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Research Paper

Relaxing effects of *Valeriana officinalis* extracts on isolated human non-pregnant uterine muscle

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Abstract

Objectives This study investigated the relaxing effects of *Valeriana officinalis* L. (Valerianaceae) on human uterine muscle. The major uses of this species in Europe are as a sedative and an anxiolytic; it is also used as a spasmolytic to treat gastrointestinal spasm.
Methods We evaluated two valerian extracts (ethanolic and aqueous) in comparison with a natural mixture of valepotriates and nifedipine on spontaneous and agonist-induced contractions in non-pregnant human myometrium *in vitro*. Qualitative and quantitative chemical analysis was used to correlate the chemical composition of extracts with their spasmolytic effects. Myometrial strips were obtained from hysterectomy specimens of premenopausal women. Longitudinal muscle strips were mounted vertically in tissue baths under physiological conditions to record their isometric contraction. The responses of cumulative concentrations of valerian extracts on spontaneous contractions in the presence and absence of the β-adrenoceptor blocker atenolol or the cyclooxygenase inhibitor indometacin, and on agonist-induced contractions, were investigated.
Key findings Valerian extracts and valepotriates inhibited uterine contractility in a concentration-dependent manner. Pretreatment with either atenolol or indometacin did not affect the uterine responses to valerian extracts. Valerian extract reduced the maximal contractile response induced by acetylcholine, phenylephrine and histamine independent of the stimulus.
Conclusions Valerian extracts may have direct inhibitory effects on the contractility of the human uterus and this justifies the traditional use of this plant in the treatment of uterine cramping associated with dysmenorrhoea.
Keywords calcium antagonists; isolated human myometrium; spasmolytic activity; uterus contractility; *Valeriana officinalis*; valepotriates

Introduction

The underground organs (roots and rhizomes) of the genus *Valeriana* (Valerianaceae), commonly referred to as valerian, are used in the traditional medicine of many cultures as rudimentary drugs. *V. officinalis* L. is the official species used in Europe. The major use is as a sedative and an anxiolytic and it is also used as a spasmolytic to treat gastrointestinal spasm.[1] A variety of preparations from this plant for oral administration are also used traditionally for the treatment of menstrual cramps, hypertension, angina, palpitations, bronchial asthma and hepatic colic.[2–4] However, some of these traditional uses have not been subject to detailed scientific study. Despite this, the use of valerian extracts for self-medication has increased dramatically.

It is known from the literature that the roots and rhizomes of *V. officinalis* contain two main groups of constituents: sesquiterpenes of the volatile oil (valerenic acid and its derivatives, valeranone, valeranal, kessyl esters) and valepotriates (valtrate, didrovaltrate, acevaltrate, isovaleroxyhydroxyvaltrate), in addition to other constituents such as flavonoids, triterpenes, lignans and alkaloids.[5] The valepotriates themselves act as prodrugs that are transformed into homobaldrinal, which has been shown to have spasmolytic activity.[6–8] The antispasmodic effect of the valepotriates was first demonstrated by Wagner & Jurgic in 1979[9] for valtrate and didrovaltrate. Further investigations revealed that valtrate and didrovaltrate probably act as musculotropic agents. Their action may be due to an influence on the influx of Ca^{2+} ions or their binding to the muscle.

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Mexican valerian *V. edulis* subsp. *Procera*, which has large roots containing as much as 8% valepotriates, is used as the source of a standardised mixture called Valmane which consists of 15% valtrate, 80% didrovaltrate and 5% acevaltrate. This mixture has been used for a large number of pharmacological studies.[10]

Previous studies have investigated the spasmolytic effects induced by aqueous and ethanolic extracts of valerian on guinea-pig vascular and bronchial smooth muscle, in order to validate some its traditional uses.[11] The results of these studies indicated that *V. officinalis* exerts important relaxing effects on the coronary and bronchial smooth muscle. The aim of the present study was to investigate whether the aqueous and ethanolic extracts of *V. officinalis* roots and the standardised mixture of valepotriates exert the same relaxing effects on human myometrial muscle. To our knowledge, our study shows for the first time the inhibitory effects of valerian on isolated human myometrium.

Materials and Methods

Plant material

Authenticated roots of V. *officinalis* L. were obtained from Aboca, Sansepolcro, (AR), Italy. A voucher specimen (DP 3221/08) is deposited in the Pharmaco-Biological Department, University of Messina, Italy.

A natural mixture of valepotriates (15% valtrate, 80% didrovaltrate and 5% acevaltrate) was supplied by Mag. Dr Till Strallhofer, Vienna-Pharmaselect Handels GmbH (Austria).

Preparation of extracts and analytical characterisation

The dried and powdered roots (100 g) were extracted in a Soxhlet extractor with 70% ethanol (600 ml) for 4 h. The extract was evaporated to dry state under reduced pressure at low temperature in a rotary evaporator. The residue yield obtained was 15%.

For preparation of the aqueous extract, the dried and powdered roots (100 g) were boiled in water (1000 ml) for 30 min. The decoction was lyophilised. The lyophilised samples were kept in sealed bottles under vacuum. The yield (dry weight) was 17.5%.

In order to prepare the test solutions, the residues were suspended in distilled water at the desired concentrations, immediately before use.

The two valerian extracts were characterised by qualitative and quantitative chromatographic analysis. An aliquot (10 g) of ethanol and aqueous residue was extracted at room temperature with dichloromethane (3 × 100 ml, 30 min each) in an ultrasonic bath. The combined extracts were filtered and evaporated to dry state and the residues dissolved in methanol (5 mg/ml) for chromatographic analysis.

Thin-layer chromatography (TLC) was mainly used for qualitative analysis of valerian extracts; quantitative analysis was by HPLC. Silica gel 60 F_{254}-precoated TLC plates (Merck, Germany) were used. TLC analysis for valepotriates was performed according to the method of Stahl and Schild.[12,13] TLC analysis for valerenic acid and its derivatives was performed using the method of Wagner & Bladt.[14] Hexane/methyl ethyl ketone (8 : 2) or hexane/ethyl acetate/glacial acetic acid (65 : 35 : 05) were used as the mobile phases. Valepotriates were detected with 2,4-dinitrophenylhydrazine (DNPH); valerenic acid and hydroxyvalerenic acid were detected with anisaldehyde-sulfuric acid.

HPLC analysis was performed using the method of Bos *et al.*[15] Valerenic acid, hydroxyvalerenic acid, valtrate, acevaltrate, didrovaltrate and isovaleroxyhydroxydidrovaltrate (IVHD valtrate) were measured. Column: Apex (Jones Chromatography, Hengoed, UK) ODS (C_{18}, 5 μm, 4.6 mm i.d. × 250 mm) Pump: Shimadzu (Columbia, MD, US) LC-10AT. Detector: Shimadzu SPD-10A, 220-221 nm (for detection of valerenic acid and hydroxyvalerenic acid), 203 nm and 255 nm (for detection of valepotriates). The injected volume was 10 μl. The eluent was methanol/water (0.5% H_3PO_4, pH 2) 80 : 20. Gradient elution was used for the determination of diene valepotriates: (A) 20 : 80 and (B) 80 : 20; initially 85% B, finally 100% B for 5 min. The flow rate was 1.5 ml/min.

The reference compounds valtrate, acevaltrate, didrovaltrate and IVHD valtrate were isolated in our laboratory using the method of Fuzzati *et al.*[16] Valerenic acid and hydroxyvalerenic acid were obtained from the Laboratoires Labservice Analitica s.r.l. (Anzola Emilia, Bologna, Italy).

The identities of the isolated valepotriates was confirmed by infrared spectroscopy, NMR and mass spectrometry and were consistent with literature data.[17,18] The purity of the valepotriates was confirmed by TLC and analytical HPLC.

Tissue collection

This study was approved by the Bioethical Committee of the Italian National Health Institute (protocol number 335/07). Samples of human uterus were obtained from hysterectomy specimens from premenopausal women without evidence of malignant uterine disease. The samples, supplied by the Department of Obstetrics and Gynaecology of the University of Messina, were immediately placed in Krebs' solution, pH 7.4 at 4°C and used within 3 h of collection.

Organ bath experiments

Longitudinal myometrial strips measuring approximately 15 × 4 × 2 mm were dissected. The strips were mounted for isometric recording under 1 g tension in an organ bath (Schuler organ bath, type 809-Hugo Sachs Elektronik, Harvard Apparatus GmbH; March-Hugstetten, Germany). Each strip was placed in a 20 ml jacketed tissue bath containing Krebs' solution at 37°C and pH 7.4. The composition of the Krebs' solution was (in mmol/l): sodium chloride 121, potassium chloride 4.5, sodium bicarbonate 15.5, sodium phosphate 1.2, calcium chloride 2.5, magnesium chloride 1.2, glucose 11.5, and was gassed continuously with 95% O_2/5% CO_2. A silk thread was used to attach the myometrial strip to a fixed hook and an isometric force transducer (HSE F30, Type 372- Hugo Sachs Elektronik, Harvard Apparatus). The strips were allowed to equilibrate for 90 min, during which time the Krebs' solution was changed every 15 min. Mechanical activity of the tissues (tonus, amplitude and frequency of contractions) was measured and data were recorded and stored digitally using HSE-ACAD W data acquisition software (Hugo Sachs Elektronik, Harvard

Apparatus), and displayed on a computer screen. Most of the strips developed spontaneous contractions within 40–90 min; strips with no spontaneous activity in this period were discarded. After development of regular phasic contractions, aqueous and ethanolic extracts of valerian, valepotriates mixture (valtrate, didrovaltrate, acevaltrate) and nifedipine were added in a cumulative manner at concentrations of 10–80, 0.5–4 and 0.01–1 μg/ml, respectively.

Experiments were further performed to explore possible mechanisms by which plant extracts alter uterine contractility. To examine whether β-adrenoceptors or prostaglandins are involved in the uterine response to extracts, concentration–response curves were obtained in the presence of a β-adrenoceptor blocker (atenolol 2 μg/ml) or a cyclooxygenase inhibitor (indometacin 3 μg/ml). Muscle strips were treated at least 25 min before applying the extracts.

In a different set of experiments, after the equilibration period and once regular contractions had developed, the spontaneous contractile activity of the uterus preparation was inhibited (quiescent preparation) by reducing the temperature of the organ bath to 30°C and modifying the physiological salt solution by reducing the calcium content to one-tenth of the level in normal Krebs' solution. After inhibition of spontaneous motility, the effects of the plant extracts, valepotriates mixture and nifedipine on maximal contractions induced by acetylcholine (1 μg/ml), adrenaline (epinephrine) (1 μg/ml) and histamine (1 μg/ml) were investigated.

Different strips from the same sample of uterus were used to test different extracts. Each treatment was tested on five strips. This number of replicates allowed us to generate a mean and SEM from each experiment and to construct the concentration–response curves. Different experiments were performed on each sample of uterus.

Statistical analysis

Results are expressed as mean ± SEM of five treatments. Differences in muscular tension were tested for significance using one-way analysis of variance followed by Dunnett's test; $P < 0.05$ was considered significant.

Results

Qualitative analysis (Table 1) under UV light (254 nm) revealed the largest zone at R_f 0.5–0.6 (equal to anisaldehyde) due to valtrate. After reaction with DNPH, a series of coloured zones were seen against a yellow background. Valtrate became blue and anisaldehyde yellow. In the lower part of the chromatogram a blue zone was found at R_f 0.2–0.3 (analogous to vanillin, yellow) due to IVHD valtrate. Two smaller and less intensely coloured zones are visible between this blue zone and the valtrate zone due to didrovaltrate (faint orange) and acevaltrate (blue).

Valerenic acids showed weak quenching at UV 254 nm. Treatment with anisaldeyde-sulfuric acid reagent revealed valerenic acids as violet zones. The largest zone was found at R_f 0.5–0.6 due to valerenic acid; in the lower part of the chromatogram a violet zone at R_f 0.2–0.3 was due to hydroxyvalerenic acid.

Quantitative analysis (Table 2) demonstrated that both ethanolic and aqueous extracts contained a reasonable amount of valerenic and hydroxyvalerenic acids: 3.4 mg/g and 1.8 mg/g, respectively in the ethanolic extract; 1.2 mg/g and 1.5 mg/g, respectively, in the aqueous extract. In contrast, the analysis confirmed a higher content of the valepotriates valtrate and IVHD valtrate in the ethanolic extract: 6.8 mg/g and 6.0 mg/g, respectively; and 3.1 mg/g and 1.1 mg/g for didrovaltrate and acevaltrate, respectively, compared with the aqueous extract (1.9 mg/g for valtrate).

Effects on spontaneous uterine contractility

In preliminary experiments, we found that uterine contractility did not significantly change with time when the strips were mounted for isometric recording under 1 g tension in the organ bath. The contractions lasted without fading for several hours in the control condition, but were progressively attenuated during application of increasing concentrations of nifedipine, used as positive control. Repeated concentration–response curves for nifedipine were statistically identical.

Valerian extracts (20–80 μg/ml) and the valepotriates mixture (1–4 μg/ml) both caused concentration-dependent decreases in uterine contractility; the aqueous extract of valerian had minor effects. The uterorelaxant effect observed seem to influence mainly the amplitude of contractions; frequency was only slightly reduced. The concentration causing 50% inhibition of the contractile amplitude was 29.5 ± 3.40 and 68.7 ± 5.20 μg/ml for the ethanolic and aqueous extracts, respectively, and 1.67 ± 0.45 μg/ml for the valepotriates. Nifedipine (0.01–1.0 μg/ml) had a concentration-dependent

Table 1 Thin layer chromatography data of valepotriates and valerenic acids in aqueous and ethanolic extracts of valerian

	R_f (10 cm)	Ethanolic extract			Aqueous extract		
		254 nm	DNPH	ASA	254 nm	DNPH	ASA
Valepotriates							
Valtrate	0.60	+	Dark-blue	ND	+	Light-blue	ND
Didrovaltrate	0.51	+	Faint-orange	ND	–	ND	ND
Acevaltrate	0.48	+	Blue	ND	–	ND	ND
IVHD valtrate	0.30	+	Blue	ND	–	ND	ND
Valerenic acids							
Valerenic acid	0.53	+	ND	Violet	+	ND	Violet
Hydroxyvalerenic acid	0.22	+	ND	Violet	+	ND	Violet

ASA, anisaldehyde-sulfuric acid; DNPH, 2,4-dinitrophenylhydrazine; IVHD, isovaleroxyhydroxydidrovaltrate; ND, not detected.

Table 2 HPLC analysis of valepotriates and valerenic acids from *V. officinalis* extracts

	Ethanolic extract	Aqueous extract
Valepotriates		
Valtrate	6.8 ± 0.49	1.9 ± 0.12
Didrovaltrate	3.1 ± 0.35	Not measureable
Acevaltrate	1.1 ± 0.11	Not measureable
IVHD valtrate	6.0 ± 0.52	Not present
Valerenic acids		
Valerenic acid	3.4 ± 0.32	1.2 ± 0.10
Hydroxyvalerenic acid	1.8 ± 0.10	1.5 ± 0.12

Values are contents (mg/g dry weight), means ± SD from three measurements.
IVHD, isovaleroxyhydroxydidrovaltrate.

inhibitory effect on spontaneous uterine activity, decreasing both amplitude and frequency of myometrial contractions, and reducing basal tone (Table 3).

Atenolol (2 μg/ml) and indometacin (3 μg/ml) did not affect the basal contractility, nor did they alter the uterine responses to valerian extracts or valepotriates (data not shown).

Effects on agonist-induced maximal contraction in quiescent uterine preparations

Quiescent myometrial strips were precontracted by application of acetylcholine (1 μg/ml), epinephrine (1 μg/ml) or histamine (1 μg/ml), and 80 μg/ml valerian extracts or 4 μg/ml valepotriates were added to determine the ability of plant extracts and valepotriates to relax a contracted uterus. Results of these experiments (Figure 1) show that plant extracts of valerian and valepotriates partially reversed the contracture of the precontracted uterine muscle independently of the stimulant. The inhibition of the maximal amplitude of agonist-induced contractions were 38–49% for the ethanolic extract. When valepotriates were applied to myometrium contracted with agonists, inhibition of the maximal amplitude of contraction was 31–42%. In the same experiments, the administration of 1 μg/ml nifedipine decreased the maximal amplitude of contraction induced by agonists by 61 ± 4.6%.

Discussion

Phytochemical analysis showed that the ethanolic and aqueous extracts were similar in composition with regard to valerenic acids. Concentrations of valepotriates were much lower in the aqueous extract than in the ethanolic extract.

This study using isolated human uterine muscle strips demonstrates that valerian extracts and the mixture of valepotriates inhibited spontaneous contractility in a concentration-dependent manner. It is known that β-adrenergic activity[19] and prostaglandins[20] modulate myometrial contractility in humans. Therefore, we evaluated whether they are responsible for the inhibitory effects of valerian extracts and valepotriates on uterine contractility. The uterine response to valerian extracts or valepotriates was not affected by atenolol or indometacin at concentrations that block the respective receptors or enzyme system in the isolated human uterus.[21]

Table 3 Inhibitory effects of ethanolic and aqueous extracts from *V. officinalis* roots, valepotriates and nifedipine on the basal tone, amplitude and frequency of spontaneous contractions in isolated human uterus strips

	Concn (μg/ml)	Basal tone (% inhibition)	Amplitude (% inhibition)	Frequency (% inhibition)
Ethanolic extract	10	2.0 ± 0.50	0	0
	20	18.5 ± 2.40*	40.0 ± 6.30*	5.0 ± 1.10
	40	22.3 ± 3.15*	78.5 ± 4.20*	10.7 ± 1.85
	80	27.0 ± 2.90*	80.0 ± 3.25*	16.0 ± 2.20*
Aqueous extract	10	0	0	0
	20	10.0 ± 1.80	18.5 ± 2.20*	3.5 ± 1.60
	40	13.5 ± 1.40*	29.0 ± 3.50*	8.7 ± 2.75
	80	17.0 ± 2.00*	52.5 ± 2.85*	9.5 ± 1.90
Valepotriates	0.5	0	0	0
	1	12.7 ± 1.70*	32.0 ± 2.60*	7.0 ± 2.40
	2	19.3 ± 2.80*	58.0 ± 3.00*	8.5 ± 2.20
	4	24.5 ± 2.75*	70.2 ± 3.40*	14.8 ± 2.50*
Nifedipine	0.01	7.2 ± 0.80	23.5 ± 1.45*	18.3 ± 1.60*
	0.1	15.0 ± 1.20*	45.7 ± 2.90*	47.0 ± 3.65*
	1	26.6 ± 1.70*	82.0 ± 3.25*	54.8 ± 4.10*

Values are means ± SEM (n = 5 per treatment). *P < 0.05 (F ratio = 3.32) vs baseline values.

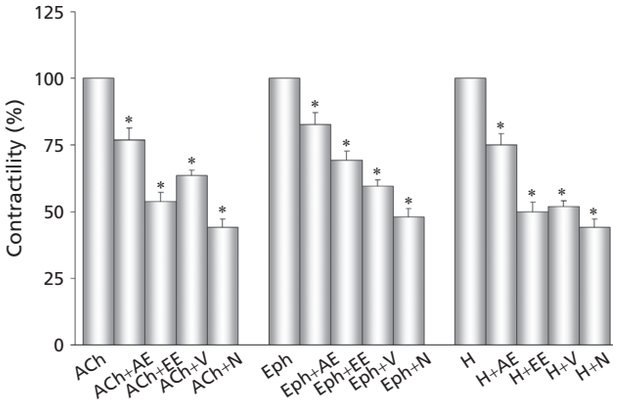

Figure 1 Effects of aqueous and ethanolic valerian extracts, valepotriate mixture and nifedipine on maximal contract response induced by acetylcholine, adrenaline (epinephrine) or histamine in quiescent uterine preparations. ACh, acetylcholine (1 μg/ml); AE, aqueous extract (80 μg/ml); EE, ethanolic extract (80 μg/ml); Eph, epinephrine (1 μg/ml); H, histamine (1 μg/ml); N, nifedipine (1 μg/ml); V, valepotriate mixture (4 μg/ml). Values are mean ± SEM (n = 5 per treatment). *P < 0.01 (F ratio = 6.35) vs agonist-treated preparations (considered to induce a maximal contractile response of 100%).

These findings suggest that the products generated by β-adrenergic nerve activity or cyclooxygenase do not play a key role in the uterine response to these plant extracts.

Spontaneous uterine contraction is closely dependent on the cytoplasmic concentration of free Ca^{2+} ions.[22] Drugs that reduce the intracellular availability of Ca^{2+} ions depress the contractile activity of isolated human uterine muscle.[21] The effects of valepotriates and valerenic acid on intracellular Ca^{2+} have been demonstrated in other tissues. Hazelhoff *et al.*[23] showed that valepotriates, valerenic acid and valeranone exert spasmolytic effects in guinea-pig ileum through direct effects on smooth muscle rather than an interaction with the autonomic nervous system. It is likely that the inhibitory effect of valerian extracts and valepotriates on spontaneous uterine contraction is also associated with their modulating effects on Ca^{2+} behaviour. This conclusion is strengthened by the findings that the relaxant effects of plant extracts observed on uterine smooth muscle did not appear to be mediated by β-adrenergic receptors or prostaglandins, as well as the finding that nifedipine produced effects similar to those of extracts in the experimental models examined. In addition, the data presented in this study demonstrate the ability of plant extracts to partially reverse the maximal contractile response of uterine tissues induced by acetylcholine, epinephrine or histamine. These data suggest that the effects of plant extracts are independent of the stimulant. It is known that, in order to induce uterine contractions, agents such as histamine, acetylcholine and epinephrine are thought to increase free intracellular Ca^{2+} (receptor-mediated) by increasing Ca^{2+} influx and/or by increasing Ca^{2+} release from cellular depots. In fact, the calcium-channel blocker nifedipine blocks acetylcholine-, epinephrine- and histamine-induced contractions in isolated human uterine strips. Thus, it is likely that the action of the extracts on pre-contracted uterus may be mediated through their ability to interfere with calcium overload, as this represents the common signal transduction pathway for all uterine contractive stimuli.

Conclusions

Our results demonstrate that the two extracts of *V. officinalis* roots possess significant uterolytic properties due to the structural features of the active principles they contain. Both extracts relaxed uterine smooth muscle, but the potency differed considerably, the relaxing effect of the ethanolic extract being superior to that of the aqueous extract. The different potencies of the ethanolic and aqueous extracts may be related to differences in the extraction procedure and therefore in their qualitative/quantitative chemical profiles. Although the spasmolytic effects of valerian extracts and valepotriates are known, this is the first report to examine their direct effect on human uterine contractility and this justifies the traditional use of this plant in the treatment of uterine cramping associated with dysmenorrhoea. Finally, these results indicate that the two extracts possess Ca^{2+}-antagonist activity. To characterise this possible Ca^{2+}-antagonist activity, further studies are in progress in Ca^{2+}-free conditions, investigating the inhibitory effect of the extracts and the various extracted compounds against contractions induced by externally administered Ca^{2+}.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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