AN INVESTIGATION, IN CATS, INTO THE ACTIVITY OF DIAZEPAM AT THE NEUROMUSCULAR JUNCTION

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SUMMARY

Diazepam, in doses of 0.2–5 mg/kg i.v., was without effect on the maximal twitch response of the flexor hallucis longus or soleus muscles of the cat. When injected 5 minutes prior to or at the time of greatest depth of block produced by gallamine or tubocurarine, diazepam was without effect on the depth or the time course of the paralysis. The commercial solvent for diazepam (Valium, Roche), in volumes that contained doses of 1–5 mg/kg of the drug, itself slightly enhanced the rate of recovery from block produced by tubocurarine or gallamine. Twitch augmentation following high-frequency tetanic stimulation of the soleus nerve is known to be prejunctional in origin, and provides a sensitive test for detecting drug activity on intramuscular nerve endings. It was unaltered by diazepam, indicating absence of prejunctional blocking activity. The unimpressive peripheral activity of diazepam in cats emphasizes the importance of spinal or supraspinal sites in its ability to produce muscle relaxation in man and animals.

Diazepam, a benzodiazepine derivative, is effective both as a tranquillizer, and for controlling the muscle rigidity and spasm of patients with tetanus and cerebral palsy (Dundee and Keilty, 1969). Spinal reflex pathways (Randall et al., 1961; Ngai, Tseng and Wang, 1966; Hamilton, 1967; Crankshaw and Raper, 1968; Olafson, Mulder and Howard, 1964; Nathan, 1970; Schlosser, 1971; Stratten and Barnes, 1971) and supraspinal structures (Hudson and Wolpert, 1970; Nakanishi and Norris, 1971) are probable sites of action for this skeletal muscle relaxant activity. However, in recent years a peripheral action has also been implicated (Hamilton, 1967; Feldman and Crawley, 1970a,b; Ludin and Dubach, 1971).

An interaction between diazepam and neuromuscular blocking drugs was first suggested by the work of Stovner and Endresen (1965) and later, more emphatically, by Feldman and Crawley (1970a,b). The ease with which non-depolarizing neuromuscular blocking drugs cumulate with repeated doses is likely to complicate studies involving these compounds, and could provide an alternative explanation for the findings of Feldman and Crawley. Therefore, this study was undertaken, in cats, to investigate any activity of diazepam at the neuromuscular junction, including any interaction with the neuromuscular blocking drugs, gallamine and tubocurarine.

A similar anaesthetic regime to that described by Feldman and Crawley (1970b) was adopted in this study. The “fast twitch” flexor hallucis longus (FHL) muscle and the “slow twitch” soleus muscle were chosen to represent the mixed population of fibres in human muscles.

METHODS

Cats of either sex, weighing between 2.5 and 3.8 kg, were used; anaesthesia was induced with thiopentone, 30 mg/kg i.p., and maintained with nitrous oxide and oxygen (70/30) and halothane 0.5–2%. The trachea was cannulated and ventilation was controlled when necessary, using a modified Ayre T-piece and Palmer Ideal respiratory pump, and routinely throughout experiments in which large doses of neuromuscular blocking drugs were administered. Drugs were injected i.v. through a polyethylene cannula in an external jugular vein. In all experiments blood pressure was monitored from a common carotid artery by means of a Bell & Howell pressure transducer coupled to a Grass (model 79) or a Servoscribe (model RE520:20) pen recorder.

The cat was laid prone on the operating table and a skin incision was made in one hind limb from the level of the Achilles tendon to the popliteal fossa.
The soleus muscle was separated from neighbouring muscles and the gastrocnemius-plantaris muscles retracted. The flexor hallucis longus (FHL) muscle was then separated from neighbouring muscles. The limb was clamped in a horizontal position by means of drills through the lower ends of the tibia and fibula, and femur. The tendon of insertion of each muscle was cut and attached to Grass (model FT10C) isometric strain gauges coupled to a Grass (model 79) curvilinear pen recorder. The skin flaps were raised to form a deep pool which was filled with warm mineral oil (heavy liquid paraffin, BP). Muscle temperature was maintained at 36–38°C by the use of a radiant heat lamp, and recorded by means of a small thermocouple (Grant Instruments, Cambridge) placed in the pool. Shielded bipolar platinum electrodes were placed on the peripheral portion of the ligated and cut sciatic nerve, the cathode of the stimulating electrode being nearest the muscle. The nerves were stimulated with rectangular pulses of 0.1 msec duration and of a strength greater than that necessary to produce maximal twitches.

When it was required to interpose regular periods of tetanic stimulation two stimulators were used, one to evoke twitches, and the other tetani. Tetani of constant frequency and duration were delivered automatically at constant time intervals by means of an arrangement of Londex clocks connected to the output of the stimulator delivering the high frequency stimulation. With the exception of diazepam, all drug solutions were made up in 0.9% NaCl solution. The formula of the solvent used in the commercial preparation of diazepam injection (Valium, Roche) is given in a previous publication (Webb and Bradshaw, 1971).

The doses of all drugs quoted in the text refer to the salts.

RESULTS

Maximal twitches of the soleus and FHL muscles were elicited at a frequency of 0.1 Hz. A constant dose of either gallamine (0.75–1 mg/kg) or tubocurarine (0.1–0.4 mg/kg) was injected intravenously at intervals of 30 min to 2 hr. A series of control experiments was carried out to determine the optimal dose interval. With the shorter dose intervals of 30 min or 1 hr marked cumulative effects were evident. By increasing the dose interval to 2 hr these cumulative effects were minimized, but, as illustrated in figure 1, occasionally such precautions proved inadequate. The dose and the interval between doses was constant in any one experiment.

Diazepam, in doses of 0.2–0.4 mg/kg i.v., was without effect on the maximal indirectly elicited twitches of either soleus or FHL muscles. These doses of diazepam, given either 5 min prior to the neuromuscular blocking drug or at the time of greatest block, did not alter in any way the depth or time course of the blocks produced by tubocurarine or gallamine in either muscle. Figure 2 exemplifies this point.

Larger doses of diazepam within the range of 1–5 mg/kg i.v. slightly hastened the rate of recovery from the neuromuscular block produced by gallamine or tubocurarine, but there was never any sign of enhancement of the block. Control experiments with the diazepam solvent showed that the whole of this weak antagonist action could be attributed to the solvent (fig. 3).

Characteristically, a fall in blood pressure was evident with tubocurarine in doses that produced a significant degree of neuromuscular block, whereas gallamine caused marked tachycardia with an accompanying rise in blood pressure. Diazepam 0.2–0.4 mg/kg i.v. produced no change in blood pressure, but larger doses resulted in a marked, but transient, fall in blood pressure.

Effect on post-tetanic twitch augmentation.

Maximal twitches of the soleus muscle were elicited
ACTIVITY OF DIAZEPAM AT THE NEUROMUSCULAR JUNCTION

FLEXOR HALLUCIS LONGUS

GALL 1 mg/kg IV
DIAZ 0.2 mg/kg IV
GALL 1 mg/kg IV

SOLEUS

GALL 0.75 mg/kg IV
DIAZ 0.4 mg/kg IV
GALL 0.75 mg/kg IV

Fig. 2. Maximal twitches of flexor hallucis longus muscle and soleus muscle elicited indirectly once every 10 sec. The upper and lower records are from different experiments. Note that diazepam (DIAZ) is without effect on the block produced by gallamine (GALL) when administered either prior to the blocking agent or at the maximal depth of block.

Vertical calibration: upper 400 g, lower 200 g; horizontal 5 min.

at a frequency of 0.4 Hz and tetani of 10 sec duration and frequency 400 Hz, were automatically interposed every 5 min (Standaert, 1963, 1964b). With this frequency of stimulation the tension of the tetanus was usually triphasic, an initial rise being followed by a rapid fall and then a slow secondary rise (fig. 4). The post-tetanic twitches were strikingly augmented in amplitude for about 2 min.

In this series of experiments diazepam, gallamine and tubocurarine were injected intra-arterially, via the sural artery, 30 sec before a tetanus. In doses below those that affected the pre-tetanic twitches, gallamine (0.005–0.05 mg) and tubocurarine (0.01–0.1 mg) depressed the post-tetanic augmentation of twitch tension (PTA) and hastened the decline of tetanic tension, but diazepam in doses of 0.05–2 mg was without effect (fig. 4).

DISCUSSION

No interaction of diazepam with the neuromuscular blocking drugs, gallamine and tubocurarine, was evident in our experiments and this is in contrast to the findings, in man, of Feldman and Crawley (1970a, b). Our findings are supported by the recent work of Dretchen, Ghoneim and Long (1971), who showed that diazepam did not alter the time course of the block produced by tubocurarine, gallamine or decamethonium in either animal preparations or in man. Similarly, Southgate and Wilson (1971) failed to find any significant alteration in the neuromuscular block produced by gallamine or suxamethonium in the presence of lorazepam or diazepam. Mougdil and Pleuvry (1970) drew a similar conclusion from results obtained in vitro.

The important aspect in the design of experiments where the animal or patient is to act as its own control, and in which repeated doses of non-depolarizing neuromuscular blocking drugs are being administered, is that the dose interval be so adjusted as to minimize the well-known cumulative effects of these compounds. Although the twitch height may have returned to control values within 20 min after a moderate i.v. dose of tubocurarine or gallamine, the tetanic response still shows evidence of peak tetanic tension depression and wane up to 1 hr afterwards. Gissen and Katz (1969) suggested that the response to repetitive stimulation in man was a more accurate index of the level of neuromuscular block than the twitch. Similarly, Hughes (1970) showed that indirectly stimulated gastrocnemius muscles of anaesthetized cats were more sensitive to paralysis by non-depolarizing agents when bursts rather than single shocks were employed.

As the enhanced recovery rate from tubocurarine...
Fig. 3. Maximal twitches of flexor hallucis longus (FHL) muscle and soleus (SOL) muscle elicited indirectly once every 10 sec.

(A) Effect of large i.v. doses of diazepam (DIAZ) on the block produced by gallamine (GALL) and on blood pressure. This dose of diazepam was contained in 0.7 ml of the commercially available solution for injection (Valium 10 mg/2 ml, Roche). In contrast to fig. 2 diazepam enhanced the recovery rate, this effect being more pronounced in the soleus muscle.

(B and C) Effect of large volumes of diazepam solvent injected i.v. at the maximal depth of block produced by intravenously administered gallamine (GALL).

Note that the solvent transiently reversed this block. Records A, B and C are different experiments. Horizontal calibration 2 min; vertical 150 g.

Fig. 4. Maximal twitches of the soleus muscle evoked at a frequency of 0.4 Hz and a tetanus at \( \tau \) (400 Hz for 10 sec) interposed. Gallamine (GALL) and tubocurarine (TC) injected intra-arterially reduced the post-tetanic augmentation in doses having no effect on the maximal twitch, whereas diazepam (DIAZ) was without effect. Horizontal calibration 1 min; vertical 100 g.

or gallamine block seen with the high doses of diazepam could be reproduced by the solvent alone, this effect must be attributed to the drug solvent. A direct action of the solvent system on the neuromuscular apparatus (pre- or post-junctionally) or induced changes in local blood flow might possibly explain such an effect. Dretchen, Ghoneim and Long (1971) also noted that the solvent for diazepam injected intra-arterially in dogs reversed the blocks produced by tubocurarine and decamethonium but discounted a blood flow effect since the peripheral vascular resistance in the muscle bed was increased; such an effect would tend to delay removal of drugs from the neuromuscular junction and thus prolong their action.

Marked solvent activity was noted by Bradshaw and Pleuvry (1971) when investigating the respiratory effects of nitrazepam and diazepam. High doses in mice and low doses in rabbits were modified by the presence of the solvent. Furthermore, the solvent itself caused a loss of the righting reflex in mice and the lethal dose of nitrazepam and diazepam was considerably reduced when the drugs were administered in the solvent. The solvent system for the commercially available diazepam injection contains approximately 50% propylene glycol, a substance which has been shown to possess significant pharmacological activity in mice (Crankshaw and Raper, 1971; Zarolsinski, Browne and Possley, 1971).

The finding that there is no interaction between diazepam and non-depolarizing neuromuscular blocking drugs suggests that diazepam is without peripheral activity on skeletal muscle in the therapeutic dose range, confirming the in-vivo findings of others (Randall et al., 1961; Hamilton, 1967; Crankshaw and Raper, 1968; Dretchen, Ghoneim and Long, 1971).
In-vitro studies may reveal a direct effect of large concentrations of diazepam on the neuromuscular apparatus, but how much relevance this bears to the in-vivo situation is questionable, since it is unlikely that blood concentrations ever reach the required value. Hamilton (1967) showed that diazepam produced a dose-related inhibition of both directly and indirectly elicited contractions of the rat diaphragm, the indirect being more susceptible, suggesting an action on neuromuscular transmission. This blocking action, exerted on both types of stimulation, was often preceded by twitch augmentation. Mougdil and Pleuvry (1970) also remarked on the twitch augmentation following pretreatment with diazepam in the rat phrenic nerve diaphragm preparation and considered this to reflect a direct action on the muscle fibres.

In the indirectly stimulated rat diaphragm preparation the characteristics of the twitch block produced by diazepam resembled those of lignocaine, and the block produced was resistant to reversal by anti-cholinesterases (Dixon, personal communication). Furthermore, diazepam was found to be equipotent with lignocaine in blocking impulse conduction in the rat phrenic nerve.

On the basis of their results, Feldman and Crawley (1970b) postulated a presynaptic site of action for diazepam, possibly limiting the synthesis or release of acetylcholine.

In the "slow twitch" soleus muscle, high frequency nerve stimulation gives rise to a post-tetanic augmentation (PTA) of the response to single shocks; this augmentation is associated with repetitive firing in both nerve and muscle (Feng et al., 1938; Standaert, 1963, 1964b; Bowman, Goldberg and Raper, 1969). The motor nerve endings are considered to be the site of origin of the repetitive firing contributing to PTA in this muscle, and this preparation provides a sensitive system for studying drug effects on motor nerve terminals.

Gallamine and tubocurarine reduced the twitch augmentation in doses smaller than those necessary to reduce the twitch tension below the pretetanic level, thus implying a prejunctional site in addition to their well documented postjunctional site of action. This is in accordance with the findings of Standaert (1964a), Segawa, Kosima, and Takagi (1967) and Bowman, Goldberg and Raper (1969). However, diazepam in a dose range of 0.05-0.2 mg i.a. was without effect on the tetanic responses or the PTA, which indicates a lack of prejunctional activity. If diazepam were to possess significant local anaesthetic activity, evidence of such an action would be expected in this test preparation, since Usubiaga and Standaert (1968) showed that the local anaesthetics procaine, lignocaine, amethocaine and cinchocaine depressed the post-tetanic augmentation of the soleus muscle in doses comparable with those necessary to block impulse conduction in sensory pathways. Possibly the failure of diazepam to alter the PTA in the soleus muscle is the result of antagonistic solvent activity as discussed earlier, especially as the i.a. route of administration was employed.

The unimpressive peripheral activity of diazepam on skeletal muscle under normal conditions emphasizes the importance of central sites, whether spinal or supraspinal, to account for the observed muscle relaxant activity in the in-vivo animal experiments, surgical patients and such isolated cases of associated drug overdose as reported by Doughty (1970).

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REFERENCES
Diazepam en dosis de 0.2-5 mg/kg por vía intravenosa no ejercerá ningún efecto sobre la respetuada contracción máxima de los músculos flexor hallucis longus o soleus del gato. Inyectada 5 minutos antes o durante el tiempo de mayor profundidad del bloqueo producido por la gallamine o la tubocurarine, el diazepam resultó insensible en disminuir la profundidad de la parálisis. El diazepam, usado en dosis de 0.2-5 mg/kg suministradas intravenosamente, no tuvo ningún efecto sobre la respuesta de la contracción máxima de los músculos flexor hallucis longus o soleus del gato. Cuando se inyecta 5 minutos antes o durante el tiempo de mayor profundidad del bloqueo producido por la gallamine o la tubocurarine, el diazepam no tuvo ningún efecto sobre la profundidad o evolución de la parálisis. El solven comercia (Valium, Roche), en voluminos que contienen dosis de 1-5 mg/kg de la droga, aumenta ligeramente el establecimiento del bloqueo producido por la tubocurarine o la gallamine. El aumento de las contracciones consiguientes a la estimulación tónica de alta frecuencia del nervio propinno fue conocido como origen presinápico. La propileno glicol como un medio de difusión periférica de diazepam proporciona un nuevo medio para el tratamiento de la parálisis y la relaxación muscular en el hombre y en los animales.