, $n = 4$; 3.5×10^{-9} g/kg IL-1β, 118.69 ± 6.42, $n = 4$; $P < 0.05$).

We also examined whether the inhibitory effect of IL-1β on LTP was mediated by the IL-1 receptor (Fig. 6). The LTP-inhibiting effect of IL-1β was diminished by pretreatment with IL-1ra in a dose-dependent manner (10^{-11}–10^{-9} g/kg). At 10^{-9} g/kg IL-1ra, the highest dose used here, the inhibitory effect of IL-1β was attenuated by 8.29% compared to the control group, and this effect represented a significant recovery from the IL-1β-induced inhibition of LTP induction (3.5×10^{-9} g/kg IL-1β alone, 109.65 ± 4.49, $n = 5$; 3.5×10^{-9} g/kg IL-1β + 10-9 g/kg IL-1ra, 154.02 ± 6.26, $n = 5$; $P < 0.01$).

3.4. Effects of metoprolol on the induction and PB-2-induced enhancement of LTP

Because the total effect of PB-2 was the enhancement of LTP, we further investigated the mechanism underlying the PB-2-induced enhancement on LTP. A growing evidence suggests that the adrenaline β-receptor contributes to the enhancement of LTP [21,33]. Thus, we focused on this type of receptor to examine the contribution to the PB-2-related enhancement (Fig. 7). An i.c.v. injection of metoprolol (7.5×10^{-6} g), an adrenaline β_1-receptor antagonist, did not show any affects on the basal response and the induction of LTP. However, a previous i.c.v. injection of metoprolol attenuated the enhancement of LTP induced by the i.v. infusion of PB-2 (PB-2 alone, 150.53 ± 9.36, $n = 5$; PB-2 + metoprolol, 121.21 ± 4.56, $n = 4$; $P < 0.05$).

3.5. Effects of IL-1ra and metoprolol on the higher dose of PB-2-induced effect on LTP

Although the enhancement of LTP induced by an i.v. infusion of 1.0 mg/kg PB-2 was related to the activation of the IL-1 receptor and the adrenaline β_1-receptor, it is not cleared the contribution of those receptors to the effect of high dose of PB-2. Because the enhancing-effect of LTP by PB-2 was in a bell-shaped manner, we further investigated whether a dose of PB-2 higher than the optimal dose to facilitate LTP activated the same receptors (Fig. 8). Although 10.0 mg/kg PB-2 induced a slight increase in LTP magnitude, a previous injection of metoprolol reduced the enhancement of LTP. Additively, a previous infusion of

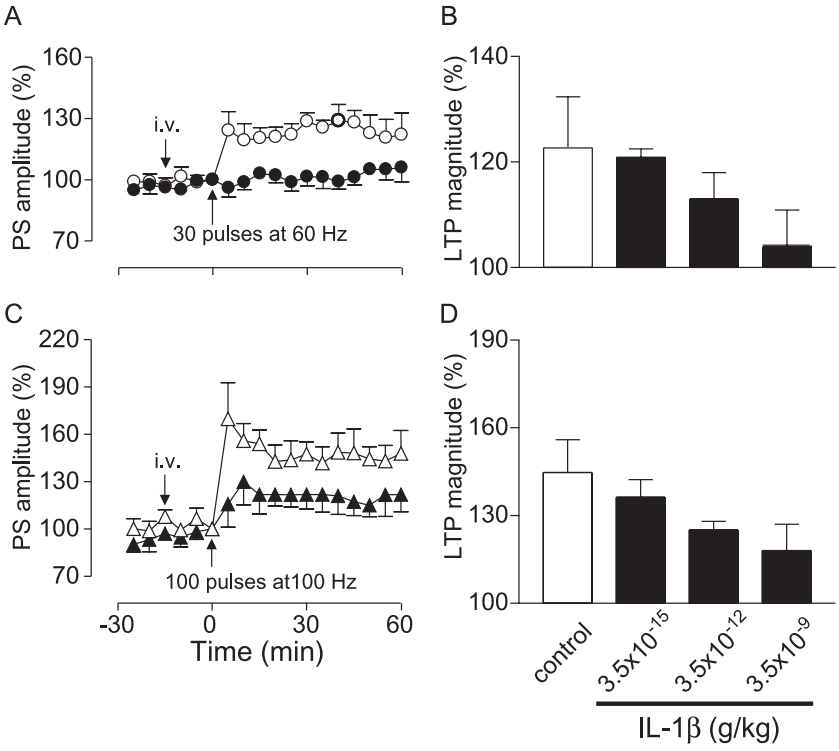

Fig. 4. No significant effect of IL-1β on the LTP induced by conventional tetanic stimulations. (A, C) Time-course changes in the PS amplitude of control (○) or 3.5×10^{-9} g/kg IL-1β-injected (●) animals in A, and control (△) or 3.5×10^{-9} g/kg IL-1β-injected (▲) animals in C. IL-1β was delivered by a single i.v. injection 15 min before tetanic stimulation consisting of 30 pulses at 60 Hz in A, and 100 pulses at 100 Hz in C. Results are expressed as percent difference from the average value 0–5 min before tetanic stimulation. Data are expressed as means ± SEM. (B, D) Comparison of the effect of IL-1β on the magnitude of LTP. Data are expressed as means ± SEM (B: control, $n = 4$; IL-1β groups, $n = 5$ each; D: all groups, $n = 5$ each). No significance was detected by a statistical analysis (analysis of variance) in both experiments (B and D).

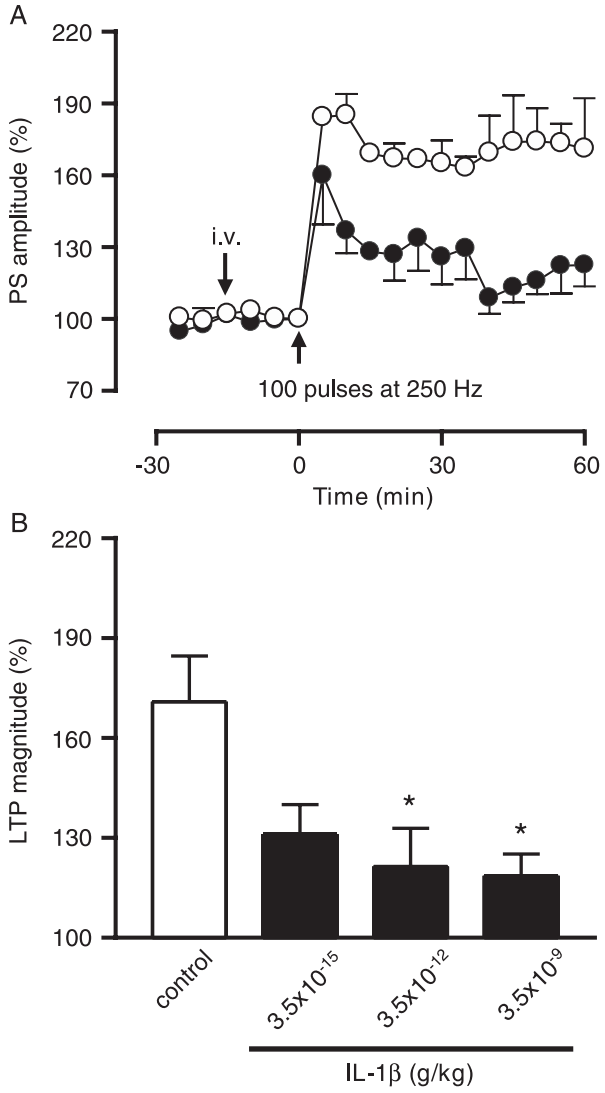

Fig. 5. Inhibitory effect of the peripheral IL-1β on the induction of LTP. (A) Time-course changes in the PS amplitude of control (○) or 3.5×10^{-9} g/kg IL-1β-injected (●) animals. IL-1β was delivered by a single i.v. injection 15 min before strong tetanic stimulation consisting of 100 pulses at 250 Hz. Results are expressed as percent difference from the average value 0–5 min before tetanic stimulation. Data are expressed as means ± SEM. (B) Comparison of the effect of IL-1β on the magnitude of LTP. Data are expressed as means ± SEM (control, $n = 5$; IL-1β groups, $n = 4$ each) $^{*}P <$ 0.05 vs. control (Tukey's test following analysis of variance).

IL-1ra facilitated the enhancement of LTP induced by 10.0 mg/kg PB-2.

4. Discussion

Previous studies from our laboratory have demonstrated that peripheral but not i.c.v. injections of PB-2, a fraction extracted from the lichen *Flavoparmelia baltimorensis* that includes α-glucan [31,32], significantly facilitated the induction of LTP in the DG of the anesthetized rat, suggesting that the primary site of action of PB-2 was not in the CNS but in the periphery [15]. In the present study, we have investigated the mechanism underlying the modulation of LTP induced by a single i.v. infusion of PB-2 in rats. We show here that PB-2 exhibits two opposing effects on LTP in this brain region, an enhancing effect and an inhibiting one, and that on balance, it causes a significant enhancing effect.

We have investigated the possible contributions of IL-1β and the IL-1 receptors to the PB-2-induced enhancement of LTP. It has been shown in an in vivo microdialysis study that peripheral administration of IL-1β increases the extracellular concentration of norepinephrine (NE) in the brain [29]. It has also been suggested that exogenous application and the synaptic release of NE enhances the induction of LTP in the DG [13,14]. Taken together, these data suggest that peripheral administration of IL-1β facilitates LTP by increasing the levels of NE.

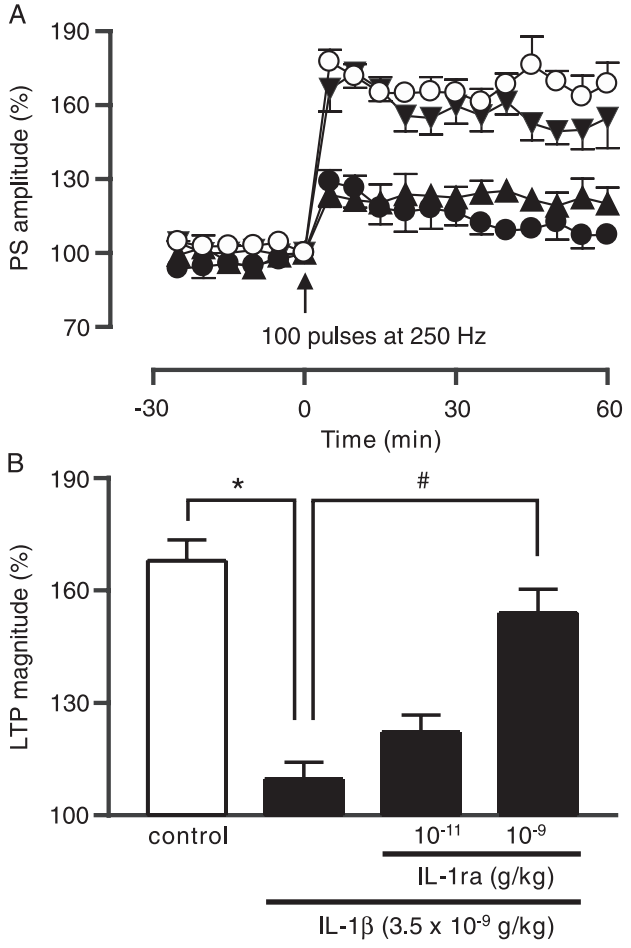

Fig. 6. IL-1β-induced inhibition of LTP via IL-1 receptor. (A) Time-course changes in the PS amplitude of control (○), 3.5×10^{-9} g/kg IL-1β- (●), 10^{-11} g/kg IL-1ra + 3.5×10^{-9} g/kg IL-1β- (▲), or 10^{-9} g/kg IL-1ra + 3.5×10^{-9} g/kg IL-1β- (▼) injected animals. IL-1β was delivered by a single i.v. injection 15 min before strong tetanic stimulation consisting of 100 pulses at 250 Hz. Results are expressed as percent difference from the average value 0–5 min before tetanic stimulation. Data are expressed as means ± SEM. (B) Comparison of the effect of IL-1ra on PB-2-induced enhancement of LTP. Data are expressed as means ± SEM (All groups, $n =$ 5) $^{*}P < 0.01$, $^{\#}P < 0.01$ (Tukey's test following analysis of variance).

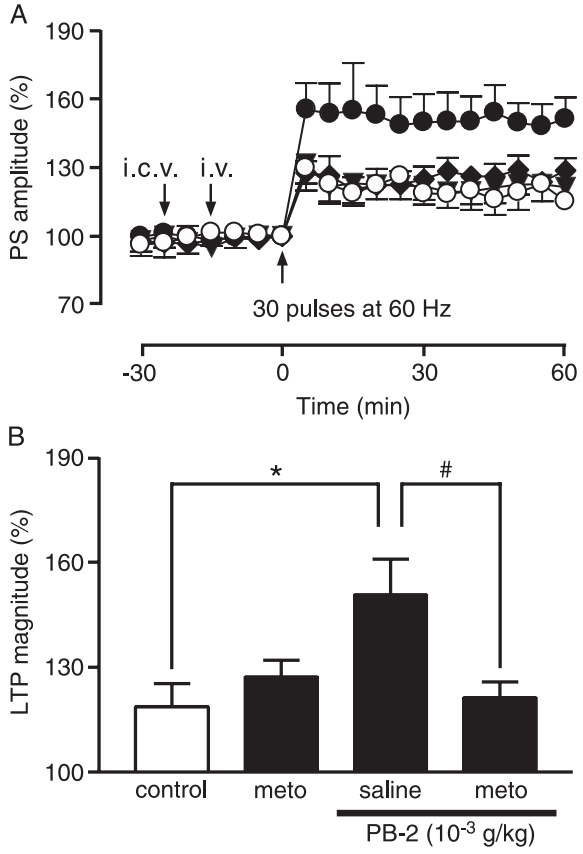

Fig. 7. Contribution of adrenaline β_1-receptor in PB-2-enhanced LTP. (A) Time-course changes in the PS amplitude of control (○), 10^{-3} g/kg PB-2- (●), 7.5×10^{-6} g metoprolol- (▼), or 7.5×10^{-6} g metoprolol- + 10^{-3} g/kg PB-2- (◆) injected animals. Metoprolol was delivered by an i.c.v. injection 25 min and PB-2 by an i.v. injection 15 min before tetanic stimulation. Results are expressed as percent difference from the average value 0–5 min before tetanic stimulation. Data are expressed as means ± SEM. (B) Comparison of the effect of IL-1ra on PB-2-induced enhancement of LTP. Data are expressed as means ± SEM (control, $n = 4$; PB-2 alone, $n = 5$; metoprolol alone, $n = 4$; metoprolol + PB-2 group, $n = 4$) $^*P < 0.05$, $^{\#}P < 0.05$ (Tukey's test following analysis of variance). The abbreviated word "meto" stands for metoprolol injection.

In the study presented here, IL-1ra, a widely used blocker of the IL-1 receptor [7], was employed to investigate whether peripheral and/or central IL-1 receptors play a role in the enhancing effect of PB-2 on LTP. Unexpectedly, i.v. and i.c.v infusions of IL-1ra significantly increased the magnitude of LTP in PB-2-treated rats, with no significant effects on the basal magnitude of LTP, suggesting that PB-2 increases an endogenous factor (or factors) that interacts with IL-1 receptors, and that this factor penetrates the CNS and attenuates LTP to some extent.

In order to investigate whether endogenous IL-1β contributes to IL-1 receptor activation, the effect of IL-1β administered at the periphery on the induction of LTP was investigated. The LTP induced by 30 pulses at 60 Hz (a weak tetanus) and 100 pulses at 100 Hz tended to be decreased by the infusions of IL-1β, and the LTP induced by 100 pulses at 250 Hz (a strong tetanus) was significantly attenuated by the infusion of IL-1β, in a dose-dependent manner (Fig. 5). These results suggest that either IL-1β does not induce NE release in the DG or that the increased level of NE was not high enough to affect LTP in this condition. Moreover, the injection of IL-1ra at a dose that significantly augmented the facilitated LTP induced by PB-2, reversed the inhibitory effect of IL-1β to almost the control level (Fig. 6), suggesting that IL-1β plays a role as an endogenous ligand of the IL-1 receptor that contributes to the inhibition of LTP.

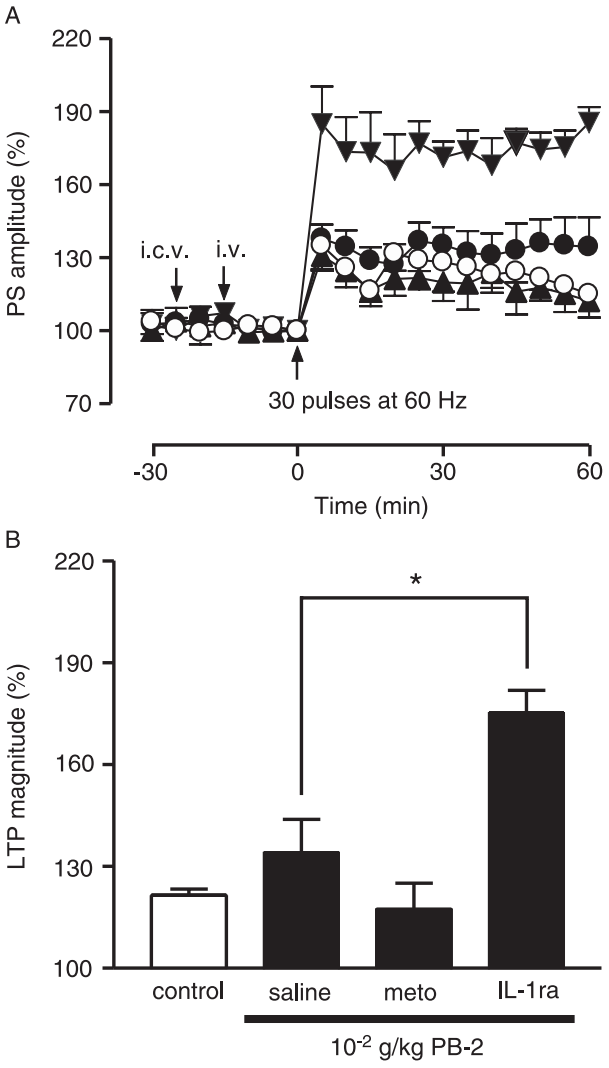

Fig. 8. Contributions of IL-1 and adrenaline β_1-receptors to the effect of a higher dose PB-2 on LTP. (A) Time-course changes in the PS amplitude of control (○), 10^{-2} g/kg PB-2- (●), 7.5×10^{-6} g metoprolol + 10^{-2} g/kg PB-2- (▲) or 10^{-11} g IL-1ra + 10^{-2} g/kg PB-2- (▼) injected animals. IL-1ra and metoprolol were delivered by an i.c.v. injection 25 min and PB-2 by an i.v. injection 15 min before tetanic stimulation. Animals in control group were injected saline (i.c.v. and i.v.). Results are expressed as percent difference from the average value 0–5 min before tetanic stimulation. Data are expressed as means ± SEM. (B) Comparison of the effect of IL-1ra and metoprolol on PB-2-induced effect of LTP. Data are expressed as means ± SEM (control, $n = 4$; PB-2, $n = 5$; metoprolol + PB-2, $n = 4$; IL-1ra + PB-2, $n = 5$) $^*P < 0.05$ (Tukey's test following analysis of variance). The abbreviated word "meto" stands for metoprolol injection.

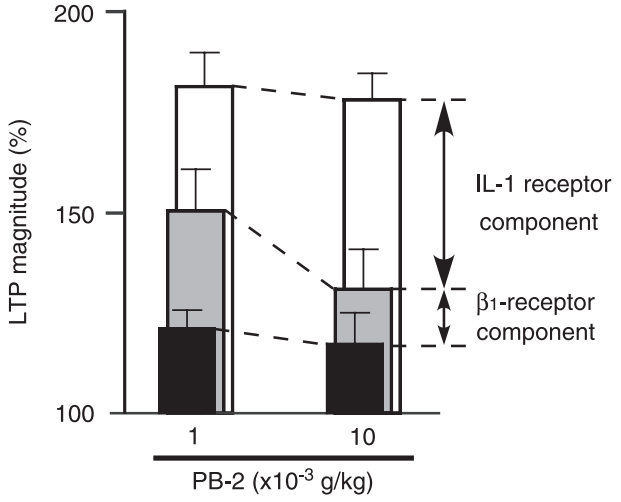

Fig. 9. Summary of contribution of IL-1 receptor and adrenaline β_1-receptor to the effect of PB-2 on LTP. Data are cited from Figs. 3, 7, and 8. In comparison of the effect of 1 and 10 mg/kg of PB-2, the increase in dose of PB-2 induced the decrease of the enhancement of LTP, accompanied by the increase in IL-1 receptor component and by the decrease in β_1-receptor component.

The activation of IL-1 receptors in the CNS contributes to the depression of synaptic function, since the treatment of IL-1β has been shown to decrease the basal synaptic responses and the induction of LTP [3,8,16,18]. However, in our observations, the IL-1 receptors did not play a role in the baseline PS amplitude or the induction of LTP in control animals (Figs. 2 and 3), which was in accordance with the results that IL-1ra did not affect the baseline response and the induction of LTP but reduce the maintenance of LTP observing in the condition only with applying the blocker after tetanic stimulation [28]. In contrast, IL-1ra facilitated the augmented LTP induced by a single i.v. infusion of PB-2, suggesting that IL-1β and the responsible IL-1 receptors play a pivotal role in the PB-2-mediated modulation of LTP in the DG.

Although the role played by the production of IL-1β in the effect of PB-2 on LTP has not been established, peripheral IL-1β is known to be important for synaptic function in the CNS. Several possible mechanisms for the peripheral effects of IL-1β on the CNS have been proposed. One possibility is that IL-1β in the peripheral circulation passes though the blood–brain barrier, thereby gaining access to the CNS. This idea is supported by reports that IL-1β can penetrate the blood–brain barrier [17] and can increase the permeability of the barrier [4]. It is therefore possible that if even a small amount of IL-1β reaches the blood–brain barrier, it can affect an increase in its permeability to subsequently administered IL-1β, which can thus easily enter the CNS, consequently reducing the magnitude of LTP. Another possibility is supported by several lines of evidence showing that some kinds of IL-1β-releasable cells [26] and a high density of IL-1 receptors [2] exist in the DG. Thus, it is possible that the IL-1β released from the IL-1β-containing cells activates IL-1 receptors in the DG, thus reducing the magnitude of LTP.

The inhibitory effect of IL-1β on the induction of LTP was significant only when strong tetanic stimulation consisting of 100 pulses at 250 Hz was used (Fig. 5). The magnitude of LTP induced by the strong tetanic stimulation was similar to that induced by the weak one (30 pulses at 60 Hz) in the PB-2- and IL-1ra-treated animals. These results suggest that the release of IL-1β was observed under conditions of higher levels of LTP.

Although an inhibitory role of IL-1β and IL-1 receptors on the PB-2-induced enhancement of LTP has been shown clearly in this study, the actual effect of PB-2 on LTP was the facilitation at doses used in this experiments. A growing evidence has suggested that the adrenaline β-receptor contributes to the increase in synaptic potentials and the induction of LTP [21,33]. Therefore, we investigated the role of the β_1-receptor in the PB-2-induced enhancement of LTP (Fig. 7). Pretreatment of metoprolol by i.c.v. injection reversibly antagonized the enhancement of LTP induced by the i.v. infusion of PB-2, suggesting that a systemic

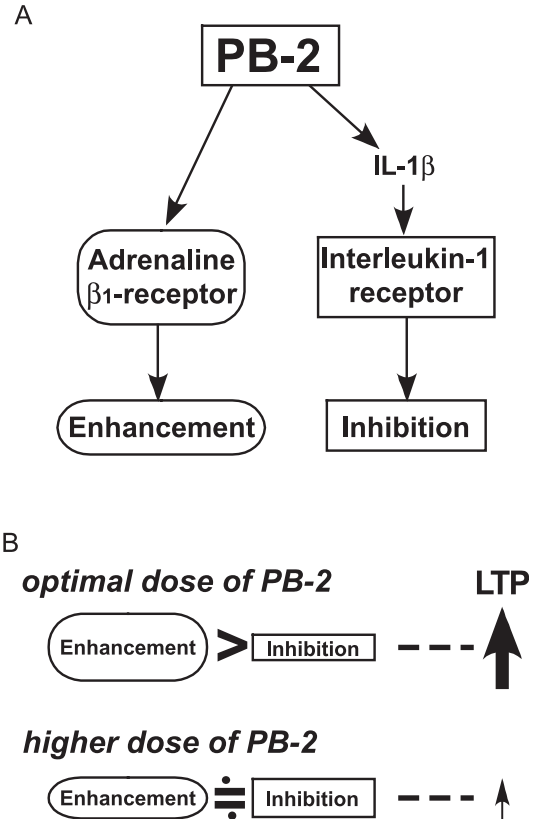

Fig. 10. Schematic illustration of the mechanism for the effect of PB-2 on LTP. (A) Simultaneous activation of the IL-1 receptor and the adrenaline β-receptor, inducing the inhibition and the enhancement of LTP, respectively. A possible endogenous ligand of the IL-1 receptor as to the effect of PB-2 on LTP is IL-1β. (B) The total effect of PB-2 is dependent on a balance between enhancement and inhibition of LTP. Optimal dose of PB-2 induced large enhancement and small inhibition, resulting in large facilitation of LTP, whereas a higher dose of PB-2 did same level of opposite effects, resulting in slight enhancement of LTP.

administration of PB-2 induces the activation of the IL-1 receptor and the adrenaline β_1-receptor simultaneously, thereby the total effect of PB-2 on LTP is dependent on a balance between the β_1-receptor-accelerated enhancement and the IL-1 receptor-induced inhibition.

Because the effect of PB-2 on LTP was in a bell-shaped manner, we further analyzed that the dual effect of PB-2 on LTP. In our previous report, a single i.v. injection of PB-2 enhanced LTP significantly at doses of 1.0 and 5.0 mg/kg and increased slightly at 10.0 mg/kg [15]. Thus, we concluded the optimal dose to facilitate LTP was 1.0–5.0 mg/kg, and 10.0 mg/kg of PB-2 was excessive dose as to the enhancement of LTP. In this study, under the IL-1ra-treated condition, 10.0 mg/kg PB-2 induced large enhancement similar to the level induced by 1.0 mg/kg PB-2- and IL-1ra-injected condition (Fig. 8). Moreover, the enhancement that induced by both 1.0 mg/kg and 10.0 mg/kg of PB-2 was reversibly antagonized by metoprolol, a β_1-selective antagonist, to the same level, suggesting that an increase in dose from 1.0 to 10.0 mg/kg of PB-2 biases the contribution ratio from the β_1-receptor-mediated enhancement to the IL-1 receptor-mediated inhibition (Fig. 9).

In summary, our data indicate that a systemic administration of PB-2 activates, at least in part, two types of receptors, that is, the adrenaline β_1-receptor and IL-1 receptor; and each mechanism induces enhancement and inhibition of LTP, respectively (Fig. 10). Moreover, the total effect of PB-2 may be dependent on a balance between the β_1-receptor-mediated enhancement and the IL-1 receptor-mediated inhibition.

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