The Gamma-Aminobutyric Acidergic Effects of Valerian and Valerenic Acid on Rat Brainstem Neuronal Activity

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Valerian is a medicinal herb that produces anxiolytic and sedative effects. It was suggested that valerian acts via gamma-aminobutyric acid (GABA)ergic mechanisms. Previous studies showed binding of valerian extract to GABA receptors, but the functional effect of the binding has not been demonstrated. In this study we evaluated the GABAergic effect of valerian extract and one of its major constituents, valerenic acid, on brainstem neuronal activity in an *in vitro* neonatal rat brainstem preparation. We first observed that muscimol, a $GABA_A$ receptor agonist, decreased the firing rate in most brainstem neurons in a concentration-related fashion; 30 μM produced a 38.9% ± 3.0% (mean ± SE) inhibition compared with control values ($P < 0.01$; 50% inhibitory concentration [IC_{50}], 2.0 ± 0.1 μM). This effect was antagonized by bicuculline (10 μM), a $GABA_A$ antagonist. Then we showed that valerian extract 3 mg/mL induced a 29.6% ± 5.1% inhibition with an IC_{50} of 240 ± 18.7 μg/mL, whereas 100 μM valerenic acid induced a 22.2% ± 3.4% inhibition with an IC_{50} of 23 ± 2.6 μM (both $P < 0.01$). Bicuculline antagonized the inhibitory effects of both the valerian extract and valerenic acid. In addition, pretreatment with valerian extract or valerenic acid decreased the brainstem inhibitory effects produced by muscimol (both $P < 0.05$), suggesting that these compounds play an important role in the regulation of GABAergic activity. Data from this study suggest that the pharmacological effects of valerian extract and valerenic acid are mediated through modulation of $GABA_A$ receptor function. Thus, valerian may potentiate the sedative effects of anesthetics and other medications that act on GABA receptors, and presurgical valerian use may cause a valerian-anesthetic interaction.

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In the United States (US), valerian is one of the most commonly used herbal medicines (1) for the treatment of anxiety and insomnia (2). Virtually all sleep-aid herbal dietary supplements contain valerian (3). It is expected that valerian use could increase because of recent liver toxicity reports of kava (4,5), another commonly used herb with similar pharmacological effects to valerian (6).

Valerian has anxiolytic, tranquilizing, and sleep-inducing effects that have been demonstrated in both animal studies and clinical trials (7–10). Valerian or its constituents could induce these effects by interacting with central gamma-aminobutyric acid (GABA) receptors (11,12). Early *in vitro* studies testing the binding of valerian extract to GABA receptors showed that the agonist muscimol was displaced, suggesting valerian binding to these receptors (12). However, the neuropharmacological effects of this binding action, which could cause agonist and/or antagonist interactions, have not been studied.

The caudal brainstem is abundant in GABA, an important inhibitory neurotransmitter, and its receptors (13–16). Both major subtypes of GABA receptors, $GABA_A$ and $GABA_B$, have an inhibitory influence on nucleus tractus solitarius (NTS) activity involved in baroreceptor inputs and chemoreceptor reflex in the rat (17), cat (18), and rabbit (19). Previously, we have used an *in vitro* neonatal rat brainstem preparation to investigate GABAergic effects of selected compounds on NTS neuronal activities (20,21). In this study, we used this *in vitro* preparation to evaluate the neuropharmacological effects of valerian extract and valerenic acid, an important constituent of the extract, on GABA receptors in the caudal brainstem.

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Methods

The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Chicago. Experiments were performed on Sprague-Dawley neonatal rats 1 to 3 days old. After the animal was deeply anesthetized with halothane, a craniotomy was performed, and the forebrain was ablated by transection at the caudal border of the pons. The caudal brainstem and cervical spinal cord were isolated by dissection in modified Krebs solution that contained (mM) NaCl 128.0, KCl 3.0, NaH_2PO_4 0.5, $CaCl_2$ 1.5, $MgSO_4$ 1.0, $NaHCO_3$ 21, mannitol 1.0, glucose 30.0, and HEPES 10.0. The stomach, connected to the esophagus, with the vagus nerves linking it to the brainstem, was kept, and all the other internal organs were removed. The preparation was then pinned with the dorsal surface upon a layer of Sylgard resin (Dow Corning) in a recording chamber. The preparation was superfused with Krebs solution at 23°C ± 1°C. The bathing solution was aerated continuously with a mixture of 95% oxygen and 5% CO_2 and adjusted to pH 7.35–7.45 (20–22).

Single tonic unitary discharges were recorded extracellularly in the NTS by glass microelectrodes filled with 3 M NaCl, with an impedance of 10–20 MΩ (unitary discharge recordings; Ref. 20). One to five neurons were recorded from each preparation. A collision test was applied by stimulating the recorded unit and the subdiaphragmatic vagal nerve to identify orthodromic inputs (23), to ensure that only second- or higher-order NTS neurons in the afferent system were used in this study. For histological identification purposes, glass microelectrodes were filled with 2% pontamine sky blue in 0.5 M sodium acetate solution. After each unitary recording, current was applied at 5 μA in cycles of 5 s on/10 s off for approximately 5 min, with the negative lead connected to the microelectrode.

To independently evaluate the brainstem effects of GABA on NTS neurons, a partition was made at the thoracic level of the preparation. An agar seal formed a recording bath chamber of the brainstem compartment. Drugs were applied only to the brainstem compartment, and their effects on the NTS neuronal activity were evaluated. After each observation, drugs were washed out from the compartment. The NTS neuronal responses observed during pretrial or pretreatment (control) were compared with posttrial (washout) to confirm that brainstem neuronal activity returned to the control level after washout. Tachyphylaxis was not evident in our experimental conditions because response to reapplication of a given compound varied by <5%.

In each experiment, the NTS unitary discharges were amplified with high-gain alternating current-coupled amplifiers (Axoprobe-1A; Axon Instruments, Burlingame, CA), displayed on a Hitachi digital storage oscilloscope (Model VC-6525; Hitachi Denshi, Ltd., Japan), and recorded on a Vetter PCM tape recorder (Model 200; AR Vetter Co., Rebersburg, PA).

Valerian extract (*Valeriana officinalis* L. species, batch 00050100) was obtained from Lichtwer Pharma AG (Berlin, Germany). The extract was standardized to 0.3% valerenic acids (which contained valerenic acid and acetyl and hydroxyvaleric acids) by the manufacturer. Valerenic acid (>98%) was obtained from ChromaDex, Inc. (Santa Ana, CA). Muscimol, baclofen, bicuculline, and saclofen were obtained from Research Biochemicals International (Natick, MA).

The data from the NTS unitary activity were analyzed on the basis of action potential discharge rate and drug concentration-related effects. The number of action potentials in a given duration was measured under pretrial, trial, and posttrial conditions (usually 50 s in each trial). The control data (pretrial) were normalized to 100%, and the NTS neuronal activities during and after trials were expressed in terms of the percentage of control activity. Results are expressed as mean ± SE. Data were analyzed with Student's *t*-test and analysis of variance for repeated measures, with $P < 0.05$ considered statistically significant.

Results

Thirty-seven tonic units were recorded in the NTS. The basal firing rate of these NTS neurons was 0.87 ± 0.13 Hz (mean ± SE). When the $GABA_A$ receptor agonist muscimol was applied to the brainstem compartment, the firing rate in most NTS neurons decreased in a concentration-related fashion. In 29 of 37 units observed, 30 μM muscimol produced an inhibitory effect of 38.9% ± 3.0% of the control level of the NTS neuronal activity ($n = 29$; $P < 0.01$; Fig. 1). The IC_{50}, defined as 50% of the observed maximum inhibition, was 2.0 ± 0.1 μM. The remaining eight units did not respond to muscimol application. None of these 8 units showed a response to baclofen, a $GABA_B$ receptor agonist, and only 15 of 29 muscimol-sensitive units responded to 30 μM baclofen, with an inhibition of 21.2% ± 2.9% compared with the control level ($n = 15$; $P < 0.01$), and the IC_{50} was 1.5 ± 0.1 μM. None of the NTS neurons recorded showed an activation response to muscimol and baclofen. Bicuculline (10 μM), the $GABA_A$ receptor antagonist, competitively antagonized these inhibitory effects by muscimol (Fig. 1). Saclofen (10 μM), the $GABA_B$ receptor antagonist, also competitively antagonized the inhibitory effects induced by baclofen. Bicuculline (10 μM) and saclofen (10 μM) did not have significant effects on the basal activity of NTS neurons.

The effects of valerian extract and valerenic acid were evaluated in another 28 muscimol-sensitive NTS units. Three milligrams per milliliter valerian extract application induced an inhibitory effect of 29.6% ± 5.1% (*n*

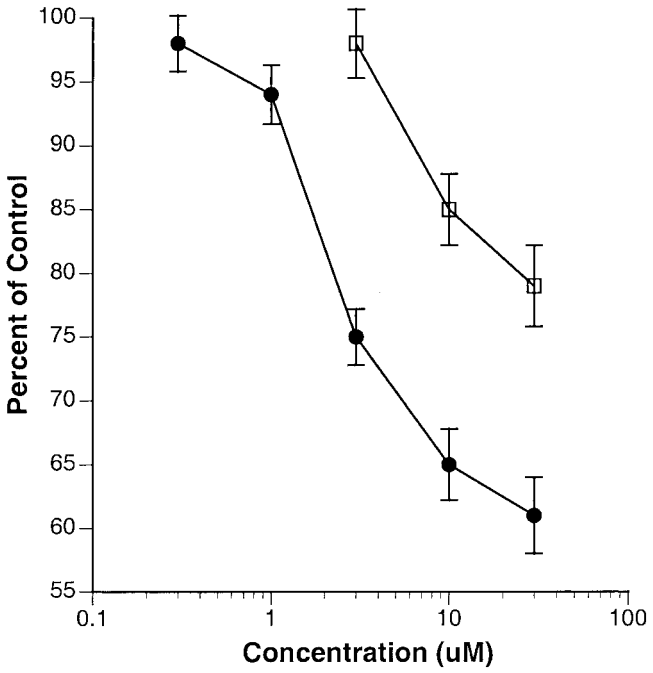

Figure 1. Effect of muscimol concentration (●) on the discharge frequency of nucleus tractus solitarius units. The discharge frequency of nucleus tractus solitarius neurons is expressed on the ordinate as percentage of control activity. The control activity is normalized to 100%. The effect of muscimol in the presence of 10 μM bicuculline is also shown (□). The difference in the unitary discharge frequency between the control recording and the recording after 30 μM muscimol application was significant ($P < 0.01$).

= 20; Fig. 2), and the IC_{50} was 240 ± 18.7 μg/mL. The inhibitory effect was also seen after 100 μM valerenic acid application (22.2% ± 3.4%; $n = 20$; Fig. 3), and the IC_{50} was 23 ± 2.6 μM. There were significant discharge rate differences between the control recordings and the recordings after 3 mg/mL valerian extract or 100 μM valerenic acid applications (both $P < 0.01$). Bicuculline (10 μM) antagonized valerian extract or valerenic acid-induced inhibitory effects (both $P < 0.05$; Figs. 2 and 3). The remaining eight muscimol-insensitive NTS units, however, did not show a significant response to valerian extract or valerenic acid applications. In addition, both valerian extract and valerenic acid induced inhibition in NTS neurons and could not be antagonized by saclofen (10 μM).

In this part of the experiment, muscimol effects were evaluated with pretreatment of valerian extract and valerenic acid. Pretreatment with valerian extract 3 mg/mL decreased the NTS inhibitory effects induced by 30 μM muscimol from 39.8% ± 3.2% to 26.5% ± 2.4% ($n = 9$). Similar results were obtained with valerenic acid, in which pretreatment with 100 μM of the compound decreased the NTS inhibitory effects induced by 30 μM muscimol from 40.2% ± 4.7% to 30.2% ± 2.6% ($n = 11$; Fig. 4). There were significant differences in the discharge rate between

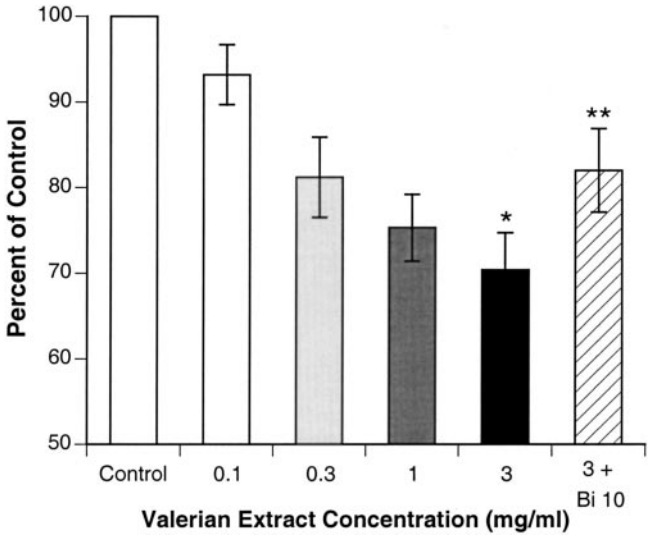

Figure 2. Effect of valerian extract on the discharge frequency of nucleus tractus solitarius units. The discharge frequency of nucleus tractus solitarius neurons is expressed on the ordinate as percentage of control activity. The control activity was normalized to 100%; the 50% inhibitory concentration was 240 ± 18.7 μg/mL. Bi 10 = bicuculline 10 μM. *$P < 0.01$ compared with control; **$P < 0.05$ compared with valerian extract 3 mg/mL.

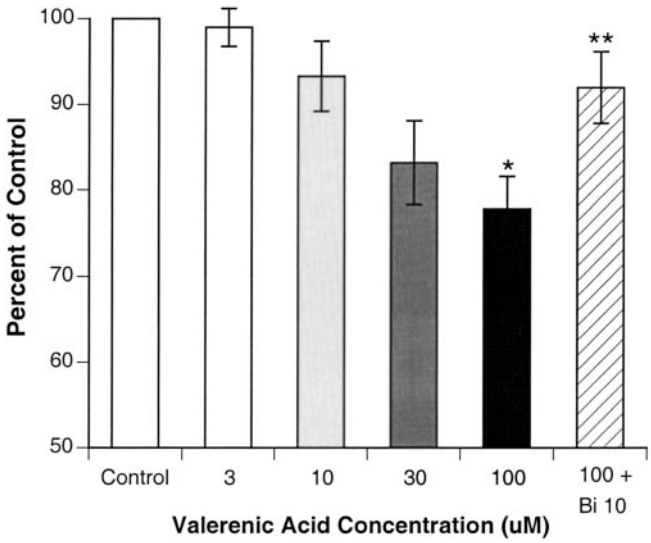

Figure 3. Effect of valerenic acid on the discharge frequency of nucleus tractus solitarius units. The discharge frequency of nucleus tractus solitarius neurons is expressed on the ordinate as percentage of control activity. The control activity was normalized to 100%; the 50% inhibitory concentration was 23 ± 2.6 μM. Bi 10 = bicuculline 10 μM. *$P < 0.01$ compared with control; **$P < 0.05$ compared with 100 μM valerenic acid.

muscimol alone and after pretreatment of valerian extract or valerenic acid (both $P < 0.05$).

Discussion

Valerian is a frequently used herbal ingredient in most sleep-aid dietary supplements (3). It is the common

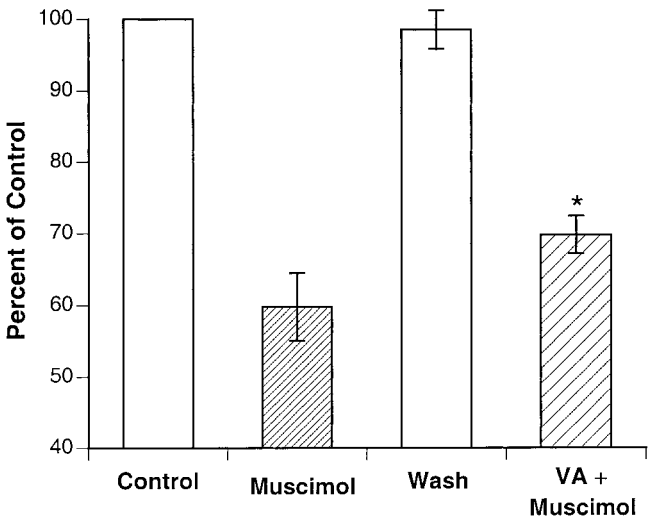

Figure 4. Effect of valerenic acid pretreatment on muscimol-induced inhibitory activity in nucleus tractus solitarius units. The discharge frequency of nucleus tractus solitarius neurons is expressed on the ordinate as percentage of control activity. Muscimol = muscimol 30 μM; VA = valerenic acid 100 μM. *$P < 0.05$ compared with muscimol alone.

name given to the herbal medication derived from the root of the *Valeriana* genus of plants, which are pink-flowered perennials native to the temperate areas of the Americas, Europe, and Asia (3,24). Different species of *Valeriana* plants are used in various parts of the world—*Valeriana officinalis* in northern Europe, *V. angstifolia* in China and Japan, and *V. wallichii* in India. In the US, *V. officinalis* L. is the most commonly used species, and we tested its effects in this investigation.

In this study, we demonstrated that treatment with valerian extract or valerenic acid caused an inhibitory effect on muscimol-sensitive NTS neurons in an *in vitro* brainstem preparation. We also observed that the inhibitory activity of both valerian extract and valerenic acid was induced via $GABA_A$, but not $GABA_B$, receptors. Previous studies showed the binding of valerian extract to $GABA_A$ receptors in rat cortical membrane preparation (12). We observed the neuropharmacological effect of $GABA_A$ activity in our experiment. The GABA agonistic activity of valerian and its positive modulation of $GABA_A$ receptors could partly explain valerian's antianxiety and sedative effects.

The dose-dependent inhibition of discharge frequency in muscimol-sensitive neurons by valerian extract suggests a $GABA_A$ agonistic activity. This activity could be mediated either by direct receptor action or by increasing the availability of GABA. It has been shown that valerian extract, aqueous or hydroalcoholic, contained GABA and other amino acids that could displace labeled muscimol (12), suggesting that specific constituents of valerian extract can directly bind to $GABA_A$ receptors. The GABA content of valerian extract could also be responsible for the stimulated release and reuptake of GABA. This could be an indirect mechanism of GABA agonistic activity of valerian extract (25,26). Additionally, derivatives of valerenic acid inhibit the local catabolism of GABA by inhibition of the enzyme GABAse, which could also increase GABA concentration (27). These mechanisms might have been operational in our *in vitro* brainstem model, but in *in vivo* models, the role of exogenous GABA in producing central nervous system (CNS) sedative effects is questionable because of the very low permeability of GABA across the blood-brain barrier (12). The significance of the inhibition of GABA catabolism by valerenic acid derivatives in *in vivo* models is not yet known.

Our study also tested the effect of valerenic acid, a constituent found only in *V. officianalis* L., on muscimol-sensitive neurons in the NTS. Earlier animal experiments showed that valerenic acid caused CNS depressant actions similar to diazepam (28), suggesting that its action could be mediated through GABA receptors. Clinical studies confirmed the sedative effects of valerenic acid and its derivatives (29). The anticonvulsant property of valerenic acid also implied a GABAergic mechanism of action (30), but it was suggested that valerenic acid did not act via direct GABA receptor binding, because valerenic acid, even at a millimolar concentration, was unable to displace labeled muscimol from $GABA_A$ receptors in a rat cortical membrane preparation (12). If valerenic acid did not bind the receptors, it could have mediated this effect by indirect mechanisms, such as inhibition of GABA catabolism to increase GABA concentration (27). Data from this study showed that 100 μM valerenic acid reduced the discharge rate in muscimol-sensitive neurons. However, we also observed that the pharmacological action of 3 mg/mL of valerian extract (contains approximately 40 μM valerenic acid) was higher than that of 100 μM valerenic acid alone, suggesting that other components in the extract could act additively or synergistically to produce GABA-agonistic effects. It appears that valerenic acid is not the only potent component of the valerian extract.

Although valerian is effective in producing depression of the CNS, the primary active ingredient of valerian remains elusive. It is believed that a combination of valerenic acid, valepotriates, and unidentified aqueous constituents may contribute to the sedative properties of valerian (31). The identified and unidentified other constituents in the extract that cause sedative effects through GABAergic mechanisms could be responsible for the effect of valerian extract in our model. Valepotriates, with at least 37 types isolated (3), may contribute to the sedative effects by interacting with the allosteric sites of GABA receptors (11). We, however, did not test valepotriates because of the complexity caused by a variety of

compounds and the unstable nature of the compound (3,29). A recently identified flavonoid, 6-methylapigenin, isolated from *V. wallichii*, interacts with the benzodiazepine-binding site on the $GABA_A$ receptor (32) and could also contribute to the GABA-agonistic activity of valerian extract.

Compared with muscimol, valerian extract and valerenic acid showed less inhibitory efficacy in our experiments, suggesting that they are weaker $GABA_A$ agonists. The IC_{50} of valerian extract was 240 μg/mL, and this concentration could be within the effective clinical range. An interesting observation in our study is that, rather than producing additive or synergistic effects, pretreatment with valerian extract or valerenic acid decreased $GABA_A$ agonist-induced NTS inhibitory effects, indicating that these compounds may play an important role in regulation of GABAergic activity. The inability of muscimol to exert the same degree of inhibition in preparations after pretreatment with valerian extract or valerenic acid suggests that valerian compounds might act as mixed agonist-antagonists of $GABA_A$ receptors. Thus, it is possible that valerian compounds may have both agonistic actions and weak GABA antagonist activity, which could hinder the receptor binding of the agonist. This property could reduce the sedative effects of valerian. However, there is also evidence that constituents of valerian extract act on other receptor systems involved in sleep regulation, such as adenosine and 5-hydroxytryptamine-1 (29,33).

Evidence of valerian's sedative effect in humans is ample (7–10,34). However, the key active constituent responsible for the sedative effect in *in vivo* models is not yet identified and will require further study, particularly since the clinical effect of valerian is obvious only after two weeks of administration (35). Treatment with valerian for a short duration or with a single dose failed to demonstrate significant sedative effects (35,36). Thus, it appears that the active constituents of valerian are susceptible to catabolism or require multiple dosing before a steady-state can be achieved. Although their effects are thought to be short-lived (PJ Houghton, personal communication, 2003), the pharmacokinetics of valerian's constituents have not been studied. Isolation of active constituents from the valerian extract with evidence of pharmacological activity *in vitro* could be used as markers for pharmacokinetic and pharmacodynamic studies.

Our data suggest that the pharmacological effects of valerian extract and valerenic acid are mediated through GABAergic activity. This is also supported by a case report in which valerian withdrawal mimicked an acute benzodiazepine withdrawal syndrome. As a result, the patient was successfully treated with benzodiazepine administration (37). Conversely, valerian should be expected to potentiate the sedative effects of anesthetics and adjuvants, such as benzodiazepines and barbiturates, which act at the GABA receptor. Thus, presurgical valerian use may cause a valerian-anesthetic interaction in surgical patients (1).

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