Neuropharmacological Studies on Ethanol Extracts of *Valeriana officinalis* L.: Behavioural and Anticonvulsant Properties

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An ethanol total extract of the roots of *Valeriana officinalis* L. in doses equivalent to 0.5–800 mg valerian root/kg b.w.i.p. was tested in male mice for possible neuropharmacological efficacy and in this respect compared with diazepam and haloperidol. The extract did not modify spontaneous motility, nociception or body temperature, and did not produce palpebral ptosis. However, it was anticonvulsant against picrotoxin (but not pentetrazol and harman) with an ED_{50} between 4.5 and 6 mg/kg and it prolonged thiopental anaesthesia. After fractionation of the crude extract, the antipicrotoxin activity was present mainly in the methylene chloride fraction ($ED_{50} = 0.25$ mg/kg). Pure valerenic acid (12.5 mg/kg b.w.i.p.) also exerted an antipicrotoxin effect.

Keywords: Valeriana officinalis L.; neuropharmacology; anticonvulsant effect; picrotoxin; harman; diazepam.

INTRODUCTION

In many European countries common valerian, *Valeriana officinalis* L., is a popular herbal remedy considered to exert sedative effects. However, the evidence that valerian is sedative in man has been equivocal for many decades. In recent years, valerian root extracts have been shown in man to improve sleep quality (Leathwood *et al.*, 1982), to reduce latency to fall asleep (Leathwood and Chauffard, 1985) and to diminish feelings of somatic arousal during a social stress situation (Kohnen and Oswald, 1988, 1992).

Only a few papers on animal experimentation with valerian have appeared during the past 20 years. In mice, sedation has been observed after administration of a tincture (Torrent *et al.*, 1972), certain extract components (Petkov and Manolov, 1974) or essential oil (Hendricks *et al.*, 1981) of *Valeriana officinalis* L; furthermore, anticonvulsant effects were found by Petkov and Manolov (1974). Ammelounx *et al.* (1978) and Holm *et al.* (1980) evaluated the EEG and evoked potentials in cats but observed thymoleptic-like rather than sedative or tranquillizing effects of a valerian extract and a mixture of three valepotriates.

By means of the [^{14}C]deoxyglucose method central effects of valerian in rats have been postulated. Certain extracts of *Valeriana officinalis* L. (but not valepotriates, valerenic acid, valeranone or the essential oil) diminished the local glucose utilization in many, albeit not all, brain parts (Grusla *et al.*, 1986; Krieglstein and Grusla, 1988). Recently, an aqueous alkaline dried extract was shown to decrease motility and to increase barbiturate anaesthesia in female mice (Leuschner *et al.*, 1993); from this finding the authors claimed sedative properties of the particular extract.

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The aim of the present study was to find out whether neuropharmacological effects in mice would be exerted by fresh valerian root extracts and some of its isolated fractions.

MATERIAL AND METHODS

Plant material and extraction methods. For all experiments valerian root from *Valeriana officinalis* L. from one commercial batch was used. Dried and chopped valerian root was extracted with 70% (v/v) ethanol (1 part drug to 5 parts solvent) in accordance with the German Pharmacopoeia (DAB 10) method for preparing tinctures. The ethanol total dry extract was obtained from the tincture in a way which prevented loss of essential oil. The resulting extract, containing no valepotriates, was then extracted successively with three different solvents to obtain three fractions: an aqueous fraction (FA), a methylene chloride fraction (FB) and a *n*-hexane fraction (FC). Each extraction was carried out for 3 h at room temperature with a dry extract–solvent ratio of 1:5 (w/w). The solutions were then filtered to remove insoluble material and concentrated to dryness using a rotary evaporator. Lactose was added to all test material in a ratio of 1:1.

Animals. Male mice (20–30 g) of the NMRI-strain were used (each animal only once). They had free access to dry food pellets and tap water and were kept in groups of 15 at an ambient temperature of 23 °C with a 12 h dark–light cycle. The experiments were performed at 22°–23 °C. When there was a waiting period between treatment and testing, the animal was put back in its home cage for this time.

Neuropharmacological testing. The following tests have been used previously to detect central effects of natural products such as aporphine alkaloids (Zetler, 1988).

Basic screening. Four parameters were observed in each animal, i.e. exploratory rearing, palpebral ptosis, threshold of nociception on a hot plate and body temperature. 25 min after pretreatment, an individual mouse was put on the filter paper-covered floor of a glass cylinder (16 cm in height, 11 cm in diameter); the number of spontaneous rearings occurring during the first 5 min was counted. At the end of this observation period the degree of ptosis was determined and scored as follows: score 0, eyes wide open; score 2, eyes half closed; score 4, eyes completely closed; scores 1 and 3 were assigned to intermediate openings (Rubin et al., 1957). Ptosis is a sign of central depression (in particular by reserpine and neuroleptics) and never shown by naive mice under the stated experimental conditions, i.e. after placement in an unfamiliar environment. The mouse was then placed (only once) on a heated (53 °C) copper plate and the latency for jumping upwards was measured; opioids and other compounds with antinociceptive activity prolong this latency. Finally, the body temperature was taken by means of an electric thermometer with the thermocouple inserted approximately 18 mm into the rectum for at least 5 s.

Locomotor activity. 25 min after the pretreatment (i.p.) the mice were placed individually on an illuminated glass disk (diameter 24 cm) with 20 photocells mounted under it. The number of crossings of a photocell was counted automatically for 5 min and the number of signals used to estimate the locomotion.

Anticonvulsant activity. The convulsants used were picrotoxin (from the seeds of Anamirta cocculus), harman (from wood and leaves of Banisteriopsis caapi) and pentetrazol (an analeptic drug). These compounds were applied 30 min after pretreatment and the animals observed for the next half-hour. Picrotoxin was given i.v. at a dose of 3–4 mg/kg, depending on the somewhat varying sensitivity of the animals as estimated with each new series of tests. Clonic-tonic convulsions occurred approximately 13 min after the injection and could be followed by death a little later. Initially, these convulsions were evaluated for the assessment of crude extracts. Later on, with more purified fractions, the number of picrotoxin-induced deaths was found to be a more dependable criterion. Presence or absence of convulsions, or death, were used as determinants of an anticonvulsant effect. The i.v. dose of harman (15 mg/kg) led after 17 s to clonic convulsions lasting 5–10 s; deaths occurred only occasionally. Pentetrazol was subcutaneously (s.c.) administered in a dose of 105 mg/kg and produced first fits after 1–2 min then clonic-tonic convulsions after 7–8 min; 80% of the animals died after 13–16 min.

Barbiturate potentiation. 25 min after pretreatment the locomotor activity (see above) of each mouse was tested for 5 min. Immediately thereafter, thiopental at a dose of 25 mg/kg was injected intravenously (i.v.) within 10 s. The time from the end of this injection until spontaneous righting was used as a measure for the duration of anaesthesia. To calculate the ED_{50} of a barbiturate-potentiating drug, a mouse was considered to show an increased effect of thiopental if the duration of its anaesthesia was at least 157 s, i.e. at least twice the mean normal duration.

Solutions, drug and dosage. Lactose and the crude extract of valerian were dispersed in an aqueous solution of 1% tylose by rubbing in a mortar and injected intraperitoneally (i.p.). In experiments with the fractions of valerian extract the materials were taken up in 0.9% sodium chloride solution (normal saline) containing 1 µL Tween 80 per mL and sonicated for 5 s. The other drugs were dissolved in normal saline. All injections had a volume of 10 mL/kg b.w. Doses refer to dry material (exluding lactose) in the case of extract and extract fractions, but in the case of other substances to the pure compounds or their salts respectively. Drugs used besides extracts of valerian were: diazepam (Hoffmann-La Roche), thiopental sodium (Byk Gulden), picrotoxin (British Pharmacopoeia 1948, Merck; Sigma), harman hydrochloride (Fluka), pentetrazol (Serva), and haloperidol (Janssen).

Statistics. Unless otherwise stated, arithmetic means ± SEM are presented. Two means were compared by the t-test. However, when (according to the preceding F-test) the variances were non-homogeneous, we used the Wilcoxon rank sum test. Multiple comparisons with one control were made with the one-way classification of Wilcoxon and Wilcox (1964). Intergroup frequencies (percentages) were compared with the aid of fourfold contingency tables, i.e. the detailed Geigy Tables (1968) for $n = 4$–60. ED_{50} values (the doses that could be expected to produce a definite effect in 50% of the animals) were calculated by the method of Litchfield and Wilcoxon (1949). A value of $p < 0.05$ was the condition for accepting statistical significance. However, the Bonferroni method (cf. Wallenstein et al., 1980) was applied when there were multiple contrasts in one group of animals as with the 'basic screening' tests (accordingly, $p < 0.05/4 = < 0.0125$ was now the critical value). Testing was one-sided when changes could occur, or could reasonably be expected to occur, in only one direction as with ptosis, inhibition of convulsions and enhancement of anaesthesia.

RESULTS

Basic screening

Figures 1–3 show that the ethanol extract of valerian, in the doses used, did not alter rearing, nociception or body temperature. On the other hand, diazepam clearly reduced exploratory rearing, lowered the body temperature and produced a weak ptosis (not shown, since this effect was not seen after valerian); nociception on the hot plate remained nearly constant. There were virtually no differences in the effects of saline and the two doses of lactose. Hence, these trials did not reveal a benzodiazepine-like tranquillizing effect of valerian.
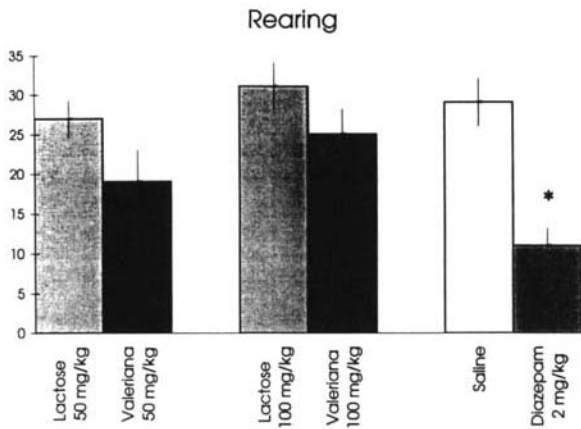
Figure 1. Results of the basic screening experiments. Rearing (number of rearings in 5 min), ten animals per treatment (i.p.). Statistical comparison of adjacent columns: * $p<0.001$.

Locomotor activity

The spontaneous running activity of the animals corresponded to 196 signals (Fig. 4) which is similar to results reported earlier (Zetler, 1984). The crude valerian extract remained ineffective, whereas diazepam enhanced the running.

Barbiturate potentiation

The valerian extract as well as diazepam prolonged thiopental-induced anaesthesia (Fig. 5). In the case of valerian, this effect occurred with doses that were (in the same animals) ineffective with respect to spontaneous motor behaviour, and in the case of diazepam with doses that increased locomotor activity. The two doses of diazepam prolonged the anaesthesia (see Methods) in 20% and 70% respectively of the animals with an ED_{50} of 0.54 (0.27–1.08) mg/kg (note that the anticonvulsant ED_{50} values were significantly smaller).

Anticonvulsant effects

As can be seen from Table 1, diazepam was very active against both picrotoxin and harman, whereas haloperidol antagonized only harman-induced convulsions. In
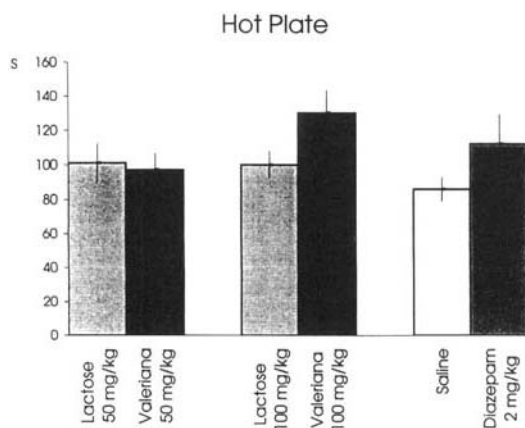

Figure 2. Results of the basic screening experiments. Hot plate (jumping latency in s), ten animals per treatment (i.p.). Statistical comparison of adjacent columns: no significant difference.
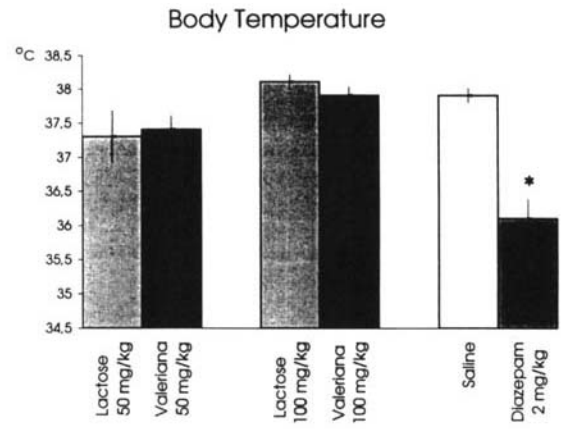

Figure 3. Results of the basic screening experiments. Body temperature, ten animals per treatment (i.p.). Statistical comparison of adjacent columns: * $p<0.001$.

contrast, the crude ethanol valerian extract was effective against picrotoxin but antagonized harman only at one dose, thus revealing in this case no clear-cut dose-response relationship; it even induced with the latter convulsant a normally absent post-convulsive state of excitation. This state lasted 15–20 min and comprised running, jumping, rolling, squeaking and motor automatisms; it was never seen in animals protected from convulsions. A very similar excitation also occurred when harman was combined with haloperidol.

The pentetrazol-induced convulsions were antagonized by diazepam with an ED_{50} of 0.13 (0.067–0.254) mg/kg. This dose is larger than the ED_{50} values against picrotoxin presented in Tables 1 and 2. Since only the difference from the value in Table 1 was statistically significant, it can be said that the anticonvulsant potency of diazepam was virtually the same against pentetrazol as against picrotoxin. Nevertheless, the crude ethanol valerian extract (the same as that used in the experiments of Tables 1 and 2), when given in doses of 100, 200 and 400 mg/kg, was completely ineffective against PTZ convulsions since not a single animal (out of 10 per dose) was protected.

Active fractions of the total extract

The antipicrotoxin effect was further used to test the fractions of the crude ethanol total extract of valerian (Table 2). These experiments were performed 6 months after those of Table 1 and yielded for both the
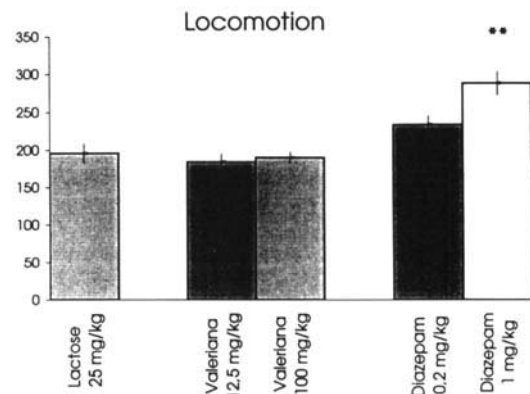

Figure 4. Locomotor activity (number of signals per 5 min). Ten mice per treatment (i.p. 25 min before testing). Statistical comparison with the lactose treated control group: ** $p<0.01$.
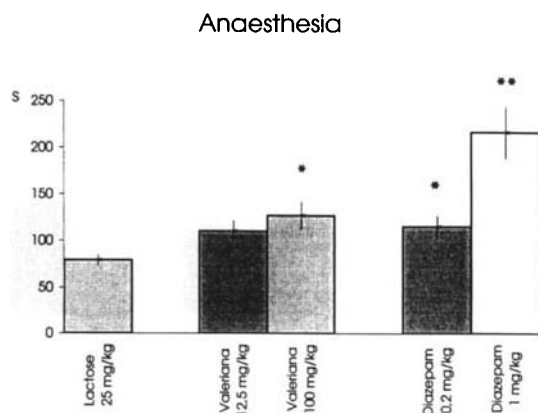
Figure 5. Duration (s) of thiopental-induced anaesthesia tested in the same mice as in the locomotor activity experiment (Fig. 4). Treatments given i.p. 25 min before motility testing; 5 min after this testing, i.v. injection of thiopental 25 mg/kg. Statistical comparison with the lactose treated control group: * $p<0.05$; ** $p<0.01$.

total extract and diazepam not exactly the same activities as before. However, the differences between the corresponding ED_{50} values in Tables 1 and 2 (6 and 4.5 mg/kg) are not statistically significant; this is important because the picrotoxin dose was now 3.5 instead of 3 mg/kg and the criterion was now death instead of convulsion. The results obtained with the aqueous fraction (FA) did not show a dependable dose-response relationship, which prevented calculation of an ED_{50}. This does not imply complete pharmacological inactivity of this fraction, since a frequency of 5 or 6 protected animals (as with the doses of 5, 20, and 40 mg/kg) makes a statistically significant difference from the control with 10 dead mice out of 10 tested. The results on the methylene chloride fraction (FB) allowed in the small-dose range the calculation of a

rather low ED_{50} of 0.25 mg/kg (note that 2.5 mg/kg protected 90% of the animals). From the n-hexane fraction (FC) we achieved a clear-cut dose-response line with an ED_{50} of 2.5 mg/kg which was, however, not significantly different from that for the total extract (the 95%-confidence ranges are widely overlapping).

We also tested the essential oil of valerian in doses of 5, 10, 20 and 40 mg/kg but detected no antipicrotoxin effect. In contrast, valerenic acid (12.5 and 25 but not 6.25 mg/kg) antagonized picrotoxin, whereas acetoxy-valerenic acid up to 25 mg/kg was ineffective in this respect.

DISCUSSION

In the present experiments valerian did not produce overt sedation or tranquillization, since we observed neither ptosis nor inhibition of rearing or locomotion. This is in agreement with corresponding negative findings of others: extracts of valerian neither lessened the motor activity of rats (Hauschild, 1958; Grusla et al., 1986) nor inhibited the EEG arousal reaction of cats (Holm et al., 1980). Accordingly, the barbiturate potentiating effect seen by us and also by Rosecrans et al. (1961) and Petkov and Manolov (1974) cannot be a non-specific consequence of sedation. The barbiturate potentiation seen by Leuschner (1993) occurred at lower doses of an aqueous valerian extract compared with a reduction of spontaneous motility with this particular extract. We do not believe that valerian enhanced the barbiturate effect by slowing the metabolism of thiopental. It is well known that thiopental owes its short-lasting anaesthetic effect to the immediate redistribution from brain into musculature rather than to rapid metabolic destruction, especially following i.v.

Table 1. Interaction of a crude ethanol valerian extract with convulsants, compared with the effects of diazepam and haloperidol

Treatment mg/kg[a] i.p.	Protected/ tested	ED_{50} mg/kg	Treatment mg/kg[a] i.p.	Protected/ tested	ED_{50} mg/kg
Convulsant:	picrotoxin 3 mg/kg i.v.		Convulsant:	harman 15 mg/kg i.v.	
Crude ethanol valerian extract (CEE)			Crude ethanol valerian extract (CEE)		
2	2/8		0.125	1/10 (5)[b]	
10	5/8		0.5	5/10 (4)	
50	7/8	6	1	2/10 (7)	
100	7/8	(2.0–18.0)[c]	2.5	2/10 (4)	
200	7/8		100	3/10 (7)	
Diazepam			Diazepam		
0.01	2/8		0.03	3/10	
0.05	5/8	0.03	0.1	5/10	0.08
0.2	7/8	(0.012–0.075)[c]	0.3	8/10	(0.04–0.16)[c]
0.5	8/8		1	10/10	
Haloperidol[d]			Haloperidol		
			1	2/10 (3)[a]	
			2	4/10 (6)	2.8
			4	6/10 (4)	(1.58–4.69)[c]
			8	8/10 (2)	

[a] 1 g CEE equivalent to 4 g valerian root.
[b] Number of mice showing a post-convulsive state of excitation (see text).
[c] 95% confidence range.
[d] Not tested as known to be ineffective against picrotoxin (Zetler, 1988).

Table 2. Inhibition of picrotoxin-induced deaths by valerian extracts as compared with diazepam

Treatment[a]	mg/kg i.p.	Protected[b]	ED$_{50}$ mg/kg
Crude ethanol valerian	2.5	3	
extract (CEE)[c]	5	5	4.5
	10	8	(2.75-7.30)[d]
	20	8	
Aqueous fraction	1.5	4	
of CEE (FA)[e]	5	5	
	10	3	
	20	6	
	30	4	
	40	6	
Methylene chloride fraction	0.1	3	
of CEE (FB)[f]	0.25	4	
	0.5	7	0.25
	0.75	5	(0.11-0.57)[d]
	1	6	
	2.5	9	
	5	6	
n-Hexane fraction	1.25	3	2.5
of CEE (FC)[g]	2.5	5	(1.30-4.80)[d]
	5	7	
Diazepam	0.05	2	0.08
	0.1	7	(0.057-0.105)[d]

[a] Treatment given 30 min before picrotoxin 3.5 mg/kg i.v.
[b] Number of mice that survived for 25 min. Ten animals per dose (the death rate of control mice was 100%).
[c] 1 g CEE equivalent to 4.2 g valerian root.
[d] 95% confidence range.
[e] 1 g FA equivalent to 5 g valerian root.
[f] 1 g FB equivalent to 140 g valerian root.
[g] 1 g FC equivalent to 135 g valerian root.

administration. A delaying interference of valerian with the pharmacokinetics of thiopental is unknown and not likely. Instead, one may think of a positive interaction of valerian at the barbiturate binding site of the GABA$_A$-benzodiazepine receptor complex (see below).

The valerian-induced sedation described by other authors is difficult to explain. Leuschner's experiments (1993) used a completely different material, i.e. an aqueous alkaline dried extract, which might lead to different pharmacological properties. In the case of Torrent *et al.* (1972) it might be a symptom of a weak intoxication; Hauschild (1958) had already suggested that subtoxic doses of valerian extracts may exert a sedation-like state and Torrent *et al.* (1972) administered i.p. half the i.v. LD$_{50}$ of valerian tincture. Hendriks *et al.* (1981) observed that the essential oil of *Valeriana officinalis* in doses of 100 to 800 mg/kg i.p. elicited in mice several symptoms of sedation, whereas Krieglstein and Grusla (1988) found the essential oil (5 mg/kg i.p.) ineffective and Hauschild (1958) pointed out that by no means can it be the cause of a sedative effect of valerian. We did not find any antipicrotoxin effect with the essential oil either and conclude that this material does not produce any neuropharmacological effects.

On the other hand, valerenic acid (but not its acetoxy derivative) was active in our experiments in a dose of 12.5 and 25 mg/kg (i.e. a quantity usually contained in 5 and 10 g of extract). Hendriks *et al.* (1981, 1985) found this compound sedative and potentiating pentobarbital in doses of 100 mg/kg. Valerenic acid's inability to reduce the cerebral glucose utilization (Kriegl-

stein and Grusla, 1988) was possibly caused by insufficient dosage (1 and 10 mg/kg i.p.). One may conclude that valerenic acid contributes to the pharmacological efficacy of our valerian extracts; its rather amphiphilic nature would support our notion that the active principle is lipophilic or amphiphilic (this can be concluded from the great potency of the methylene chloride fraction (FB); see Table 2).

Antipicrotoxin efficacy is a remarkable feature that is by no means shared by all centrally depressant drugs; for example, haloperidol is devoid of it (Table 1). Only a few corresponding observations exist in the literature: valerian antagonized the cocaine-induced convulsions of rats (Eichholtz and Krauth, 1937) and the convulsions caused in mice by pentetrazol and strychnine (Pfeifer and Zechner, 1953; Petkov and Manolov, 1974). The inhibition of picrotoxin by valerian in our study is specific, since it was not paralleled by an antagonism of harman and pentetrazol. The absence of a clear-cut antiharman effect of valerian suggests a missing interaction at the benzodiazepine receptor to which β-carbolines such as harman and β-carboline-3-carboxylate bind (Braestrup and Nielsen, 1980; Rommelspacher *et al.*, 1981). The valerian-induced excitation following harman convulsions cannot be explained; similar side effects occurred with haloperidol which, nonetheless, dose-dependently antagonized the main effects of harman (Table 1). The absence of antipentetrazol effects in our study and the weak activity of an alkaline dried extract (Leuschner *et al.*, 1993), in contrast to the studies where valerian preparations significantly antagonized the pentetrazol convulsions (Pfeifer and Zechner, 1953; Petkov and Manolov, 1974), point out the necessity of a detailed description of the method of preparation of the plant extracts. Whereas our valerian preparations were devoid of valepotriates (see Material and Methods section), Pfeifer and Zechner (1953) used alcoholates (1:1) from fresh valerian root known to contain valepotriates. Petkov and Manolov (1974) also observed pharmacological activity, namely anticonvulsant effects, against pentetrazol and strychnine in a fraction of valerian roots containing mainly valepotriates. Thus, it may be concluded that only valerian extracts with valepotriates show an antipentetrazol activity.

The anticonvulsant effect of valerian extracts based on ethanol extraction (free of valepotriates) differed from that of diazepam as it solely concerned picrotoxin; its mechanism may thus be located at the chloride channel of the GABA$_A$-benzodiazepine receptor complex. This would correspond to the barbiturate-enhancing effect, since barbiturates potentiate GABA by an interaction at the picrotoxin site of the GABA$_A$ receptor complex (Simmonds and Turner, 1987).

The lack of effect of valerian on rearing and locomotion is also in contrast to diazepam; the latter drug, in low non-sedative doses, reduces rearing and increases locomotion (Figs 1 and 4) as a consequence of lessened anxiety (Minck *et al.*, 1974; Thiébot *et al.*, 1976; Soubrié *et al.*, 1977; Simiand *et al.*, 1984; Rägo *et al.*, 1988). We may conclude that valerian does not exert the same type of antianxiety effect as diazepam.

Taken together, our results suggest that the observed effects of ethanol valerian extracts are caused by an unknown interaction with the GABA$_A$-benzodiazepine receptor complex; this interaction may differ from that

of diazepam. A similar conclusion was drawn by Krieglstein and Grusla (1988) from their experiments on the local cerebral glucose utilization, which reacted to valerian extracts in a way similar to the analogous trials of Cudennec *et al.* (1985) with the GABA agonist, progabide. In comparison with valerian, this GABA-mimetic antiepileptic drug was much less active against picrotoxin (ED_{50} in mice: 105 mg/kg i.p.), but antagonized many other types of convulsions such as those by pentetrazol (Worms *et al.*, 1982). Finally, it is worth mentioning that *Valeriana officinalis* was found to contain material binding to the central benzodiazepine receptor although being without other benzodiazepine-like properties (Medina *et al.*, 1989). The amount of GABA, around 0.4 mg/g, which is genuinely present in valerian root (Lapke *et al.*, 1993) is too small to account for significant effects on the brain, since only much higher doses are able to increase their brain GABA concentration (Frey and Löscher, 1980). Further studies are necessary to evaluate the significance of interaction of valerian as ethanol extract and pharmacologically active subfractions with the $GABA_A$/benzodiazepine receptor/Cl^- ionophore complex.

Acknowledgements

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