J. Pharm. Pharmacol. 1981, 33: 267–268
Communicated January 29, 1981

0022–3573/81/040267–02 $02.50/0
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Lack of effect of benzodiazepines on bicuculline-insensitive GABA-receptors in the field-stimulated guinea-pig vas deferens preparation

A. DOBLE*, M. J. TURNBULL†, *MRC Neurochemical Pharmacology Unit, Medical Research Council Centre, Medical School, Hills Road Cambridge CB2 2QH, †Imperial Chemical Industries Limited Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG U.K.*

The benzodiazepines have been shown to enhance GABA-mediated inhibition at several sites in the central nervous system (Schmidt et al 1967; Dray & Straughan 1976; Okamoto & Sakai 1979) and it has been suggested that these compounds exert their psychotropic action by a 'GABA amplification' mechanism (Tallman et al 1980). Although this amplification seems to occur at the level of the GABA receptor, the precise molecular nature of the interaction is not understood. Some evidence suggests that the benzodiazepines increase the affinity of the GABA receptor for GABA (Costa & Guidotti 1979; Guidotti et al 1979) whilst other evidence points to an interaction at the level of the chloride ionophore that is coupled to the GABA receptor (Costa et al 1979; Simmonds 1980).

We have recently described a novel GABA receptor that depresses depolarization-evoked transmitter release from monoamine neuroterminals in the peripheral and central nervous systems (Bowery et al 1979, 1980). These receptors show an atypical pharmacology, in that they are insensitive to the competitive GABA antagonist bicuculline and have an anomalous agonist specificity characterized by the high potency of β-substituted congeners such as baclofen and the lack of potency of carboxyl-modified congeners such as 3-amino propane sulphonic acid. Furthermore, these receptors do not appear to operate through the opening of chloride ion channels.

In the present study we have examined the ability of benzodiazepines to affect the responses to GABA at these chloride-independent GABA receptors on sympathetic neuroterminals, using the isolated field-stimulated vas deferens.

Guinea-pigs (ca 350g) were killed by cervical dislocation and the vasa deferentia quickly dissected. The tissue was suspended in Krebs' solution (mM Na^+ 145; K^+ 3·5; Ca^{2+} 2; Mg^{2+} 1·2; Cl^- 124; $H_2PO^-_4$ 1·2; HCO^-_3 25; SO^{2-}_4 1·2 and glucose 1) in a 5 ml organ bath and gassed with 95% O_2/5% CO_2 at 37°C. The tissue was field stimulated with 100 ms trains of 1 ms pulses at 50 Hz and 60 V every 10 s (Grass S88 stimulator) using 6 mm platinum ring electrodes above and below the tissue. The contractions were measured using a Palmer isotonic transducer (under a tension of 200 mg) linked to a Kipp and Zonen flatbed chart recorder. Cumulative dose-response curves to GABA were obtained before and 5 min after addition of the appropriate concentration of benzodiazepine dissolved in 5 μl ethanol, or with 5 μl ethanol alone. This volume of ethanol had no effect either on the contractile response of the tissue or its sensitivity to GABA.

GABA depressed the evoked twitch height in a dose-dependent manner (mean max % reduction = 30 ± 6(7)) with an EC50 of 5·6 ± 0·6 × 10^{-6} M. This partial depression of contraction contrasted with the total depression of contraction seen with 3 μM tetrodotoxin or 3 μM leucine-enkephalin. Diazepam (10^{-5} M) produced, by itself, an inhibition of contraction of 21·3 ± 1·1% (n = 7) (see below). This effect was additive to that of GABA but the dose-response curve to GABA in the presence of 10^{-5} M diazepam was not significantly different from that obtained in the absence of benzodiazepine (Fig. 1). Nor was any effect on the response to GABA observed in the presence of 10^{-5} M nitrazepam, flunitrazepam, RO-6983 or RO-6986 (n = 3). Diazepam

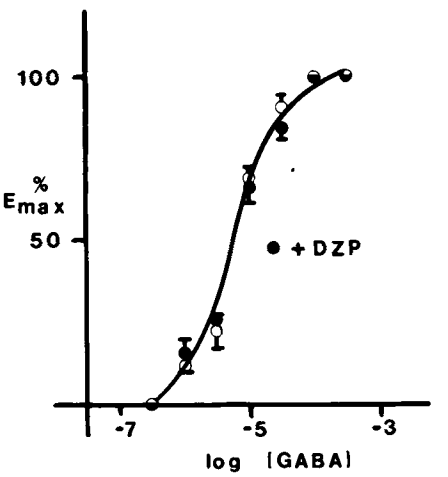

FIG. 1. Lack of effect of diazepam on the depressant responses to GABA on the field-stimulated guinea-pig vas deferens preparation. Inhibition of contraction is plotted as a percentage of the maximal inhibition by GABA obtained on that tissue. Each point is the mean ± s.e.m. of seven independent determinations. The dose-response curves are compared in the absence (○) and presence (●) of 10^{-5} M diazepam. EC50 values for GABA were 5·6 ± 0·6 μM in the absence of diazepam and 5·5 ± 1·0 μM in the presence of diazepam.

* Correspondence.

had no effect on the inhibition of contraction by L-baclofen nor by the α_2-adrenoceptor agonist clonidine. The recently described 'endogenous ligand' for the benzodiazepine receptor—β-carboline-3-carboxylic acid ethyl ester (Braestrup et al 1980)—did not affect the responses to GABA or baclofen after preincubation for 5 min at 10^{-4} M, nor did it have any direct depressant effect itself.

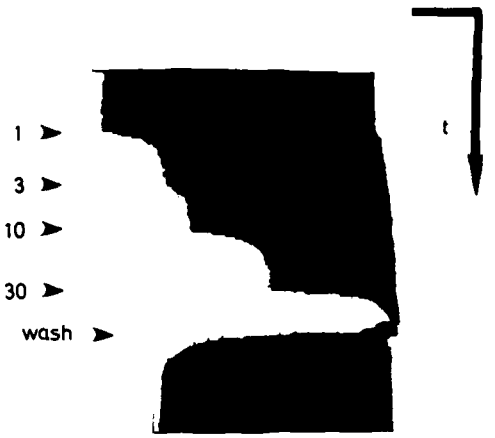

FIG. 2. Direct depressant effect of diazepam on the field-stimulated guinea-pig vas deferens preparation. Time runs from top to bottom. Diazepam was added in 5 μl ethanol at the arrows to give an accumulated concentration as indicated to the left of the trace ($\times 10^{-5}$ M).

In contrast to the small maximum depression of contraction seen with GABA (30%), the contraction of the guinea-pig vas deferens could be totally abolished by high concentrations of diazepam. A typical response to increasing concentrations of diazepam is shown in Fig. 2. The EC50 for this direct effect of diazepam was $3{\cdot}7 \pm 0{\cdot}9 \times 10^{-4}$ M ($n = 3$). Direct depressant effects were also elicited by nitrazepam, but not by flunitrazepam, RO-6893 or RO-6896.

This direct depressant effect of diazepam is unlikely to be related to an effect at c.n.s.-type benzodiazepine receptors for two reasons. Firstly, the concentrations of diazepam required to demonstrate these direct effects are much higher than those required to saturate the c.n.s. benzodiazepine binding-site (Braestrup & Squires 1978) and are incompatible with the amounts of benzodiazepines required to modify electrophysiological responses to GABA in the c.n.s. (Okamoto & Sakai 1979; Simmonds 1980). Furthermore, these concentrations of diazepam are much higher than those seen in the plasma following dosage sufficient to produce psychotropic effects, which are in the micromolar range (Hillestad et al 1974). Secondly, no direct depressant effect was observed with flunitrazepam, although this compound is more potent than diazepam in behavioural tests (Randall et al 1974) and in in vitro measurements of benzodiazepine receptor activity (Braestrup & Squires 1978).

Hamilton (1967) has reported that similar large doses of diazepam depress transmission at the neuromuscular junction. It has been proposed by De Groof & Bianchi (1978) that this effect of high concentrations of diazepam may result from changes in calcium mobility in the neuroterminal and the muscle cells. On one preparation we examined the effect of 10^{-3} M diazepam on the contractions elicited from the guinea-pig vas deferens by 10^{-4} M noradrenaline or acetylcholine, and found the effect of both spasmogens to be depressed; this suggests that direct effects on the muscle contribute to the depressant effects of diazepam seen in the field-stimulated preparation.

The finding that benzodiazepines and the postulated 'endogenous ligand' for the benzodiazepine receptor fail to potentiate the response of the guinea-pig vas deferens to GABA at behaviourally active concentrations suggests that, in contrast to bicuculline-sensitive chloride-linked GABA receptors in the c.n.s., the chloride-independent GABA receptors that modulate transmitter release at sympathetic neuroeffector junctions are not coupled to benzodiazepine receptors.

L-Baclofen was kindly donated by CIBA-GEIGY Ltd, and the benzodiazepines by Roche Products Ltd.

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