Psychotropic Effects of Japanese Valerian Root Extract

Tatsuya SAKAMOTO,* Yoko MITANI and Kazu NAKAJIMA

New Product Division, Rohto Pharmaceutical Co., Ltd., 8-1, Tatsumi-nishi 1-chome, Ikuno-ku, Osaka 544, Japan. Received August 7, 1991

The psychotropic effects of "Hokkai-Kisso", *i.e.* roots of Japanese valerian, were compared with those of diazepam and imipramine. Both 30% EtOH extract of valerian root (11.2 g/kg) and diazepam (3 mg/kg) significantly prolonged hexobarbital-induced sleep in mice. Spontaneous ambulation and rearing during an open field test were significantly decreased by valerian extract (11.2 g/kg), but kessyl glycol diacetate (KGD, 400 mg/kg) and diazepam (3 mg/kg) significantly increased ambulation. Diazepam (10 mg/kg) significantly decreased approach-avoidance conflict in mice in a water-lick conflict test, but valerian extract and KGD did not.

By contrast, valerian extract (4.1 g/kg) and imipramine (20 mg/kg) significantly inhibited immobility induced by a forced swimming test in rats, but did not increase spontaneous motor activity during an open field test just before the forced swimming test.

In addition, valerian extract and imipramine significantly reversed reserpine-induced hypothermia in mice. These results indicate that valerian extract acts on the central nervous system and may be an antidepressant.

Keywords Hokkai-Kisso; valerian; *Valeriana*; kessyl glycol diacetate; antidepressant; forced swimming test; reserpine-induced hypothermia; imipramine; diazepam

Introduction

Valerian is the common name given to genus *Valeriana*, herbaceous perennial plants widely distributed in the temperate regions of North America, Europe and Asia.

The underground part of valerian has a long history of use in north-western Europe as a sedative.

The label "sedative" is generally taken to mean that an agent has a calming effect in stress situations with little attendant hypnosis or loss of coordination, and the principal use of sedative drugs is to produce drowsiness and promote sleep. Investigations of valerian's effects have focused on defining the sedative, but the scientific basis for the use of valerian as a mild sedative has not been elucidated completely. Nowadays, sedative drugs are usually regarded as general central nervous system depressants, and have largely been replaced by benzodiazepines and other compounds. Nonetheless, valerian remains a popular over-the-counter sedative in many European countries and in Japan. We therefore hypothesized that the sedative action of valerian includes some psychotropic effect.

Chemical and pharmaceutical investigation of valerian constituents have concentrated on the two major groups of constituents present, the iridoids and the sesquiterpenoids. The iridoids named valepotriates were isolated as analgesic and sedative principles from Indian valerian roots.[1] On the other hand, Takamura *et al.*[2] reported that the sesquiterpenoids named kessyl glycol diacetate (KGD) containing Japanese valerian root is an active principle for prolonging hexobarbital-induced sleeping time in mice. Hikino *et al.*[3] reported that Japanese valerian containing low amounts of valepotriates show a greater effect on hexobarbital-induced sleeping time than Chinese and Nepalese valerian containing high amounts of valepotriates. Consequently, we took notice of the Japanese valerian root.

In this report we present the results of a study of the psychotropic effects of "Hokkai-Kisso", *i.e.* Japanese valerian root.

Materials and Methods

Animals Five to 7 weeks old ddY mice and 8 weeks old Wistar rats, all male, were purchased from SLC, Japan Inc. They were kept in a barrier-sustained room at $23 \pm 3\,°C$, $55 \pm 5\%$ humidity and a 12 h light-dark cycle. There were about 15 changes of air each hour.

Effect on Hexobarbital-Induced Sleeping Time Four to 5 ddY mice (5 weeks old) in each group, weighing 22—34 g, were used. Drugs were administered orally 30 min before intraperitoneal injection of hexobarbital (100 mg/kg). The time required to regain the righting reflex was measured.

Effect on Open Field Activities Five weeks old ddY mice, weighing 20—33 g were used. The open field apparatus was a wooden box $(75 \times 75 \times 30\,cm)$. Its floor was divided by black lines into 25 compartments. The numbers of ambulation and rearing in a 5 min period after placing the mice in the apparatus were recorded. Mice were divided into groups of 8 to 10 each according to their ambulation, which was measured on the day before drug administration, so that differences between average scores were minimal. The drug effects were measured 0.5, 1, 2.5 and 4 h after a single oral administration of the drugs.

Effect on Approach-Avoidance (Water-Lick) Conflict Test Six weeks old ddY mice, weighing 25—34 g were used. The apparatus was a clear Plexiglas box $(35 \times 20 \times 25\,cm)$ with a black Plexiglas compartment $(10 \times 10 \times 10\,cm)$ attached to one wall. An opening $(1.5 \times 3\,cm)$ led from the large box to the small compartment. The entire apparatus had a metal grid floor. A water bottle with a metal drinking tube was fitted to the outside of the small compartment. An electrostatic sensor and electrostimulator circuit were connected between the drinking tube and the grid floor. The mice were deprived of water for 24 h, and then allowed to drink the water from the tube for 5 min once a day in the apparatus. Food was available in the home cage at all times. The mice were divided into groups of 4—7 each according to their drinking times, which were measured on day 3 in the apparatus, so that differences between average times were minimal. The drugs effects were measured on the day after the third session. Sixty min after oral administration of the drug, each mouse was placed in the apparatus. Water was available at the tube for 15 s. Then electric shocks (80 V, 10 Hz) were administrated between the drinking tube and the grid floor following each 3 s lick. The number of shocks delivered during the 5 min was counted for each mouse. The method of Vogel *et al.*[4] was used with modification.

Effect on Forced Swimming Test Eight weeks old Wistar rats, weighing 207—236 g were used. The water tank was a clear Plexiglas cylinder (diameter; 17 cm, height; 40 cm). Water at 25 °C was filled to a depth of 20 cm in the tank. Rats were forced to swim once each day for 15 min and for the first 5 min the amount of time during which the rat was immobile was measured. The drug effects were measured on the day after the fourth session. Rats were divided into groups of 5 each according to their immobile time, which was measured on day 4, so that differences between average immobile times were minimal. The drugs were given orally 24, 12 and 2 h before the test session. The doses were those given at each administration. Spontaneous motor activity was measured by the open field test for 5 min just before forced swimming on the fifth day. The method of Shimazoe *et al.*[5] was used with slight modification.

Effect on Reserpine-Induced Hypothermia[6a] Five ddY mice (6 weeks old) in each group, weighing 26—32 g, with a 36.5—38.6 °C rectal tem-

perature, were used. The drugs were administered orally 18 h after a subcutaneous injection of reserpine (5 mg/kg). The rectal temperature was measured with a thermistor before and 0 to 6.5 h after administration of the drugs.

Preparation of Sample Drugs One hundred grams of Japanese valerian root (Hokkai-Kisso, purchased from Tochimoto Tenkaidō Co., Osaka, Japan) were refluxed three times with 700 ml of 30% ethanol, respectively 60 °C for 1 h and the extract was combined and freeze dried under vacuum for 2 d. KGD was isolated from Japanese valerian roots according to the method of Hikino et al.[3] Diazepam and imipramine (Wako Pure Chemical Co., Osaka, Japan) were used as control drugs. The extracts of valerian, KGD, diazepam and imipramine were suspended in 3% gum arabic, provided that the drugs for anticonflict effect were suspended in saline with 3% gum arabic. Hexobarbital (Wako Pure Chemical Co., Osaka, Japan) was suspended in saline and 10% NaOH was added dropwise until it dissolved. Reserpine was dissolved in saline with 20% propylene glycol which acidified to pH 3—5 with acetic acid.

Analysis of KGD in Extracts of Japanese Valerian Roots One gram of valerian extract was suspended in water (10 ml) and extracted with benzene (4 ml × 3). The combined benzene solution was evaporated in vacuo to give a residue. The residue was dissolved in 0.5 mg/ml of n-eicosane (internal standard) acetone solution and adjusted to a volume of exactly 10 ml. A 2 μg aliquot was applied to gas-liquid chromatography (Shimadzu GC-7AG). The gas-liquid chromatography conditions were as follows. Column: 3% Apiezon grease L Gas chrom Q 60/80 mesh (2 m × 3 mm i.d.); column temperature: 190 °C; injector and detector temperature: 210 °C; carrier gas and flow rate: N_2, 40 ml/min; detector: FID.

Quantitative analysis of KGD was carried out by using standard lines of authentic samples.

Data Analysis 1) The data on sleeping time and on reserpine-induced hypothermia were analyzed first by ANOVA, followed by Dunnett's multiple comparison test.

2) The data on open field activity and approach-avoidance conflict were analyzed first by the Kruskal–Wallis H-test, followed by Dunnett's multiple comparison test.

3) The data from the forced swimming test was analyzed by paired Student's t-test.

Results and Discussion

Oral administration of valerian extract dose-dependently prolonged hexobarbital-induced sleep. At 11.2 g/kg the effect was significant and was almost the same as that of 3 mg/kg diazepam (Table I).

In the open field test, the mice in the control group adapted to the test situation with repeated exposure, and there was a progressive decrease in ambulation and rearing. Valerian extract dose-dependently decreased ambulation and rearing from 2.8 to 11.2 g/kg 1 h after oral admin-

istration, and the effect was significant at 11.2 g/kg, but valerian extract tended to increase ambulation 2.5—4 h after administration. Diazepam at 3 mg/kg caused a significant increase in ambulation 0.5 h after administration and tended to increase ambulation 1.0—2.5 h after administration (Fig. 1). KGD at 400 mg/kg caused a significant increase in ambulation 1 h after administration and tended to increase ambulation 0.5 and 2.5 h after administration (Fig. 2). This effect was similar to that of diazepam.

An 11.2 g dose of valerian extract contained 400 mg of KGD, as determined by gas-liquid chromatography. Takamura et al.[2] and Hikino et al.[3] reported that KGD is the main active principle for prolonging hexobarbital-induced sleeping time in mice. Therefore, the effect of valerian extract on hexobarbital-induced sleeping may have been caused by KGD. However, 11.2 g/kg of valerian extract caused a significant decrease in ambulation, but at 400 mg/kg of KGD significantly increased ambulation 1.0 h after administration. This indicates that the decrease in ambulation after administration of valerian extract is not caused by KGD. In animals which are in a state of freezing due to an intense fear of the environment, ambulation can be easily induced by giving benzodiazepines.[7] KGD probably has an antianxiety effect similar to that of diazepam. We therefore investigated the anticonflict effects of KGD, valerian extract and diazepam. The results are shown in

TABLE I. Effect of Valerian and Diazepam on Hexobarbital-Induced Sleep in Mice

Drug	Dose (g/kg)	Sleeping time (min) (mean ± S.E.)	(n)
Vehicle		34.3 ± 6.2	(4)
Diazepam	0.003	$73.8 \pm 8.8^{a)}$	(4)
Valerian	2.8	42.6 ± 7.2	(4)
	5.6	48.3 ± 9.9	(4)
	11.2	$85.8 \pm 6.1^{a)}$	(5)

Drugs were administered orally 30 min before intraperitoneal hexobarbital injection. a) $p<0.01$ significantly different from vehicle by Dunnett's multiple comparison test.
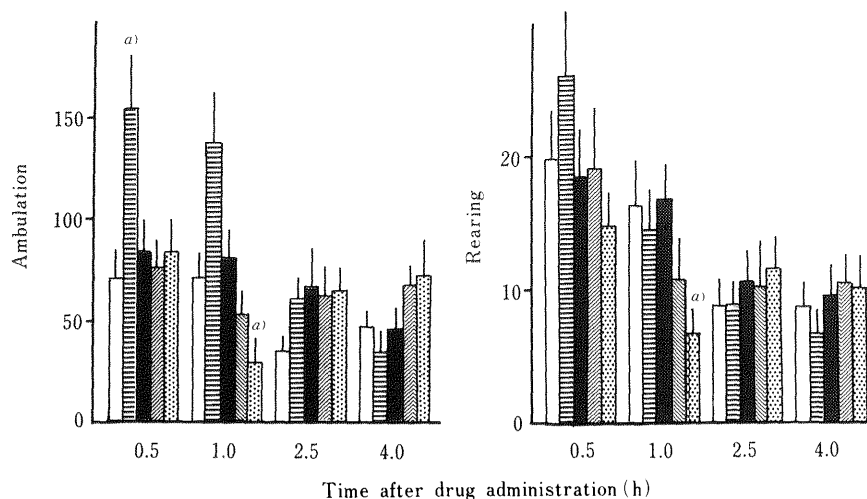

Fig. 1. Effects of Valerian Extract and Diazepam on Ambulation and Rearing in Mice; Open Field Test (5 min Period)

The drugs were administered orally. □, vehicle; ▥, diazepam 3 mg/kg; ■, valerian 2.8 g/kg; ▨, valerian 5.6 g/kg; ▨, valerian 11.2 g/kg. Each bar shows a mean ± S.E. of 10 mice. a) $p<0.01$ significantly different from the vehicle by Dunnett's multiple comparison test.
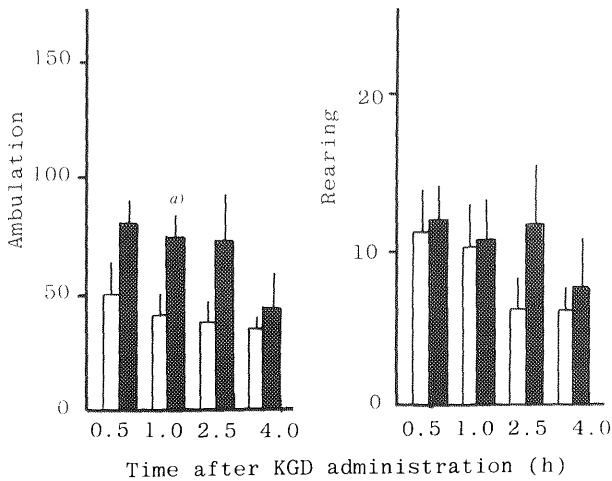
Fig. 2. Effect of KGD on Ambulation and Rearing in Mice; Open Field Test (5 min Period)

KGD was administered orally. The open column shows vehicle and the dotted column shows KGD (400 mg/kg). Each bar shows a mean ± S.E. of 8 mice. a) $p < 0.05$ significantly different from the vehicle by Mann–Whitney's U-test.
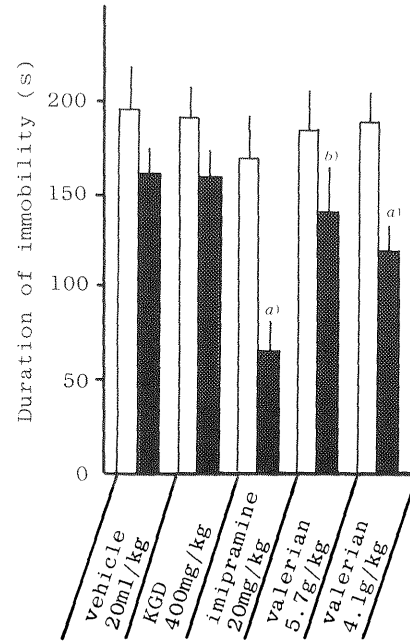

Fig. 3. Effects of Valerian Extract and Imipramine on the Total Duration of Immobility in Rats; Forced Swimming Test

The drugs were given orally 24, 12 and 2 h before the test session. Each dose shown was given three times. The open column shows the duration of immobility during the swimming session on the fourth day, and the dotted column shows the duration of immobility during the test session. Each bar shows a mean ± S.E. of 5 rats. a) $p < 0.05$ and b) $p < 0.01$ significantly different from the respective control values obtained 24 h before drug administration (paired Student's t-test).

TABLE II. Effect of Valerian, KGD and Diazepam on Conflict Behavior in the Mouse in a Water-Lick Conflict Procedure

Drug	Dose (g/kg)	Number of shocks/5 min (mean ± S.E.)	(n)
Exp. I			
Vehicle		7.0 ± 0.4	(4)
Diazepam	0.003	12.0 ± 2.0	(5)
	0.01	25.2 ± 5.9[a]	(5)
KGD	0.2	10.8 ± 1.8	(5)
Valerian	5.6	7.8 ± 2.1	(5)
Exp. II			
Vehicle		3.7 ± 0.5	(7)
KGD	0.284	4.7 ± 1.1	(6)
	0.4	4.5 ± 0.5	(6)

Drugs were administered orally 60 min before the test session. a) $p < 0.01$ significantly different from vehicle by Dunnett's multiple comparison test.

TABLE III. Effects of Valerian and Imipramine on Ambulation and Rearing in Rats in an Open Field Test just before Forced Swimming on Day 5

Drug	Dose (g/kg)	Frequency of ambulation (mean ± S.E.)	Frequency of rearing (mean ± S.E.)	(n)
Vehicle	—	57.4 ± 10.8	15.0 ± 2.8	(5)
KGD	0.4	63.6 ± 10.9	16.4 ± 3.4	(5)
Imipramine	0.02	47.8 ± 14.1	14.2 ± 4.2	(5)
Valerian	5.7	38.0 ± 10.2	7.6 ± 2.2	(5)
Valerian	4.1	73.8 ± 16.9	16.8 ± 5.1	(5)

Open field activity was measured for 5 min. There were no significant differences from vehicle by Dunnet's multiple comparison test.

Table II, the effects of KGD, valerian extracts and diazepam on the number of shocks delivered during the 5 min session. At 10 mg/kg diazepam significantly increased the number of shocks delivered. However, KGD at 200—400 mg/kg and valerian extract at 5.6 g/kg did not increase the number of shocks. This indicates that KGD and valerian extract have not as much antianxiety effect as diazepam.

Next, we compared the antidepressant effect of valerian extract with that of imipramine. Porsolt et al.[8] devised a "behavioral despair test" for rats and mice. They described that animals forced to swim in water caused a characteristic immobile posture, which is reduced by antidepressants.

As shown in Fig. 3, giving imipramine at 20 mg/kg or valerian extract at 4.1 and 5.7 g/kg resulted in significantly shorter durations of immobility as compared with the previous day. However, neither KGD at 400 mg/kg nor 3% gum arabic solution altered the duration of immobility. In contrast, spontaneous motor activity, when measured just before forced swimming on the fifth day, was unaffected by imipramine, valerian extract and KGD (Table III).

The forced swimming test serves as a useful model for the study of antidepressants. However, a lack of specificity in this screening of antidepressants has been pointed out.[9]

Psychostimulant, anticholinergic and antihistaminergic agents can also affect the results. However, Porsolt et al.[8] and Shimazoe et al.[5] reported that these durgs decreased the immobile time and increased the locomotor activity, although most antidepressants decrease immobile time without increasing spontaneous motor activity. Therefore, the results of the forced swimming test indicate that valerian extract has an antidepressant action. Dose-dependent suppression during immobility was not observed in valerian extract. This is a necessary consideration in the possibility that a reduction in immobility at high dose valerian extract was accompanied by sedation.

We also investigated the effect of valerian extract on reserpine-induced hypothermia in mice. This method is widely used to evaluate antidepressants. Most antidepressants have an anti-reserpine effect because they antagonize monoamine uptake.[6] In this experiment, the body temperature dropped below 27 °C 18 h after the subcutaneous administration of reserpine (5 mg/kg). Vale-
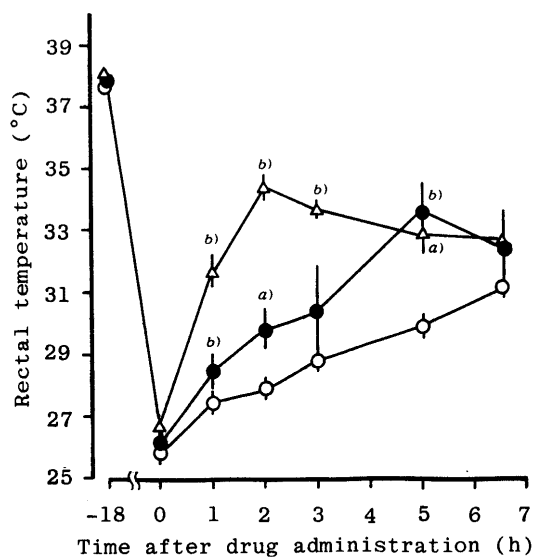
Fig. 4. Effects of Valerian and Imipramine on Reserpine-Induced Hypothermia in Mice

Drugs were administered orally 18 h after subcutaneous injection of reserpine (5 mg/kg). Each dot and bar shows a mean $\pm$ S.E. of 5 mice. $\bigcirc$, vehicle; $\bullet$, valerian 11.2 g/kg; $\triangle$, imipramine 20 mg/kg. a) $p < 0.05$ and b) $p < 0.01$ significantly different from the vehicle by Dunnet's multiple comparison test.

rian extract at 11.2 g/kg and imipramine at 20 mg/kg significantly reversed the reserpine-induced hypothermia (Fig. 5). This suggests that the valerian extract has antagonized the monoamine uptake. The effect of imipramine peaked 2 h after oral administration and then gradually decreased. In contrast, the effect of valerian extract peaked 5 h after oral administration. This difference in timing may be related to the potency after oral

administration, which is dependent on the absorption rates of these drugs from the gastrointestinal tract. It is also possible that valerian extract and imipramine antagonize monoamine uptake by different mechanisms. In either case, the reversal of reserpine-induced hypothermia by valerian extract can be considered to be an antidepressant effect.

In conclusion, these experiment results show that valerian extract acts on the central nervous system and may be an antidepressant. As we have not yet identified the active component, further investigation is necessary.

References
1) K. W. von Eickstedt and S. Rahman, Arzneim.-Forsch., 19, 316 (1969).
2) K. Takamura, M. Kakimoto, M. Kawaguchi and T. Iwasaki, Yakugaku Zasshi, 93, 599 (1973).
3) H. Hikino, Y. Hikino, H. Kobinata, A. Aizawa, C. Konno and Y. Oh'izumi, Shoyakugaku Zasshi, 34, 19 (1980).
4) J. R. Vogel, B. Beer and D. E. Clody, Psychopharmacologia, 21, 1 (1971).
5) T. Shimazoe, S. Shibata and S. Ueki, J. Pharmacobio-Dyn., 11, 639 (1987).
6) a) B. M. Askew, Life Sci., 10, 725 (1963); b) A. Carlsson, K. Fuxe and U. Ungerstedt, J. Pharm. Pharmacol., 20, 151 (1968); c) J. Glowinski and J. Axelrod, Nature (London), 26, 1318 (1964).
7) S. Ueki, S. Watanabe, T. Yamamoto, S. Shibata and K. Shibata, Jpn. J. Pharmacol., 35, 287 (1984).
8) R. D. Porsolt, G. Anton, N. Blavet and M. Jalfre, Eur. J. Pharmacol., 47, 379 (1978).
9) M. D. Schechter and W. T. Chance, Eur. J. Pharmacol., 60, 139 (1979); R. G. Browne, ibid., 58, 331 (1979); M. B. Wallach and L. R. Hedley, Comm. Psychopharmacol., 3, 35 (1979).