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Change of Hyperexcitability of Hippocampus by Cyclosporin A and Its Modulatory Action by Fentanyl

Cyclosporin A is used to treat patients with immune-mediated diseases, chronic diseases requiring organ transplantation, or malignancies. These conditions often require higher cyclosporin A doses, which may be toxic to the central nervous system. Fentanyl is also used in clinical conditions that have a risk of hypoxic neurosusceptiblity, which suggests that the drug may be a neuroprotective agonist against brain ischemic injury. Fentanyl is an opioid agonist and appears to play an important role in regulating the excitability of the hippocampus under electroconvulsion. In this study, the effects of fentanyl on modulating cyclosporin A-induced neurotoxicity was investigated. Treatment with 3 μM of cyclosporin A was found to reduce the electroconvulsive activity threshold. Fifty ng/mL of fentanyl reduced the electroconvulsive activity, and 1 μM of DAGO ([D-Ala^2, N-Me-Phe^4, Gly-ol]-enkephalin) also decreased the electroconvulsive activity. Fifty ng/mL of fentanyl was also found to reduce cyclosporin A-induced electroconvulsive activity. Although cyclosporin A neurotoxicity may be observed in various conditions, the opioid effect of neuroprotection may be involved in an interrelated mechanism. The exogenous opioid agonist suppressed cyclosporin A-induced electroconvulsive activity. Furthermore, there may be a functional anticonvulsant effect on cyclosporin A-induced neurotoxicity with an increased opioid agonist concentration.

Key Words : *Neurotoxicity; Cyclosporin A; Fentanyl*

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INTRODUCTION

Cyclosporin A is an immune-suppressive drug used in patients with immune-mediated diseases, chronic diseases requiring organ transplantation, or malignancy. These diseases often require higher cyclosporin A doses, which may cause central nervous system (CNS) problems (1-3). Seizures frequently poses a physical threat to patients, who often suffer from pain requiring appropriate control. Fentanyl is a commonly used analgesic agent that is used in that situation. However, interactions between fentanyl and cyclosporin A, especially in the CNS, must be considered. Although cyclosporin A-induced neurotoxicity is well recognized, the exact mechanism is still unclear. Cyclosporin A neurotoxicity may be due to the effect of cyclosporin A per se or to the combined effect of cyclosporin A and other associated clinical conditions, such as hypomagnesemia, hypocalcemia, the effect of multiple high-dose drugs already administered, antihypertensives, analgesics, steroid, CNS infarct, CNS hemorrhage, CNS vascular injury, brain ischemia, CNS hypoxia, CNS infection, and cardiopulmonary dysfunction.

Moreover, only 28.5% patients experiencing a neurological complication during cyclosporin A administration showed the cyclosporin A and its metabolites in the cerebrospinal fluid (CSF) (4). Therefore, the main question to be answered is whether cyclosporin A neurotoxicity also include other endogenous factors of the CNS.

Fentanyl is an opioid agonist that is used in clinical conditions where there is a risk of hypoxic neurosusceptiblity. Fentanyl may be neuroprotective against brain ischemic injury and cyclosporin A. Frey reported that fentanyl protects against some types of brain injury (5).

Therefore, whether or not fentanyl has an influence on the common side effects of cyclosporin A-inducing seizures and the neuroprotective effects on cyclosporin A neurotoxicity is the main focus of this study.

MATERIALS AND METHODS

Sprague-Dawley rats (14-21 days) weighing 28.7-49.2 g were used. The animals were kept in groups with their mothers at room temperature with access to food and tap water ad libitum.

Prior to decapitation, the animals were anesthetized with halothane. The whole brains were carefully and rapidly removed, the blood was rinsed off, and placed in a cold oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 120; KCl 3.3; NaH_2PO_4 1.23; $MgSO_4$ 0.9; $NaHCO_3$ 25; $CaCl_2$ 1.3; and dextrose 10 under humidified gas (95% O_2, 5% CO_2) for 30-45 sec. The pH of ACSF was 7.3-7.4 and the osmolarity was 315-325 mOsm/kg. Subsequently, the brain was placed on filter paper and dissected. The left-side of the brains were placed in clean oxygenated cold ACSF and the right-side of the brains were placed on the tissue chopper (Mc Ilwain). The first three hippocampal slices were discarded and the next 3-8 slices were collected (650 μm in thickness). The slices were placed in a chamber at room temperature and superfused with oxygenated ACSF. After equilibration for at least 1-hr incubation with the undisturbed superfusion, the extracellular recordings were started. The slices were placed on a nylon mesh in an incubation well at 29.5-30.5°C under humidified gas, then perfused with ACSF at a rate of 3.5-4 mL/min.

The hippocampal slices were stimulated with a platinum-coated tungsten bipolar stimulation electrode (A-M Systems) by single shock pulses of 0.1-msec duration with 60 Hz, for a total of 2-sec duration, every 10 min (Grass S88 Stimulator). The recording electrode with a single barrel glass microelectrode with a thin wall diameter of 1.5×1.12 mm (A-M systems) filled with 3M NaCl was placed in the dentate gyrus (Duo 773 electrometer, World Precision Instrument).

Prior to exposure to the drugs, the slices were placed in a stable baseline environment for at least 15 min using the ground to control artifacts and the response was observed in an oscilloscope (Nicolet 410, Nicolet Instrument Corporation). The recording electrode was subsequently placed in the pyramidal layer of the CA1 region under visual control. The continued control of the ACSF was recorded for every 10-min electric stimulation, for at least 60 min, 6 times. The slices were stimulated in conditions of the above drug-induced conditions, every 10-min, at least eight times. In the 50 ng/mL fentanyl-treatment experiment after applying 3 μM of cyclosporin A, 50 ng/mL of a fentanyl-containing solution was perfused immediately after finishing at least eight stimulations every 10 min with 3 μM of cyclosporin A. In the 50 ng/mL fentanyl-treatment experiment, 50 ng/mL of a fentanyl-containing solution were perfused immediately after finishing at least six stimulations of the control, which was stimulated every 10 min at least eight times. Subsequently, 10 μM of a naloxone-containing solution was perfused and stimulated every 10 min at least eight times. In the 1 μM of DAGO ([D-Ala^2, N-Me-Phe^4, Gly-ol]-enkephalin)-treatment experiment, 1 μM of a DAGO-containing solution was perfused immediately after finishing at least six stimulations of the control, which was stimulated every 10 min at least eight times. Thereafter, 1 μM of a CTAP-containing solution was perfused and stimulated every 10 min at least eight times.

The frequency and duration of the electroconvulsive activities were then examined eight times, and the mean of 8 observations were obtained. Although five to eight slices were obtained from one rat, only one slice result from one rat in the experiments containing cyclosporin A was selected. This is because the first 3 slices were also discarded in order to obtain stable results. The slices were usually not suitable for recording the drug effect after 10 hr, and slices showing the cyclosporin A-induced continuous electroconvulsive activity after 6 stimulation with no resting phase were excluded. The frequency, duration, and the latency of onset observing different types of electrical activity under each experimental condition were analyzed by a Student's t-test and using the advisor wizard of Sigmastat. Quantitative variables are expressed as a mean±SD.

RESULTS

The effect on the ictal and interictal activity of the electroconvulsive activities on the hippocampus by fentanyl treatment after cyclosporin A

Treatment with 3 μM of cyclosporin A increased both the duration and frequency of the electroconvulsive activity (Fig. $1A_2$). The mean duration and frequency of 3 μM of cyclosporin A-induced ictal activity were 35.5±1.4 sec (Fig. 1B) (n=15) and 133.0±17.9 (Fig. 1C) (n=15), respectively. There were significant differences in the duration and frequency between the 3 μM cyclosporin A-induced ictal activity and the control group. 3 μM of cyclosporin A on the hippocampal slices showed interictal activity (Fig. $2A_2$). The mean duration and frequency in the 3 μM of cyclosporin A-induced interictal activity were 116.4±44.4 sec (Fig. 2B) (n=9) and 63.6±35.8 (Fig. 2C) (n=9), respectively. There was no significant difference in the duration and frequency of onset between the 3 μM of cyclosporin A-induced interictal activity and the control group showing interictal activity. The mean latency time of onset was 166.2±29.8 sec (Fig. 2D) (n=9). There was a significant difference in latency between the 3 μM of cyclosporin A-induced interictal activity and the control showing interictal activity. However, there were nine 3 μM of cyclosporin A-induced slices showing interictal activity.

Treatment with 50 ng/mL fentanyl shortened the duration and decreased the frequency of the 3 μM cyclosporin A-induced ictal activity (Fig. $1A_3$). The mean duration and frequency of the elecrtoconvulsive activity of the 50 ng/mL fentanyl-induced ictal activity were 24.9±1.9 sec (Fig. 1B) (n=4) and 90.3±16.5 (Fig. 1C) (n=4), respectively. There were significant differences in both the duration and frequency between the 50 ng/mL fentanyl-induced and the 3 μM cyclosporin A-induced ictal activity.

50 ng/mL of fentanyl on the 3 μM of cyclosporin A-induced

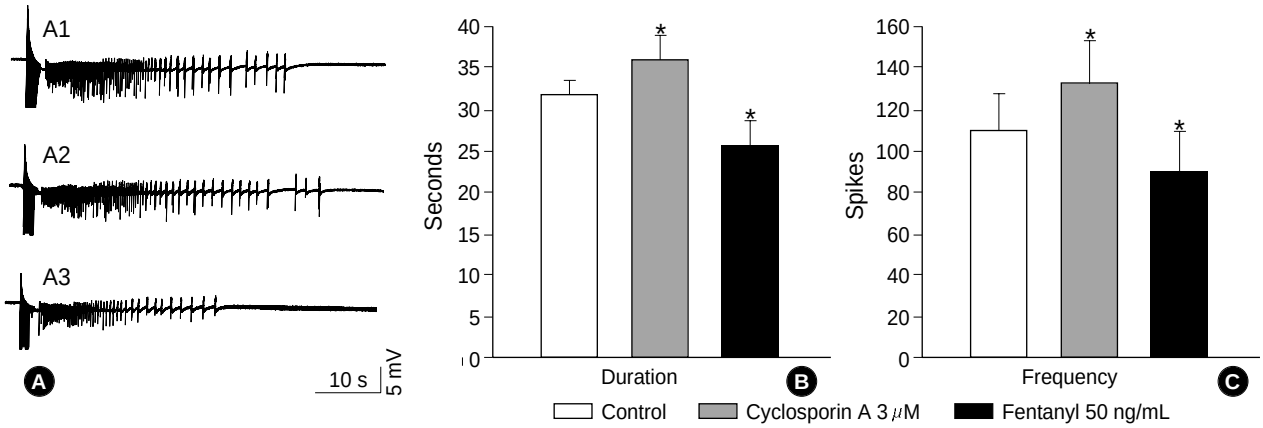

Fig. 1. Effect of fentanyl on cyclosporin A-induced electroconvulsive ictal activity. (A1) Control electroconvulsive ictal activity. (A2) 3 μM of cyclosporin A increased the electroconvulsive ictal activity. (A3) 50 ng/mL of fentanyl decrease the cyclosporin A-induced electroconvulsive activity. (B) 3 μM of cyclosporin A prolong the duration of the electroconvulsive ictal activity and 50 ng/mL of fentanyl shorten the duration of 3 μM of cyclosporin A-induced ictal activity. (C) 3 μM of cyclosporin A increase the frequency of the electroconvulsive ictal activity and 50 ng/mL of fentanyl decrease the frequency of 3 μM of cyclosporin A-induced electroconvulsive activity. *p<0.05.

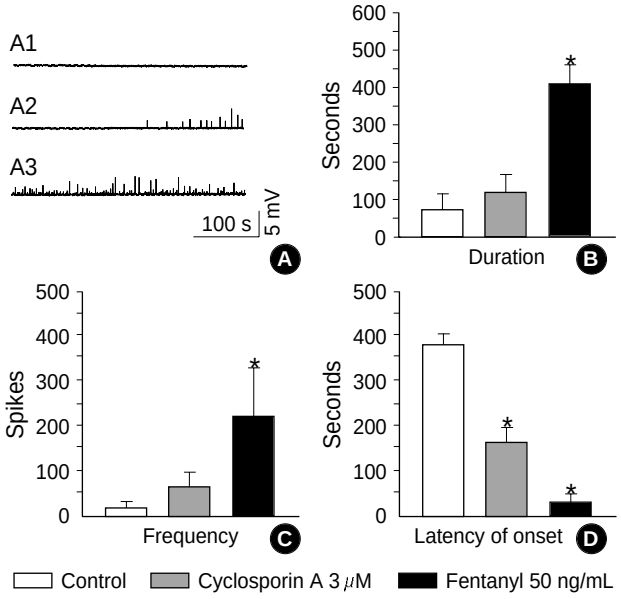

Fig. 2. Effect of fentanyl on cyclosporin A-induced electroconvulsive interictal activity. (A1) Control electroconvulsive interictal activity. (A2) 3 μM of cyclosporin A show some electroconvulsive interictal activity. (A3) 50 ng/mL of fentanyl show numerous electroconvulsive interictal activity. (B) 3 μM of cyclosporin A prolong the duration of control electroconvulsive interictal activity and 50 ng/mL of fentanyl show a great increase of duration of 3 μM of cyclosporin-induced interictal activity. (C) 3 μM of cyclosporin A increase the frequency of control electroconvulsive interictal activity and 50 ng/mL of fentanyl show a great increase of frequency of 3 μM of cyclosporin A-induced electroconvulsive interictal activity. (D) Latency time of onset of interictal activity in 3 μM of cyclosporin A and 50 ng/mL of fentanyl is greatly shortened compared to that in the control. The numbers of slices showing the interictal activity are not significantly different between the control and 3 μM of cyclosporin A-induced groups, but are significantly different between 3 μM of cyclosporin A- and 50 ng/mL of fentanyl-induced groups. *p<0.05.

slices showed the interictal activity (Fig. 2A$_3$) (n=4). The mean duration and frequency of the 50 ng/mL fentanyl-induced interictal activity were 405.3±46.6 sec (Fig. 2B) (n=4) and 218.0±100.5 (Fig. 2C) (n=4), respectively. There were significant differences in both the duration the frequency between the 50 ng/mL fentanyl-induced interictal activity and the 3 μM cyclosporin A-induced interictal activity. The mean duration of latency was 36.0±11.9 sec (Fig. 2D) (n=4), and there was a significant difference in the latency between the 50ng/mL fentanyl-induced interictal activity and the 3 μM cyclosporin A-induced interictal activity. Four slices with the 50 ng/mL fentanyl-treatment showing interictal activity was significantly different from that of the cyclosporin A-induced slices showing interictal activity.

The effects of 50 ng/mL of fentanyl on the electroconvulsive ictal and interictal activity in the control hippocampal slices

Treatment with 50 ng/mL of fentanyl shortened both the duration and decreased the frequency of electroconvulsive ictal activity in the control hippocampal slices (Fig. 3A$_2$). The mean duration and frequency of the 50 ng/mL fentanyl-induced electroconvulsive activity were 23.1±2.4 sec (Fig. 3B) (n=10) and 85.9±8.1 (Fig. 3C) (n=7), respectively. There was a significant difference in duration between the 50 ng/mL fentanyl-induced ictal activity and the control, but there was no significant difference between the two groups.

50 ng/mL fentanyl on the control hippocampal slices showed the interictal activity (Fig. 4D$_2$) and the mean duration and frequency of the 50 ng/mL fentanyl-induced interictal activity were 518.5±64.0 sec (Fig. 4A) (n=5), and 132.0±14.0 (Fig. 4B) (n=5), respectively. There were significant differences in both the duration and frequency between the 50 ng/mL fentanyl-induced interictal activity and the con-

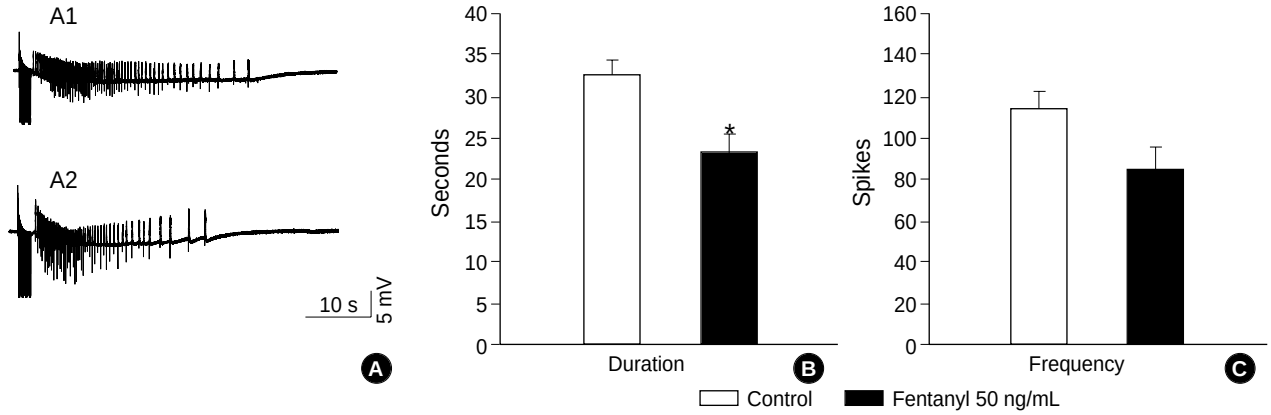

Fig. 3. Effect of fentanyl on electroconvulsive ictal activity of control hippocampal slices. (A1) Control electroconvulsive ictal activity. (A2) 50 ng/mL of fentanyl show a decrease of control electroconvulsive ictal activity. (B) 50 ng/mL of fentanyl shorten the duration of control electroconvulsive ictal activity. (C) 50 ng/mL of fentanyl decrease the frequency of control electroconvulsive ictal activity. *p<0.05.

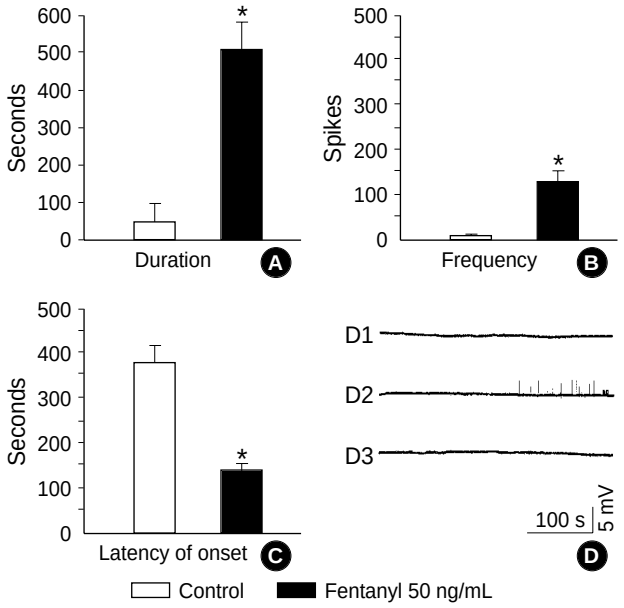

Fig. 4. Effect of fentanyl on control electroconvulsive interictal activity. (A) 50 ng/mL of fentanyl increase duration of control electroconvulsive interictal activity. (B) 50 ng/mL of fentanyl increase frequency of control electroconvulsive interictal activity. (C) Latent time of onset of interictal activity in 50 ng/mL of fentanyl-induced group is greatly shortened compared to that in the control. The numbers of slices showing the interictal activity are significantly different between control and 50 ng/mL of fentanyl-induced groups. (D1) Control hippocampal slices show no interictal activity. (D2) 50 ng/mL of fentanyl show interictal activity. (D3) The interictal activity by 50 ng/mL fentanyl is reversed by 10 μM of naloxone. *p<0.05.

trol interictal activity. The mean latency in the 50 ng/mL fentanyl-induced groups was 143.0±11.3 sec (Fig. 4C) (n=5), and there was a significant difference in the latency between the 50 ng/mL fentanyl-induced interictal activity and the control hippocampal slices showing interictal activity. Five slices with 50 ng/mL of fentanyl-treatment showing interictal activity were significantly different than the control hippocampal slices showing interictal activity. The interictal activity from 50 ng/mL of fentanyl on the control hippocampal slices was reversed by 10 μM naloxone (Fig. 4D3).

The effects of 1 μM of DAGO on ictal and interictal activity in hippocampal slices

1 μM DAGO shortened the duration of ictal activity in the hippocampal slices and decreased the frequency (Fig. 5A2). The mean duration and frequency of the 1 μM of DAGO-induced electroconvulsive activity were 12.7±2.8 sec (Fig. 5B) (n=7) and 87.3±11.1 (Fig. 5C) (n=7), respectively. There were significant differences in both the duration and frequency between the 1 μM DAGO-induced ictal activity and the control hippocampal slices.

1 μM of DAGO perfusion into the hippocampal slices showed interictal activity (Fig. 6D2) and the mean duration and frequency of the 1 μM DAGO-induced interictal activity were 464.6±42.5 sec (Fig. 6A) (n=5) and 64.2±23.0 (Fig. 6B) (n=5), respectively. There were significant differences in both the duration and frequency between the 1 μM DAGO-induced interictal activity and the control hippocampal slices showing interictal activity. The mean latency in the 1 μM DAGO-induced slices was 28.4±9.4 sec (Fig. 6C) (n=5), and there was a significant difference in the latency between the 1 μM DAGO-induced interictal activity and the control hippocampal slices showing interictal activity. The number of 1 μM DAGO-induced slices showing interictal activity differed significantly from the control hippocampal slices showing interictal activity. The interictal activity of hippocampal slices treated with 1 μM DAGO was reversed by 1 μM of D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (CTAP) (Fig. 6D3).

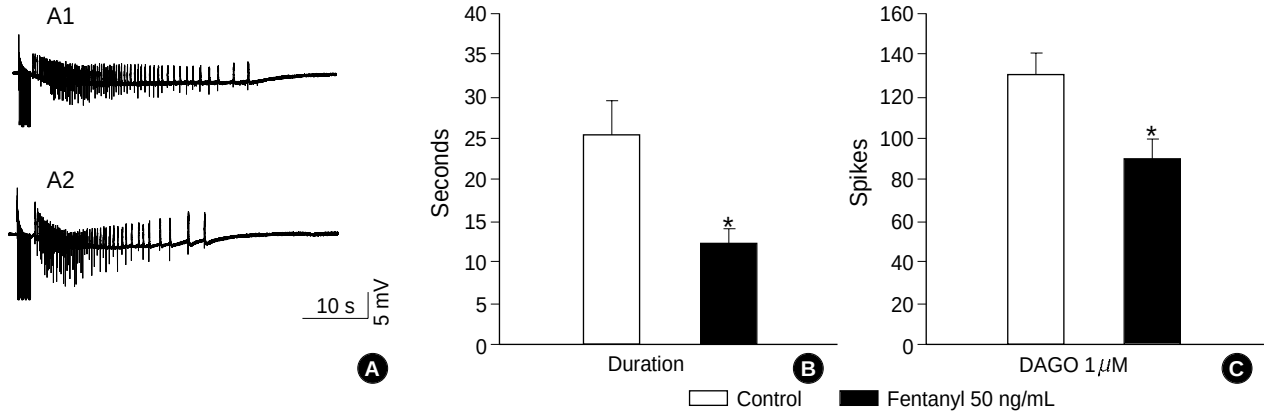

Fig. 5. Effect of DAGO on electroconvulsive ictal activity of control hippocampal slices. (A1) Control electroconvulsive ictal activity. (A2) 1 μM of DAGO show a decrease of control electroconvulsive ictal activity. (B) 1 μM of DAGO shorten the duration of control electroconvulsive ictal activity. (C) 1 μM of DAGO decrease the frequency of control electroconvulsive ictal activity. *p<0.05.

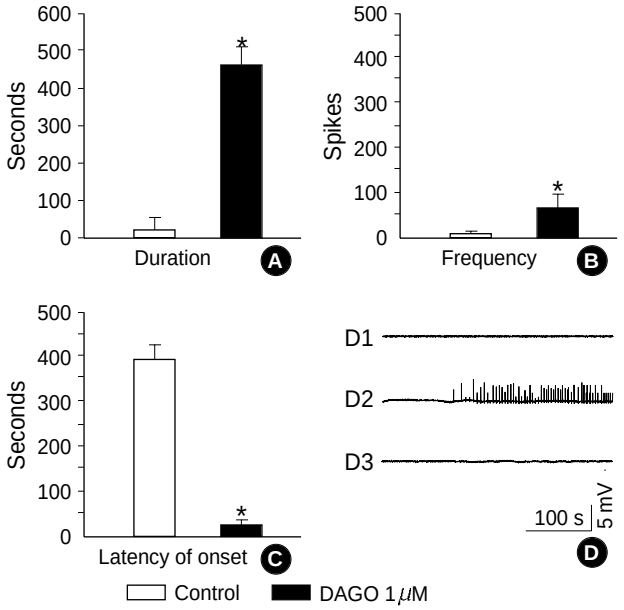

Fig. 6. Effect of DAGO on control electroconvulsive interictal activity. (A) 1 μM of DAGO increase duration of control electroconvulsive interictal activity. (B) 1 μM of DAGO increase frequency of control electroconvulsive interictal activity. (C) Latency time of onset of interictal activity in 1 μM of DAGO is greatly shortened compared to that in the control. The numbers of slices showing the interictal acti-vity are significantly different between control and 1 μM of DAGO-induced groups. (D1) Control hippocampal slices show no interictal activity. (D2) 1 μM of DAGO show an interictal activity. (D3) The interictal activity by 1 μM of DAGO is re-versed by 1 μM of CTAP. *p<0.05.

DISCUSSION

This study showed that 3 μM cyclosporin A elongated the duration of electroconvulsive activity on a hippocampal slice under 1.3 mM of calcium, and decreased the electroconvulsive threshold. An in vivo study of electroshock-induced seizure suggested that cyclosporin A lowers the seizure threshold (8). However, another in vitro study did not show any cyclosporin A neurotoxicity (9) whereas an in vitro cyclosporin A model showed that the cyclosporin A-induced electroconvulsive activity with hypomagnesemia (10). Because the in vitro model is only a reflection of the applied cyclosporin A effects under ACSF without other systemic and metabolic interplay, our results reflected the effect of the low calcium level. Both hypocalcemia and hypomagnesemia cause cyclosporin A toxicity (1, 11). As suggested by the presence of many clinical conditions associated with cyclosporin A neurotoxicity, its toxicity is markedly exaggerated under certain concurrent factors, including hypocalcemia.

The present study showed that 1 μM cyclosporin A caused no changes in the convulsion threshold, while 3 μM of cyclosporin A decreased the electroconvulsive threshold. Although cyclosporin A neurotoxicity cannot be predicted in a dose-dependent manner, it is generally associated with serum concentrations higher than 1 μM (12-14). These cyclosporin A serum concentrations at the equilibrium status are trough levels; a higher peak level may be achieved temporarily even in the same patient under certain conditions (2). Moreover, cyclosporin A is lipophilic and has a high affinity to cyclophilin and calcineurin, which are highly concentrated in cortical neurons (15, 16). Cyclosporin A also has a high affinity for lipoproteins including low-density lipids and its active cellular uptake via the low-density lipoprotein receptors may have a concentrating effect (17). As a result, the higher cyclosporin A concentration showed an increasing likelihood of neurotoxicity (18). Cyclosporin A exhibited concentration-dependent damage: neuronal damage occurred from 5 to 30 μM cyclosporin A; astrocyte damage occurred from 5 to 60 μM cyclosporin A; and oligodendrocyte damage occurred from 1 to 15 μM cyclosporin A (18). In these experiments, a higher dose of cyclosporin A might have increased the like-

lihood of detecting cyclosporin A neurotoxicity.

It has been reported that various opioid agonists increase the seizure threshold and have anticonvulsant effects (19). Opioids have a protective role against seizure activity by cyclosporin A that is mediated by the opioid receptors, and the mu and delta opioid agonists have an anticonvulsant effect (20, 21). Fentanyl is an opioid agonist bearing activity at the mu and delta agonists with greater activity at the former. The anticonvulsant effect of fentanyl and enhanced anticonvulsant effect of phenytoin and phenobarbitone by fentanyl have been reported (6).

This study demonstrated that 50 ng/mL of fentanyl shortened the cyclosporin A-induced electroconvulsive activity and increased the threshold of electroconvulsive activity. Fentanyl may play an important role in regulating the hippocampal excitability, especially in a hippocampus under seizure. These findings show that fentanyl has a protective role against cyclosporin A-induced electroconvulsive activity and the cyclosporin A-induced electroconvulsive activity were also modulated by fentanyl. The anticonvulsant action of fentanyl on the cyclosporin A-induced electroconvulsive activity may be mediated primarily by the mu or delta opioid receptors. The potential importance of activating opioid substances by seizures usually involves the modulation and regulation of seizure (22) and the most effective anticonvulsant appears to act at the mu and delta binding sites (23).

Opioid-induced seizure protection is related to endogenous opioid substances and is also selectively mediated by the mu and delta receptors (19).

The mu opioid receptor agonists increase the seizure threshold and have anticonvulsant activity (24). Opioid receptors subtypes are localized in the rat hippocampus (25) and the number of opioid substances in the hippocampus are greatly changed by seizure (26). Fentanyl and DAGO were then used to examine the electroconvulsive activity, especially in the hippocampus. The results showed a shortening of the electroconvulsive activity duration and an elevated electroconvulsive threshold after fentanyl, as has been reported elsewhere (5), and anticonvulsant effect of DAGO, which has also been reported (19). The shortening induced by both fentanyl and DAGO was reversed by 10 μM of naloxone and 1 μM of the highly specific mu receptor antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2). This suggests the anticonvulsant effect of fentanyl and DAGO is mediated by the opioid receptors and the effect is stronger on mu opioid receptors.

The interictal activity by fentanyl and DAGO also disappeared with a perfusion of 10 μM of naloxone and 1 μM of CTAP, respectively. Fentanyl and DAGO may act postictally on the spontaneous arrest of seizures and modulate postictal inhibition. There is an inherent ability of a seizure to inhibit the recurrence of subsequent seizure episodes, and this opioid is included in post-seizure inhibition (7).

Although cyclosporin A neurotoxicity can be influenced by various factors, the opioid effect of neuroprotection is involved in the interrelated mechanism. These exogenous opioids suppress the cyclosporin A-induced electroconvulsive activity, and with an increased level of opioids, there may be a functional anticonvulsant effect on cyclosporin A-induced neurotoxicity. These results suggest that opioid substances may play a role in the pathogenesis of cyclosporin A-induced neurotoxicity and these results may be a positive clue to the relationship between cyclosporin A-induced neurotoxicity and the opioid system.

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