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Valproate attenuates the development of morphine antinociceptive tolerance

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ABSTRACT

Morphine is a potent opioid analgesic. Repeated administration of morphine induces tolerance, thus reducing the effectiveness of analgesic treatment. Although some adjuvant analgesics can increase morphine analgesia, the precise molecular mechanism behind their effects remains unclear. Opioids bind to the mu opioid receptor (MOR). Morphine tolerance may be derived from alterations in the intracellular signal transduction after MOR activation. Chronic morphine treatment activates glycogen synthase kinase 3β (GSK3β), whose inhibition diminishes morphine tolerance. Valproate is widely prescribed as an anticonvulsant and a mood stabilizer for bipolar disorders because it increases the amount of γ-aminobutyric acid (GABA) in the central nervous system. Although the activation of GABAergic neurons may be responsible for the chief pharmacologic effect of valproate, recent studies have shown that valproate also suppresses GSK3β activity. We examined the effect of valproate on the development of morphine antinociceptive tolerance in a mouse model of thermal injury. Mice were treated with morphine alone or with morphine and valproate twice daily for 5 days. The resulting antinociceptive effects were assessed using a hot plate test. While mice treated with morphine developed tolerance, co-administration of valproate attenuated the development of tolerance and impaired the activation of GSK3β in mice brains. Valproate alone did not show analgesic effects; nevertheless, it functioned as an adjuvant analgesic to prevent the development of morphine tolerance. These results suggest that the modulation of GSK3β activity by valproate may be useful and may play a role in the prevention of morphine tolerance.

Morphine is a potent opioid analgesic that is widely used for acute and chronic pain control [24]. Repeated administration of morphine induces tolerance, which reduces its effectiveness as an analgesic agent. Opioids bind to the mu opioid receptor (MOR) to activate various signaling molecules through heterotrimeric guanine nucleotide-binding proteins (G proteins). Chronic morphine tolerance may arise from adaptations in the intracellular signal transduction after MOR activation, as morphine does not effectively induce MOR phosphorylation or internalization [9]. Persistent activation of MOR on the cell surface may cause altered signal transduction including changes in MOR-coupled G proteins from Giα to Gsα [5], increased activity of protein kinase C [11], and upregulation of *N*-methyl-D-aspartate receptor signaling [26]. Chronic morphine treatment also activates the cyclin-dependent kinase 5-glycogen synthase kinase-3β (GSK3β) signaling pathway, while inhibition of this pathway diminishes morphine tolerance and restores the efficacy of analgesic in rat [20] and mouse models [7].

Valproate is widely prescribed as an anticonvulsant and a mood stabilizer for bipolar disorders. It increases the amount of γ-aminobutyric acid (GABA) in the central nervous system [18]. Although the activation of GABAergic neurons may be responsible for the central pharmacologic effect of valproate, recent studies have shown that valproate also suppresses the activity of GSK3β [2,14]. Since valproate use is frequent in clinical discourse, we examined the effect of valproate as an inhibitor of GSK3β on the development of morphine antinociceptive tolerance in a mouse model of thermal injury.

All animal experimental procedures were in accordance with a protocol approved by the Institutional Animal Care Committee of Chiba University, Chiba, Japan. C57BL/6 male mice (22–27 g; aged 10–15 weeks; 69 mice) were used. All mice were provided with food and water *ad libitum* before the experiment.

The following reagents were used: sodium valproate (Sigma Chemical Co., Irvine, UK), morphine hydrochloride (Takeda Pharmaceutical Co., Tokyo, Japan), and SB216763 (Biomol International, Plymouth Meeting, PA, USA). The following antibodies were used: rabbit polyclonal antibody against MOR-1 (Chemicon, Temecula, CA, USA), mouse monoclonal antibody (mAb) against phospho-GSK3β (Tyr279/Tyr216) (Upstate Biotechnology, Chicago, IL, USA), Cy-3-conjugated donkey antibody against rabbit IgG, and Cy-2-conjugated donkey antibody against mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA, USA).

Abbreviations: MOR, mu opioid receptor; G proteins, heterotrimeric guanine nucleotide-binding proteins; GSK3β, glycogen synthase kinase 3β; GABA, γ-aminobutyric acid.

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A hot plate test was carried out to assess the effects of a pharmacologic agent on the thermal nociceptive threshold of mice. Mice were placed on a 54.5 °C hot plate (Socrel hot-plate model DS37, Ugo Basile, Italy). The response latency to either a hind paw lick or a jump was recorded. In the absence of a response, the animals were removed from the hot plate after 60 s to avoid tissue injuries, and a 60 s latency was assigned as the response. Before drug administration, the hot plate latency was measured 3 times, and the average of the second and third measurements was used as the pre-drug response latency at 0 min. The hot plate latency was also measured at 5, 15, 30, 45, and 60 min following intraperitoneal drug injection. Each reagent was dissolved in 100 μl saline for intraperitoneal injection. To confirm analgesia with morphine, the hot plate latency was measured at 5, 15, 30, 45, and 60 min after a single intraperitoneal injection with morphine (20 mg/kg, $n = 25$). To obtain control data, mice were injected with the vehicle (saline, $n = 2$) or valproate (150 mg/kg; $n = 5$) twice a day for 5 days, and the hot plate tests were performed on day 5 after the pharmacological agents were administered intraperitoneally. To evaluate the effect of valproate on morphine analgesia, 20 mg/kg morphine alone ($n = 12$) or morphine with valproate (50, 150, 300 mg/kg; $n = 5$, 14, 5, respectively) were administered intraperitoneally twice a day for 5 days. On day 5, more than 6 h after drug administration, mice were administered 20 mg/kg of morphine and then subjected to the hot plate test.

To analyze the effects of these drugs on animal performance in the hot plate test, the %MPE was calculated, where %MPE = ([post-drug maximum response latency − pre-drug response latency]/[cut-off time {60 s} − pre-drug response latency]) × 100. The post-drug maximum response latency was defined as the single longest response latency during the entire time course of the hot plate test. A higher %MPE represented a better analgesic effect.

Mice were deeply anesthetized with pentobarbital (Dainippon Sumitomo Pharma, Osaka, Japan) and fixed by transcardial perfusion with 4% paraformaldehyde in phosphate-buffered saline (PBS). Their brains were further immersion-fixed for 12 h in 4% paraformaldehyde at 4 °C. After fixation, the brains were dehydrated by immersing in increasing concentrations of ethanol, and subsequently embedded in paraffin wax. For immunofluorescence, sections (8 μm) were incubated with 10% normal goat or bovine serum in PBS for 30 min to block nonspecific antibody binding and then incubated with a primary antibody in PBS for 1 h at room temperature. The sections were rinsed with PBS and then incubated with a mixture of Cy-3-conjugated anti-rabbit IgG and Cy-2-conjugated anti-mouse IgG in PBS for 1 h at room temperature. Subsequently, the sections were rinsed with PBS and mounted on glass slides with Perma Fluor (Immunon, Pittsburgh, PA, USA). Immunolocalization was observed with a fluorescence microscope using FITC/rhodamine filters and Plan-Neofluar 20× and 40× NA 0.75 objectives (Axiovert 200 M; Carl Zeiss, Oberkochen, Germany). The brightness and contrast were optimized using AxioVision 4.4 software (Carl Zeiss), and immunofluorescence images were captured with a digital camera (AxioCam MRm, Carl Zeiss). The mean grey values of cells with the background subtraction were used for densitometry.

To compare the hot plate %MPE, latencies, and other values between groups, one-way or two-way ANOVA was used followed by the Bonferroni post hoc test (GraphPad Prism 4.0, GraphPad Software, San Diego, CA, USA).

We evaluated analgesia induced with administration of a single dose of morphine (20 mg/kg) administration in our mice model with the hot plate test (Fig. 1A). The response latencies reached the 60 s cut-off point 30 min after injection. The response latencies of the valproate injection group and the saline control group (NS) did not differ significantly (Fig. 1A). Thus, valproate alone did not affect the nociceptive threshold of mice.

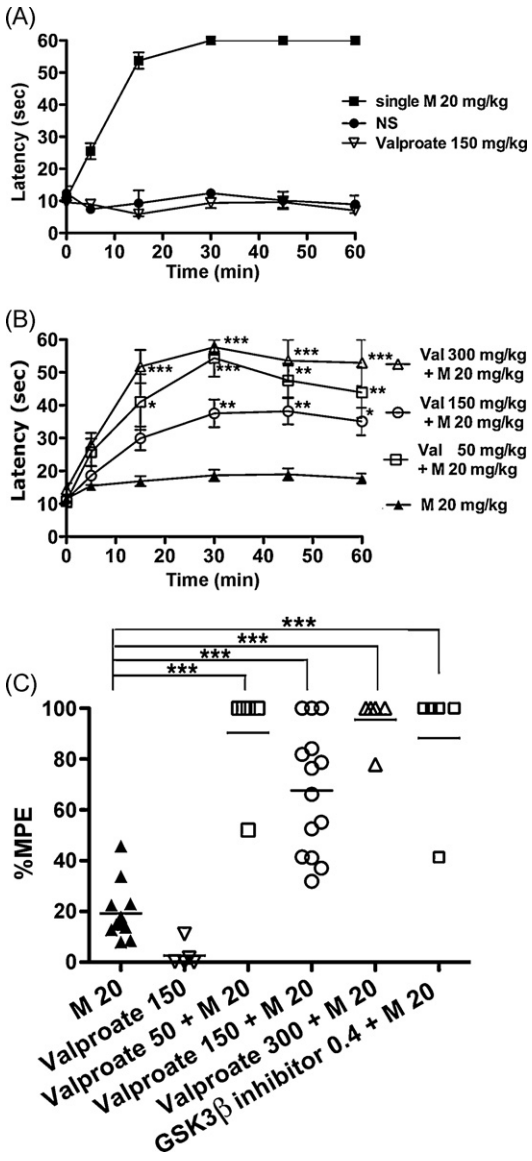

Fig. 1. Valproate attenuated the development of morphine tolerance. (A) The graph represents the latent responses (0–60 s) of mice administered morphine once (single M), saline twice a day for 5 consecutive days (NS), or 150 mg/kg of valproate twice a day for 5 consecutive days (mean ± SEM). (B) The graph represents the latent responses (0–60 s) of mice administered morphine alone (M, $n = 12$), 50 mg/kg of valproate and morphine, 150 mg/kg valproate and morphine, and 300 mg/kg valproate and morphine twice a day for 5 consecutive days (mean ± SEM; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, two-way ANOVA with the Bonferroni post hoc test). (C) The distribution of %MPE after the repetitive drug treatment for 5 days. The mean %MPEs of the mice that received valproate and morphine were significantly greater than those of mice treated with morphine alone. The mean %MPE of mice receiving GSK3β inhibitor (0.4 mg/kg of SB216763) and morphine ($n = 5$) was also significantly greater than that of mice treated with morphine alone ($^{***}P < 0.001$, one-way ANOVA with the Bonferroni post hoc test).

Subsequently, to evaluate the effect of valproate on morphine tolerance, mice were administered morphine alone or a combination of morphine and valproate intraperitoneally twice a day for 5 consecutive days. On day 5, more than 6 h after the drug administration, mice were administered morphine alone (20 mg/kg) and then subjected to the hot plate test (Fig. 1B). We found that the response latencies were significantly reduced, indicating that morphine tolerance was induced by repeatedly injecting morphine (Fig. 1B, M20). On the other hand, the latent responses of mice co-administered valproate and morphine were significantly longer