Full Paper

Studies on Somnolence in the Daytime Caused by Drugs Used for Neuropathic Pain

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Abstract. In the present study, the characteristics of the sleep features of amitriptyline, mexiletine, and *N*-(4-tertiarybutylphenyl)-4-(3-cholorpyridin-2-yl)tetrahydropyridine-1(2*H*)-carbox-amide (BCTC) were studied. Electrodes were chronically implanted into the frontal cortex and the dorsal neck muscles of rats for electroencephalogram (EEG) and electromyogram (EMG) measurements, respectively. EEG and EMG were recorded with an electroencephalograph, and SleepSign ver. 2.0. was used for sleep–wake state analysis. Recordings were performed from 11:00 to 17:00. Amitriptyline caused significant decreases in sleep latency and total wake time and an increase in total non-rapid eye movement (non-REM) sleep time. Mexiletine caused a significant decrease in sleep latency, but no significant effect was observed in total wake time and total non-REM sleep time. On the other hand, BCTC, which is an antagonist of transient receptor potential vanilloid 1 (TRPV1), showed no significant effect on sleep latency, total wake time, total non-REM sleep time, and total REM sleep time. From these results, it can be concluded that a TRPV1 antagonist may become a useful drug for neuropathic pain without sedative side effects in the daytime, different from amitriptyline and mexiletine.

Keywords: amitriptyline, mexiletine, TRPV1 antagonist, somnolence

Introduction

Neuropathic pain is a refractory and chronic clinical pain disorder, and this symptom is produced by diabetes mellitus, herpes infection, and direct nerve injury (1–3). On the other hand, it is well known that non-steroidal anti-inflammatory drugs (NSAIDs) and opiates are inactive against neuropathic pain; therefore, anti-depressants, Na⁺-channel blockers, and anti-convulsants are used empirically for patients with neuropathic pain (1, 3), and the effectiveness of these drugs has been reported in many studies (4–6). However, because these drugs have some adverse clinical effects, the use of these drugs is limited and particular attention should be paid when these drugs are used. One of the adverse events occurring with the clinical use of anti-

depressants, Na⁺-channel blockers, and anti-convulsants is central depressant effects, for example, somnolence and dizziness. These effects influence the social life of the patients, for example, by decreasing daytime functioning and the quality of life (QOL).

On the other hand, it is well recognized that transient receptor potential vanilloid 1 (TRPV1) is a non-selective cation channel activated with acidic stimulation or treatment with capsaicin, and it has been reported that this receptor is associated with pain reactions (7, 8).

N-(4-Tertiarybutylphenyl)-4-(3-cholorpyridin-2-yl) tetrahydropyridine-1(2*H*)-carbox-amide (BCTC), which is a potent and selective antagonist of TRPV1, is reported to attenuate the pain reaction induced by nerve injury or inflammation in animal studies (9, 10); however, there is no report on how a TRPV1 antagonist affects the central nervous system.

In this study, therefore, we studied the central depressant effects of various drugs for neuropathic pain, including amitriptyline (antidepressant), mexiletine (Na⁺-

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channel blocker), and BCTC (candidate therapeutic compound) in rats.

Materials and Methods

Animals

Male Sprague-Dawley (SD) rats weighing 330–400 g (Charles River, Yokohama) were used. All animals were maintained in an air-conditioned room with controlled temperature ($24 \pm 2^\circ\text{C}$) and humidity ($55 \pm 15\%$). They were housed in aluminum cages with sawdust and kept under a 12-h light-dark cycle (lights on from 7:00). The animals were allowed free access to food and water, except during the experiments and except animals used for the study of BCTC; these animals were fasted 18 h before starting the experiment. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Surgery

The animals were anesthetized with pentobarbital sodium (Nembutal®, 50 mg/kg, i.p.; Abbott Laboratories, North Chicago, IL, USA) and then fixed to a stereotaxic apparatus (SR-5N; Narishige, Tokyo). For electroencephalogram (EEG) recording, a stainless steel screw electrode (800 μm) was chronically implanted into the right frontal cortex (A: 0.5, L: 3.0), according to the atlas of Paxinos and Watson (11). A stainless steel screw fixed in the left frontal bone served as a reference electrode. To record the electromyogram (EMG), stainless steel wire electrodes (200 μm) were implanted into the dorsal neck muscle. The electrodes were connected to a miniature receptacle, and the whole assembly was fixed to the skull with dental cement. At least 7 days were allowed for recovery from surgery.

EEG and EMG recordings

EEG and EMG were recorded with an electroencephalograph (Model EEG 5113; Nihon Kohden, Tokyo) for 6 h (11:00–17:00). The recording was carried out according to a previously described (12–14), which showed that 6-h recording is reasonable to assess the somnolence caused by the drug. The signals were amplified and filtered (EEG, 0.5–30 Hz; EMG, 16–128 Hz) and then digitized at a sampling rate of 128 Hz and recorded using the data acquisition program SleepSign ver. 2.0 (Kissei Comtec, Nagano). EEG and EMG of the rat were measured in a cylindrical plastic cage (diameter, 26 cm; height, 31 cm), placed on a stainless steel grid. The grid floor was placed inside the plastic cage. The stainless steel rods of the grid (3-mm-wide) were set 2-cm-apart. The observation cage was

placed in a soundproof and electrically shielded box ($70 \times 60 \times 60$ cm).

Sleep-wake state analysis

The sleep-wake states were automatically classified into 10-s periods as waking, non-rapid eye movement (non-REM), or rapid eye movement (REM) sleep by SleepSign ver. 2.0, according to the criteria previously described (13, 14). As a final step, the defined sleep-wake stages were examined visually, and corrected if necessary. Each state was characterized as follows: waking, low-amplitude EEG, and high-voltage EMG activities; non-REM sleep, high-amplitude slow, or spindle EEG and low-voltage EMG activities; REM sleep, low-voltage EEG, and EMG activities. Sleep latency was defined as the time from the start of experiment up to the first 12 consecutive 10-s periods of non-REM and REM sleep.

Drugs

The following drugs were used: amitriptyline hydrochloride (Sigma), mexiletine hydrochloride (Pfizer), and BCTC (Pfizer). Amitriptyline was dissolved in distilled water. Mexiletine was dissolved in physiological saline. BCTC was suspended in 5% SBEDC solution and Tween 80 (99.5:0.5). The drugs, except for mexiletine, were administered orally and mexiletine was injected subcutaneously at 11:00. When BCTC was administered, rats were fasted for 18 h before administration of BCTC. EEG and EMG were measured for 6 h immediately after drug administration. Drugs were administered at intervals of 7 days when the same rats were used for repeated experiments. The doses of drugs used in this study were higher than those used for nociceptive action.

Data analysis and statistics

Values are each a mean $\pm$ S.E.M. One-way analysis of variance (ANOVA) with Dunnett's test was used to estimate the drug effects. A *P*-value less than 0.05 was considered significant.

Results

Effect of amitriptyline on sleep

Amitriptyline caused dose-dependent decreases in sleep latency and total wake time and an increase in non-REM sleep time. Significant effects were observed at doses of 30 and 100 mg/kg, p.o. in sleep latency, and a dose of 100 mg/kg, p.o. affected both the total wake time and total non-REM sleep time; however, no remarkable effect was observed in total REM sleep at all doses used in the present study (Fig. 1).

Fig. 1. Effect of amitriptyline on sleep. Columns and vertical bars represent the means $\pm$ S.E.M. ($N = 8$). Drugs were administered orally. * and **: Significantly different from the control group at $P < 0.05$ and $P < 0.01$, respectively (ANOVA With Dunnett's Test).

Effect of mexiletine on sleep

Sleep latency was significantly decreased by the subcutaneous injection of mexiletine at doses of 30 and 100 mg/kg; however, no significant effects were observed in total wakefulness time, non-REM sleep time, and REM sleep time (Fig. 2).

Effect of BCTC on sleep

No remarkable effect in sleep latency was observed by oral administration of BCTC at the doses used in this study. Moreover, no significant effects were shown in

Fig. 2. Effect of mexiletine on sleep. Columns and vertical bars represent the means $\pm$ S.E.M. ($N = 8$). Drugs were injected subcutaneously. *: Significantly different from the control group at $P < 0.05$ (ANOVA With Dunnett's Test).

the total wake time, total non-REM sleep time, and total REM sleep time even at a dose of 100 mg/kg (Fig. 3).

Discussion

In the present study, we studied the characteristic sleep features of drugs for neuropathic pain. As a result, amitriptyline caused a significant decrease in sleep latency and the total awake time and an increase in the total non-REM sleep time. Mexiletine also showed a significant decrease in sleep latency, but no significant

Fig. 3. Effect of BCTC on sleep. Columns and vertical bars represent the means $\pm$ S.E.M. ($N = 8$). Drugs were administered orally.

effect was observed on the sleep-wake cycle in rats. On the other hand, BCTC caused no change to sleep latency and sleep-wake cycle in rats.

Amitriptyline is the major prescribed classical anti-depressant, and the drug has also been used empirically for patients with neuropathic pain. Recent reports have been published about the effectiveness of amitriptyline on neuropathic pain in clinical and animal studies (15, 16). On the other hand, a central depressant effect was observed frequently when amitriptyline was used clinically. As shown in the present results, amitriptyline caused a significant somnolence effect at doses of 30 and

100 mg/kg, p.o., doses that are effective in neuropathic pain model rats. In the United States, amitriptyline is used for patients with insomnia in about 20% of all prescribed hypnotics (17). Furthermore, there are reports that this drug affects serotonergic, histaminergic, and cholinergic neurotransmitter receptors and so on (18). It is well known that there is an interaction between anti-histaminergic and anti-cholinergic effects and an hypnotic effect in humans and animals.

Mexiletine is a Na^+ -channel blocker used clinically as an anti-arrhythmic drug and is also used for patients with neuropathic pain. There is a report about a contributing factor to neuropathic pain, which is hyperactivity of the Na^+ channel (19). Studies on the analgesic action of mexiletine were also reported in both animals and humans (5, 19). On the other hand, it has been reported that patients treated with mexiletine occasionally exhibit somnolence and dizziness as adverse reactions (20). As shown in the present study, mexiletine caused a significant decrease in sleep latency, that is, mexiletine has a sleep-inducing effect. As is clear from the above findings, the rat model in the present study may be used to detect slight somnolence induced by analgesics such as amitriptyline and mexiletine.

BCTC is a potent and selective TRPV1 antagonist, and this compound is effective against the various types of pain in animal studies (9, 10, 21); therefore, BCTC can be used as a new analgesic for neuropathic pain in humans. TRPV1 is expressed not only in the primary sensory nerves and kidney but also in the brain (7, 8). In addition, it has been reported that BCTC can pass through the blood brain barrier (22); thus, it is reasonable to presume that BCTC has some effect on the central nervous system; however, as shown in the present text, BCTC had no hypnotic effect in rats even at a dose of 100 mg/kg, a dose that is higher than that showing an analgesic effect.

From these findings, it seems likely that BCTC has a potent therapeutic effect on any type of pain, including neuropathic pain, without causing sleepiness, different from amitriptyline and mexiletine. Furthermore, it is suggested that a TRPV1 antagonist may be a useful analgesic without causing daytime sleepiness.

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