Effect of Japanese Angelica Root Extract on Pentobarbital-Induced Sleep in Group-Housed and Socially Isolated Mice: Evidence for the Central Action

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ABSTRACT—We investigated the effect of the aqueous extract of Japanese angelica root (JAR) on pentobarbital (PB) sleep in group-housed and socially isolated mice. The JAR extract (1.25–2.5 g/kg, p.o.) dose-dependently reversed the decrease in PB sleep caused by isolation stress without affecting PB sleep in group-housed mice. The JAR extract (2.5 g/kg, p.o.) also antagonized the decrease in PB sleep caused by the α2-adrenoceptor antagonists yohimbine and idazoxan (1 mg/kg, i.p.) and the α1-adrenoceptor agonist methoxamine (200 nmol, i.c.v.) in group-housed mice. These results suggest that the JAR extract reverses changes in the arousal level caused by isolation stress and the activation of central noradrenergic systems.

Keywords: Angelicae radix, Social isolation stress, Pentobarbital sleep

Japanese angelica root (JAR) is a medicinal plant, included in number of traditional Sino-Japanese herbal prescriptions, and it is used as a decoction. The extract of this plant has been clinically used to improve gynecological diseases such as menoxenia and anemia via its hemogenic, analgesic and sedative activities.

Several in vivo and in vitro studies elucidated the pharmacological activity of the aqueous extract and chemical components of JAR in the peripheral tissues and demonstrated vasodilative and immunostimulating effects (1, 2). However, very little information is available on the action of this plant on the central nervous system (CNS) of rodents. Hayashi showed that the water extract of JAR had no effect on the CNS in mice and rats (1). Ohta et al. demonstrated (3) that the orally administered JAR extract, as well as the peony root extract, improved the scopolamine-induced working memory impairment in rats. The latter findings suggest the ability of systemically administered JAR extract to exert an activity in the CNS.

It has been reported that the arousal level of animals can be modulated by stressful manipulations dominantly via corticotropin-releasing factor (CRF) (4) and noradrenergic systems in the brain (5, 6). The changes in the arousal level caused by stressors also affect the activity of hypnotic drugs (7, 8). We reported previously that the social isolation stress causes a decrease in the duration of pentobarbital-induced sleep in mice and that the hyperactivity of central noradrenergic and CRF systems is involved in this decrease (9). It is conceivable that these pathophysiological changes in the CNS caused by social isolation stress are an adequate model to investigate the action of traditional Sino-Japanese herbal medicines on the CNS since a number of traditional medicines have been considered to improve an imbalance in the homeostatic condition of the human body.

In the present study, to pharmacologically elucidate the activity of JAR at the CNS, we investigated the effect of the aqueous extract prepared from JAR on pentobarbital sleep in group-housed and socially isolated mice. We also examined the effects of the aqueous extracts prepared from peony root and rehmannia root since these plants, as well as JAR, have been clinically believed to have nourishing effects on the blood or hemogenic actions.

Male ddY mice weighing 18–20 g (Japan SLC Co., Shizuoka) were obtained at the age of 28 days. Animals were housed in groups of 5–6 per cage (24 × 17 × 12 cm) or socially isolated by being housed individually for 4–6 weeks before the start of the experiments (9). The mice were given free access to food and water. The housing was thermostatically maintained at 24 ± 1°C with a constant humidity (65%) and a 12-hr light-dark cycle (lights on: 0700–1900 hr).
Drug-induced sleep in group-housed and isolated mice was measured as previously reported (8). Briefly, pentobarbital-Na (45 mg/kg) was injected intraperitoneally (i.p.), and the sleeping time was taken as the period between the loss of the righting reflex and its return.

Japanese angelica root (Angelica acutiloba KITAGAWA), peony root (Paeonia lactiflora PALLAS) and rehmannia root (Rehmannia glutinosa LIBOSCHITZ) were purchased from Tochimoto-Tenkai-Doh, Co., Ltd., Osaka. To prepare the aqueous extract of each, 120 g of the dried herb was boiled with 900 ml distilled water at 100°C for 90 min. After filtration, the filtrate was free-dried. The yield of JAR, peony root and rehmannia root extracts were 41.1%, 35.4% and 52.2%, respectively.

The following drugs were used: pentobarbital-Na (Tokyo Kasei, Tokyo), idazoxan HCl and methoxamine HCl (Sigma, St. Louis, MO, USA), and yohimbine HCl (Nacalai Tesque, Kyoto). Drugs, except for the JAR extract, were dissolved in saline. The JAR extract was dissolved in distilled water and administered orally (p.o.) 60 min before pentobarbital injection. Idazoxan and yohimbine (1 mg/kg) were injected intraperitoneally (i.p.), and methoxamine (200 nmol) was injected intracerebroventricularly (i.c.v.). The p.o. and i.p. administrations were performed in a constant volume of 10 ml/kg body weight 60 min before pentobarbital injection. The i.c.v. injections were performed in a constant volume of 5 µl/mouse, as previously described (2-mm-lateral, 2-mm-caudal to the bregma and 3-mm-deep) (9–11).

The present studies were conducted in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals of Toyama Medical and Pharmaceutical University.

The data were analyzed with one-way or two-way analysis of variance followed by the Student-Newman-Kuels test. Differences with P<0.05 were considered significant.

Consistent with our previous findings (9, 11), the duration of sleep caused by pentobarbital (45 mg/kg, i.p.) was significantly shorter than that in the animals housed in a group for the same period as socially isolated animals (Fig. 1). JAR extract administered at doses of 1.25–2.5 g/kg had no effect on pentobarbital sleep in the group-housed mice, but it dose-dependently antagonized the social isolation stress-induced decrease in pentobarbital sleep without affecting the pentobarbital sleep in group-housed mice (Fig. 1A: Fhousing condition x drug (3,75)=3.564, P<0.05). There was a significant interaction between the housing condition and peony root extract administration (F(2,50)=3.945, P<0.05), but the post-hoc test revealed no significant effect of the peony root extract on pentobarbital sleep in group-housed and socially isolated mice. The extract prepared from rehmannia root failed to change the duration of pentobarbital sleep in these animals at the same dose range as the JAR extract (Fig. 1C: Fhousing condition x drug (2,44)=0.238, P>0.05).

As shown in Fig. 2, systemically administered α2-adrenoceptor antagonists yohimbine and idazoxan (1 mg/kg, i.p., each) and i.c.v. injected α1-adrenoceptor agonist methoxamine (200 nmol/mouse) significantly decreased the duration of pentobarbital sleep in group-
housed mice. We have confirmed in our previous and preliminary experiments that the doses and administration routes of these drugs used in this study exhibit no effect on the hypnotic activity of pentobarbital in the socially isolated mice and that the suppressive effect of i.p. yohimbine on pentobarbital sleep in group-housed mice is qualitatively the same as that of i.c.v. yohimbine (Ref. 9 and Matsumoto et al., unpublished data). The oral administration of the JAR extract significantly reversed the decrease in pentobarbital sleep caused by i.p. yohimbine and idazoxan and i.c.v. methoxamine at the dose (2.5 g/kg) that had not affect the pentobarbital sleep by itself.

The present results demonstrated that the aqueous extract of JAR attenuated the increase in the arousal level of mice caused by social isolation stress and by stimulation of central noradrenergic systems. This finding strongly indicates that the JAR extract is able to modulate the activity of CNS.

When administered orally, the aqueous extract prepared from JAR had no effect on pentobarbital-induced sleep in group-housed mice at the dose range of 1.25-2.5 g/kg. This finding agrees with the previous report that the aqueous extract of JAR did not affect hexobarbital-induced sleep in rats (1). However, this extract caused the pentobarbital sleep in socially isolated mice to return to the level of group-housed mice without affecting the pentobarbital sleep in group-housed animals. Ojima et al. (9) reported previously that the social isolation stress-induced decrease in pentobarbital sleep is partly mediated by the hyperactivity of central noradrenergic and CRF systems. In addition, we recently found that the decrease in the γ-aminobutyric acid (GABA)A-receptor function also plays an important role in the decrease in pentobarbital sleep caused by social isolation stress in mice since both neuroactive steroids with ability to enhance the GABA A-receptor function and the benzodiazepine receptor antagonist flumazenil antagonized the decrease in pentobarbital sleep caused by social isolation stress but had no effect on the sleep in group-housed mice (10, 11). Taken together, the present results suggest that chemical components of the aqueous extract of JAR can reverse the hyperactivity of central noradrenergic and CRF systems and/or the decrease in the GABA A-receptor function caused by social isolation stress.

This idea can be supported by the present findings that the JAR extract almost completely antagonized the decrease in pentobarbital sleep caused by the a2-adrenoceptor antagonists yohimbine and idazoxan and the a1-adrenoceptor agonist methoxamine. In fact, it has been reported that the blockage of a2-adrenoceptors located on noradrenergic cell bodies and nerve terminals and the stimulation of a1-adrenoceptors enhance the activity of noradrenergic neurons (12, 13) and affect the sleep/arousal mechanisms (6, 14). Thus, it is likely that the JAR extract may attenuate the activation of the central noradrenergic system caused by yohimbine, idazoxan and methoxamine in group-housed mice and that this attenuating action may account for the antagonistic effect of the JAR extract on the social isolation stress-induced decrease in pentobarbital sleep.

The present results do not exclude the possibility that other neuronal mechanisms are implicated in the at-
tenuating action of the JAR extract on the \(\alpha_2\)-adrenoceptor antagonist-induced and \(\alpha_1\)-adrenoceptor agonist-induced decrease in pentobarbital sleep in group-housed mice. A recent finding in this laboratory demonstrated that flumazenil, a selective benzodiazepine receptor antagonist, apparently antagonized the decrease in pentobarbital sleep caused by i.c.v. yohimbine and i.c.v. methoxamine in group-housed mice at the dose that had no effect on pentobarbital sleep by itself. This finding suggests that putative endogenous benzodiazepine receptor ligands with ability to suppress the \(\mathrm{GABA}_A\) receptor function (15) are involved in the decrease in the hypnotic activity of pentobarbital caused by stimulation of central noradrenergic systems (Matsumoto et al., unpublished data). Thus, it is possible that the JAR extract may be able to modulate the function of the \(\mathrm{GABA}_A\)ergic system in the brain.

In the present study, the aqueous extracts of peony root and rehmannia root had no effect on pentobarbital-sleep in group-housed or socially isolated mice at the similar dose range to the JAR extract, suggesting that differences in chemical components among these medicinal plants contribute to the difference in the effects on pentobarbital sleep in mice. Essential oils such as ligustilide and butylidenephthalide are known to be characteristic chemical components in the JAR extract, and they do not exist in the aqueous extracts of peony root and rehmannia root. Interestingly, in our preliminary experiments, we found that the aqueous extract of cnidium rhizome (Cnidium officinale MAKINO) which contains essential oils similar to those included in the JAR extract also reversed the decrease in pentobarbital sleep caused by social isolation stress and by the \(\alpha_2\)-adrenoceptor antagonist yohimbine. Thus, it could be speculated that essential oils commonly included in the JAR and cnidium rhizome extracts play important roles in the actions of these plants in mice.

In conclusion, the present results indicate the central action of the aqueous extract of JAR. However, further investigations are required to clarify the exact mechanisms underlying the action of the JAR extract on the pentobarbital sleep decreased by social isolation stress and stimulation of central noradrenergic systems and to identify the chemical component(s) implicated in the central action of the JAR extract.

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