BENZODIAZEPINE Ro 15-1788: ELECTROPHYSIOLOGICAL EVIDENCE FOR PARTIAL AGONIST ACTIVITY

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The effects of diazepam and Ro 15-1788 were assessed upon responses of mouse spinal cord (SC) neurons in cell culture to the amino ac ' neurotransmitters 4-aminobutyric acid (GABA) and S-glutamic acid. Diazepam (100 nM) enhanced GABA responses by 65 ± 3% (113 cells), while Ro 15-1788 (100 nM) failed to alter GABA responses but reduced their enhancement by diazepam. Higher Ro 15-1788 concentrations (1 μM or 10 μM) enhanced GABA responses to a moderate extent, while blocking further enhancement of GABA by diazepam. Neither diazepam nor Ro 15-1788 affected glutamate responses or resting membrane potential or conductance of spinal cord neurons. These results provide electrophysiological support for partial agonist, rather than pure antagonist, activity of Ro 15-1788.

The imidazobenzodiazepine Ro 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate) is a potent displacer of benzodiazepine (BZ) binding [6] and has been shown to antagonize the behavioral and electrophysiological actions of diazepam [1, 6, 12, 18] but not anticonvulsants which did not bind to BZ receptors [6, 12, 18]. Low concentrations of Ro 15-1788 blocked the in vitro enhancement by diazepam of [^{3}H]GABA or [^{3}H]muscimol binding to rat brain membranes [8, 17].

While initial studies in rodents and humans failed to reveal any intrinsic activity of Ro 15-1788 [4, 6], subsequent behavioral studies have demonstrated apparent partial agonist activity. At high doses, Ro 15-1788 reduced the convulsant activity of pentylenetetrazole, bicuculline and Ro 5-3663 [5, 12, 19] and, like diazepam, Ro 15-1788 antagonized the development of amygdaloid kindled seizures in rats [1, 15]. Using drug discrimination techniques, animals generalized the cue for the BZ agonist chlorazepate to Ro 15-1788 [3]. These behavioral results, while suggesting that Ro 15-1788 has BZ-like activity, do not necessarily confirm that such activity arises from an agonistic effect at BZ receptors.

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We have investigated the effects of Ro 15-1788 upon GABA responses on mouse spinal cord (SC) neurons in cell culture and the enhancement of such responses by diazepam. We report that low concentrations of Ro 15-1788 (100 nM) antagonized diazepam enhancement of GABA responses, while higher concentrations (1 μM and 10 μM) enhanced GABA responses, suggesting that Ro 15-1788 is a partial agonist at BZ receptors.

Cell cultures were prepared from SCs with attached dorsal root ganglia from 12-14-day-old mouse embryos, as described earlier [11, 14], and SC neurons (5-8 weeks in vitro) were penetrated on the modified stage of an inverted phase-contrast microscope, using high resistance (25-40 MΩ) glass micropipettes filled with either 4 M potassium acetate (KAc) or 3 M potassium chloride (KCl). Cultures were bathed in a phosphate-buffered saline (composition in mM: NaCl 143.4, KCl 4.2, CaCl$_2$ 0.9, MgCl$_2$ 10.0 and glucose 5.6 in 9.5 mM sodium phosphate buffer at pH 7.35-7.40), and maintained at 33-34°C. Using a conventional bridge circuit, simultaneous current injection and membrane potential measurement were made with a single recording micropipette; data were recorded on a 6-channel Gould polygraph.

GABA (0.5 M, pH 3.2) or S-glutamate (0.5 M, pH 10.0) were applied ion-tophoretically using 400 ms rectangular current pulses (+ 0.5 to + 20 nA for GABA, − 5 to − 80 nA for S-glutamate) at 4 s intervals. Diazepam, Ro 15-1788 or vehicle (0.1% or less dimethylsulfoxide) were applied by miniperfusion (0.2 psi, 30 s) from a pipette 15-100 μm from the cell soma. Medium containing vehicle only did not alter responses to GABA or S-glutamate. GABA responses (KCl recordings) of 6-9 mV amplitude were evoked following membrane hyperpolarization to − 80 to − 90 mV, and glutamate responses (KAc recordings) of similar amplitude obtained near resting membrane potential (− 60 to − 70 mV) were used for assessment of drug actions. Data were accepted only if the amino acid responses returned to control levels within 5 min following removal of the drug-containing pipette from the vicinity of the cell under study. Results are expressed as mean ± standard error of the mean. When KAc recording micropipettes are used, application of GABA to SC neurons produces membrane hyperpolarization and an increase in membrane conductance [11]. Use of KCl-containing recording micropipettes allows chloride ions to enter the cell, changing the equilibrium potential for chloride ions from about − 65 mV to − 20 mV, and GABA responses become depolarizing [11].

GABA responses were enhanced (65.0 ± 3.0%) over control by 100 nM diazepam (n = 113 cells) in a reversible fashion (Fig. 1A1), and responses to S-glutamate were unaffected by 1 μM diazepam (responses 98.9 ± 1.0% control, 10 cells, Fig. 1A2), confirming previous studies demonstrating concentration-dependent enhancement of GABA responses by BZs in cultured avian and mouse SC and mouse cortical neurons [2, 9, 10, 20]. Diazepam (0.1 nM-10 μM) did not alter membrane potential or conductance.

Ro 15-1788 (100 nM) failed to alter GABA responses, while inhibiting (by 28.9

± 7.0%, $P<0.01$, Student's t-test) enhancement of GABA responses by 100 nM diazepam (Fig. 2). At 1 μM, Ro 15-1788 reversibly enhanced GABA responses by more than 10% in 14 of 15 cells studied (mean enhancement 21.9 ± 2.9%, $P<0.001$ from control; Figs. 1A3, 1B), but did not alter S-glutamate responses (responses 101.6 ± 0.6% control, 6 cells; Fig. 1A4). At 1 μM, Ro 15-1788 also abolished further enhancement of responses by diazepam (Figs. 1B and 2). Similar effects on basal and diazepam-enhanced GABA responses were seen with 10 μM Ro 15-1788, and at this concentration the enhancement of GABA responses by Ro 15-1788 was no greater than that observed at 1 μM Ro 15-1788 (Fig. 2). This would suggest that
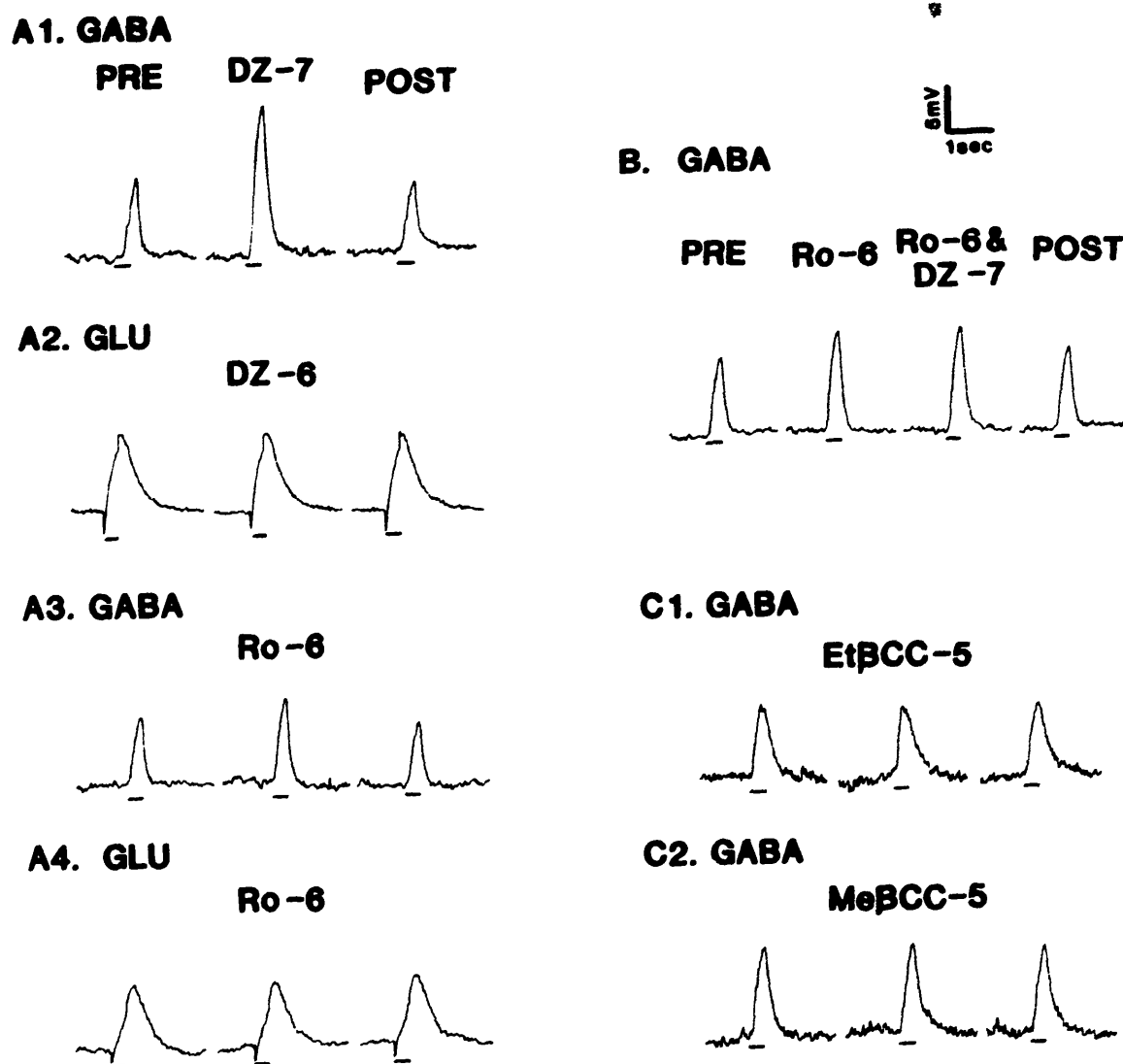

Fig. 1. Effects of diazepam and Ro 15-1788 on amino acid responses of mouse SC neurons in culture. A1: effects of diazepam (10^{-7} M) on GABA responses (membrane potential (MP) = -80 mV). A2: effects of diazepam (10^{-6} M) on glutamate responses (MP = -71 mV). A3, 4: effects of Ro 15-1788 (10^{-6} M) on GABA (MP = -75 mV) and glutamate (MP = -75 mV) responses. B: blockade of diazepam (100 nM) enhancement of GABA responses by Ro 15-1788 (10^{-6} M) (MP = -80 mV). C: effects of βCCMe (10^{-5} M, C1, MP = -90 mV) and βCCEt (10^{-5} M, C2, MP = -90 mV) on GABA responses.

the agonistic action of Ro 15-1788 is indeed 'partial' in nature. Ro 15-1788 (0.1 μM-10 μM) did not alter membrane potential or conductance. Enhancement of GABA but not S-glutamate responses with absence of direct membrane actions suggests some selectivity in the effects of these BZs. In contrast, another anticonvulsant, phenobarbital, has been shown to inhibit S-glutamate responses and to produce membrane hyperpolarization by increasing chloride conductance, a GABAmimetic action [10]. Other BZ antagonists β-carboline-3-carboxylic acid methyl ester (βCCMe) and the ethyl ester (βCCEt) [7, 12] failed to affect GABA responses at 10 μM (Fig. 1C1, 1C2) (βCCMe GABA responses 106.4 ± 3.4% control, 6 cells; βCCEt GABA responses 101.9 ± 3.9% control, 8 cells). Thus, not all compounds with BZ antagonist activity exhibit agonist activity at high concentrations.

Ro 15-1788 has been demonstrated to block diazepam enhancement of GABA effects in cat SC in vivo and isolated rat superior cervical ganglion [12, 13]. In the latter preparation very high Ro 15-1788 concentrations (167 μM and greater) enhanced GABA depolarization by approximately 20% [12].
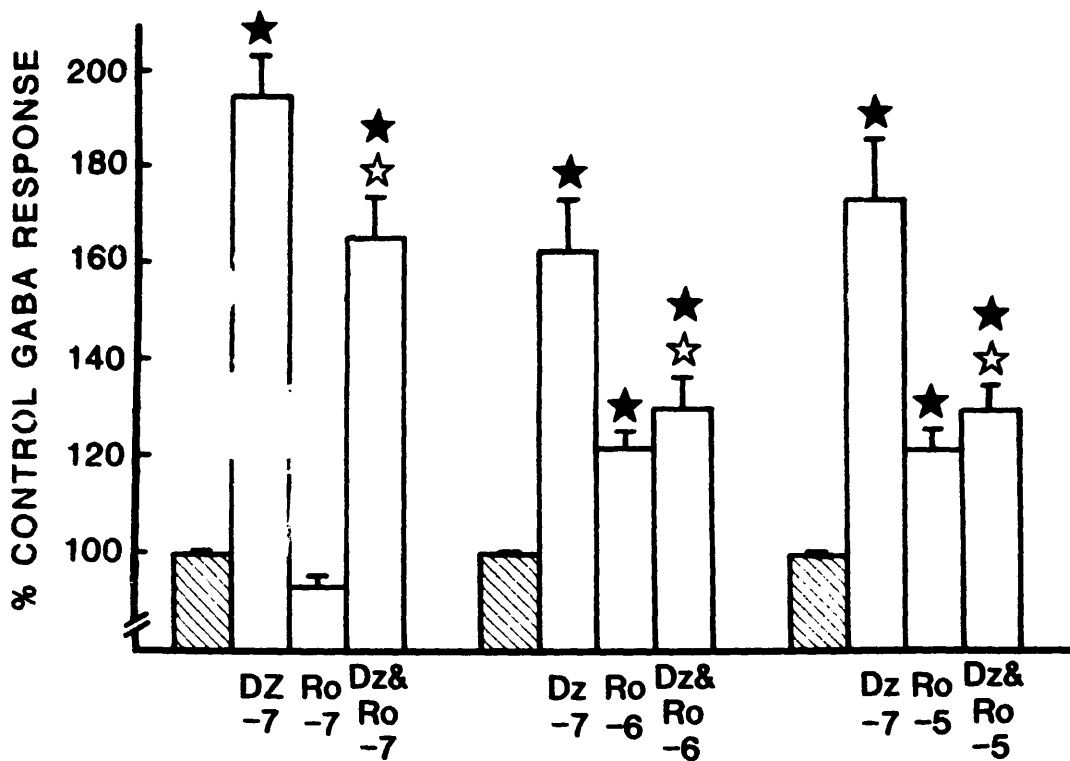

Fig. 2. Enhancement of GABA responses by diazepam and Ro 15-1788. Data shown are from 5-15 cells for each drug or combination. The effects of 100 nM diazepam, the particular Ro 15-1788 concentration studied and the Ro 15-1788-diazepam combination were examined on each cell studied, with 2-3 cycles of application of each drug or combination. By Student's t-test: empty stars $P<0.02$, significant difference from 100 nM diazepam; filled stars $P<0.01$, significant difference from vehicle alone (0.1% dimethylsulfoxide in buffer, hatched bars). Drug concentrations shown on the figure are logarithm molar.

Neurochemical studies have shown a potent antagonism of diazepam-enhanced GABA binding by Ro 15-1788 (IC_{50} = 5 nM) but have not revealed enhancement of GABA binding [8, 17]. These assays were performed at 2°C; since the potency of diazepam as an enhancer of GABA binding increases severalfold at 25°C [16], it may be possible that high concentrations of Ro 15-1788 would enhance GABA binding upon assay at physiological temperature.

A recent study [7] has examined the relationship between in vivo receptor occupancy by various BZ receptor ligands and their activity against audiogenic seizures in DBA/2 mice. Ro 15-1788, but not βCCMe or βCCEt, was found to have anticonvulsant activity. Further, the doses required for the anticonvulsant activity of Ro 15-1788 were some 150-fold higher than for diazepam. Whereas classical BZ agonists had anticonvulsant activity in this model at low receptor occupancy (< 10%), almost complete occupancy by Ro 15-1788 was required for its anticonvulsant activity. These results, together with the differing concentration-dependencies of GABA enhancement by diazepam and Ro 15-1788, suggest that different levels of receptor occupancy by the two compounds may be required for the enhancement of GABA receptor affinity.

The partial agonist activity of Ro 15-1788 at high doses or concentrations should be taken into account when the compound is used experimentally and clinically. Ro 15-1788 has been proposed as combination therapy to counteract the sedative effects of the antischistosomal BZ 3-methylclonazepam [6] and may be useful in BZ overdose cases. While Ro 15-1788 lacks sedative activity in man at doses of 600 mg/day and lower [4], higher doses could possibly cause sedation or tolerance.

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