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A new class of potent centrally acting muscle relaxants: pharmacology of oxazolidinones in rat decerebrate rigidity

M. Masaki & H. Shinozaki*[1]

Research Laboratory of Nippon Chemiphar, 1-22-1, Hikokawado, Misato, Saitama 341 and The Tokyo Metropolitan Institute of Medical Science*, 3-18-22, Honkomagome, Bunkyo-ku, Tokyo 113, Japan

1 The severity of anaemic decerebrate rigidity was quantitatively determined by measuring the frequency of electromyographic potentials in the rat.

2 Some oxazolidinones markedly reduced the severity of this decerebrate rigidity in a dose-dependent manner, (4**S**,5**R**)-4-(2-methylpropyl)-3-[3-(perhydroazepin-1-yl)propyl]-5-phenyl-1,3-oxazolidin-2-one (MLV-6976) being the most potent. In addition to the oxazolidinones, an aminoalcohol derivative, (1**RS**,2**SR**)-5-methyl-1-phenyl-2-(3-piperidinopropylamino)hexan-1-ol (MLV-5860) also reduced the rat decerebrate rigidity.

3 In the oxazolidinone series, the optical isomers with absolute configuration (S) at the 4-position were more potent than the corresponding (4**R**)-isomers, while there was no significant difference in their LD_{50} values.

4 Normal rats and mice receiving MLV-6976 at doses which reduced decerebrate rigidity showed no behavioural changes, impairment of motor coordination only appearing at extremely high doses. MLV-6976 and its derivatives did not affect spinal reflex potentials in cats.

5 MLV-6976 reduced the severity of harmaline-induced tremor in mice in a dose-dependent manner, but slightly augmented tremorine-induced tremor.

6 The frequency of the spike discharges induced by iontophoretically applied glutamate was reduced by MLV-6976 in a dose-dependent manner in rat cortical neurones.

7 The amplitude of miniature endplate potentials of the rat diaphragm was decreased by MLV-6976 only at concentrations greater than 0.1 mM.

8 It is concluded that MLV-6976 acts on the brainstem or/and higher levels of the brain rather than on the spinal cord or the peripheral nervous system to reduce the excessive activities of the nervous system.

Introduction

Decerebrate rigidity is a condition which appears when the brain stem of an animal is transected or damaged above the vestibular nuclei which exert a strong facilitatory action on ipsilateral extensor motoneurones. Most clinically useful muscle relaxants reduce the extent of the rigidity by acting on the central nervous system (CNS) or directly on skeletal muscle. After ligating both carotids and the basilar artery (so-called anaemic decerebration), the animal presents an intense extensor rigidity in which more than half the cerebellum and a considerable part of the pons are destroyed (Pollock & Davis, 1930; Fukuda *et al.*, 1974a). Animals with decerebrate rigidity have proved useful for the study of the mechanisms of action of the centrally acting muscle relaxants and some CNS depressants (Maxwell & Read, 1972; Sontag & Wand, 1973; Maxwell & Sumpter, 1974; Fukuda *et al.*, 1974a,b; Anderson & Raines, 1976; Wand *et al.*, 1977; Ochiai & Ishida, 1982; Goto *et al.*, 1982; 1983; Raines *et al.*, 1985).

Using this animal model to search for new central muscle relaxants, MLV-208 (5-[N-benzyl-N-(3-piperidinopropyl)]amino-3-phenylisoxazole) has been shown to have an inhibitory action on decerebrate rigidity in rats, as well as on drug-induced tremor in mice (Shinozaki & Hirate, 1986). Furthermore, MLV-

[1]Author for correspondence.

208 demonstrated a powerful inhibitory action on the glutamate-induced response at the crayfish neuromuscular junction (Shinozaki & Ishida, unpublished observations). This compound contains a constituent moiety of alkylenediamine, which is contained in some CNS depressants. Therefore, we have designed and synthesized a series of compounds with the chemical moiety of alkylenediamine, which reduce both decerebrate rigidity in the rat and responses to glutamate at the crayfish neuromuscular junction (Masaki *et al.*, 1985). In the present paper, we describe the effects of such drugs on decerebrate rigidity in the rat and summarize some of their neuropharmacology. In particular, we have examined those of (4S,5R)-4-(2-methylpropyl)-3-[3-(perhydroazepin-1-yl)propyl]-5-phenyl-1,3-oxazolidin-2-one (MLV-6976), which is the most potent relaxant of this series.

Methods

Decerebrate rigidity in rats

The methods used in the present study were essentially similar to those described by Fukuda *et al.* (1974a). Male albino rats (Wistar strain) weighing 280 to 350 g had food and water *ad libitum* before the experiments. Under ether anaesthesia, a trephined hole, 5 mm in diameter, was made in the central part of the occipital bone, and the basilar artery ligated under microscopic observation after both carotid arteries had been ligated and cut. Immediately after the ligation of the basilar artery, anaesthesia was discontinued (only the small inferior part of the cerebellum, the medulla, and the spinal cord survived after these operations, therefore, the anaesthesia was unnecessary: Fukuda *et al.*, 1974a). The decerebrate rats were restrained on their backs on a hand-made plastic panel. In order to maintain a constant severity of forelimb rigidity, a bar about 2.5 cm in height was put under the shoulder of the decerebrate rat. Arterial blood pressure was simultaneously monitored through a pressure transducer via a polyethylene cannula inserted in the femoral artery. To inject the drug sample, another polyethylene cannula was inserted in the femoral vein of the other leg.

Experiments were carried out at a constant room temperature of about 24°C. Body temperature of the decerebrate rat was maintained at 37°C with a heat-lamp. The severity of the rigidity was determined from the frequency of electromyographic discharges recorded from the muscle (M. triceps brachii) of the forelimb of the decerebrate rat with a coaxial needle electrode. The frequencies of electromyographic potentials of amplitudes above 0.4 mV (this was the most suitable value in the signal to noise ratio of the recording system) were counted with a pulse integrator, and the integrated count recorded as a histogram with 10 s epochs on a pen-writer and digital counters. The efficacy of drugs was expressed as a decrease in frequency of electromyographic potentials. Only those results from rats which demonstrated a complete recovery from the drug action were used to assess the significance of differences between the different groups of experiments. The results are presented as the mean ± s.e.mean for n experiments. Differences were analysed using Student's t test. All the test samples were dissolved in 0.9% NaCl solution and given intravenously at a rate of 0.1 ml $100\,g^{-1}$ body weight, spending 10 s per injection in infusing the solution. The value of LD_{50} was estimated by the up-and-down method (Brownlee *et al.*, 1953) when test samples were given intravenously. Drugs were used as fumarate salts except in the electrophysiological experiments in which hydrochloride salts were used. MLV-5860 was used as a hydrochloride salt. The hydrochloride salt of MLV-6976 has the code number NC-1200.

General symptoms and impairment of motor coordination in mice and rats

General symptoms were observed in mice after intraperitoneal injection of MLV compounds at various doses, and in some experiments the spontaneous locomotor activity of male rats (Wistar strain) and mice (ICR strain) was measured by use of an Animex counter (Svensson & Thieme, 1969). The impairment of motor coordination was measured in the Rotorod test according to a procedure similar to that described by Dunham & Miya (1957). Male albino rats (Wistar strain) weighing 90–125 g ($n = 104$) had food and water *ad libitum* before the experiments. Animals were placed on a horizontal rotating rod having a diameter of 60 mm which rotated at 8 r.p.m. Animals that remained on the rod for 2 min or more in successive trials were selected and used in experiments. Test drugs or vehicle were administered intraperitoneally to groups of 8 rats, and every 15 min rats were placed on the rotating rod. The number of rats failing to remain on the rotating rod for 2 min was determined.

Antagonism of drug-induced tremor in mice

The methods were essentially similar to those described previously (Shinozaki, 1984; Shinozaki *et al.*, 1985). Male albino mice (ICR strain, 9 weeks old) had food and water *ad libitum* before the experiments. To induce tremor, tremorine and harmaline were injected subcutaneously immediately before the mouse was released on the experimental field. The severity of the tremor was determined quantitatively in terms of the sum of the mean square value using a power spectral analysis of the random current induced by the

movement of a magnet (0.4 g, 2600 gauss) attached to a mouse on a wire coil (40 cm in diameter, 204 kΩ) (see Shinozaki, 1984). The mean square value was calculated at every tremor component from the total area under the plots of the power spectral densities versus frequency. Test drugs and vehicle were administered intraperitoneally 5 min before the application of harmaline (50 mg kg^{-1} s.c.) and simultaneously with tremorine (70 mg kg^{-1} s.c.)

Spinal reflex potentials in cats

Experiments were performed on 9 spinal cats (2.3–3.6 kg), immobilized with gallamine triethiodide (Sigma, 5 mg kg^{-1}, i.v. plus 10 mg kg^{-1}, s.c. every 2 h) and artificially respired. Under ether anaesthesia, the spinal cord was transected at the atlanto-occipital junction. To maintain the blood pressure, 5% glucose Ringer solution was usually infused during the experiment at a rate of 1 ml min^{-1} kg^{-1}, and as a result, the diastolic blood pressure was kept at a level above 50 mmHg. The left L_7 dorsal root was electrically stimulated (0.2 Hz, 0.3 ms) and the reflex potential was extracellularly recorded from the L_7 ventral root. Bipolar platinum electrodes were used for both stimulation and recording. Dorsal root reflex potentials were also recorded from the ipsilateral L_6 or S_1 dorsal root. Spinal and peripheral tissues were covered with a liquid paraffin pool thermoregulated with heat-lamps to maintain body temperature from 36 to 38°C. Test samples were injected into the femoral vein by a catheter.

Reduction of glutamate-induced excitation of rat cortical neurones

Experiments were performed on cortical neurones in the rat. Albino rats (Wistar strain, 250–350 g) were anaesthetized by intraperitoneal injection of urethane-chloralose (urethane 750 mg kg^{-1} and α-chloralose 50 mg kg^{-1}). A small hole about 3 mm in diameter was drilled in the middle of the rat parietal bone, and through it a seven barrel glass micropipette with a tip diameter of about 4 μm was inserted into the cerebral cortex (less than 2.0 mm in depth from the surface). Iontophoretic ejection was effected by an apparatus incorporating automatic balancing at the electrode tip. On some sites iontophoretic current pulses of glutamate induced spike discharges from cortical neurones. Action potentials of single neurones were recorded by means of the centre barrel (2 M NaCl) of seven barrel micropipettes and were monitored on an oscilloscope and either photographed, or electronically counted with a frequency counter and displayed on a pen recorder trace. The outer barrels of the seven barrel micropipettes contained aqueous solutions of the following agents: acetylcholine Cl (1 M, pH 7.0), Na L-glutamate (1 M, pH 7.0), N-methyl-D-aspartate (NMDA) (0.1 M, pH 7.0), Na L-α-kainate (0.1 M, pH 7.0), Na quisqualate (0.1 M, pH 7.0), γ-aminobutyric acid (GABA, 1 M, pH 5.0), L-glutamate diethyl ester (GDEE, 0.1 M, pH 5.0) and MLV compounds (hydrochloride salt, 0.05 M, pH 7.0).

Endplate responses

The methods used were similar to those described in earlier papers (Fatt & Katz, 1951). The nerve-diaphragm preparation of male rats (Wistar strain) weighing 230–270 g was used, immersed in an isotonic solution containing (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.6, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11 (pH 7.4) equilibrated with 95% O_2 – 5% CO_2 at a bath temperature of about 30°C. Potential changes of the muscle membrane were recorded with a 3 M KCl-filled microelectrode. The usual procedure was to locate a suitable site with the intracellular electrode and record spontaneous miniature endplate potentials (m.e.p.ps) on moving film. Test drugs were administered by bath application. In some cases the repetitively induced twitch tension of the diaphragm and gastrocnemius muscle fibre of the rat was isometrically measured *in vitro* and *in situ*, respectively. In the latter case, drugs were administered via a polyethylene cannula inserted in the descending aorta of the rat which was anaesthetized by intraperitoneal injection of urethane (1 g kg^{-1}) and α-chloralose (25 mg kg^{-1}).

Materials

The following substances were used: α-chloralose (Tokyo Kasei), chlorpromazine hydrochloride (Sigma), diazepam (Horizon inj., Yamanouchi), ether (Junsei Kagaku), gallamine triethiodide (Sigma), harmaline HCl (Sigma), tolperisone hydrochloride (Nippon Chemiphar), tremorine dihydrochloride (Sigma), urethane (Tokyo Kasei), γ-amino-*n*-butyric acid GABA (Tokyo Kasei), L-glutamate diethyl ester (Sigma), monosodium L-glutamate (Wako), L-α-kainic acid (Fujisawa), quisqualic acid (Nippon Kayaku), N-methyl-D-aspartic acid (NMDA, Sigma) and MLV-compounds.

Results

Anaemic decerebrate rigidity in rats

Within 15 min after ligation of both carotids and the basilar artery of the rat, intense extensor rigidity appeared in the forelimb, the neck, the abdomen, the hindlimb and the tail. Extension of the forelimb was particularly marked and lasted for more than 1.5 h. The tips of the forelimb trembled finely. External

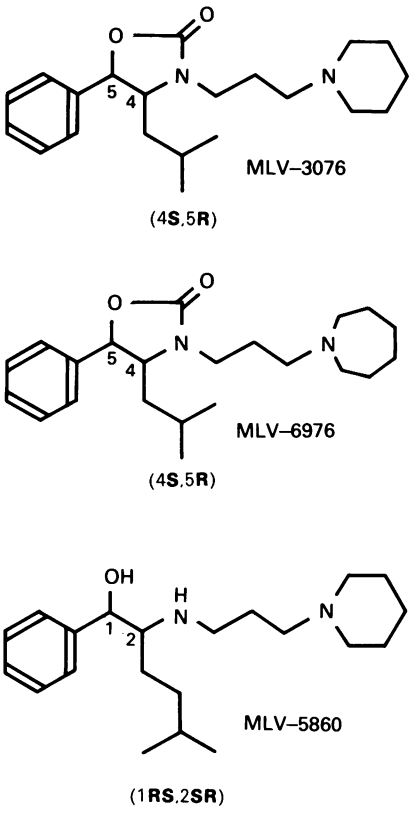

Figure 1 The structures of MLV-3076, MLV-6976 and MLV-5860; compounds which demonstrated a potent inhibitory action on the rat decerebrate extensor rigidity.

stimuli such as touching the body or pinching the tail transiently increased the rigidity of the forelimb and the frequency of electromyographic potentials, but infusion of the physiological saline into the femoral vein did not affect the severity of the rigidity. The mean frequency of electromyographic potentials with amplitudes above 0.4 mV, derived from the forelimb muscle, was 2528 ± 26 spikes $(10\,s)^{-1}$ ($n = 145$) immediately before test samples were given, and remained almost constant until the decerebrate rat died. There was no statistical difference in frequencies of electromyographic potentials between the control values of each experimental group (decerebrate rats) before the application of test samples. The rate of respiration was significantly increased in all decerebrate rats. The blood pressure was slightly hypertensive, its mean value (146 ± 1 mmHg, $n = 145$) being about 20 mmHg higher than that of intact animals.

Among many test samples of alkylenediamine derivatives, some oxazolidinones and aminoalcohols demonstrated a potent inhibitory action on rat decerebrate extensor rigidity (Figure 1). A compound, which has a hexamethylene-iminopropyl group and an isobutyl group at the 3- and 4-position of the oxazolidinone ring, respectively, has so far been the most powerful in reducing the severity of the rigidity. The potency at reducing the rigidity was changed to some extent by replacing the terminal imino group with various other tertiary amines and significantly altered by replacing the isobutyl group at the 4-position with other alkyl groups (Masaki *et al.*, 1985). (4S,5R) -4- (2-methylpropyl) -3- [3-(perhydroazepin-1-yl)propyl]-5-phenyl-1,3-oxazolidin-2-one (MLV-

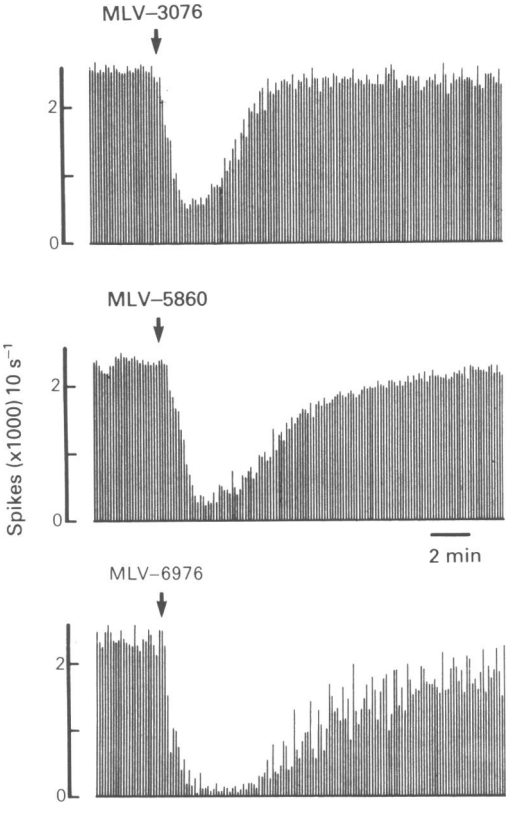

Figure 2 A comparison of the inhibitory action of various MLV compounds on rat decerebrate rigidity. MLV-3076, -5860 and -6976 were given intravenously to the decerebrate rat at a dose of $2\,mg\,kg^{-1}$. The vertical bars represent the frequency of electromyographic potentials with amplitudes above 0.4 mV, in 10 s epochs. The electromyographic potentials were derived from the muscle of the forelimb (M. triceps brachii). The traces show typical responses which are almost equivalent to the average. Arrows indicate the intravenous injection of the MLV compound.

6976) has so far been found to be the most effective compound and (4**S**,5**R**)-4-(2-methylpropyl)-5-phenyl-3-(3-piperidinopropyl)-1,3-oxazolidin-2-one (MLV-3076) also has a potent action on the rigidity. In addition to these oxazolidinones, an aminoalcohol derivative, (1**RS**,2**SR**)-5-methyl-1-phenyl-2-(3-piperidinopropylamino)hexan-1-ol (MLV-5860), has been found to be very potent at reducing the severity of the rigidity. Two to 5 min after an intravenous injection of the same dose of MLV-6976 and MLV-5860 the frequency of electromyographic potentials was reduced to a similar extent as that to MLV-3076, but the duration of action of MLV-6976 was much longer than that of the other test samples (Figure 2).

When MLV-6976 was given intravenously at various doses, the frequency of electromyographic potentials was reduced in a dose-dependent manner (Figure 3). An intravenous injection of 2 mg kg^{-1} MLV-6976 caused a reduction of electromyographic activity which reached a maximum within 3 min after the injection. The frequency of electromyographic potentials gradually increased, and was restored exponentially to the control level in a dose-dependent manner (Figures 2 and 3). At intravenous doses higher than 8 mg kg^{-1}, the decerebrate rigidity was significantly reduced and electromyographic activity almost disappeared for a while, but complete recovery was observed. When various doses of other MLV compounds were given intravenously to decerebrate rats, the frequency of electromyographic potentials was reduced in a similar manner to that of MLV-6976. Figure 4 represents dose-response curves for these compounds. For the experiments shown in Figure 4 the limit of the dose-range was 8 mg kg^{-1}, as at this dose MLV-5860 produced respiratory arrest and all three rats died. MLV-6976 was more potent and longer lasting than tolperisone.

Since these oxazolidinones possess two asymmetric carbon atoms at the 4- and 5-position, four optically

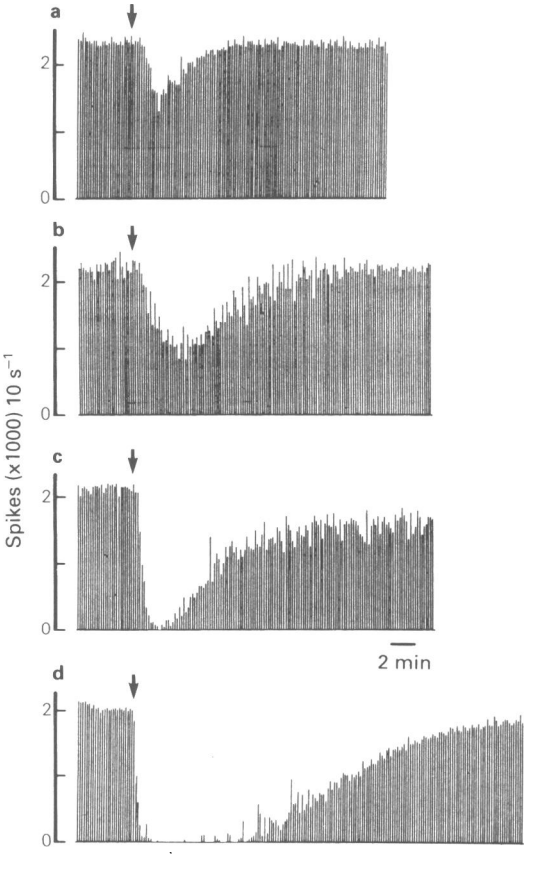

Figure 3 Dose-dependent effects of intravenously administered MLV-6976 (a) 0.5 mg kg^{-1}, (b) 1 mg kg^{-1}, (c) 2 mg kg^{-1}, (d) 4 mg kg^{-1} on rat decerebrate rigidity. The experiments were similar to those described in the legend to Figure 2.

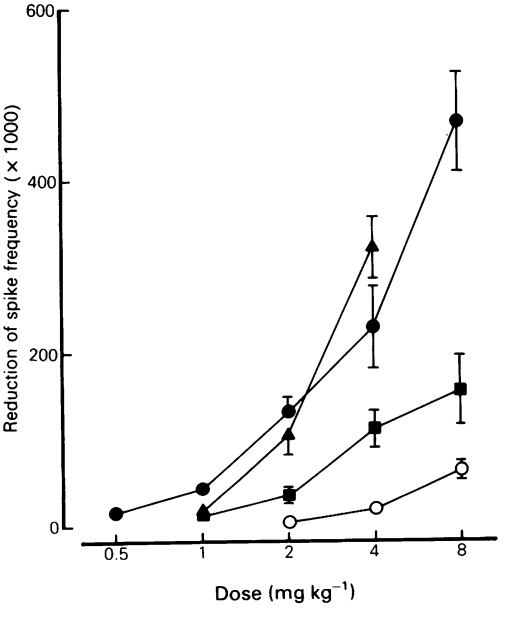

Figure 4 Dose-response curves for the reduction in rat decerebrate rigidity induced by the MLV compounds and tolperisone. Ordinates: responses expressed as a decrease in frequency of electromyographic potentials. Abscissa scale: log intravenous dose of test compound. Each point represents the mean value and the vertical lines indicate s.e.mean ($n = 6$). (●) MLV-6976; (▲) MLV-5860; (■) MLV-3076; (○) tolperisone.

Table 1 A comparison of the decrease in frequencies of electromyographic potentials caused by isomers of MLV compounds

Compound	*Configuration*	*Dose* (mg kg^{-1})	n	*Response* (×1000)	LD_{50} (mg kg^{-1})
Group A					
MLV-6976	(4S,5R)	2	6	132.2 ± 18.6	38.0
MLV-6976D	(4R,5S)	2	6	64.0 ± 14.2[a]	32.9
MLV-6977	(4S,5S)	2	6	95.9 ± 6.8	25.5
MLV-6977D	(4R,5R)	2	6	11.7 ± 2.2[b]	26.4
Group B					
MLV-3076	(4S,5R)	4	6	112.1 ± 21.7	49.1
MLV-3076D	(4R,5S)	4	6	39.0 ± 11.1[c]	42.4
MLV-3077	(4S,5S)	4	6	106.5 ± 10.9	30.6
MLV-3077D	(4R,5R)	4	6	13.5 ± 4.3[d]	42.4

Responses were expressed as decreases in frequency of electromyographic potentials recorded from the muscle of the forelimb (M. triceps brachii) of the decerebrate rat after the intravenous injection of MLV compounds. The doses were chosen by referring to the results shown in Figure 4 in order that the responses to MLV-6976 and MLV-3076 were at a similar level. Values shown are means ± s.e.mean. LD_{50} values were determined by the up-and-down method from ten animals when samples were administered intravenously. [a]$P < 0.05$ and [b]$P < 0.01$, compared with the response to MLV-6976. [c]$P < 0.05$ and [d]$P < 0.01$, compared with the response to MLV-3076. MLV-6979D, MLV-6977 and MLV-6977D in group A are optically active stereoisomers of MLV-6976. MLV-3076D, MLV-3077 and MLV-3077D in group B are optically active stereoisomers of MLV-3076.

active stereoisomers exist; (4S,5R), (4R,5S), (4S,5S) and (4R, 5R). The first and the second correspond to the geometric *cis* isomer and the third and the last to the *trans* isomer. It is of great interest to examine the relationship between the configuration of the isomers and their activities on the rigidity. Table 1 shows a comparison of the decrease in frequencies of electromyographic potentials caused by two representatives powerful series, MLV-6976 and MLV-3076, and their isomers. In both series, the optical isomers in which the absolute configuration at the 4-position was (S) demonstrated more potent effects than the corresponding (4R)-isomers, while there was no significant difference in their LD_{50} values.

The blood pressure was transiently reduced by MLV compounds in a dose-dependent manner. Since the anaemic decerebrate rigidity is very sensitive to changes in blood pressure, the relationship between the reduction of the rigidity and the blood pressure changes induced by MLV-6976 was examined. Within 1 min after the injection of MLV-6976 at a dose of 4 mg kg^{-1}, the blood pressure changed from a control level of 138 ± 9 mmHg to a minimum level of 98 ± 9 mmHg ($n = 6$) but it rapidly returned to the control level within 1–2 min, while the rigidity was still reduced significantly at that time. Thus, the time courses of the reduction of the rigidity and the blood pressure did not coincide. The reduction of blood pressure caused by MLV-6976 was smaller and more transient than that caused by tolperisone at the same dose, while the former reduced the rigidity more markedly.

Behavioural effects in mice and rats

When MLV-6976 was injected intraperitoneally to the normal animal at doses of less than 25 mg kg^{-1}, which were enough to reduce the rat rigidity, behavioural signs were not different from those of the control group. Table 2 shows the effect of MLV-6976 on spontaneous locomotor activity. Other inhibitory actions on the CNS were not observed in rats at the doses which showed a significant reduction of the rigidity. Impairment of motor coordination was observed at extremely high doses only. Thus, at 50 mg kg^{-1} i.p. which is near the minimum lethal dose, some mice showed convulsive behaviour and severe respiratory depression. High doses (greater than 30 mg kg^{-1} i.p.) of MLV-6976 produced a transient and dose-dependent sedation in almost all mice about 5 min after its administration.

Antagonism of drug-induced tremor in mice

Mice receiving tremorine at a subcutaneous dose of 70 mg kg^{-1} demonstrated severe tremor, diminished normal locomotor activity, and profuse salivation, lachrymation and diarrhoea. The results were quite similar to those described previously (Shinozaki *et al.*, 1985; Shinozaki & Hirate, 1986). Gross observations of the test groups receiving MLV-6976, revealed that neither the tremor nor the other effects induced by tremorine seemed to be reduced. In addition, harmaline-induced tremor did not appear to be reduced by MLV-6976. However, since it is possible to analyse

Table 2 The effect of MLV-6976 on the spontaneous locomotor activity of the rat

	Dose (mg kg^{-1})	*Cumulative counts* 10 min	20 min	30 min	40 min
Control	–	455 ± 32	847 ± 47	1231 ± 88	1617 ± 139
MLV-6976	25	517 ± 30	994 ± 69	1421 ± 115	1817 ± 127
Diazepam	4	129 ± 15	172 ± 23	223 ± 45	267 ± 56
Chlorpromazine	4	240 ± 64	298 ± 77	333 ± 73	357 ± 82

The spontaneous locomotor activity was measured in 4 rats with the Animex counter. Drugs were given intraperitoneally to the rat immediately before the measurement of the locomotor activity, and the counts were determined cumulatively every 10 min. Diazepam and chlorpromazine showed a statistically significant difference from the control ($P < 0.001$) at all determinations, but there was no difference between the control and the MLV-6976 group.

quantitatively the severity of the tremor in the experimental animal by using the power spectral method (Shinozaki, 1984; Shinozaki *et al.*, 1985), the severity of drug-induced tremor was quantitatively determined (see Methods). The power spectral data fitted the theoretical curve well regardless of the presence or absence of MLV-6976, although the magnitude of the power spectral density varied in each animal. The mean square values of the tremor component (the severity of tremor) varied depending upon doses of tremorogenic agents. Figure 5 shows the time course of the sum of mean square values of the tremor component after the subcutaneous administration of tremorine and harmaline in the presence of MLV-6976 (5 and 10 mg kg^{-1}). Harmaline-induced tremor was reduced by MLV-6976 in a dose-dependent manner, on the other hand, its tremor frequency was not affected (control: 14.0 ± 0.2 Hz ($n = 5$) and treatment (10 mg kg^{-1} i.p.): 14.2 ± 0.2 ($n = 5$)). The ability of MLV-6976 to reduce the harmaline-induced tremor was significantly weaker than that of other established anti-tremor agents (Shinozaki *et al.*, 1985). Also the tremorine-induced tremor was not reduced by MLV-6976 at doses less than 10 mg kg^{-1} and its onset was to some extent enhanced by doses greater than 10 mg kg^{-1}. During the enhancement of this tremorine effect another tremor component with a different frequency appeared.

Spike discharges of single cortical neurones in rats

The effects of iontophoretically applied MLV-6976 on responses to glutamate were examined on 42 neurones in the cerebral cortex. MLV-6976 significantly reduced glutamate-induced excitation of rat cortical neurones on 39 cells studied in a dose-dependent manner (Figure 6). Spontaneous firing of the rat cerebral neurone was sometimes gradually depressed by iontophoretic application of MLV-6976. The drug also reduced significantly excitations evoked by kainate in 7 of 8 cells, quisqualate in 7 of 7 cells, NMDA in

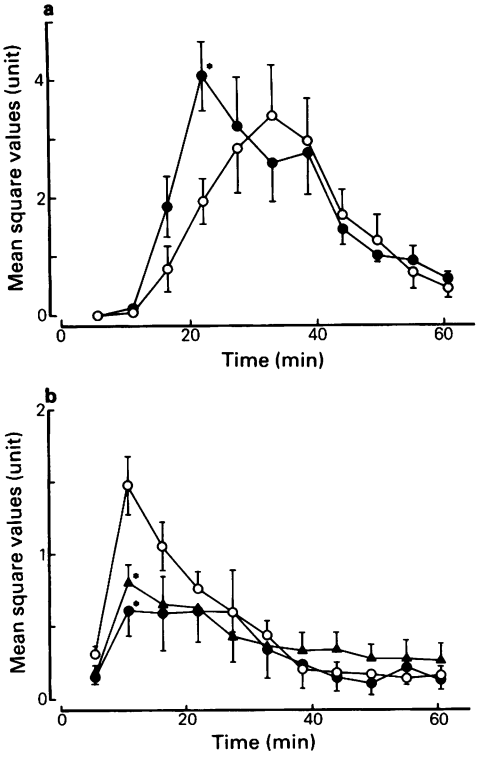

Figure 5 The time courses of (a) tremorine- and (b) harmaline-induced tremors in the absence and presence of MLV-6976. The severity of the tremor was determined in terms of the mean square value of the total area under the calculated power spectrum curve. Tremorine and harmaline were administered at doses of 70 mg kg^{-1} and 60 mg kg^{-1}, respectively. Vertical lines show s.e.mean. The number of animals was 5 in each group. Ordinate scales: the sum of the mean square value in the tremor component. Abscissa scales: time after injection of a tremorogenic agent (min). (○) Control; MLV-6976 (▲) 5 mg kg^{-1}, (●) 10 mg kg^{-1}. *$P < 0.01$ in (a) and $P < 0.05$ in (b).

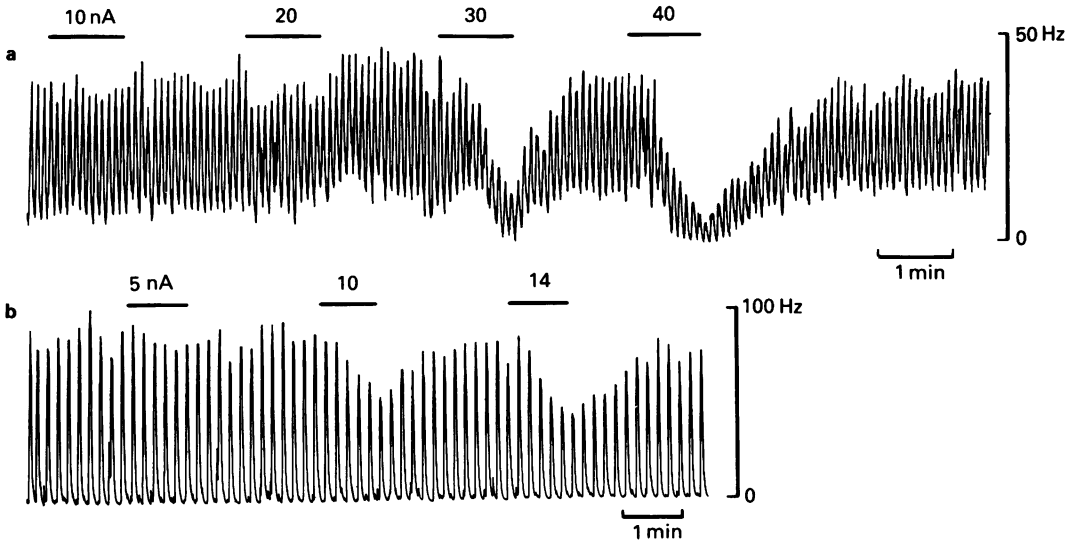

Figure 6 Dose-dependent reduction of glutamate-induced spike discharges. The two ratemeter records, which were derived from different neurones, illustrate excitatory responses to L-glutamate, (a) 24 nA, every 5 s for a period of 2 s; (b) 21 nA, every 10 s for 2 s, in each rat cortical neurone. The numbers in the figure represent the current in nA of MLV-6976 which was applied iontophoretically for the period indicated.

5 of 5 cells and acetylcholine (ACh) in 5 of 7 cells, but the effects of MLV-6976 on these different excitants were not tested on the same cells. Responses to ACh and NMDA seemed to be less reduced by MLV-6976 than those to glutamate, but this analysis is not yet complete. Recovery from drug action was relatively rapid. When MLV-6976 was administered 5 s before the application of glutamate, the firing rate gradually declined after the rate had attained a level similar to the control, suggesting that the inhibitory action of MLV-6976 was activity-dependent. The details of the electrophysiological experiments will appear elsewhere.

Spinal reflex potentials in cats

Since the muscle tone depends on the activity of the motoneurones and many muscle relaxants reduce the spinal reflex potentials, in particular, polysynaptic reflexes, the action of MLV-6976 on spinal reflex potentials was examined in the spinal cat. MLV-6976 hardly affected such spinal reflexes, when administered intravenously at doses of 1, 2, 4, and 8 mg kg^{-1}. Other MLV compounds such as MLV-5860 (5 mg kg^{-1}, i.v.), MLV-3076 (5 and 8 mg kg^{-1}, i.v.) and MLV-3077 (5 mg kg^{-1}, i.v.) had no effect on the cat spinal reflex.

Endplate responses

Intra-arterial injection of MLV-6976 to the descending aorta of the rat at a dose of 8 mg kg^{-1} did not cause any change of the twitch tension of the gastrocnemius muscle induced by the nerve stimulation. On the other hand, tubocurarine chloride (0.1 mg kg^{-1}, i.a.) abolished completely the amplitude of twitch responses.

Muscle twitch of the rat diaphragm *in vitro* induced by direct or nerve stimulation was reduced in a dose-dependent manner but was not affected at concentrations less than 0.1 mM. At a concentration of 0.1 mM, the amplitude of the twitch response induced by nerve stimulation was slightly reduced but that induced by direct stimulation was not affected. At a concentration of 0.3 mM, the amplitude of the twitch responses induced by repetitive stimulation of the nerve fibre was abolished completely within 10 min after application of MLV-6976. When the preparation was immersed in the solution containing MLV-6976 at concentrations greater than 0.3 mM, the twitch tension induced by direct stimulation of the muscle was gradually reduced and finally abolished within 30 min but it recovered and returned to the control level after washing the preparation for more than 1 h.

MLV-6976 did not affect or very slightly reduced the amplitude of spontaneous m.e.p.ps at a concentration of 0.1 mM ($n = 4$), which did not affect the resting membrane potential of the muscle. The values of the mean amplitude of spontaneous m.e.p.ps were lowered as the concentration of MLV-6976 increased. Histograms of the amplitude distribution of the spontaneous m.e.p.ps demonstrated that the mean amplitude of the spontaneous m.e.p.p. was reduced to

75% of the control 7 min after the application of 0.3 mM MLV-6976. The frequency of spontaneous m.e.p.ps was not affected by MLV-6976 at concentrations less than 0.1 mM, but it was inclined to increase as the concentration of MLV-6976 was increased.

Discussion

MLV-6976 markedly reduced rat anaemic decerebrate extensor rigidity in a dose-dependent manner, and to a lesser extent harmaline-induced tremor. Harmaline-induced tremor can be effectively controlled by a variety of drugs (Hara & Kawamori, 1954; Zetler, 1957; Tseng & Wang, 1971; Kelly & Naylor, 1974; Guidotti *et al.*, 1975; Robertson, 1980) including anti-parkinsonian drugs, ganglion blockers, sedatives, ataraxics, benzodiazepines, antiadrenergic drugs, anticonvulsants, 5-hydroxytryptamine and its antagonists. Most of these compounds also reduce tremorine-induced tremor, but MLV-6976 did not reduce the tremorine-induced tremor but rather exaggerated it. It has been suggested that glutamate, which is believed to be an excitatory neurotransmitter in the CNS, is involved in the harmaline-induced tremor (Guidotti *et al.*, 1975). In the present study iontophoretically applied MLV-6976 reduced the glutamate-induced firing of rat cerebral cortical neurones in a dose-dependent manner, and this inhibitory action was produced by a relatively low iontophoretic current for drug application, although ACh responses were also reduced to a lesser extent by this drug. In the new-born rat spinal cord preparation, the glutamate-induced depolarizing response was more effectively reduced by MLV-6976 than the ACh-induced one (Shinozaki *et al.*, unpublished observation). MLV compounds have also been found to reduce glutamate-induced responses at the crayfish neuromuscular junction (Masaki *et al.*, 1985; Shinozaki & Ishida, 1986). The neuromuscular system of the crayfish has some properties in common with mammalian central synapses, and provides us with an insight into the chemical transmission processes at many synapses as it can be used as a model for studying the mechanism of action of drugs on synaptic transmission in the mammalian CNS (Katz, 1966; Shinozaki, 1980). MLV-5860 is one of the most potent blockers of glutamate at the crayfish neuromuscular junction where glutamate is believed to be the transmitter (Shinozaki & Ishida, 1986). Other MLV compounds also reduced responses to glutamate. The mechanism of action of MLV-5860 at the crayfish neuromuscular junction appears to be due to the block of the glutamate channel, rather than competitive antagonism at glutamate receptors (Shinozaki & Ishida, 1986). In addition to their glutamate blocking action, MLV compounds inhibited the cholinergic response in the rat diaphragm preparation but at high concentrations. The threshold concentration to reduce the cholinergic response at the rat diaphragm endplate was about 0.1 mM, not dissimilar to concentrations of other open channel blockers at an ACh-gated channel (Adams, 1976; Shinozaki & Ishida, 1984), but more than 10 times higher than that required to reduce the glutamate response at the crayfish neuromuscular junction.

The spontaneous locomotor activity of the rat was not affected by MLV-6976, which suggests that MLV-6976 has little or no tranquillizing effect. In the *in situ* experiment, MLV-6976 did not cause any change of the twitch tension induced by nerve stimulation, when administered intra-arterially at a dose of 8 mg kg^{-1}, which completely reduced the electromyographic activity in the decerebrate rat. MLV compounds reduced to some extent the severity of nystagmus induced by electrical stimulation of lateral geniculate body in the rabbit, but exhibited hardly any anticonvulsant or analgesic properties (Shinozaki *et al.*, unpublished observations). Centrally acting muscle relaxants, in general, affect spinal polysynaptic reflexes. New type muscle relaxants, such as tolperisone, baclofen and afloqualone, depress both the mono- and poly-synaptic reflex potentials (Davidoff & Sears, 1974; Ochiai & Ishida, 1982). On the other hand, MLV compounds, which markedly reduced the severity of the rat decerebrate rigidity, hardly affected spinal reflex potentials. In view of this and because the actions of the MLV compounds on the endplate and muscle were extremely weak, it is thought that MLV compounds act on the brainstem and/or higher levels of the brain rather than on the spinal cord to reduce the muscle tone. It seems probable that MLV compounds act on the CNS in a manner different from that of other central muscle relaxants, and further experiments need to be undertaken to characterize the neuropharmacological profile of MLV-6976.

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