Pharmacological Properties of Traditional Medicines. XXVI.1) Effects of Sansohnin-to (酸棗仁湯) on Pentobarbital Sleep in Stressed Mice

Ken-ichi SAITO, * Sumiyo UMEUDA, Keiko KAWASHIMA, and Yoshihiro KANO

Department of Kampo Medicinal Science, Hokkaido College of Pharmacy, 7-1 Kataruoka-cho, Otaru, Hokkaido 047-0264, Japan. Received July 13, 1999; accepted September 21, 1999

We investigated the effect of Sansohnin-to (酸棗仁湯, SAT) on changes of duration in sodium pentobarbital (PB)-induced sleeping time caused by five types of stress.

SAT reversed shortened PB sleep in repeated cold stress or 45 min-restraint stress tests and the prolonged PB sleep in 120 min-restraint stress. SAT did not reverse the shortened PB sleep in the specific stress state caused by an alternating rhythm in temperature stress or social isolation stress. In addition, SAT influenced both shortened PB sleep in 45 min-restraint stress and prolonged PB sleep in 120 min-restraint stress. SAT had no effect on PB sleep in unstressed control mice. These findings suggest that SAT has unusual activity, different from synthetic narcoleptics such as benzodiazepine. This is because SAT had no effect on PB sleep in unstressed mice, and it reverses stress-induced decrease and/or increase in PB sleep by improving stress-induced functional changes in the central nervous system, rather than by acting like a synthetic hypnotic on the γ-aminobutyric acid (GABA) receptor.

Key words: Sansohnin-to; stress; insomnia; pentobarbital sleep; Kampo medicine

It is generally said that modern times are very stressful. With the increase of various psychological problems due to the complexity and high stress of modern society, patients with insomnia are increasing. Synthesized chemical narcoleptics are generally used for treatment of insomnia, however, due to their toxicity, side effects grow more and more apparent.

On the other hand, Kampo medicine uses natural substances which generally have low toxicity and few side effects, and treats disease as unique to the individual, and balances homeostasis and increasing immunity to diseases. In traditional prescriptions used to treat sleep disorders, Sansohnin-to (SAT), a well-known sedative traditional prescription, is used for patients with weakness and fatigue, annoyance, insomnia, amnesia, and neurotic symptoms. However, the active components of SAT and their pharmacological mechanism of action, have not been clarified yet.

In the present study, the pharmacological properties of SAT were investigated using disease-model animals induced by various stresses.

MATERIALS AND METHODS

Drugs and Chemicals Sodium pentobarbital (PB) was purchased from the Dainippon Pharmaceutical Co., Ltd. Sansohnin (seeds of Zizyphus spinosa Hu) was supplied by Mikuni Co., Ltd., (Japan), and Licorice (roots of Glycyrrhiza spp.). Chimo (rhizomes of Anemarrhena asphodeloides Bunge), Senkyu (rhizomes of Cnidium officinale Makino) and Bukuryo (sclerotium of Poria cocos Wolf) were purchased from Tochimoto-Tenkaido Co., Ltd., (Japan).

Animals Male ddY mice (4 weeks old, 18—22 g) were used in this study were purchased from Nihon SLC Co., Ltd., (Japan). They were separated in groups of 10—11 per cage in the breeding room and kept for at least one week before the experiments. They were fed a commercial diet (MF, Oriental Yeast Co., Tokyo) and allowed tapwater ad libitum. Housing conditions were thermostatically maintained temperature at 24±1°C under a 12 h dark—light cycle. All procedures involving the mice were performed using protocols approved by our Institutional Animals Care and Use Committee.

Preparation of Extracts of SAT Sansohnin (5.0 g) was added to 200 ml of distilled water and boiled until the volume was reduced to 150 ml. Then, Licorice (0.5 g), Chimo (1.5 g), Senkyu (1.5 g) and Bukuryo (2.5 g) were added, and the whole was boiled until the volume was reduced to 75 ml. The filtered mixture was freeze-dried and the obtained powder (1 g corresponds to 5.9 g crude drugs) stored in a refrigerator. The powder was dissolved in water at the desired concentration just before use.

Repeated Cold Stress The procedures of repeated cold stress were according to the methods of Matsumoto et al.3) Mice were exposed to a cold environmental temperature of 4°C. This stress application was usually carried out twice a day at 9—11 a.m. and 2—4 p.m., respectively, for 3 d, and the last stress was induced at 9—11 a.m. after three overnight (7 times in total). SAT was orally administered 15 min after the last stress application. PB-sleeping time was measured 30 min after oral administration.

SART Stress (Specific Stress State Caused by Alternating Rhythm in Temperature) Procedures for SART stress were essentially according to the methods of Kurashi et al.4) Mice were exposed to a cold environmental temperature of 4°C from 5:30 p.m. to 11:00 a.m. and then alternately to room temperature (24°C) and the cold one at 4°C at 30 min intervals from 11:00 a.m. to 5:30 p.m. Such SART stress was delivered for 3 d and ceased at 11:00 a.m. after exposure to four overnight cold stresses. SAT was orally administered 15 min after the last stress application. PB-sleeping time was measured 30 min after oral administration.

Social Isolation Stress Procedures for social isolation stress were according to the methods of Matsumoto et al.5) Mice were housed in groups of 5 per cage (24×17×12 cm) or social isolation by being housed individually for 7 weeks before the start of the experiments. SAT was orally administered to the animals at the end of the stress. PB-sleeping time was measured 30 min after oral administration.

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* To whom correspondence should be addressed.
FIG. 1. Changes of PB-Induced Sleeping Time 30, 60 and 120 min after Oral Administration of SAT to Unstressed Mice
SAT: 850 mg/kg, p.o., PB: 40 mg/kg, i.v. ANOVA: (F3, 33, 0.01)=2.95, Dunnett's test; N.S.: non significantly.

FIG. 2. Effects of SAT on PB-Induced Sleeping Time Changed by Repeated Cold Stress
SAT: 850 mg/kg, p.o., PB: 40 mg/kg, i.v. ANOVA: (F2, 25, 0.01)=23.29, Scheffe's test; *p<0.05.

Restraint Stress  Procedures for restraint stress were accorded according to the methods of Shibaoka et al. Mice were placed and kept in restraint cages for 45 or 120 min. SAT was orally administered to the animals 30 min before restraint. PB-sleeping time was measured at the end of stress.

Measurement of PB-Sleeping Time  PB (40 mg/kg) was injected i.v. and the duration of PB-induced sleeping time was measured as the period between the loss of the righting reflex and its return.

Statistical Analysis  The data were analyzed with one-way analysis of variance (ANOVA) followed by the Scheffe’s test. Differences with p<0.05 were considered significant.

RESULTS

Figure 1 shows the changes in PB-induced sleeping time 30, 60 and 120 min after oral administration of SAT to unstressed mice. SAT had no effect on PB sleep in unstressed mice for 30—120 min after oral administration.

Figure 2 shows the effects of SAT on PB-induced sleeping time changed by repeated cold stress. The PB-induced sleeping time of mice exposed to repeated cold stress was significantly shorter than that of unstressed control (52% of unstressed control). SAT significantly prolonged PB sleep shortened by stress (137% of stress control) without affecting PB sleep in unstressed control mice.

Figure 3 shows the effects of SAT on PB-induced sleeping time changed by SART stress. The PB-induced sleeping time of mice exposed to SART stress was significantly shorter than that of unstressed control (54% of unstressed control). The SART stress-induced decrease in PB sleep was not significantly different from those of mice orally administered SAT.

Figure 4 shows the effects of SAT on PB-induced sleeping time changed by social isolation stress. The PB-induced sleeping time of mice exposed to social isolation stress was significantly shorter than that of unstressed control (66% of unstressed control). SAT did not affect the PB sleep shortened by social isolation stress.

Figure 5 shows the effects of SAT on PB-induced sleeping time changed by 45 min-restraint stress. The PB-induced sleeping time of mice exposed to 45 min-restraint stress was significantly shorter than that of unstressed control (82% of
unstressed control). SAT significantly returned the 45 min-restraint stress-induced decrease in PB sleep to the level of unstressed control.

Figure 6 shows the effects of SAT on PB-induced sleeping time changed by 120 min-restraint stress. The PB-induced sleeping time of mice exposed to 120 min-restraint stress was significantly longer than that of unstressed control (135% of unstressed control). SAT significantly returned the 120 min-restraint stress-induced increase in PB sleep to the level of unstressed control.

DISCUSSION

Stressful stimuli such as pain, fever, anxiety and novelty cause various responses in both humans and animals. Stressful stimuli also increase arousal because physical stimulus such as pain and psychological stimulus such as anxiety have been found to cause insomnia. It is known that animals exposed to physical and psychological stress have responses such as functional changes in the central nervous system and/or peripheral system, changes in nociceptive response and changes in the hypnotic effect of barbiturates. The present study demonstrated the effect of SAT on changes in PB sleep of mice caused by five types of stress, and helps to clarify possible pharmacological properties of SAT.

Repeated cold stress significantly decreased the PB-induced sleeping time in mice. It has been reported that repeated cold stress decreases the duration of PB sleep by inducing functional changes in the central nervous system and subacute changes in the GABAergic system, leading to decreased sensitivity to PB. SAT reversed the shortened PB sleep resulting from repeated cold stress (Fig. 2). It is concluded that SAT influences the functional changes in the GABAergic system induced by this stress.

45 min-restraint stress significantly decreased the PB-induced sleeping time in mice (Fig. 5). 120 min-restraint stress significantly prolonged the PB-induced sleeping time in mice (Fig. 6). In rat, arousal increases by 60 min-restraint stress and decreases by 120 min-restraint stress. Brain corticotropin-releasing hormone (CRH) and opioid are involved in the mechanism by which stress modulates arousal. CRH, the main secretagogue of adrenocorticotropic-related peptides in stress, plays an important role to integrate changes in various stress, such as stimulation of the sympathetic nervous system, inhibition of gastric acid secretion, stimulation of colonic transit, suppression of food intake, and induction of aggression. The action of brain CRH in increasing arousal is predominant in 60 min-restraint, and that of opioid in decreasing arousal predominant in 120 min-restraint in rats.

Meanwhile, there is the possibility that the activity of the metabolic system for PB could be induced by stress. However, it has been reported that the metabolism of PB is not influenced by repeated cold stress or restraint stress and that changes in PB-induced sleeping time by these stress types are not due to a change in PB metabolism. Therefore, these reports indicate that functional changes in the central nervous system are responsible for the decrease or increase in PB sleep.

SAT reversed the shortened PB sleep in the 45 min-restraint stress and the prolonged PB sleep in 120 min-restraint stress. SAT may affect functional changes in the brain CRH-opioid system (the state of imbalance) induced by restraint stress.

SAT stress significantly decreased the PB-induced sleeping time in mice. SAT stress has been shown to shorten latency of response to thermal stimulation and activate the cholinergic system. SAT had no effect on the shortened PB sleep in SART stress (Fig. 3). SAT may have no effects on PB sleep with hyperalgesia.

Social isolation stress significantly decreased the PB-induced sleeping time in mice (Fig. 4). Functional changes in noradrenergic, corticotropin-releasing-factor and GABAergic systems in the brain are involved in the this stress-induced decrease in the hypnotic activity of PB. SAT had no effect on the shortened PB sleep in social isolation stress. Thus, the above findings indicate that functional changes in the brain induced by repeated cold stress and restraint stress are different from those with SART stress and social isolation stress in quality and/or quantity.

On the other hand, SAT had no effect on PB sleep in unstressed control. SAT may be more effective in the pathophysiological state caused by stress. From these findings, we conclude that SAT has unusual activity, different from synthetic narcoleptics such as benzodiazepine, since SAT had no effect on PB sleep in unstressed mice. In other words, SAT reverses a decrease or increase in PB sleep by improving stress-induced functional changes in the central nervous system, rather than by acting like a synthetic hypnotic.

Further studies to clarify active compounds and the pharmacological mechanism of SAT will be performed in the future.

CONCLUSIONS

1. SAT reversed the decrease in PB sleep induced by repeated cold stress or 45 min-restraint stress and the increase in PB sleep induced by 120 min-restraint stress.
2. SAT did not reversed the decrease in PB sleep induced by SART stress or social isolation stress.
3. SAT had no effect on PB sleep in unstressed control mice.
4. SAT has unusual activity, different from synthetic hypnotics.
REFERENCES AND NOTES


