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Evaluation of the muscle relaxant properties of a novel β-carboline, ZK 93423 in rats and cats

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1 The muscle relaxant action of ZK 93423 (6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate ethyl ester), a novel β-carboline with agonistic properties at the benzodiazepine receptor, was examined by assessing its effect on the tonic electromyogram (EMG) activity of the gastrocnemius-soleus (GS) muscle of genetically spastic rats and on ventral root reflexes, presynaptic inhibition and fusimotor activity in the spinal cord of decerebrate cats.

2 ZK 93423 ($0.1-10.0\,mg\,kg^{-1}$) depressed the tonic EMG activity in mutant rats in a dose-dependent manner. This effect was reversed by the benzodiazepine antagonist Ro 15-1788 ($5.0\,mg\,kg^{-1}$).

3 Both ZK 93423 ($0.5\,mg\,kg^{-1}$) and diazepam ($0.3\,mg\,kg^{-1}$) enhanced the presynaptic inhibition of the GS muscle and associated dorsal root potentials in decerebrate cats in an almost identical manner. The actions of both drugs were reversed by Ro 15-1788 ($5.0\,mg\,kg^{-1}$).

4 ZK 93423 ($0.5\,mg\,kg^{-1}$) and diazepam ($0.3\,mg\,kg^{-1}$) depressed the activity of both static and dynamic fusimotor neurones, as deduced from changes in the afferent responses of muscle spindle primary endings to low frequency ($1\,s^{-1}$) sinusoidal stretching. The depressant action of diazepam ($0.3\,mg\,kg^{-1}$) was weaker than that of ZK 93423 ($0.5\,mg\,kg^{-1}$). The actions of both drugs were reversed by Ro 15-1788 ($5.0\,mg\,kg^{-1}$).

5 ZK 93423 ($0.5\,mg\,kg^{-1}$) failed to alter the magnitude of monosynaptic ventral root reflexes evoked by electrical stimulation of a flexor and an extensor nerve, whereas diazepam ($0.3\,mg\,kg^{-1}$) had a depressant effect on both types of monosynaptic reflexes, which was antagonized by Ro 15-1788 ($5.0\,mg\,kg^{-1}$).

6 Neither ZK 93423 ($0.5\,mg\,kg^{-1}$) nor diazepam ($0.3\,mg\,kg^{-1}$) depressed polysynaptic ventral root reflexes evoked by electrical stimulation of a flexor and a cutaneous nerve.

7 The present results demonstrate that ZK 93423 exerts a potent muscle relaxant action due to a specific interaction with benzodiazepine receptors. However, its profile of actions on spinal motor mechanisms is not identical to that of diazepam.

Introduction

In 1977, evidence for the existence of saturable, stereospecific, high-affinity binding sites for benzodiazepines in the mammalian central nervous system, which are thought to mediate the anxiolytic, anticonvulsant, sedative and muscle relaxant action of benzodiazepines, was described in two separate studies (Möhler & Okada, 1977; Squires & Braestrup, 1977). In the search for an endogenous ligand at benzodiazepine receptors, Braestrup *et al.* (1980) extracted from human urine β-carboline-3-carboxylate ethyl ester (β-CCE) which potently bound to benzodiazepine binding sites. Subsequent pharmacological studies revealed that β-CCE and related β-carbolines not only antagonized the actions of benzodiazepines, but also possessed pharmacological properties of their own which were opposite to those of benzodiazepines (Tenen & Hirsch, 1980; Cowen *et al.*, 1981; File *et al.*, 1982; Polc *et al.*, 1982; Braestrup *et al.*, 1983; Croucher *et al.*, 1984; Prado de Carvalho *et al.*, 1984; Turski *et al.*, 1984). The finding that the pharmacological actions of both, benzodiazepines and β-CCE, were subject to an antagonism by the im-

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idazobenzodiazepine Ro 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo(1,5a)-(1,4)-benzodiazepine-3-carboxylate) led to the characterization of β-CCE and related β-carbolines as inverse agonists of the benzodiazepine receptor (Nutt *et al.*, 1982; Polc *et al.*, 1982).

Recently a novel series of β-carboline derivates has been synthesized which represent the whole spectrum of action on the benzodiazepine receptor including inverse agonists, pure antagonists, partial agonists and full agonists (Braestrup *et al.*, 1983; 1984; Meldrum *et al.*, 1983; Petersen *et al.*, 1983; 1984; Jensen *et al.*, 1984 a, b; Petersen & Jensen, 1984; Stephens *et al.*, 1984 a, b; Klockgether *et al.*, 1985). Among these compounds 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate ethyl ester (ZK 93423) has been characterized as a full agonist at the benzodiazepine receptor because of its anticonvulsant, anxiolytic and sedative properties and its increased affinity for benzodiazepine binding sites in the presence of muscimol (Petersen *et al.*, 1983; Braestrup *et al.*, 1984; Stephens *et al.*, 1984a,b).

Since no information is yet available about possible muscle relaxant properties of ZK 93423, the action of this compound was investigated in two animal models relevant for the study of centrally acting muscle relaxants. First, experiments were performed on mutant Han-Wistar rats developing a progressive paresis of their hindlimbs associated with increased muscle tone (Pittermann *et al.*, 1976). The depression of sustained activity in the electromyogram (EMG) of the gastrocnemius-soleus (GS) muscle in these rats has been shown to be a valid and reliable tool for studying the muscle relaxant properties of benzodiazepines (Turski *et al.*, 1982; 1984; Schwarz *et al.*, 1983a,b; 1984). Second, neurophysiological experiments were carried out in cats decerebrated at the intercollicular level. Since benzodiazepines are thought to exert their muscle relaxant action by depressing spinal reflexes and fusimotor activity and enhancing presynaptic inhibition (Ghelarducci *et al.*, 1966; Ngai *et al.*, 1966; Swinyard & Castellion, 1966; Schmidt *et al.*, 1967; Przybyla & Wang, 1968; Crankshaw & Raper, 1970; Hudson & Wolpert, 1970; Stratton & Branes, 1971; Tseng & Wang, 1971; Polc *et al.*, 1974; Takano & Student, 1978), it seemed appropriate to investigate the effect of ZK 93423 on these mechanisms in decerebrate cats and to compare it with the action of diazepam.

Methods

EMG recording in mutant rats

Experiments were performed on mutant Han-Wistar rats (Han-Wist, spa/spa; Zentralinstitut für Versuchstierzucht, Hannover, F.R.G.) of either sex at the age of 10 to 12 weeks, 100 to 150 g in weight, as described elsewhere (Turski *et al.*, 1982). Briefly, spontaneous EMG activity was recorded from the GS muscle of unanaesthetized mutant rats with pairs of teflon-insulated stainless steel wire electrodes inserted percutaneously (Cooner Wire, Chabworth, CA, U.S.A.). Rats were placed individually in ventilated Plexiglas boxes and their hindlimbs which were gently secured with adhesive tapes were allowed to hang through slots in the bottom of the boxes. The electrical signals were amplified, band-pass filtered and integrated. The EMG was recorded continuously and the mean activity over 5 min segments was calculated. Spontaneous pre-drug control activity in the EMG of the GS muscle of mutant rats was measured in two 10 min segments. The changes in EMG activity after drug administration were calculated as a fraction of the activity before and after the administration of the drugs under study.

Neurophysiological experiments in decerebrate cats

Experiments were performed on cats of either sex 2.5 to 4.0 kg in weight. Under halothane anaesthesia tracheal, arterial and venous catheters were inserted. Both hindlimbs were completely denervated, unless otherwise stated, and hindlimb nerves and muscles were prepared, as indicated below. In all animals the spinal cord was exposed by a dorsal laminectomy from L4 to S2. All exposed tissues were covered with pools of warmed mineral oil maintained at 37°C. After decerebration at the intercollicular level the halothane anaesthesia was discontinued. Experiments were started 2 h after decerebration. Animals in which the blood pressure fell below 90 mmHg during the experiments were excluded from this study.

For the evaluation of drug effects on monosynaptic and polysynaptic ventral root reflexes, dorsal root potentials and presynaptic inhibition of monosynaptic reflexes the proximal stumps of the left GS, common peroneal (PC) and sural (SU) nerve were mounted on bipolar platinum electrodes for electrical stimulation. The ventral roots L7 and S1 were cut and mounted on bipolar electrodes for recording of reflex activity. Afferent nerve action potentials were recorded from the intact dorsal roots L7 and S1 with the help of monopolar platinum electrodes. A filament of the dorsal root L6 was cut and mounted on a bipolar platinum electrode for recording of dorsal root potentials.

GS, PC and SU reflexes were elicited every 5 s by electrical stimulation of the nerve stumps with single square electrical shocks of 0.2 ms duration at different stimulus strengths expressed as multiples of the afferent nerve threshold (T). Presynaptic inhibition of the monosynaptic GS reflex (1.6 T) was obtained by a conditioning train of 3 electrical shocks ($300\,s^{-1}$, 1.2 T)

delivered to the PC nerve at various intervals (20 to 400 ms) before the test shock. Dorsal root potentials were recorded from the dorsal root filament L6 following conditioning stimulation of the PC nerve using the same stimulation parameters as for presynaptic inhibition. All signals were amplified, band-pass filtered, displayed on an oscilloscope, stored on magnetic tape and later digitized by a laboratory computer PDP 11/23 (Digital Equipment Corporation, Maynard, MA, U.S.A.).

Ten consecutive responses were averaged before (control) and after application of solvent or drugs. The magnitude of reflexes was evaluated by measuring the area bounded by the averaged response and the baseline. The dorsal root potentials were evaluated by fitting a biexponential function to the data using a VAX 11/730 digital computer (Digital Equipment Corporation, Maynard, MA, U.S.A.) (Figure 3). This non-linear fit provided values for the amplitude and the rising and falling time constants. These three parameters led to a measure for the area, the amplitude and the half-time of decay of the dorsal root potential. All parameters obtained were expressed as a percentage of the corresponding control parameters.

Fusimotor activity was assessed indirectly by measuring the response of muscle spindle primary endings to sinusoidal stretch (Hulliger *et al.*, 1977a). For this purpose the left soleus muscle was freed from its surrounding tissue leaving its nerve and blood supply intact. The tendon of the isolated muscle was attached to a servo-controlled vibrator (Pye-Ling, V 50, Ling Dynamic Systems Ltd, Royston, Herts.). Single afferents of the soleus muscle were isolated in dorsal root filaments and classified as muscle spindle primary endings if they were silenced during the rising phase of a maximal muscle twitch and had conduction velocities exceeding 75 ms^{-1}. Discharge of muscle spindle primary afferents were monitored during single sinusoidal stretches of the soleus muscle at 1 s^{-1}, amplitude 75 μm (low) and 0.75 mm (large), which were repeated every 5 s. Afferent responses to a number of single cycles were averaged and displayed in a cycle histogram. Histograms were constructed of 25 trials with 40 ms bin width, 4 ms dwell time, 10 dwells per bin. A VAX 11/730 digital computer fitted the histogram by the method of least squares with a sine curve and printed out its parameters (mean level of fitted sine, depth of modulation).

Changes in fusimotor action were deduced from changes in fitted mean and modulation depth of spindle primary endings, when compared to the de-efferented preparation. As demonstrated by Matthews and coworkers (Goodwin *et al.*, 1975; Hulliger *et al.*, 1977a,b), fusimotor action whether of dynamic, static or mixed (combined static and dynamic) type, enhances the fitted mean of primary endings. On using sinusoidal stretch of low amplitude fusimotor action reduces the modulation depth, irrespective of which kind of fusimotor action is predominant. In contrast, sinusoidal stretching of large amplitude seems to be a reasonable test for separating dynamic and static fusimotor action, since within this range dynamic fusimotor action invariably increases the modulation depth of primary endings, whereas static fusimotor action reduces it. Mixed fusimotor action is characterized by small or absent changes of the modulation depth. Statistical evaluation of the results was carried out by means of Student's *t* test.

Drugs

ZK 93423, diazepam (Roche, Grenzach-Whylen, F.R.G.) and Ro 15-1788 were dissolved in a 3% solution of Tween 80 (Serva, Heidelberg, F.R.G).

In rats, the drugs were administered intraperitoneally. EMG recording started 5 min after the injection of the solvent or ZK 93423 and was continued for 2 h. Ro 15-1788 was injected 10 min before ZK 93423. Each

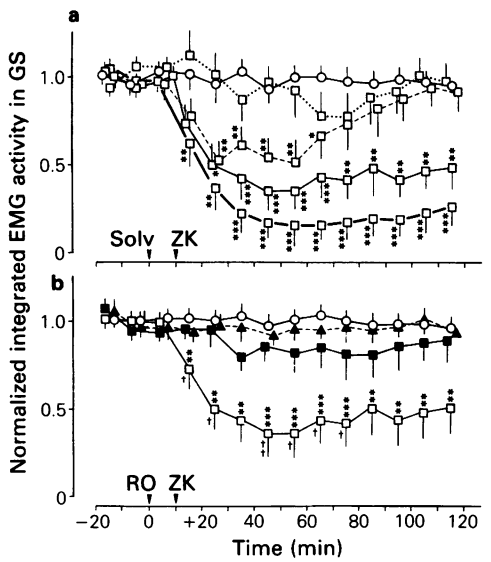

Figure 1 Time course of the action of different doses of ZK 93423 (a) and of Ro 15-1788 + ZK 93423 (b) on spontaneous electromyogram (EMG) activity in the gastrocnemius-soleus (GS) muscle of mutant rats. In (a), (○——○) solvent, (□ · · · · □) ZK 93423 (0.1 mg kg^{-1}), (□– –□) ZK 93423 (0.5 mg kg^{-1}), (□——□) ZK 93423 (2.0 mg kg^{-1}), (□ — □) ZK 93423 (10.0 mg kg^{-1}). In (b), (○) solvent, (▲) Ro 15-1788 (5.0 mg kg^{-1}), (□) ZK 93423 (2.0 mg kg^{-1}), (■) Ro 15-1788 (5.0 mg kg^{-1}) + ZK 93423 (2.0 mg kg^{-1}). Abscissa scale: time (min); ordinate scale: normalized mean EMG activity in the GS. Points show means and vertical lines s.e.means of results from 6–9 animals. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus the solvent. $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$ versus ZK 93423 (Student's *t* test).

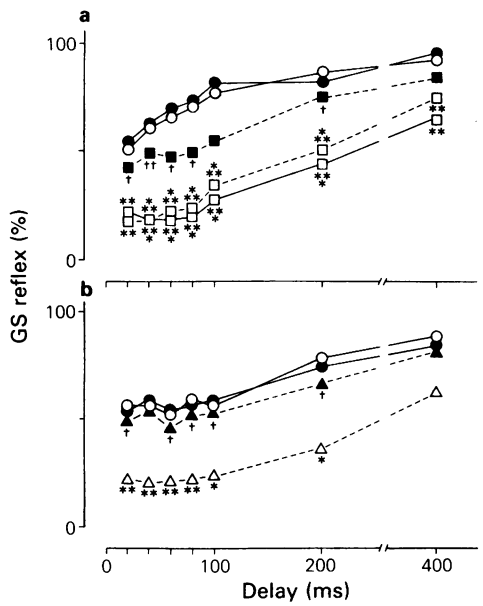

Figure 2 (a) Time course of the presynaptic inhibition of the electrically-evoked monosynaptic gastrocnemius-soleus (GS) reflex (1.6 T) after conditioning stimulation of the common peroneal nerve with 3 electrical shocks ($300\,s^{-1}$, 1.2 T) in 8 decerebrate cats before (●——●) and after injections of the solvent (○——○), ZK 93423 ($0.5\,mg\,kg^{-1}$) (□ – – □), ZK 93423 ($2.0\,mg\,kg^{-1}$) (□——□) and ZK 93423 ($0.5\,mg\,kg^{-1}$) + Ro 15-1788 ($5.0\,mg\,kg^{-1}$) (■ – – ■). (b) Time course of the presynaptic inhibition in 6 decerebrate cats before (●) and after injections of the solvent (○), diazepam ($0.3\,mg\,kg^{-1}$) (△) and diazepam ($0.3\,mg\,kg^{-1}$) + Ro 15-1788 ($5.0\,mg\,kg^{-1}$) (▲). Abscissa scale: delay (ms) between conditioning stimulation and test reflex. Ordinate scale: area of the conditioned GS reflex expressed as a % of the unconditioned GS reflex. Points represent the means of results from 8 or 6 animals. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus the solvent. $\dagger P<0.05$, $\dagger\dagger P<0.01$, $\dagger\dagger\dagger P<0.001$ versus ZK 93423 ($0.5\,mg\,kg^{-1}$) or diazepam, respectively (Student's *t* test).

drug treatment was tested in a separate group of rats consisting of 6 to 9 animals. In decerebrate cats, the drugs were administered intravenously. Neurophysiological experiments were performed 15 to 30 min after the injection of the solvent, ZK 93423 or diazepam and 10 to 25 min after the injection of Ro 15-1788. Ro 15-1788 was injected 30 min after the injection of ZK 93423 or diazepam.

Results

ZK 93423 in doses of 0.1, 0.5, 2.0 and $10.0\,mg\,kg^{-1}$ dose-dependently depressed the tonic EMG activity of the GS muscle in mutant rats, whereas the solvent was ineffective (Figure 1a). The depressant effect of ZK 93423 reached its maximum 15 to 40 min after the injection and with 2 and $10\,mg\,kg^{-1}$ this effect lasted throughout the measurement period. Following the dose of $0.5\,mg\,kg^{-1}$ the EMG activity slowly increased to control values after its maximal depression (Figure 1a). The effect of ZK 93423 ($2.0\,mg\,kg^{-1}$) could be completely antagonized by pretreatment with Ro 15-1788 ($5.0\,mg\,kg^{-1}$). Ro 15-1788 ($5.0\,mg\,kg^{-1}$) did not affect tonic EMG activity, when given alone (Figure 1b).

In decerebrate cats, ZK 93423 ($0.5\,mg\,kg^{-1}$) markedly potentiated the presynaptic inhibition of the GS reflex induced by conditioning stimulation of the PC nerve, when compared to either pre-drug control or the solvent (Figure 2a). Higher doses of ZK 93423 ($2.0\,mg\,kg^{-1}$) did not further enhance this effect (Figure 2a) suggesting that $0.5\,mg\,kg^{-1}$ ZK 93423 is a maximally effective dose in this respect. Subsequent injection of Ro 15-1788 ($5.0\,mg\,kg^{-1}$) reversed the effect of ZK 93423 ($0.5\,mg\,kg^{-1}$) on the inhibition of the GS reflex (Figure 2a). These changes in the extent of GS reflex inhibition were paralleled by an augmentation of dorsal root potentials and a reversal of this effect by subsequent administration of Ro 15-1788 (Figure 3). Table 1 demonstrates that the solvent was

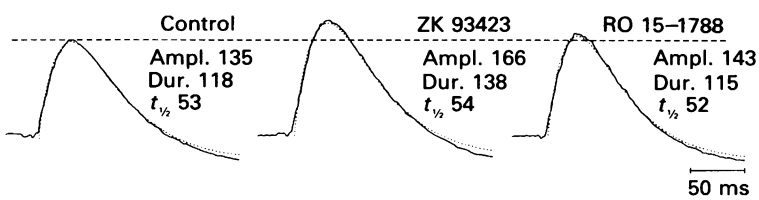

Figure 3 Dorsal root potentials recorded from a dorsal root L6 filament following conditioning stimulation of the common peroneal nerve with 3 electrical shocks ($300\,s^{-1}$, 1.2 T) in a decerebrate cat before (control) and after consecutive injections of ZK 93423 ($0.5\,mg\,kg^{-1}$) and Ro 15-1788 ($5.0\,mg\,kg^{-1}$) (averaged from 10 responses). Inset values give amplitude (Ampl., μV), overall duration (Dur., ms) and half-time of decay ($t_{1/2}$, ms) of the dorsal root potentials. Dotted lines represent the fitted biexponential function; broken line represents the amplitude of the pre-drug control potential.

Table 1 Magnitude of dorsal root potentials

	Solvent	*ZK 93423*	*ZK 93423 + Ro 15-1788*	*Diazepam*	*Diazepam + Ro 15-1788*
Area	93 ± 5(7)	127 ± 11*(6)	93 ± 18†(6)	132 ± 12*(6)	100 ± 13†(6)
Ampl.	98 ± 6(7)	131 ± 14*(6)	97 ± 14†(6)	134 ± 14*(6)	98 ± 11†(6)
$t_{1/2}$	107 ± 4(7)	99 ± 5 (6)	107 ± 10 (6)	99 ± 7 (6)	101 ± 11 (6)

Area, amplitude (ampl.) and half-time of decay ($t_{1/2}$) of dorsal root potentials, recorded from a dorsal root L6 filament after conditioning stimulation of the common peroneal nerve with 3 electrical shocks (300 s^{-1}, 1.2 T), are expressed as a % of the respective pre-drug control values after injection of the solvent, ZK 93423 (0.5 mg kg^{-1}), ZK 93423 (0.5 mg kg^{-1}) + Ro 15-1788 (5.0 mg kg^{-1}), diazepam (0.3 mg kg^{-1}) and diazepam (0.3 mg kg^{-1}) + Ro 15-1788 (5.0 mg kg^{-1}) in decerebrate cats. Ro 15-1788 was injected 30 min after the injection of ZK 93423 or diazepam. Results are presented as means ± s.e.mean. Number of experiments are given in parentheses. * $P < 0.05$ versus the solvent. † $P < 0.05$ versus ZK 93423 or diazepam, respectively (Student's t test).

without effect on dorsal root potentials and that the augmenting effect of ZK 93423 was due to an increase in the amplitude without a concomitant increase in the half-time of decay of the dorsal root potentials. These changes led to an increase in the overall duration of the dorsal root potentials (Figure 3).

Diazepam (0.3 mg kg^{-1}) potentiated the presynaptic inhibition of the GS reflex (Figure 2b) and increased the area and amplitude of dorsal root potentials, without affecting the half-time of decay (Table 1). These effects were antagonized by subsequent administration of Ro 15-1788 (5.0 mg kg^{-1}). The potencies of ZK 93423 (0.5 mg kg^{-1}) and diazepam (0.3 mg kg^{-1}) at enhancing presynaptic inhibition and dorsal root potentials were approximately the same.

In untreated cats, decerebrated at the intercollicular level, muscle spindle primary endings were found to be predominantly under static fusimotor control, as deduced from their fit parameters in comparison to the de-efferented preparation. A small number of endings was isolated and they were found to be under mixed or predominantly dynamic control. Injection of the solvent did not affect fusimotor activity, whereas ZK 92423 (0.5 mg kg^{-1}) potently removed fusimotor drive from the muscle spindles (Table 2A). The cycle histogram of the response of a representative muscle spindle primary ending predominantly under static fusimotor control is depicted in Figure 4A. ZK 93423 (0.5 mg kg^{-1}) potently removed static fusimotor drive from this muscle spindle, as indicated by the decrease

Table 2 Fit parameters of afferent responses of muscle spindle primary endings

		Small amplitude		*Large amplitude*	
		F.M.	*M.D.*	*F.M.*	*M.D.*
A	Control	17.3 ± 2.2	8.9 ± 1.5	21.9 ± 2.0	27.3 ± 3.1
	Solvent	17.6 ± 1.8	9.0 ± 1.9	20.9 ± 2.1	28.0 ± 3.2
	ZK 93423	7.5 ± 2.7***	14.2 ± 1.6**	2.1 ± 1.1***	36.6 ± 3.6*
	Ro 15-1788	14.5 ± 2.2††	8.8 ± 1.5†	14.0 ± 2.0†††	22.9 ± 3.3†
	De-eff.	4.7 ± 2.1***	19.0 ± 1.6***	0.1 ± 0.4***	46.7 ± 3.3*
B	Control	17.2 ± 1.4	7.4 ± 1.1	21.4 ± 3.0	25.2 ± 3.6
	Solvent	17.4 ± 1.5	7.3 ± 1.0	21.6 ± 3.4	25.0 ± 3.4
	Diazepam	13.2 ± 0.9**	11.6 ± 1.4*	12.0 ± 3.8**	33.0 ± 3.5*
	Ro 15-1788	16.9 ± 0.9†	7.1 ± 1.2†	19.4 ± 3.0†	25.0 ± 3.2†
	De-eff	4.6 ± 0.9***	19.2 ± 1.3***	2.0 ± 2.0***	50.9 ± 2.6**

(A) Fitted mean (F.M.) and modulation depth (M.D.) in s^{-1} of 12 muscle spindle primary endings during small (75 μm)-and large (0.75 mm)-amplitude low frequency (1 s^{-1}) sinusoidal stretching before (control) and after consecutive injection of the solvent, ZK 93423 (0.5 mg kg^{-1}) and Ro 15-1788 (5.0 mg kg^{-1}) and after de-efferentation (de-eff.) in decerebrate cats. (B) Fitted mean and modulation depth of 18 endings before (control) and after consecutive injection of the solvent, diazepam (0.3 mg kg^{-1}) and Ro 15-1788 (5.0 mg kg^{-1}) and after de-efferentation. Results are presented as means ± s.e.means. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus the solvent. † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ versus ZK 93423 or diazepam, respectively (Student's t test).

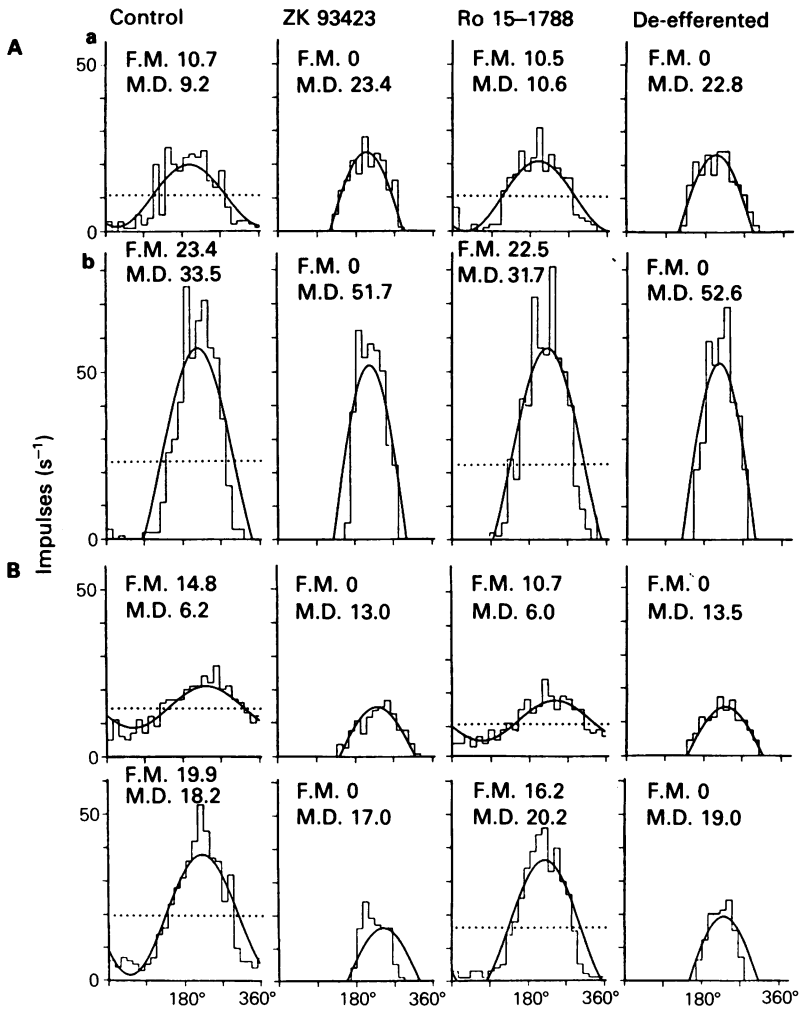

Figure 4 Cycle histograms showing mean responses of (A) a soleus muscle spindle primary ending under predominant static and (B) a second soleus muscle spindle under mixed fusimotor drive, to (a) small (75 μm)- and (b) large(0.75 mm)-amplitude sinusoidal stretching of low frequency (1 s^{-1}) in decerebrate cats before (control) and after consecutive injections of ZK 93423 (0.5 mg kg^{-1}) and Ro 15-1788 (5.0 mg kg^{-1}) and after de-efferentation (de-eff). Abscissa scale: phase of stretch cycle (deg); 270 deg corresponds to the maximum extension of the muscle; ordinate scale: impulse rate (s^{-1}). Inset values give fitted mean (F.M.) indicated by dotted lines and modulation depth (M.D.) of the fitted sinusoid in s^{-1}.

in the fitted mean and the increase in the modulation depth. The effect of ZK 93423 which made the response look very similar to the response of the de-efferented spindle was reversed by Ro 15-1788 (5.0 mg kg^{-1}). However, the depressant effect of ZK 93423 was not confined to static fusimotor neurones, as documented in Figure 4B, in which the response of a representative ending under mixed fusimotor control is shown. ZK 93423 (0.5 mg kg^{-1}) decreased the fitted mean, slightly increased the modulation depth during low-amplitude stretching and had no effect on the modulation depth during large-amplitude stretching, indicating a complete removal of both static and dynamic fusimotor drive. Ro 15-1788 (5.0 mg kg^{-1}) antagonized this effect. It was consistently found that ZK 93423 (0.5 mg kg^{-1}) induced an almost complete pharmacological de-efferentation of muscle spindle primary endings irrespective of whether they had originally been found to be under static or dynamic control.

Table 3 Magnitude of ventral root reflexes

	Solvent	*ZK 93423*	*ZK 93423 + Ro 15-1788*	*Diazepam*	*Diazepam + Ro 15-1788*
GS_{mono}	108± 8(6)	93±7 (13)	89±10(5)	75±10*(6)	100± 7†(6)
PC_{mono}	96± 5(5)	105±9 (11)	84±10(7)	74± 9*(6)	107±16†(6)
PC_{poly}	95± 8(5)	122±7*(12)	115±18(6)	97±16 (6)	101±14 (6)
SU	98±10(6)	105±5 (6)	100± 5(6)	101± 8 (6)	112±10 (6)

Area of the monsynaptic gastrocnemius-soleus reflex (1.6 T, GS_{mono}), monosynaptic common peroneal reflex (1.6 T, PC_{mono}), polysynaptic component of the common peroneal reflex (5.0 T, PC_{poly}) and polysynaptic sural reflex (5.0 T, SU_{poly}), expressed as a % of the respective pre-drug control values, after injection of the solvent, ZK 93423 (0.5 mg kg^{-1}), ZK 93423 (0.5 mg kg^{-1}) + Ro 15-1788 (5.0 mg kg^{-1}), diazepam (0.3 mg kg^{-1}) and diazepam (0.3 mg kg^{-1}) + Ro 15-1788 (5.0 mg kg^{-1}) in decerebrate cats. Ro 15-1788 was injected 30 min after the injection of ZK 93423 or diazepam. Results are presented as means ± s.e.mean. Number of experiments are given in parentheses. * $P < 0.05$ versus the solvent, † $P < 0.05$ versus diazepam (Student's t test).

Diazepam (0.3 mg kg^{-1}) had a depressant effect on fusimotor activity which was reversed by Ro 15-1788 (5.0 mg kg^{-1}) (Table 2B). The depressant effect of diazepam (0.3 mg kg^{-1}) was smaller than that of ZK 93423 (0.5 mg kg^{-1}). However, like ZK 93423 (0.5 mg kg^{-1}), diazepam (0.3 mg kg^{-1}) removed fusimotor drive from endings under static, mixed or dynamic fusimotor control, indicating an action of this compound on both types of fusimotor neurones.

Figure 5 shows that ZK 93423 (0.5 mg kg^{-1}) failed to affect the ventral root reflexes evoked by stimulation of the GS and SU nerve. As summarized in Table 3 the solvent, ZK 93423 (0.5 mg kg^{-1}) and Ro 15-1788 (5.0 mg kg^{-1}) administered consecutively did not alter the magnitude of the monosynaptic GS reflex (1.6 T), the monosynaptic PC reflex (1.6 T) and the polysynaptic SU reflex (5.0 T). ZK 93423 (0.5 mg kg^{-1}) slightly enhanced the magnitude of the polysynaptic component of the PC reflex (5.0 T), an effect that could not be reversed by Ro 15-1788 (5.0 mg kg^{-1}).

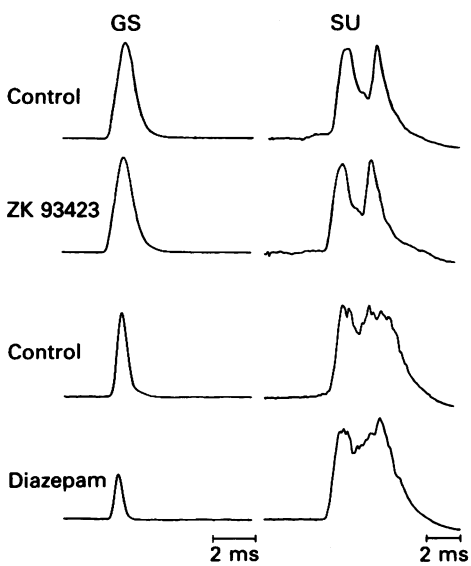

Figure 5 Recordings of ventral root reflexes evoked by electrical stimulation of the gastrocnemius-soleus nerve (GS) (1.6 T) (left side) and sural nerve (SU) (5.0 T) (right side) before (control) and after injection of either ZK 93423 (0.5 mg kg^{-1}) or diazepam (0.3 mg kg^{-1}) in decerebrate cats (averaged from 10 responses).

In contrast to ZK 93423 (0.5 mg kg^{-1}), diazepam (0.3 mg kg^{-1}) decreased the magnitude of the monosynaptic GS reflex (Figure 5) and the monosynaptic PC reflex. Subsequent injection of Ro 15-1788 (5.0 mg kg^{-1}) reversed these effects (Table 3). Diazepam (0.3 mg kg^{-1}) failed to affect the polysynaptic component of the PC reflex (Table 3) and the SU reflex (Figure 5 and Table 3).

Discussion

ZK 93423 dose-dependently depressed tonic EMG activity in the GS muscle of mutant rats indicating a muscle relaxant action of this compound. This action was evidently due to an interaction of ZK 93423 with benzodiazepine receptors since it was antagonized by the benzodiazepine antagonist Ro 15-1788 in a dose that was devoid of an action of its own. Although Ro 15-1788 may exert intrinsic actions in higher doses it can be considered as a neutral antagonist at the low doses used throughout this study (Hunkeler *et al.*, 1981; Polc *et al.*, 1981; Nutt *et al.*, 1982). Comparison of the effect of ZK 93423 with that of diazepam in the same animal model reveals that these drugs are approximately equipotent at exerting a muscle relaxant effect (Schwarz *et al.*, 1984).

Experiments in rodent models like the mutant rat employed in the present study or the spastic mouse (Chai, 1961; Biscoe & Fry, 1982) allow the estimation

of the net effect of muscle relaxant compounds on overactive motor units and may, therefore, be of great value for the pharmacological investigation of these compounds. However, little information is provided by such experiments on the mechanisms underlying the action of these drugs. This disadvantage can be overcome by parallel studies in decerebrate cats. This preparation enables the action of muscle relaxant drugs on spinal motor mechanisms, which are essential for the regulation of muscle tone, to be investigated.

Both, ZK 93423 and diazepam potently enhanced the presynaptic inhibition of the GS reflex and increased the area and amplitude of dorsal root potentials without affecting the half-time of decay. The effects of both drugs on presynaptic inhibition and dorsal root potentials were susceptible to antagonism by Ro 15-1788, suggesting the benzodiazepine specificity of their action. The present results are in good agreement with earlier accounts of the action of diazepam (Schmidt *et al.*, 1967; Stratten & Barnes, 1971; Polc *et al.*, 1974). The finding of Schmidt *et al.* (1967) that diazepam increases the half-time of decay of dorsal root potentials could not be confirmed. This difference is probably due to methodological reasons since a computer assisted method was used in the present study which allowed the parameters of the recorded potentials to be evaluated in a very reliable way.

ZK 93423 and diazepam had a depressant action on fusimotor activity, as deduced from changes in the afferent response of muscle spindle primary endings to low frequency sinusoidal stretching, an effect that was found to be benzodiazepine specific. Although the doses of ZK 93423 and diazepam used were equipotent in enhancing presynaptic inhibition and dorsal root potentials, the depressant effect of diazepam on fusimotor activity was considerably smaller than that of ZK 93423 suggesting a greater potency of the latter drug in this case. The present experiments are of special interest since for the first time in pharmacological studies a paradigm has been used that allows the differentiation of effects on static and dynamic fusimotor neurones. Our results strongly suggest that both ZK 93423 and diazepam depress the activity of both types of fusimotor neurones. So far, only indirect evidence had been accumulated suggesting a depressant action of diazepam on both static and dynamic fusimotor neurones (Polc *et al.*, 1974; Takano & Student, 1978).

Electrically-evoked monosynaptic ventral root reflexes of both flexors and extensors were unaffected by ZK 93423. In contrast, diazepam exerted a depressant effect on electrically-evoked monosynaptic ventral root reflexes, confirming earlier results (Schmidt *et al.*, 1967; Polc *et al.*, 1974). However, the present study provides additional information on the benzodiazepine specificity of this effect.

Polysynaptic reflexes evoked by stimulation of a flexor and a cutaneous nerve were found to be resistant to ZK 93423. ZK 93423 even slightly increased the polysynaptic component of the PC reflex. This latter finding, however, may be a non-specific effect arising from the variability of polysynaptic reflex responses, since it was neither antagonized by Ro 15-1788 nor accompanied by parallel changes of the magnitude of the SU reflex. Diazepam completely failed to affect polysynaptic reflex responses in the present study. The latter finding seems surprising since several studies have been published which describe a powerful depressant action of benzodiazepines on polysynaptic reflexes (Ngai *et al.*, 1966; Przybyla & Wang, 1968; Crankshaw & Raper, 1970; Hudson & Wolpert, 1970; Tseng & Wang, 1971; Polc *et al.*, 1974). However, in other studies such an action of diazepam and other benzodiazepines was not confirmed. Swinyard & Castellion (1966) did not observe a depression of spinal polysynaptic reflex after diazepam in spinalized cats. Ghelarducci *et al.* (1966), using nitrazepam, observed a slight depression of polysynaptic reflexes in spinalized cats after high doses but no such effect in decerebrate cats. Schmidt *et al.* (1967) found only a negligible effect of diazepam on polysynaptic reflexes in barbiturate anaesthetized spinalized cats.

In conclusion, the β-carboline ZK 93423 acts as a potent muscle relaxant, as predicted from its biochemical characterization as a full agonist of the benzodiazepine receptor. However, the profile of its action on spinal motor mechanisms is not identical to that of benzodiazepines, the most prominent difference being its failure to depress spinal monosynaptic reflexes and its higher efficacy in depressing fusimotor activity.

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