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Mephenesin, methocarbamol, chlordiazepoxide and diazepam: actions on spinal reflexes and ventral root potentials

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Summary

1. Dose levels of mephenesin, methocarbamol, chlordiazepoxide and diazepam which abolished polysynaptic reflex contractions had no effect on monosynaptic knee-jerk reflexes in chloralose anaesthetized cats.

2. Ventral root potentials were recorded following stimulation of the corresponding dorsal root (L7 or S1), and the areas of the mono- and polysynaptic components were measured by planimetry.

3. Dose levels of the drugs which abolished polysynaptic reflex contractions reduced the areas of the polysynaptic component of the ventral root potentials by about 50%. Mephenesin and methocarbamol reduced the area of the monosynaptic component to a similar extent. Chlordiazepoxide was less potent in this respect while diazepam was without effect at this dose level.

4. Linear regression lines were calculated for the reduction in the mono- and polysynaptic components of ventral root potentials with increasing doses of each of the four drugs. With methocarbamol and mephenesin the lines were parallel and coincident. With chlordiazepoxide and diazepam they were parallel but not coincident. Large doses of diazepam were required to reduce the area of the monosynaptic component, this drug being the only one of the four tested to have a differential action on the two components which was statistically significant.

5. The results are discussed in terms of depressant actions of the drugs on α-motorneurones, effects of the drugs at higher centres concerned with motor function, and the lack of evidence that spinal interneurones represent a specific site of action for centrally acting skeletal muscle relaxants.

Introduction

Mephenesin (Berger & Bradley, 1946) may be regarded as the forerunner of the class of drugs known as centrally acting skeletal muscle relaxants. Berger (1947) suggested that mephenesin acted predominantly on the spinal cord to produce skeletal muscle relaxation. After more detailed experimentation, Henneman, Kaplan & Unna (1949) suggested that mephenesin had a specific action on spinal interneurones because polysynaptic reflex contractions were abolished while monosynaptic knee-jerk reflexes were unaffected. The capacity of mephenesin to abolish

polysynaptic reflex contractions at dose levels which had little or no effect on monosynaptic reflex contractions was confirmed by Kaada (1950) and extended to many other drugs which produce muscle relaxation by a central action (see Domino, 1956, and Smith, 1965, for references). Smith (1965), however, has rightly stated that there is little direct evidence for specific actions of mephenesin and other drugs of this class on spinal interneurones.

Rosenberg & Cooke (1967) have shown that mephenesin prolongs the refractory period of skeletal muscle. We have shown (Crankshaw & Raper, 1968) that both mephenesin and methocarbamol produce this effect by a direct action on skeletal muscle fibres. This action may be of significance in the relief of muscle spasm associated with high frequency repetitive discharges. It was suggested that these changes in excitability might be of significance at other sites involved in the genesis of muscle contraction, such as the spinal interneurones or the α-motorneurones. In these experiments the muscle relaxant diazepam was found to be without peripheral action on skeletal muscle.

Little quantitative work has been performed on the effect of centrally acting muscle relaxants on ventral root potentials. Henneman *et al.* (1949) have stated that mephenesin reduced the polysynaptic component of the ventral root potential while leaving the monosynaptic component unaffected. Kaada (1950), on the other hand, has stated that this differential effect on the two components was not absolute, and noted that the monosynaptic spike, as well as the polysynaptic component of the ventral root potential, was reduced with higher doses of mephenesin. No supporting quantitative data were given by either author.

In the present paper we have studied the effects of four drugs, mephenesin, methocarbamol, chlordiazepoxide and diazepam, on the amplitude of ventral root potentials in the spinal cord of the anaesthetized cat.

Methods

Experiments were performed on adult cats anaesthetized by the intraperitoneal injection of α-chloralose (100 mg/kg). The trachea was cannulated and the animals respired, keeping the end tidal pCO_2 within the range 32–38 mm Hg. Arterial blood pressure was monitored continuously from the cannulated right carotid artery, using a Statham p23Db transducer coupled to a pen-recorder (Offner Dynograph 504A). The body temperature of the cat was monitored continuously and maintained between 37° and 38° C by an electric heating pad. Drugs were administered by slow intravenous injection into a cannulated forelimb vein. Experiments were performed only in cats where the systolic blood pressure remained consistently above 100 mm Hg.

In twelve animals reflex contractions were elicited. The animals were placed in a supine position and monosynaptic stretch reflexes were elicited in one leg by striking the patellar tendon with an automatic knee-jerk hammer (C. F. Palmer) at a frequency of 0·1 Hz. Reflex contractions of the contralateral tibialis anterior muscle were elicited at a frequency of 0·1 Hz by stimulation of the exposed ipsilateral femoral nerve with square wave pulses of 1 ms duration and an intensity sufficient to cause a maximal withdrawal response. The reflex contractions were recorded on a smoked drum using flat spring myographs.

In twelve animals ventral root potentials were recorded. A dorsal laminectomy was performed to expose both the lumbar portion of the spinal cord and the cauda equina. Either the L7 or S1 dorsal and ventral nerve roots were dissected free on one side of the spinal cord and cut distally. The cat was fixed in a rigid frame and the exposed spinal cord was covered by a pool of liquid paraffin maintained at 37° C. A bipolar platinum stimulating electrode was placed on either the L7 or S1 dorsal nerve root. Continuous stimulation was applied for the duration of the experiment using square wave pulses of 50 μs duration. These were passed through a stimulus isolation unit (A.E.L.104A) at a frequency of 1 Hz, the voltage used being twice that required to produce a maximal polysynaptic discharge (4–6 V). Ventral root potentials were recorded using a bipolar platinum electrode placed on the corresponding ventral root. After amplification (Tektronix 122 preamplifier) the potentials were recorded photographically from an oscilloscope (Tektronix 502A).

The drugs used were mephenesin (Sigma), methocarbamol (A. H. Robins), chlordiazepoxide and diazepam (Roche). Mephenesin and methocarbamol were dissolved in 50% v/v polyethylene glycol 300 (Shell) in distilled water to produce a 100 mg/ml. solution. Chlordiazepoxide, which is unstable in aqueous solution (Randall, 1961), was dissolved in distilled water immediately before administration. Diazepam was dissolved in a mixture of glycofurol (Roche), benzyl alcohol and ethyl alcohol of the same composition as that used in the commercially available injectable preparation (Valium, Roche). Solutions were diluted in a suitable volume of isotonic sodium chloride solution before injection. Control experiments showed that solvent effects were not significant at the highest dose levels of the drugs used in these experiments.

Results

Reflex contractions

Polysynaptic flexor reflexes and monosynaptic knee-jerk reflexes were elicited during the cumulative administration of mephenesin (5–155 mg/kg), methocarbamol (5–155 mg/kg), chlordiazepoxide (0·4–100 mg/kg) and diazepam (5–315 μg/kg). Doses of the drugs were given intravenously at 10 min intervals and measurements were taken 4 min after each dose was administered.

All four drugs reduced and finally abolished flexor reflex contractions at dose levels which had little or no effect on knee-jerk reflexes. Effects of mephenesin and diazepam on reflex contractions are shown in Figs. 1 and 2. With all the drugs used the maximum effect produced at each dose level was reached within 2–3 min

TABLE 1. *Doses of the drugs (mg/kg) required to reduce flexor reflex contractions by* 95% *and the areas of the mono- and polysynaptic components of the ventral root potential by* 50%

	Reflex contractions		Ventral root potentials	
	Flexor reflex (95%)	Knee-jerk	Monosynaptic component (50%)	Polysynaptic component (50%)
Mephenesin	80 (mg/kg)	—	70 (mg/kg)	70 (mg/kg)
Methocarbamol	100	—	70	100
Chlordiazepoxide	10	—	70	10
Diazepam	0·10	—	5	0·16

At dose levels which produced a 95% reduction of the flexor contractions, knee-jerk reflexes were unaffected.

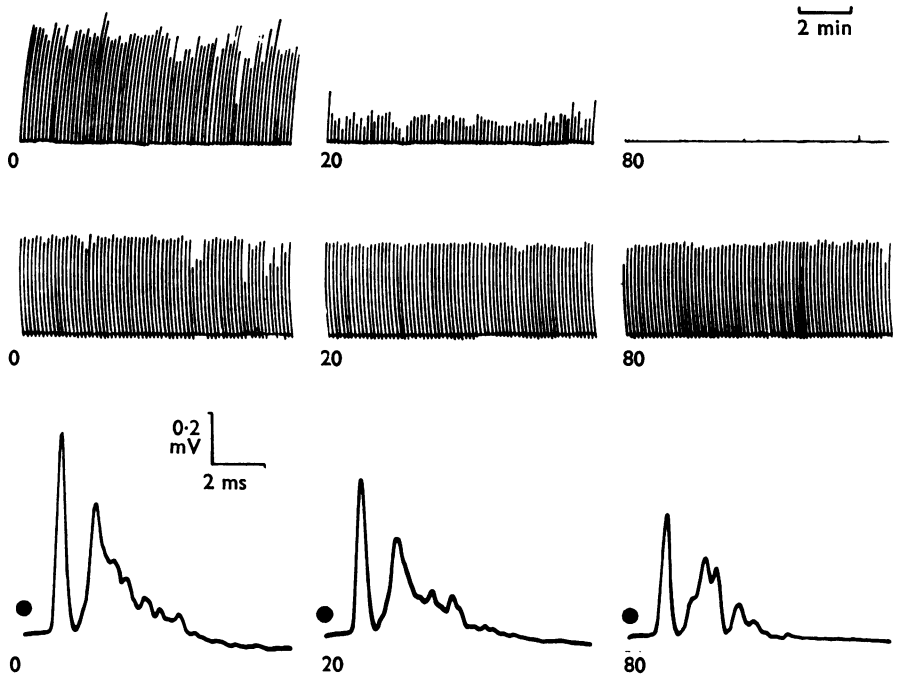

FIG. 1. Upper and middle records show kymograph tracings of the flexor reflex and the knee jerk reflex elicited at a frequency of 0·1 Hz in a chloralose anaesthetized cat before and after the intravenous injection of mephenesin at the dose levels indicated (mg/kg). Lower record, ventral root potentials elicited by stimulation of the corresponding dorsal root in a chloralose anaesthetized cat before and after intravenous injections of mephenesin at the dose levels indicated (mg/kg).

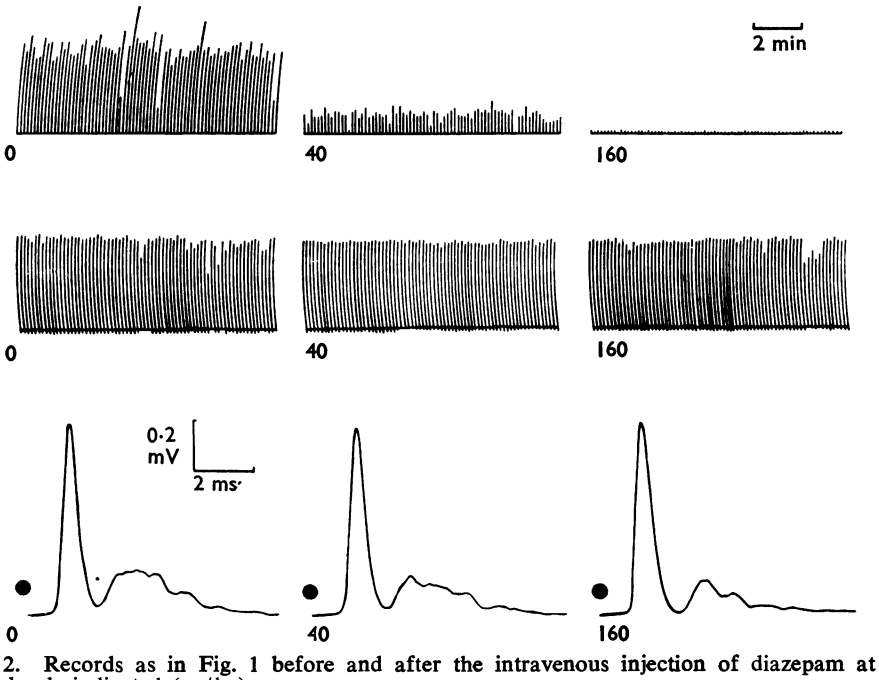

FIG. 2. Records as in Fig. 1 before and after the intravenous injection of diazepam at the dose levels indicated (μg/kg).

of injection and thereafter the effect stabilized until the next dose in the series was administered. The mean dose of each drug required to produce a 95% reduction in the flexor contractions was obtained from dose/effect curves, and these are shown in Table 1.

Ventral root potentials

Recordings of ventral root potentials elicited by stimulation of the corresponding dorsal roots were monitored continuously during the cumulative administration of mephenesin (5–155 mg/kg), methocarbamol (5–155 mg/kg), chlordiazepoxide (0·4–100 mg/kg) and diazepam (5 μg–2·5 mg/kg). Three experiments were performed with each drug and the dose cycle used was the same as that described for the experiments where reflex contractions were recorded.

Ventral root potentials (Figs. 1 and 2) consist of a monosynaptic spike of short latency (1–2 ms), followed by a polysynaptic component, composed of a more prolonged asynchronous discharge (Patton, 1965). In our experiments the areas under the two components of the ventral root potentials were measured by planimetry.

Mephenesin (Fig. 1), diazepam (Fig. 2), chlordiazepoxide and methocarbamol all reduced the polysynaptic component of the ventral root potential. At the dose levels of the drugs which abolished polysynaptic reflex contractions in the previous experiments, however, the areas of the polysynaptic components of the ventral root potentials were only reduced to approximately half the control values (Table 1).

Similar dose levels of mephenesin (Fig. 1) and methocarbamol, which had been noted to have no effect on knee-jerk reflexes, reduced the area of the monosynaptic component of the ventral root potential to approximately half. Chlordiazepoxide was less potent than mephenesin and methocarbamol in this respect while diazepam was without effect on the monosynaptic potential at dose levels that markedly reduced the polysynaptic component (Fig. 2, Table 1).

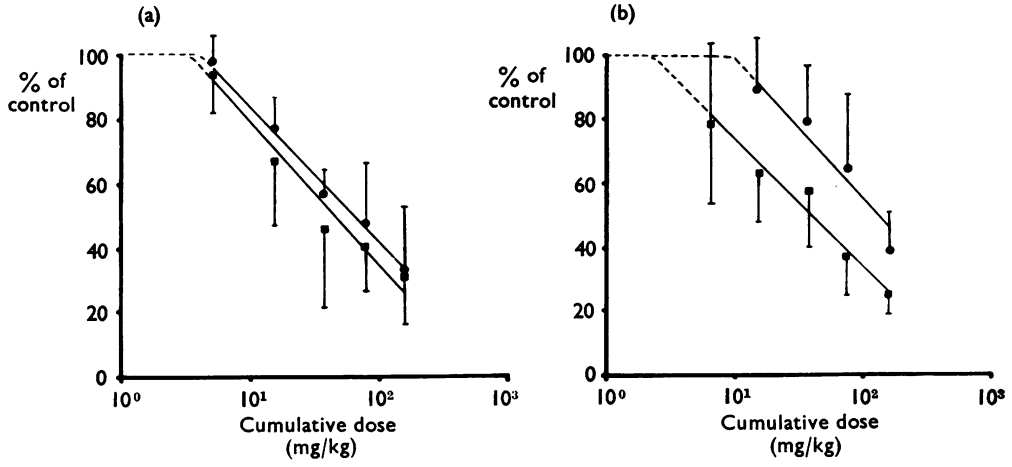

FIG. 3. Changes in the areas of the monosynaptic and polysynaptic components of the ventral root potentials produced by cumulative doses of mephenesin (a) and methocarbamol (b). Solid lines represent the calculated regression lines. Individual points indicate the mean (±S.E.) of the changes in area of the monosynaptic (■) and polysynaptic (●) components expressed as a percentage of control values.

The effect of increasing doses of mephenesin, methocarbamol, chlordiazepoxide and diazepam on the areas of the monosynaptic and polysynaptic components of the ventral root potential are shown in Figs. 3 and 4. In each experiment the relevant area was measured from five observations at each dose level and a mean calculated. Each point represents the mean (±S.E.) of the respective areas under the ventral root potential from three animals with each drug, expressed as a percentage of control values (Figs. 3 and 4). Linear regression equations for the dose response curves were calculated for each drug and are shown in Table 2, together with a statistical evaluation of the results. For each drug, the lines representing the change in area of the monosynaptic and polysynaptic components of the ventral root potential with increasing dose were tested for linearity and coincidence.

With mephenesin and methocarbamol (Fig. 3, Table 2) there was no significant difference in either the slope or the position of the regression lines representing the monosynaptic and the polysynaptic components of the ventral root potential. Similar results were obtained with chlordiazepoxide (Fig. 4, Table 2); however, the effect was not as marked. Diazepam (Fig. 4, Table 2) was the only drug which showed a differential action which was significant. In doses of 5–155 μg/kg, diazepam reduced the area of the polysynaptic component of the ventral root potential without affecting the area of the monosynaptic component. At higher dose levels (0·2–2·5 mg/kg) the monosynaptic spike was reduced in amplitude. The slope of the regression line for the monosynaptic component at these higher dose levels was not significantly different from that calculated for the polysynaptic component of the ventral root potential, but there was a significant difference in the position of the lines (Table 2).

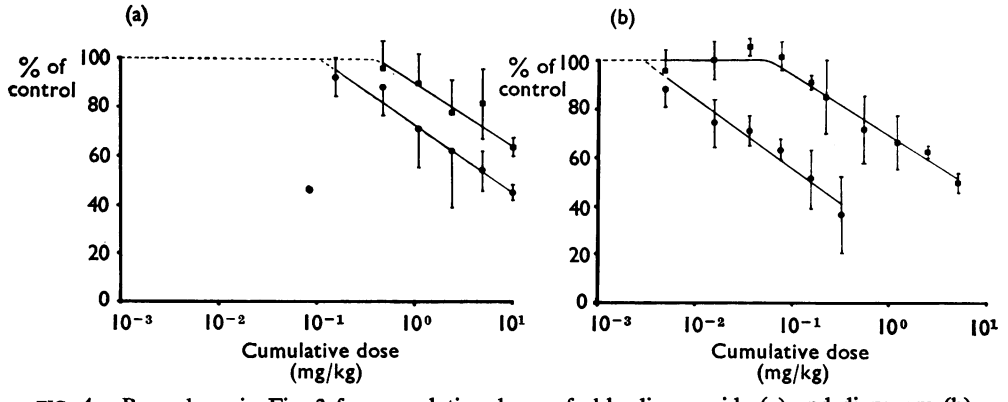

FIG. 4. Records as in Fig. 3 for cumulative doses of chlordiazepoxide (a) and diazepam (b).

TABLE 2. *Linear regression equations for the changes in the area of the monosynaptic and polysynaptic component of the ventral root potential at different doses of each drug*

	Linear regression equation		Comparison of regression lines (*m* : *p*)			
	Monosynaptic component (*m*)	Polysynaptic component (*p*)	Parallelism ($P>0.05$)	Coincidence ($P>0.05$)	Differential sensitivity (*m* : *p*)	Significance ($P>0.05$)
Mephenesin	$y=128-41.5x$	$y=116-40.4x$	Yes	Yes	0·55	No
Methocarbamol	$y=126-35.0x$	$y=114-42.3x$	Yes	Yes	0·25	No
Chlordiazepoxide	$y=73-29.0x$	$y=91-23.7x$	Yes	No	5·4	No
Diazepam	$y=144-24.9x$	$y=108-26.7x$	Yes	No	33	Yes

The lines were tested for parallelism, coincidence and significant difference in position as shown. All lines were tested and found to be linear ($P>0.05$).

Discussion

Mephenesin, methocarbamol, chlordiazepoxide and diazepam displayed a specificity of action in abolishing polysynaptic reflex contractions at dose levels which had no effect on the monosynaptic knee-jerk reflex. The ability of these drugs to abolish polysynaptic reflex contractions at dose levels which leave the monosynaptic stretch reflexes unaffected confirm the findings of other workers who have used mephenesin (Henneman *et al.*, 1949), methocarbamol (Truitt & Little, 1958), chlordiazepoxide (Randall, Schallek, Heise, Keith & Bagdon, 1960) and diazepam (Randall, Heise, Schallek, Bagdon, Banziger, Boris, Moe & Abrams, 1961). This differential action was evident in the recorded ventral root potentials in the case of diazepam, but was not apparent in the case of mephenesin and methocarbamol. Results obtained with chlordiazepoxide were intermediate between the two groups.

An anomaly exists in the case of methocarbamol and mephenesin, where dose levels of the drugs which are without effect on the knee-jerk reflex cause a significant decrease in the area on the monosynaptic component of the ventral root potential. Methocarbamol has been shown to be devoid of a local anaesthetic action both *in vivo* and *in vitro* (Crankshaw & Raper, 1968). Mephenesin has a local anaesthetic action *in vitro* (Crankshaw & Raper, 1968), and also following local infiltration (Feinstein & Libet, 1953), but has insignificant local anaesthetic action *in vivo* at the dose levels used in these experiments (Rosenberg & Cooke, 1967 ; Crankshaw & Raper, 1968). A local anaesthetic action at the site of the recording electrode can therefore be excluded. The reduction in the nerve action potential must represent a decrease in the number of actively conducting nerve fibres. This in turn must indicate a decrease in the number of α-motoneurones discharging in response to the applied stimulus (Patton, 1965). Whether these changes represent effects on prejunctional excitatory processes or changes in the electrical excitability and membrane characteristics of the α-motoneurone itself is uncertain.

There is conclusive evidence that the knee-jerk reflex and the short latency spike recorded from the ventral root when stimulating the dorsal root are both monosynaptic systems (Lloyd, 1943 ; Renshaw, 1940). In the electrically elicited response, motoneurones are bombarded by a synchronous stimulus. The knee-jerk reflex, however, involves stretch receptors which produce brief but asynchronous stimulation of the α-motoneurones. This asynchronous afferent input certainly results in facilitation of the α-motoneurones (Lloyd, 1946). It is possible that this facilitation, occurring during the knee-jerk reflex, counteracts the drug induced depression of α-motoneurone firing, an effect which is unmasked when the monosynaptic pathway is stimulated by a single synchronous stimulus.

A single α-motoneurone is influenced by many interneurones (Lorente de Nó, 1938), and the polysynaptic component of the ventral root potential represents α-motoneurone firing in response to stimulation from these interneurones. The polysynaptic component of the ventral root potential occurs between 4 and 10 ms after application of the stimulus at a time when facilitation is well established (Lloyd, 1946). In this case, as in the physiological withdrawal reflex, interneurones provide stochastic stimulation of the α-motoneurones.

The significant parallelism and coincidence of the regression lines for the reduction of the mono- and polysynaptic components of the ventral root potential suggests a common site of action on the α-motoneurone. The maximal rate of firing of an

α-motoneurone in response to the convergent stimuli from interneurones will depend on the nature of the stimuli and the responsiveness of the α-motoneurone itself. It is possible that small changes in α-motoneurone excitability would be of greater importance in the rate of firing of an α-motoneurone than similar changes in the many interneurones converging on this cell. The explanation of the differing effects of mephenesin and methocarbamol on the mono- and polysynaptic reflexes might therefore lie in the characteristics of the reflex pathway involved; an important factor being the temporal as well as the spatial pattern of the mono- and polysynaptic reflex rather than a differential action on specific nerve cells within the spinal cord.

Mephenesin and methocarbamol have both been shown to prolong the refractory period of skeletal muscle, within the dose ranges required to abolish the flexor reflex (Crankshaw & Raper, 1968). As the withdrawal reflex necessitates repetitive firing of the skeletal muscle, an impairment of its ability to respond to this type of stimulation may result in a diminution of the withdrawal reflex and leave the knee-jerk reflex unaffected. It should be noted that the reduction in the ventral root potentials was at no stage comparable with the reduction in the withdrawal reflex at the same dose level. This suggests that the peripheral actions of mephenesin and methocarbamol may be of significance in the abolition of the flexor reflex.

In the case of the two benzodiazepines, diazepam and chlordiazepoxide, the position of the regression lines for the effects on the mono- and polysynaptic components of the ventral root potential revealed a degree of specificity of action. The selective action of diazepam was highly significant. Diazepam has been shown to be without action on peripheral nerve or skeletal muscle (Crankshaw & Raper, 1968). At dose levels which abolish the flexor reflex, depression of the α-motoneurone and other components of the monosynaptic reflex was not apparent in our experiments. This specificity, in which there is no apparent change in the excitability of α-motoneurones, must therefore be interpreted as an action on either spinal interneurones, supraspinal centres or both.

Chlordiazepoxide, at dose levels that reduce the polysynaptic component of the ventral root potential, produces some depression of α-motoneurones, as judged from the reduction in the monosynaptic component of the ventral root potential. Ngai, Tseng & Wang (1966) have shown that, after spinal transection, the ability of chlordiazepoxide and diazepam to abolish the flexor reflex is impaired, demonstrating that the actions of these drugs at supraspinal levels plays a role in their ability to block reflex contractions.

The experiments described in this paper indicate that the ability of a drug to abolish polysynaptic reflexes while leaving monosynaptic reflexes unimpaired does not necessarily imply a specific action on spinal interneurones.

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