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Effect of some benzodiazepines on peripheral neuromuscular function in the rat in-vitro hemidiaphragm preparation

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The effect of equimolar cumulative concentrations of 11 different benzodiazepines on the indirectly evoked twitch contraction was investigated in the rat in-vitro phrenic nerve-hemidiaphragm preparation. Depending on the pattern of the concentration-response curves two groups of benzodiazepines can be distinguished: (i) a first group with a biphasic action, e.g. potentiation of twitch tension in low concentrations and depression of twitch tension in high concentrations, and (ii) a second group with primary depression of twitch tension with increasing concentrations. All of the tested compounds ultimately caused a 100% depression of twitch tension at concentrations ranging from 0·175 to 0·35 mmol litre^{-1}. Although this peripheral effect of benzodiazepines on neuromuscular function is not the main site of action of these compounds, there are enough arguments to state that it is not a simple toxic effect. There is some evidence from this study that the peripheral component of the benzodiazepine effect on muscle relaxation may involve a multi- rather than one single receptor system.

The benzodiazepines are a group of psychotropic drugs with several pharmacological actions such as: anxiolysis, sedation, hypnosis, amnesia, anticonvulsant action and muscle relaxation (Harvey 1980). The skeletal muscle relaxing effect is generally considered to originate in the central nervous system (Greenblatt & Shader 1974). Conflicting results are reported in the literature regarding the effect of benzodiazepines on the peripheral neuromuscular function and the interaction of benzodiazepines with neuromuscular blocking drugs (Haefely et al 1981). Mainly chlordiazepoxide and diazepam have been studied regularly in in-vitro neuromuscular preparations (Haefely et al 1981). Several newer benzodiazepines however, are now widely used in medical practice. The aim of this study was to study in a comparative manner the effect of cumulative concentrations of different structurally related benzodiazepines on the peripheral neuromuscular function with the in-vitro rat phrenic nerve-hemidiaphragm preparation.

MATERIALS AND METHODS

Phrenic nerve-hemidiaphragm preparations (Bülbring 1946) of Wistar rats, 250–300 g, were suspended in double walled baths containing 45 ml Krebs solution (mmol litre^{-1} NaCl 113, KCl 4·7, $CaCl_2$, 2·5, $MgSO_4$ 1·2, $NaHCO_3$ 25, NaH_2PO_4 2·5, glucose 11·5). They were aerated with 5% carbon dioxide in oxygen. The temperature was kept constant at 37 °C and the pH was adjusted between 7·35–7·45. The phrenic nerve was stimulated by a Grass S48 stimulator with square wave supramaximal stimuli of 0·2 ms duration at a rate of 0·1 Hz. The resulting twitch concentration was quantitated by an isometric force displacement transducer (Grass F 0·3 C) and recorded on a polygraph. The control contractive force was between 20 and 50 g. For each experiment six hemidiaphragms from three rats were studied. The results were expressed as percentage of control values, i.e. the twitch tension recorded before the addition of any compound to the bath.

In a pilot study, control experiments were made in which the twitch tension was measured over 3–4 h without adding drugs to the bath in order to evalute the stability of the preparation.

Most benzodiazepines derivatives are poorly water-soluble and cannot be dissolved in Ringer solution even after warming up to 37 °C and automatic stirring. Therefore stock solutions of pure substance in ethanol 96% (5 mg ml^{-1} ethanol) were first made and then further diluted with Ringer solution. In the first group of experiments the effect of ethanol on the twitch tension was tested. Amounts of ethanol up to twice those necessary for dissolving the highest concentrations of benzodiazepines used in the following groups were added to the bath.

In the second group of experiments the effect on twitch tension of cumulative concentrations of different benzodiazepines was studied. After the twitch

* Correspondence.

tension was stable for 10 min, cumulative equimolar concentrations (0·0035, 0·035, 0·0875, 0·175, 0·2625, 0·35 mmol litre^{-1}) of the benzodiazepines were added to the bath at 15 min intervals. All of the benzodiazepines studied belong to the group of 1,4-benzodiazepines. The pure substances were available commercially.

In all the experiments the drugs were washed out with Krebs solution when 100% depression of the twitch tension was reached. The recovery of the twitch tension was expressed in percentage of the control values.

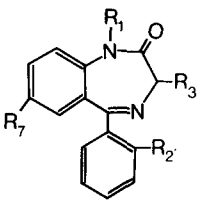
RESULTS

The structural formulae of the benzodiazepines investigated in this study are depicted in Fig. 1.

In the pilot experiments, when no drugs were added to the bath, no significant changes in twitch tension occurred during 240 min. None of the following experiments had a longer duration. In the first group of experiments, when ethanol was added to the bath, no significant effect on the twitch tension was observed at concentrations well above those used to dissolve the highest concentrations of benzodiazepines in the main experiments. In much higher concentrations a reduction of the twitch tension could be observed. When 75 to 100% depression of the twitch tension was reached, a contracture of the muscle developed as demonstrated by an elevation of the baseline.

The results of the second group of experiments are summarized in Table 1. All benzodiazepine derivatives ultimately caused a 100% depression of twitch tension at concentrations ranging from 0·175 to 0·35 mmol litre^{-1}. Some benzodiazepines (diazepam, temazepam, desmethyldiazepam, flurazepam, flunitrazepam, nitrazepam) initially caused an increase in twitch tension which was followed by depression of the twitch tension at higher concentrations. Others (oxazepam, lorazepam, lormetazepam, prazepam, halazepam) showed a primary and dose-dependent depression.

After reaching 100% twitch depression a shift of the baseline, caused by an apparent muscle contracture, was observed with diazepam, temazepam, flunitrazepam, nitrazepam and lormetazepam. Table 1 also shows the degree of recovery of the



1,4-BENZODIAZEPINE-2-ONES

Drug	R_7	R_1	R_3	$R_{2'}$
Diazepam	Cl	CH_3	H	H
Desmethyldiazepam	Cl	H	H	H
Oxazepam	Cl	H	OH	H
Temazepam	Cl	CH_3	OH	H
Lorazepam	Cl	H	OH	Cl
Lormetazepam	Cl	CH_3	OH	Cl
Flunitrazepam	NO_2	CH_3	H	F
Nitrazepam	NO_2	H	H	H
Flurazepam	C	CH_2-CH_2-N<C_2H_5 C_2H_5	H	F
Halazepam	H	CH_2-CF_3	H	H
Prazepam	Cl	CH_2-cyclopropyl	H	H

FIG. 1. Structural formulae of some 1,4-benzodiazepines.

Table 1. Effect of various 1,4 benzodiazepines on a, twitch tension; b, on baseline shift and c, the wash-out recovery in rat phrenic nerve-hemidiaphragm.

	a. Change of twitch tension in percentage of control values at cumulative concentration of benzodiazepines* (mmol litre^{-1})						b. Baseline shift	c. Wash out recovery
Drug	0·0035	0·035	0·0875	0·175	0·2625	0·35		
Diazepam	+0·5 ± 0·2	+10 ± 2	+28·5 ± 5	−100 ± 0			yes	50–75%
Temazepam	0	+2·7 ± 2·8	+9 ± 3·9	+13·3 ± 8·7	−95·8 ± 2·5	−100 ± 0	yes	50–75%
Desmethyldiazepam	0	0	0	+22·5 ± 5·5	−100 ± 0		no	75–100%
Flurazepam	+2·2 ± 1·2	+7·6 ± 1·6	+20·6 ± 5·3	−100 ± 0			no	75–100%
Flunitrazepam	+0·2 ± 0·2	+19·5 ± 3·8	+34·8 ± 5·7	−21·8 ± 28	−100 ± 0		yes	0–25%
Nitrazepam	0	+9·7 ± 1·7	+16·3 ± 1·9	+12 ± 1	−100 ± 0		yes	25%
Oxazepam	0	−2·3 ± 0·7	−7·3 ± 1	−100 ± 0			no	50–75%
Lorazepam	0	−16 ± 4	−46·2 ± 12	−100 ± 0			no	0–25%
Lormetazepam	−5·5 ± 3·2	−46 ± 3·5	−78·5 ± 7·5	−100 ± 0			yes	0–25%
Prazepam	0	−4·3 ± 1·8	−17·6 ± 11	−100 ± 0			no	50–75%
Halazepam	0	0	−1·5 ± 1·5	−86·5 ± 10·7			no	25–50%

* Mean ± s.e., n = 6.

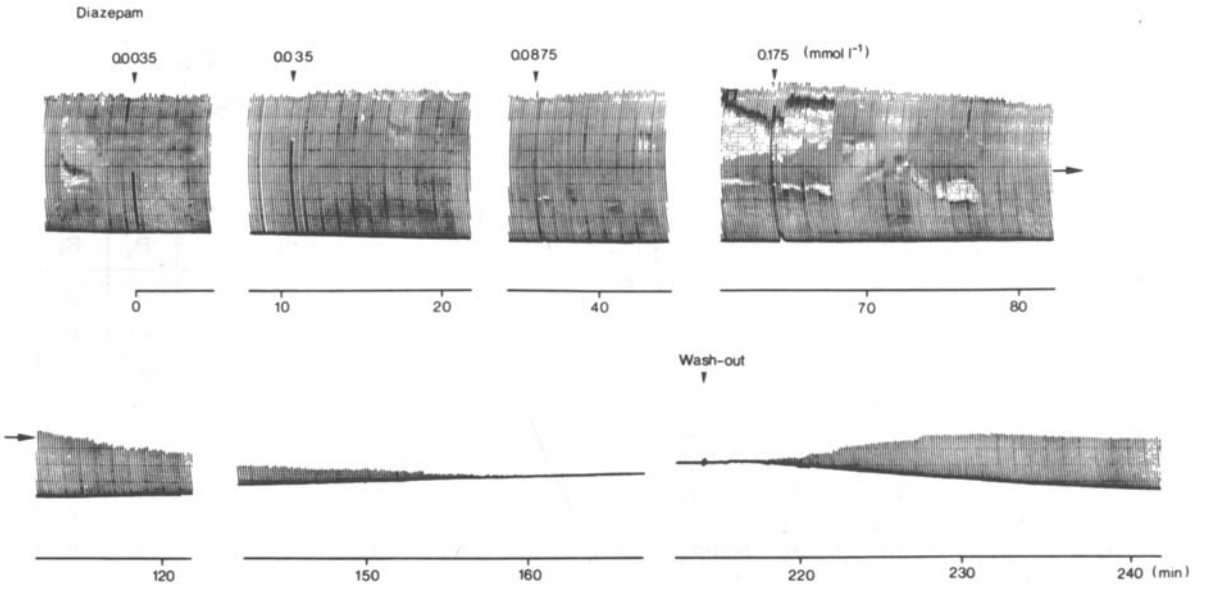


FIG. 2. The effect of cumulative concentrations of diazepam on the twitch tension of the in-vitro phrenic nerve-hemidiaphragm preparation of the rat.

twitch tension after washing-out the bath with fresh Krebs solution. In general the recovery is about 75%, but is markedly lower with nitrazepam, flunitrazepam, lorazepam and lormetazepam (0–25%).

Typical recordings of the two types of concentration-response curve are shown in Figs 2 (diazepam) and 3 (oxazepam). They demonstrate also that the onset of the effect is slow i.e. that the drugs must be present for about 15 min before the effect is fully developed.

DISCUSSION

The results demonstrate that the benzodiazepines investigated have a different effect on the twitch tension of the in-vitro phrenic nerve-hemidiaphragm preparation. Depending on the pattern of the

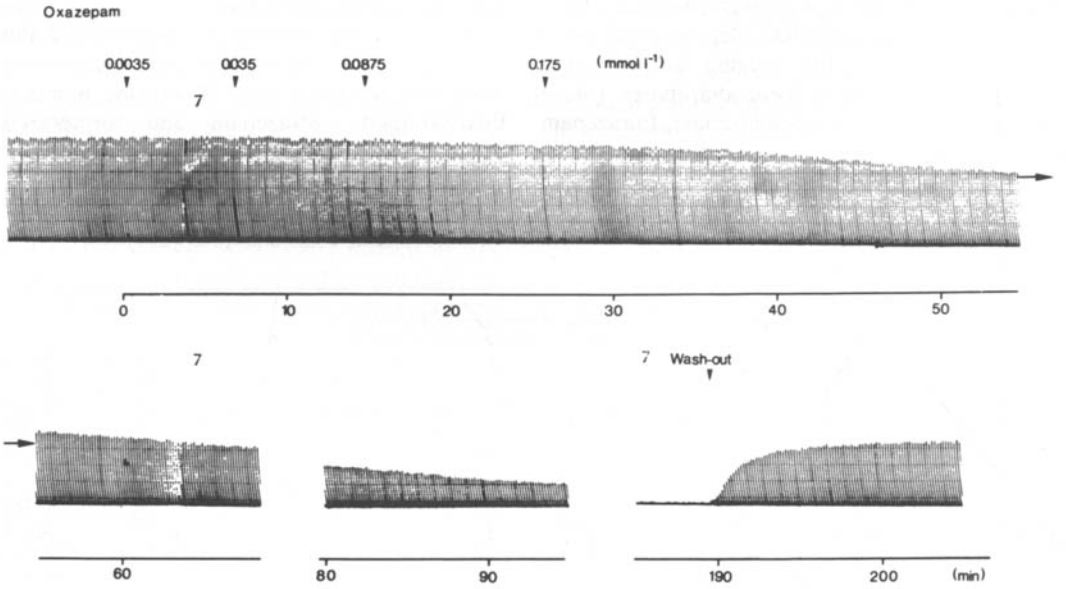


FIG. 3. Effect of cumulative concentrations of oxazepam on the twitch tension of the in-vitro phrenic nerve-hemidiaphragm preparation of the rat.

concentration-response curve, two groups of benzodiazepines can be distinguished. In the first group a biphasic action can be observed, e.g. increase of twitch tension in lower concentrations and depression of twitch tension in higher concentrations. In the second group there is no initial increase in twitch tension, but a direct depression of twitch tension.

The effects of diazepam on the in-vitro peripheral neuromuscular function have been reported as absent (Crankshaw & Raper 1968; Whittaker 1980), potentiating (Moudgil & Pleuvry 1970; Vergano et al 1969) or inhibitory (Hamilton 1967; Dasgupta et al 1969; Dretchen et al 1971; Vyskocil 1977, 1978). The concentrations of diazepam were different in all these studies. These conflicting results can be explained by the biphasic pattern of the concentration-response curve as found in our study for diazepam as well as for some other 1,4-benzodiazepines. Depending on the actual concentration of diazepam, no effect, an increase, or a depression of twitch tension can be observed. Earlier, Hamilton in his studies of the effect of diazepam (Hamilton 1967) and flurazepam (Hamilton & Stone 1982) on the in-vitro rat phrenic nerve-hemidiaphragm preparation, also observed this biphasic effect. The mechanism underlying this biphasic pattern of the concentration-response curve is still unclear, but Torda & Gage (1977) and Torda & Murphy (1979) showed that diazepam not only reduced the duration of the end-plate currents and hence the amplitude of the postsynaptic potentials in the mouse hemidiaphragm, but also acts presynaptically where it increases the quantal content of the end-plate potential in the mouse sternomastoid nerve muscle preparation. Torda and his colleagues suggested that this increase in quantal content is likely to mask some of the postsynaptic depression of the amplitude of the end-plate potential by diazepam.

Several mechanisms of action for the depression of muscle contraction by diazepam have been proposed: desensitization of the acetylcholine receptor (Vyskocil 1978), involvement of purine receptors (Hamilton et al 1982), changes in the permeability of the muscle membrane for chloride ions (Vyskocil 1977) and changes in the calcium kinetics of the muscle (De Groof et al 1980).

The concentrations necessary to produce neuromuscular impairment are rather high. However, the observed effects, with the possible exception for the contracture of the diaphragm at higher concentrations, should not be regarded as toxic effects since there is a fairly good recovery of the twitch tension after wash-out, even after 4 h exposure to benzodiazepines. This would not be the case with a toxic effect. Furthermore, recent work by Wilkinson et al (1982) demonstrated that crude membrane preparations of rat hemidiaphragm possess binding sites for [^{3}H]flunitrazepam suggesting that the effects of benzodiazepines on mammalian skeletal-muscle preparations may be mediated through specific high affinity binding sites. The high concentrations required for neuromuscular and/or muscular blockade might then be due to a lower sensitivity of the peripheral neuromuscular system to these drugs than the central nervous system, which is also suggested for chlorpromazine (Su & Lee 1960). Our results also suggest that some benzodiazepines are probably more likely to potentiate the effects of neuromuscular blocking drugs. Some benzodiazepines of the second group can depress twitch tension in concentrations which are significantly lower than those for diazepam. Further interaction studies, both in-vitro and in-vivo, should be made with these benzodiazepines.

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