The Effect of Diazepam on Presynaptic Inhibition in Patients with Complete and Incomplete Spinal Cord Lesions

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SUMMARY: *The effect of diazepam on presynaptic inhibition in man has been examined in 5 patients with complete spinal transections and 7 patients with incomplete lesions. The inhibition of the H reflex by vibration applied to the tendo Achilles was used to assess presynaptic inhibition of the Ia monosynaptic pathway. Diazepam increased this inhibition in the patients with incomplete lesions, but had no significant effect on the inhibition in the patients with complete spinal transections.*

Evidently diazepam can enhance presynaptic inhibition in man. The effect, however, cannot be demonstrated in patients with longstanding complete spinal lesions possibly because of some alteration in the segmental presynaptic inhibitory mechanism in this group.

RÉSUMÉ: *L'effet du diazepam sur l'inhibition présynaptique chez l'homme a été examiné chez 5 patients avec transections spinales complètes et chez 7 patients avec lésions incomplètes. L'inhibition du réflexe H par vibration appliquée au tendon d'Achille était utilisée comme mesure de l'inhibition présynaptique du faisceau Ia monosynaptique. Le diazepam augmentait cette inhibition chez les patients avec lésions incomplètes, mais n'avaient pas d'effet significatif sur l'inhibition chez les patients avec transections spinales complètes.*

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INTRODUCTION

Diazepam has been shown to reduce muscle tone in patients with motor disorders. The pertinent studies have been reviewed by Greenblatt and Shader (1974). How this effect is produced in man, however, remains unclear.

In the experimental animal the principal site of action of diazepam on the motor system may be the reticular formation. Thus diazepam reduces the excitability of segmental reflex pathways in the intact animal (Hudson and Wolpert, 1970; Nakanishi and Norris, 1971) and decerebrate animal (Tseng and Wang, 1971) but not in the spinal animal (Ngai et al., 1966; Hudson and Wolpert, 1970; Tseng and Wang, 1971) except in extremely high doses. Diazepam reduces the rate of firing of neurons in the reticular formation (Pryzbyla and Wang, 1968) and these neurons appear to be more sensitive to diazepam than certain spinal interneurons (Tseng and Wang, 1971). Finally, the diminished excitability of segmental reflex pathways produced by systemic diazepam can be achieved by injecting minute quantities of diazepam directly into the vertebral artery (Pryzbyla and Wang, 1968).

Studies in the acute spinal preparation have suggested an additional action on the spinal cord. Thus diazepam has been shown to increase primary afferent depolarization and to produce an augmentation of the dorsal root potential that can be antagonized by picrotoxin (Schmidt et al., 1967; Stratten and Barnes, 1971; Schlosser, 1971; Polc et al., 1974). These findings imply that diazepam is capable of enhancing presynaptic inhibition at segmental levels.

It has also been postulated that diazepam may have a direct action on the spinal cord in man.

Neill (1964) reported improvement in the clinical status of patients with complete spinal lesions receiving diazepam and Cook and Nathan (1967) observed clinical changes in patients with physiologically complete spinal lesions who were given diazepam 20 mg IV. Nathan (1970) later postulated that enhanced presynaptic inhibition at segmental level could be an important site of action of the drug in motor disorders in man.

The present study examines this hypothesis. Diazepam was administered to patients with complete spinal lesions and incomplete lesions of the nervous system. The inhibition of the H reflex by vibration was used as an estimate of presynaptic inhibition of the Ia monosynaptic pathway.

METHODS

Studies were carried out on 13 patients with neurological deficits resulting in spasticity. One study was rejected because of persistent clonus which interfered with the recording of the control data. In 7 patients the lesion was incomplete resulting from spinal trauma (1), transverse myelitis (1), and multiple sclerosis (5). The other 5 patients had complete, or virtually complete, spinal transection in each case resulting from trauma. In 4 of these there was loss of all sensory and motor function below the level of the lesion. In the 5th (study 10) there was complete motor paralysis and sensory

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loss except for a small area on the dorsum of one foot in which sensory stimuli could be vaguely perceived. If the patient had been taking diazepam to control spasticity it was discontinued 3 days prior to the study.

Studies were performed with the subject lying prone. The leg to be examined was immobilized in a padded frame with padded clamps gripping the malleoli. The sole of the foot rested against a foot board pivoted at the level of the malleoli. The ankle was fixed as close to 90° as possible.

Square wave electrical stimuli, 1 ms duration, generated by a Grass S 88 stimulator equipped with a 1:4 step-up transformer were delivered to the popliteal nerve in the popliteal fossa using bipolar surface electrodes. These were positioned to produce the electrically elicited monosynaptic reflex (H reflex) at the lowest possible threshold. The electrode was then immobilized with a rubber strap. The stimulator was triggered by a digital PDP 12 computer programmed to deliver 10 impulses at random intervals of between 2 and 3 seconds. The stimulus voltage was increased in increments to recruit, in turn, the low threshold H reflex and the higher threshold direct motor response (M response). When a maximum M response had been obtained a further increase of at least 30% in the stimulus voltage ensured that a supramaximal stimulus had been delivered.

Vibration was applied to the tendo Achilles 1 cm above its insertion into the calcaneum with a Wahl Jumbo vibrator (frequency 60 Hz; undamped amplitude 4 mm).

The compound action potential of the soleus muscle was recorded with 2 cm diameter disc surface electrodes. The active electrode was placed over the belly of the soleus, in the midline, approximately 2 cm below the insertions of the medial and lateral heads of gastrocnemii and the reference electrode was placed 8 cm distally over the tendo Achilles. A 4 cm square lead plate over the upper gastrocnemius served as a ground.

Action potentials were amplified by a Tektronix (type 2A-60) differential amplifier, with filter settings 0.1 Hz and 0.1 MHz, and monitored on a Tektronix (RM-564) storage oscilloscope. The data was digitized at a sampling rate of 1000 Hz by the computer and 10 responses were averaged. The responses were then plotted with an incremental plotter (complot 7) and the peak to peak amplitude of the H or M response was measured from the plotting paper. The H-M recruitment curve was obtained by plotting the amplitudes of the averaged H and M responses against the stimulus voltage.

When the experimental conditions appeared stable the H-M recruitment curve was recorded. At each stimulus level control runs and runs with vibration were alternated. After each run with vibration an interval of 90 seconds was allowed to elapse to avoid the prolonged depression of the monosynaptic reflex that may follow vibration (Arcangel et al., 1971). The data for a complete H-M recruitment curve was recorded again after administration of diazepam. The ratio of the maximum H (vibration) to the maximum H (control) was used as an estimate of presynaptic inhibition of the Ia monosynaptic pathway. At the beginning of each study, 15 ml of blood was taken for control measurement of serum concentration of diazepam and its major metabolite desmethyldiazepam. These and subsequent samples were assayed for diazepam and desmethyldiazepam by a minor modification of the gas liquid chromatography, electron capture method described by Zingales, (1973). Diazepam was given intravenously over a period of 10 to 23 minutes in a dose sufficient to cause subjective relaxation but not sleep (15-30 mg). Dosage ranged from 0.25 to 0.66 mg/kg with a mean value of 0.39 mg/kg. Following completion of the diazepam infusion, 5 ml blood samples were drawn through an indwelling plastic catheter, in most instances at 5 minute intervals, until completion of the study. Samples were heparinized and immediately centrifuged. The plasma obtained was frozen and later assayed within 2 months.

The levels of diazepam in the blood samples taken immediately before, during and immediately after the recording of the H-M recruitment curve were averaged to obtain the "mean blood level." The standard "t" test and a modification of the Student's "t" test (Snedecor and Cochran, 1967) were used to compare the findings in the patients with complete and incomplete lesions. The relationship between the dose of diazepam and the blood level was determined using the correlation coefficient. Probabilities less than 0.05 (two tailed) were considered significant.

RESULTS

The two patient populations appear to be comparable for the purposes of this study (table 1). Those with incomplete lesions were somewhat older than those with complete spinal lesions but, although this may affect the H vibration/H control ratio (Delwaide, 1973), there was no statistical difference between the initial ratios in the two groups. Similarly there was no significant difference between the mean duration of the lesions.

Blood levels were examined in 10 of the 12 patients. In spite of the 3 day washout period residual amounts of diazepam were detected in the sera of 3 patients (studies 5, 8, 9). Administration of the drug produced a substantial rise in the concentration of diazepam in the sera of all 10 patients (table 1). There was a clear correlation between the dose in mg/kg and the "mean blood level" ($r = 0.74$; $0.01 > p > 0.001$).

There was no significant difference between the mean dose in mg/kg or the "mean blood level," in the complete and incomplete groups. So, in this respect as well, the two populations are comparable.

The metabolite desmethyldiazepam was detected in the control sera of 3 patients (studies 5, 8, 9) and these levels remained unchanged throughout the study. Desmethyldiazepam was not detected in the samples of any of the other patients so it is unlikely to be responsible for the findings that follow.

In each patient with an incomplete

TABLE 1

	Patient	Sex	Age	Diagnosis	Duration months	Dose mg	DZ mg/kg	Serum DZ µg/ml Initial	Serum DZ µg/ml mean	H vib/H control % before DZ	H vib/H control % after DZ	Difference
				INCOMPLETE LESIONS								
1	SS	M	54	trauma T 5	6	15	.26	0	1.40	93.8	0.0	93.8
2	JC	F	30	transverse myelitis	26	15	.36	—	—	104.0	80.0	24.0
3	DC	M	43	multiple sclerosis	192	20	.27	0	2.14	40.0	16.7	23.3
4	BN	F	44	multiple sclerosis	216	20	.41	0	2.36	57.7	18.2	39.5
5	RP	M	33	multiple sclerosis	60	30	.53	.35	2.71	6.7	6.1	0.6
6	VJ	F	42	multiple sclerosis	120	20	.42	0	1.91	20.0	0.0	20.0
7	FS	F	52	multiple sclerosis	216	20	.37	—	—	73.6	61.5	12.1
	Mean		42.5		119	18.5	.37	.07	2.03	56.5	26.1	30.4
											S D	30.3
											t	2.66
											0.05 > p > 0.025	
				COMPLETE SPINAL LESIONS								
8	LB	M	25	trauma C 5	84	30	.54	.17	2.19	43.1	54.4	—11.3
9	CD	F	31	trauma C 6	72	25	.66	.25	4.17	4.0	7.0	— 3.0
10	MB	M	27	trauma T 6	65	20	.25	0	2.22	93.1	90.8	2.3
11	JZ	M	26	trauma C 5	136	20	.29	0	1.86	0.0	4.5	— 4.5
12	HF	M	29	trauma C 5	3	20	.30	0	2.48	8.2	3.9	4.3
	Mean		27.6		72	23	.41	.08	2.58	29.7	32.1	— 2.4
											S D	6.14
											t	0.9
											not significant	

Table 1 — The effects of diazepam (DZ) on the H vibration/H control ratio in patients with incomplete and complete spinal lesions. Diazepam produces a significant decrease in this ratio only in the group with incomplete lesions. The mean difference in the H vibration/H control ratio in the patients with incomplete lesions (30.4) and those with complete spinal lesions (—2.4) was significantly different at the 5% level ($t^1 = 2.46 < t = 2.78$; Snedecor and Cochran, 1967).

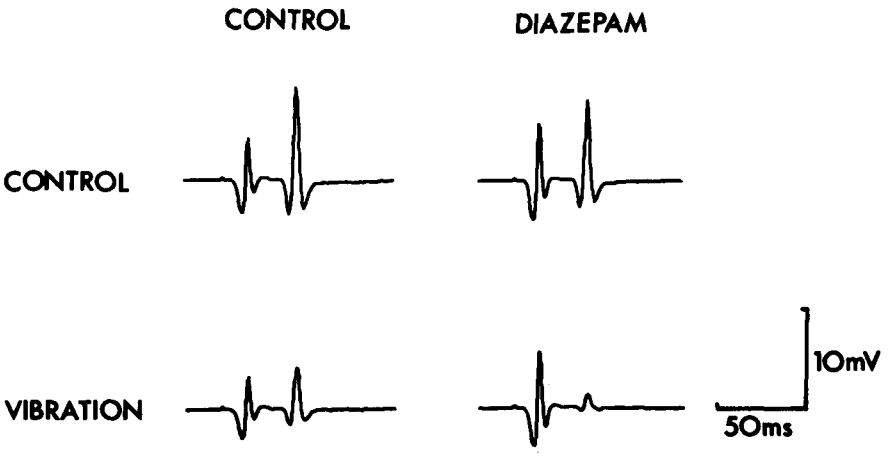
lesion diazepam enhanced the inhibition of the H reflex by vibration (Fig. 1, 2). The mean difference of 30.4% is significant ($t = 2.66$; $0.05 > p > 0.025$). These findings indicate that diazepam can enhance presynaptic inhibition in man.

In the patients with physiologically complete spinal transections however, diazepam had no consistent effect on the inhibition of the H reflex by vibration (Fig. 3).

The mean difference in the H vibration/H control ratio in the patients with incomplete lesions (30.4%) and those with complete spinal lesions (—2.4%) was significantly different at the 5% level ($t^1 = 2.46 < t = 2.78$; Snedecor and Cochran, 1967).

Diazepam also produced significant alterations in the H/M ratio, the direct muscle response and the twitch tension which will be reported separately.

Figure 1 — Effect of diazepam 20 mg IV on the inhibition of the H reflex by vibration in a patient with spasticity from an incomplete lesion (study 3). The initial deflection is the M response and the second deflection is the H reflex. Diazepam increased the inhibition of the H reflex by vibration implying that presynaptic inhibition of the Ia monosynaptic pathway is enhanced (bottom right). Each trace represents the average of 10 electromyographic responses.



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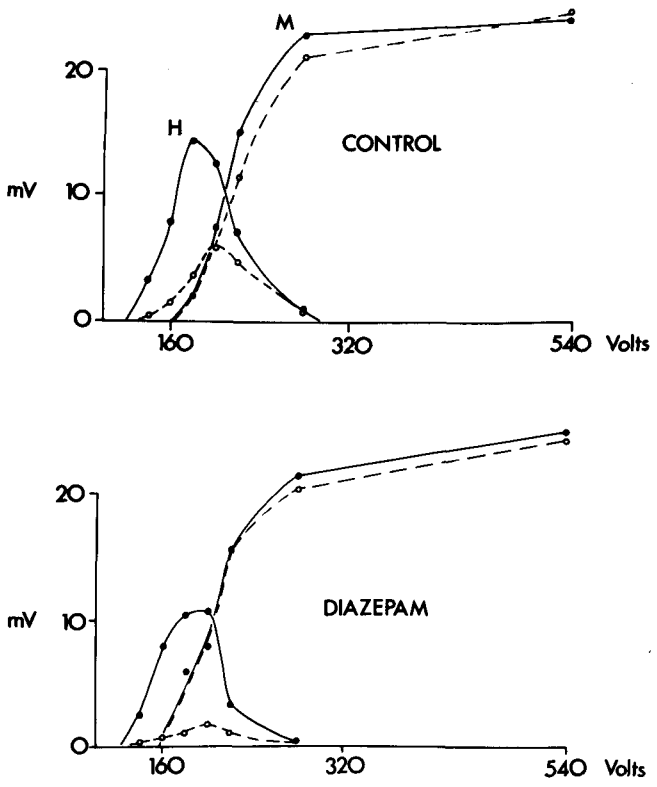

Figure 2 — Effect of diazepam 20 mg IV on the inhibition of the H reflex by vibration in a patient with spasticity from an incomplete lesion (study 3). The H-M recruitment curve is obtained by increments in the stimulus voltage (absissa). Each point represents the average amplitude of 10 responses in mv (ordinate). The dotted line represents the responses obtained with vibration. Diazepam exaggerates the inhibition of the H reflex produced by vibration implying that presynaptic inhibition of the Ia monosynaptic pathway is enhanced.

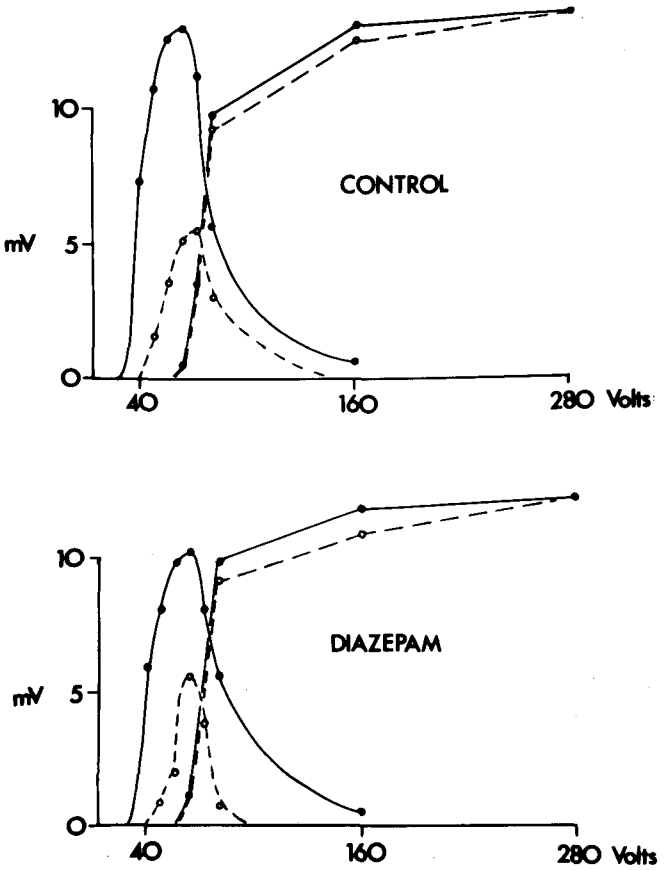

Figure 3 — Effect of diazepam 30 mg IV on the inhibition of the H reflex by vibration in a patient with spasticity from complete spinal transection (study 8). Each point represents the average of 10 responses. The dotted line represents the responses obtained with vibration. Diazepam did not affect the inhibition implying that presynaptic inhibition of the Ia monosynaptic pathway is not enhanced.

DISCUSSION

The monosynaptic reflex can be inhibited by muscle vibration in both animals and in man (Lance et al., 1973). This effect has been attributed to presynaptic inhibition of the Ia afferents, since it can be blocked by picrotoxin, is accompanied by depolarization of the Ia nerve terminals, and can occur while the excitability of the motoneurons to direct stimulation is unchanged (Gillies et al., 1969; Barnes and Pompeiano, 1970). The evidence that the same phenomenon in man is due to presynaptic inhibition is less direct. However the inhibition cannot be attributed to occlusion in the Ia pathway (Lance et al., 1973), or to postsynaptic inhibition from the spread of vibration to antagonists (Dindar and Verrier, 1975), and can occur when the excitability of the motoneurons to other inputs is unchanged (Delwaide, 1973). The inhibition of the H reflex by vibration applied to the tendo Achilles has therefore been used to estimate presynaptic inhibition in man (Delwaide, 1973; Pedersen et al., 1974).

Diazepam is apparently capable of increasing presynaptic inhibition in man. This has been demonstrated in the present study and confirms the preliminary findings of Delwaide (1971; quoted by Pedersen, 1974). This appears to be directly attributable to diazepam and not its immediate metabolite desmethyldiazepam.

There appears to be an important difference between patients with complete spinal lesions and those with incomplete lesions as, in those with complete lesions, diazepam had no consistent effect upon presynaptic inhibition. Possible explanations for this finding must be considered.

The two populations appear to be comparable for the purpose of this study. The administered dose of diazepam and the "mean blood level" were similar for both groups. Although the mean H vibration/H control ratio was larger in the incomplete group this difference between the means is not statistically significant. The difference in the response to diazepam, therefore, can not be attributed to these factors.

Although all patients had discontinued medication for at least 3 days prior to the study, diazepam in small quantities, was detected in the initial blood samples in 3 of the patients, again from both groups. The

diazepam administered during the study produced substantial increases in the serum concentrations, so it is unlikely that the small residual quantities detected in a few patients could have obscured the effect of the comparatively large administered dose.

It is possible that the drug acts solely on supraspinal centres. There is considerable evidence indicating that diazepam has powerful effects on the reticular formation (Ngai et al., 1966; Pryzbyla and Wang, 1968; Hudson and Wolpert, 1970; Nakanishi and Norris, 1971; Tseng and Wang, 1971) and that it may affect both excitatory and inhibitory systems (Pryzbyla and Wang, 1968; Nakanishi and Norris, 1971). Presynaptic inhibition of spinal pathways in animals is subject to supraspinal control and Lundberg and Vyklicki (1966) have deliniated a reticulo-spinal system in the cat capable of inhibiting presynaptic inhibition of the Ia monosynaptic pathway. It could be postulated, therefore, that presynaptic inhibition of the Ia monosynaptic pathway in man is also subject to such a supraspinal controlling mechanism and that this may be modified by the administration of diazepam.

However, the present findings in patients with complete spinal lesions are in contrast to the findings in the acutely spinalized animal. Schmidt et al., (1967), Stratten and Barnes (1971), Schlosser (1971) and Polc et al., (1974) found that diazepam increased primary afferent depolarization and produced an augmentation of the dorsal root potential that could be antagonized by picrotoxin or bicuculline implying that diazepam enhances presynaptic inhibition at spinal level. Chin et al., (1974) found that this effect was greater in the intact animal but that it remained after spinalization. Why is this effect not demonstrable in patients with complete spinal lesions? The dose administered in this study would appear to be adequate. Enhancement of dorsal root potentials was observed in spinal animals with doses as low as 0.05 mg/kg (Schmidt et al., 1967) whereas the mean dose administered in this study was 0.41 mg/kg. The acutely spinalized cat may not be directly comparable to patients with longstanding quadriplegia or paraplegia. For example the presynaptic inhibitory mechanism could have failed in some way in the patient group. Delwaide (1973) has suggested that failure of presynaptic inhibitory mechanisms could occur in patients with longstanding neurological disease. Diazepam is no longer effective in the spinal cat when gamma-amino-butyric acid (GABA), the presumed transmitter of presynaptic inhibition, is depleted (Polc et al., 1974). In this context it would be of interest to know the effect of diazepam on the dorsal root potential of the chronic spinal cat.

There are therefore two possible reasons why diazepam had no apparent effect on presynaptic inhibition in patients with longstanding complete spinal lesions. One, that the drug acts principally upon supraspinal centres and two, that in patients with longstanding spinal lesions, some alteration in the segmental presynaptic mechanism has occurred which renders diazepam ineffective.

In conclusion diazepam is apparently capable of enhancing presynaptic inhibition in man but this effect could not be demonstrated in patients who had suffered longstanding complete spinal lesions. These findings, in spinal man, of course, do not exclude the action of diazepam on other segmental mechanisms (an effect on the glycine receptor has been proposed by Young et al., 1974) but offer no support for the postulate that enhancement of presynaptic inhibition at segmental level is one of the drug's major effects in spinal man.

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