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Differential Modulatory Effects of Cholera Toxin and Pertussis Toxin on Pain Behavior Induced by TNF-α, Interleukin-1β and Interferon-γ Injected Intrathecally

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The present study was designed to characterize the possible roles of spinally located cholera toxin (CTX)- and pertussis toxin (PTX)-sensitive G-proteins in pro-inflammatory cytokine induced pain behaviors. Intrathecal injection of tumor necrosis factor-a (TNF-α; 100 pg), interleukin-1β (IL-1β; 100 pg) and interferon-γ (INF-γ; 100 pg) showed pain behavior. Intrathecal pretreatment with CTX (0.05, 0.1 and 0.5 mg) attenuated pain behavior induced by TNF-α and INF-γ administered intrathecally. But intrathecal pretreatment with CTX (0.05, 0.1 and 0.5 μg) did not attenuate pain behavior induced by IL-1β. On the other hand, intrathecal pretreatment with PTX further increased the pain behavior induced by TNF-α and IL-1β administered intrathecally, especially at the dose of 0.5 μg. But intrathecal pretreatment with PTX did not affect pain behavior induced by INF-γ.Our results suggest that, at the spinal cord level, CTX- and PTX-sensitive G-proteins appear to play important roles in modulating pain behavior induced by pro-inflammatory cytokines administered spinally. Furthermore, TNF-α, IL-1β and INF-γ administered spinally appear to produce pain behavior by different mechanisms.

Key words: Cholera toxin, Pertussis toxin, Pain behavior, TNF-α, IL-1β, INF-γ Spinal cord

INTRODUCTION

The possible mechanisms at molecular level for the effect of pertussis toxin (PTX) could involve ADP-ribosylation of the alpha-subunit of the guanine nucleotide binding regulatory protein (Gi) catalyzed by the toxin (Hildebrandt *et al.*, 1983; Sekura *et al.*, 1983). Cholera toxin (CTX), on the other hand, elevates cAMP level by acting on the Gs unit of the GTP binding regulatory protein (Imaizumi *et al.*, 1987; Osugi *et al.*, 1987). Previous studies have reported that the modulation of nociception by the injection of PTX or CTX alone into the spinal level appears to be different, when different types of pain models are employed. For example, intrathecal (i.t.) injection of PTX showed a hyperalgesic response or allodynia as measured tail water-immersion test (DeLapp *et al.*, 1997; Womer *et al.*, 1997). And this hyperalgesic response was still manifested even after 3 month, suggesting that deficiencies in inhibitory systems, as compared with increases in excitatory systems, may play a role in the pathophysiology of at least some central or neuropathic pain states. However, some groups have reported that i.t. injection of PTX did not affect the basal nociceptive latency in the tail-flick test (Chung *et al.*, 1994; Song *et al.*, 1994). In contrast to the studies of PTX, the regulatory role of CTX-sensitive G-proteins in perceiving nociception at the spinal cord level has not been known.

Several lines of evidence have demonstrated that intrathecal injection of pro-inflammatory cytokine cause the hyperalgesic response (Choi *et al.*, 2003; Han *et al.*, 2003; Patel *et al.*, 2000; Reeve *et al.*, 2000). For example, pro-inflammatory cytokine has been proved to be involved in the pathophysiology of several illness responses and pathological pain states (Patel *et al.*, 2000; Reeve *et al.*, 2000; Watkins *et al.*, 1994; Wiertelak *et al.*, 1994). In addition, the hyperalgesia induced by subcutaneous formalin is significantly enhanced by the spinal administration of IL-

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1β (Takano *et al.*, 1996). On the other hand, intrathecal injection of IL-1β receptor antagonists produces antinociception in the hyperalgesic models (Namioka *et al.*, 1999; Tadano *et al.*, 1999). Furthermore, the hyperalgesia induced by subcutaneous formalin is significantly reduced by spinal administration of IL-1 receptor antagonist (Maier *et al.*, 1995; Watkins *et al.*, 1995). Intrathecal injection of mouse INF-γ evoked biting behavior, whereas mice with disruption of the functional gene for INF-γ receptor antagonist did not respond (Robertson *et al.*, 1997; Xu *et al.*, 1997). Thus, these studies let us know that the central application of inflammatory mediators such as IL-1β and TNF-α can produce hyperalgesia and allodynia.

In previous study, we found that intrathecal pretreatment with CTX attenuated hyperalgesic response induced by glutamate, NMDA, AMPA and kainic acid administered intrathecally in a dose-dependent manner, but intrathecal pretreatment with PTX further increased the hyperalgesic response (Chung *et al.*, 2000). Recently, it has been reported that pro-inflammatory cytokines may play an important role in the development of inflammatory and neuropathic pain or hyperalgesia in the spinal cord (Patel *et al.*, 2000; Reeve *et al.*, 2000; Robertson *et al.*, 1997; Xu *et al.*, 1997). Cytokines can also have indirect non-neuronal effects which may sensitize responses since they give rise to the endogenous production and release of mediators from glial cells, including glutamate, NO synthetase and prostaglandins, all of which are capable of altering pain processing within the CNS (Basarsky *et al.*, 1994; Malmberg *et al.*, 1992, 1995; Meller *et al.*, 1993; Misra *et al.*, 1996; Parpura *et al.*, 1994; Simmons *et al.*, 1992; Stanfa *et al.*, 1996).

Thus, it led us to speculate that spinal CTX or PTX-sensitive G-proteins may modulate the hyperalgesic response induced by intrathecally injected pro-inflammatory cytokines. Thus, the present study was designed to examine the effect of CTX or PTX pretreated spinally on the pain behavior induced by TNF-α, IL-1β and INF-γ administered intrathecally.

MATERIALS AND METHODS

These experiments were approved by the University of Hallym Animal Care and Use Committee. All procedures were conducted in accordance with the Guide for Care and Use of Laboratory Animal, published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain.

Experimental animals

Male ICR mice (Daehan Lab., Korea) weighing 23-25 g were used for all the experiments. Animals were housed 5 per cage in a room maintained at 22 ± 0.5°C with an alternating 12 h light-dark cycle for at least 5 days before the experiments were started and food and water were available ad libitum. Each animal was used only once. All hyperalgesic measures were recorded between 10:00 and 17:00.

Intrathecal injection

Intrathecal administration was performed in conscious mice (Hylden and Wilcox *et al.*, 1980) using a 30-gauge needle connected to a 25 μL Hamilton syringe with polyethylene tubing. The i.t. injection volume was 5 μL and the injection site was verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the spinal cord. The dye injected i.t. was distributed both rostrally and caudally but with short distance (about 0.5 cm) and no dye was found in the brain. The success rate for the injections was consistently found to be over 95%, before the experiments were done.

Proinflammatory cytokines induced nociceptive behavior test

One group of mice were pretreated intrathecally once with either saline (5 μL) or CTX (0.05, 0.1or 0.5 mg) for 24 h. Another group of mice were pretreated intrathecally once with either saline (5 μL) or PTX (0.05, 0.1 or 0.5 μg) for 6 days. These pretreatment times were determined in previous studies in which the effects reached a maximum after injection. And then, mice were injected intrathecally with tumor necrosis factor-a (TNF-α; 100 pg), interleukin-1β (IL-1β; 100 pg) and interferon-γ (INF-γ; 100 pg). The doses of TNF-α, IL-1β or INF-γ were selected on the basis of findings in our preliminary studies, which showed that pain behaviors induced by TNF-α, IL-1β or INF-γ administrated intrathecally were near-maximally occurred at this dose. Immediately after the intrathecal injection with pro-inflammatory cytokines, the mice were placed in a glass cylinder chamber (20 cm high, 20 cm diameter) and its behavior response was recorded for 30 min. The cumulative response time(s) of scratching and biting episodes were measured by means of a stop-watch. This test was performed in a temperature- and humidity-controlled (22 ± 1, 55 ± 0.5%) room.

Drugs

TNF-α, IL-1β and INF-γ were purchased from R and D Systems Inc. (Minneapolis, MN, U.S.A.). Cholera toxin and pertussis toxin were purchased from Research Biomedicals Inc. (Natick, MA). All the drugs were dissolved in sterile saline (0.9% NaCl solution).

Statistical analysis

Statistical analysis was carried out by one-way analysis

of variance (ANOVA) with post-hoc test. P values less than 0.05 were considered to indicate statistical significance. All values are expressed as the mean ± S.E.M.

RESULTS

Mice were injected i.t. with 5 μL of saline or CTX (0.05, 0.1 or 0.5 μg) 24 h before the intrathecal injection of pro-inflammatory cytokine. As shown Fig. 1, 2, and 3 TNF-α, IL-1β and INF-γ manifested pain behavior. Intrathecal pretreatment with CTX, at the dose which had no intrinsic effect, attenuated pain behavior induced by TNF-α and INF-γ (Fig. 1a, 3a). But intrathecal pretreatment with CTX did not affect pain behavior induced by IL-1β (Fig. 2a). In another group, mice were injected i.t. with 5 μL of saline or PTX (0.05, 0.1 or 0.5 μg) 6 days before the intrathecal injection of TNF-α, IL-1β or INF-γ. In contrast to the results with CTX, intrathecal pretreatment with PTX at higher

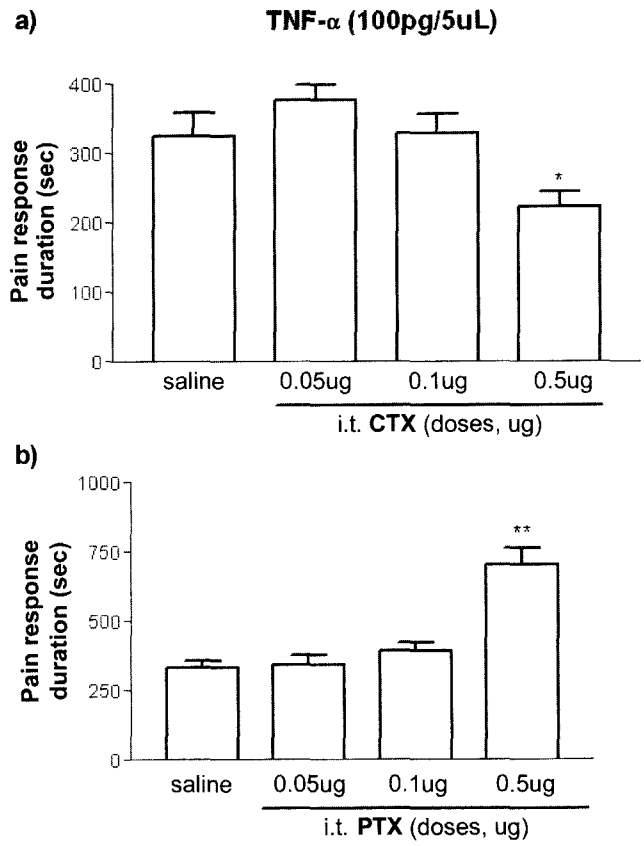

Fig. 1. The effect of cholera toxin (CTX; a) or pertussis toxin (PTX; b) on pain behavior induced by TNF-α administered intrathecally. Mice were once pretreated intrathecally either CTX (for 1 day) or PTX (for 6 days) before intrathecal administration of TNF-α (100 pg). The pain behavior induced by TNF-α was observed for 30 min after TNF-α injection. The vertical bars indicate the standard error of the mean. The number of animal used for each group was 7 (*, $p<0.05$ compared with control group).

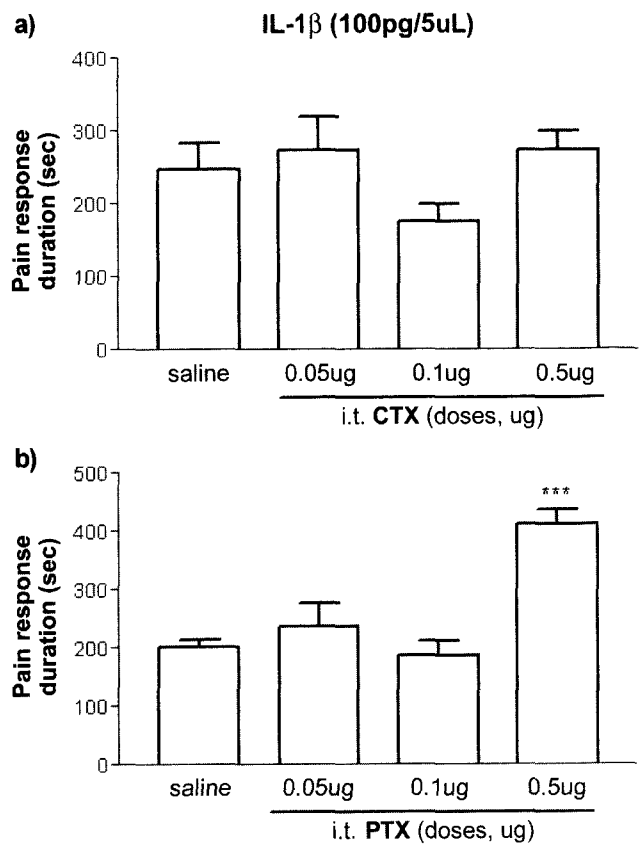

Fig. 2. The effect of cholera toxin (CTX; a) or pertussis toxin (PTX; b) on hyperalgesic response induced by IL-1β administered intrathecally. Mice were once pretreated intrathecally either CTX (for 1 day) or PTX (for 6 days) before intrathecal administration of IL-1β (100 pg). The pain behavior induced by IL-1β was observed for 30 min after IL-1β injection. The vertical bars indicate the standard error of the mean. The number of animal used for each group was 7 (*, $p<0.05$ compared with control group).

dose (0.5 μg) which does not affect basal latency for the nociception further enhanced pain behavior induced by TNF-α and IL-1β (Fig. 1b, 2b). But intrathecal pretreatment with PTX did not affect pain behavior induced by INF-γ (Fig. 3b). At lower doses of PTX (0.05 and 0.1 μg) did not alter pain behavior induced by TNF-α, IL-1β, and INF-γ (Fig. 1b, 2b, and 3b).

DISCUSSION

The results of the present study clearly demonstrated, for the first time, that CTX- and PTX-sensitive G-proteins play important roles in the regulation of pain behavior induced by pro-inflammatory cytokines such as TNF-α, IL-1β and INF-γ at the spinal cord level. Furthermore, the pain behavior induced by TNF-α, IL-1β and INF-γ appear to be differentially modulated by CTX and PTX pretreated intrathecally.

Intrathecal pretreatment with CTX attenuated pain

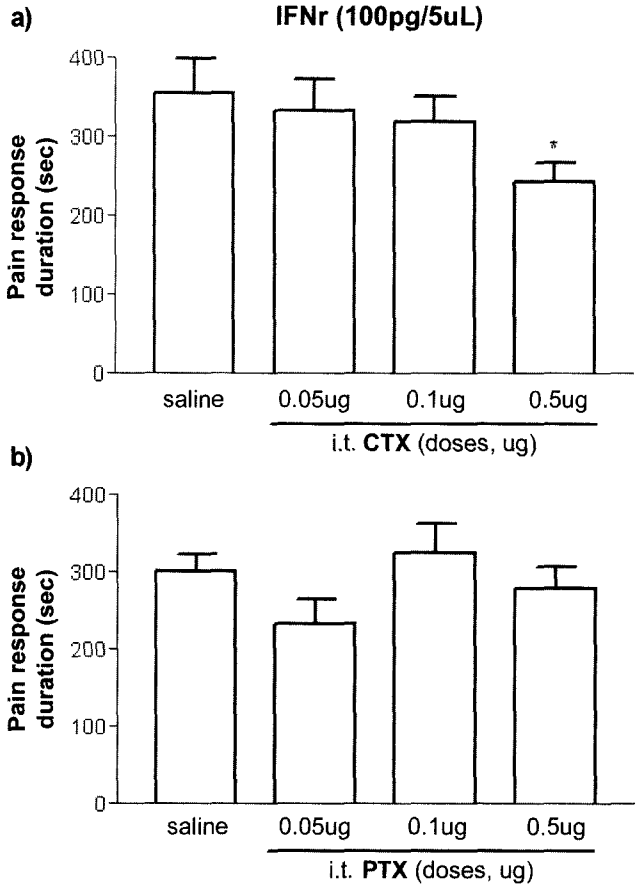

Fig. 3. The effect of cholera toxin (CTX; a) or pertussis toxin (PTX; b) on hyperalgesic response induced by INF-γ administered intrathecally. Mice were once pretreated intrathecally either CTX (for 1 day) or PTX (for 6 days) before intrathecal administration of INF-γ (100 pg). The pain behavior induced by INF-γ was observed for 30 min after INF-γ injection. The vertical bars indicate the standard error of the mean. The number of animal used for each group was 7 (*, $p<0.05$ compared with control group).

behavior induced by TNF-α administered intrathecally. On the other hand, intrathecal pretreatment with PTX caused an enhancement of pain behavior induced by TNF-α administered intrathecally. These results suggest that spinally located CTX and PTX-sensitive G-proteins play opposite action against spinally injected TNF-α-induced pain behavior.

Pain behavior induced by IL-1β administered intrathecally was not attenuated by intrathecal pretreatment with CTX. On the other hand, intrathecal pretreatment with PTX increased pain behavior induced by IL-1β administerd intrathecally. It suggests that spinally located PTX-sensitive G-proteins play an important action in the transmission of nociception mediated by IL-1β administered intrathecally. However, CTX-sensitive G-proteins may not be involved in IL-1β-induced pain behavior.

In contrast to IL-1β, pain behavior induced by INF-γ administered intrathecally was attenuated by CTX pretreated intrathecally. On the other hand, intrathecal pretreatment with PTX did not affect pain behavior induce by INF-γ administered intrathecally, suggesting that spinally located CTX-sensitive G-proteins exert the inhibitory action against the transmission of nociception mediated by INF-γ. However, spinally located PTX-sensitive G-proteins appear not to be involved in spinally injected INF-γ-induced pain behavior.

In addition to cytokines, we found in previous studies that CTX- and PTX-sensitive G-proteins exert their modulatory action against pain behavior induced by excitatory amino acids, substance P or capsaicin injected intrathecally (Chung *et al.*, 2001; Lee *et al.*, 2001). For example, intrathecal pretreatment with CTX attenuated pain behavior induced by glutamate, NMDA, AMPA and kainic acid administered intrathecally in a dose-dependent manner, whereas intrathecal pretreatment with PTX further increased the hyperalgesic response (Chung *et al.*, 2000). In addition, intrathecal pretreatment with CTX suppressed both the first and second phases of the formalin-induced nociceptive behavior (Chung *et al.*, 2001; Lee *et al.*, 2001). On the other hand, pretreatment with PTX did not affect the formalin-induced response. Capsaicin-and substance P-induced nociceptive behavior were attenuated by the pretreatment with CTX. In addition, SP-induced nociceptive response was also attenuated by the pretreatment with PTX. However, the capsaicin-induced nociceptive response was not influenced by PTX pretreatment. These previous finding also suggest that, at the spinal cord level, CTX-sensitive G-protein are involved in the formalin-, capsaicin-, and substance P-induced nociceptive behavior responses, whereas PTX-sensitive G proteins are involved in SP-induced nociceptive response.

In conclusion, although the role of CTX- and PTX-sensitive G proteins in the regulation of the pain response induced by pro-inflammatory cytokines given i.t. has been delineated clearly, we found in the present study that intrathecal pretreatment with CTX selectively reduced pain behavior induced by TNF-α and INF-γ administered intrathecally, without affecting IL-1β-induced pain behavior. On the other hand, intrathecal pretreatment with PTX further enhanced pain behavior induced by TNF-α and IL-1β administered intrathecally, without affecting IFN-γ-induced pain behavior. Although the exact reason for these differential effects of PTX and CTX administered spinally on nociceptive process induced by pro-inflammatory cytokines spinally located are currently unclear, our results clearly suggest that TNF-α, IL-1β and INF-γ administered intrathecally produce pain behavior in a different manner. The detailed underlying mechanism involved in pain behavior induced by TNF-α, IL-1β and INF-γ administered intrathecally should be further investigated in the future study.

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