Table 2 Effect of Ro 15-1788 on the changes in seizure threshold produced by diazepam, other anticonvulsants and β-CCE

Drug treatment	Seizure threshold to PTZ (mg per kg) Vehicle (Tween)	Ro 15-1788 (10 mg per kg)	P value
Saline	34±2 (5)	35±3 (6)	N/S
β-CCE (1 mg per kg, i.v.)	26±3 (5)	35±3 (6)	<0.001
Diazepam (5 mg per kg, i.p.)	62±14 (7)	39±4 (5)	<0.001
Phenobarbitone (40 mg per kg, i.p.)	52±5 (6)	56±9 (6)	N/S
Valproate (400 mg per kg, i.p.)	57±14 (5)	53±10 (6)	N/S
Progabide (200 mg per kg, i.p.)	50±6 (7)	55±11 (7)	N/S

Diazepam, phenobarbitone, sodium valproate and progabide were given in the doses shown. Concentrations of solutions were diazepam 5 mg ml^{-1} in commercial vehicle (Roche), phenobarbitone 10 mg ml^{-1} and sodium valproate 100 mg ml^{-1} in normal saline, and progabide 100 mg ml^{-1} in Tween. Saline was given in a volume of 4 ml per kg. 15 min later Ro 15-1788, 10 mg per kg, was injected i.p. and a further 15 min later PTZ was infused (see Table 1 legend). With β-CCE, Ro 15-1788, 10 mg per kg in Tween, was given 10 min before an i.v. injection of 1 mg per kg β-CCE (1 mg ml^{-1} solution), with PTZ infused 5 min later. Seizure thresholds were expressed as mean ±s.d. with the number of animals given in parentheses. The *P* value refers to significance of differences between Ro 15-1788 and Tween vehicle-treated animals. There were no differences in seizure threshold between animals pretreated with saline, Tween, β-CCE vehicle or diazepam vehicle. Statistical analysis performed by Student's *t*-test. N/S, not significant.

while higher concentrations of Ro 15-1788 (167–835 μM) showed intrinsic activity in that they increased the responses to GABA (Fig. 1). The ratio of agonist and antagonist doses of Ro 15-1788 in this system was similar to that displayed in the seizure threshold model.

Barbiturates also increase the responses to GABA in the ganglion preparation[12], but as in the seizure threshold model, the effect of phenobarbitone (40% increase in the amplitude of GABA responses at 787 μM) was not antagonized by Ro 15-1788 at either 3.34 or 33.4 μM. These concentrations of Ro 15-1788 alone had no effect on the response to GABA.

When applied to the GABA–bicuculline-pretreated ganglion, 1 μM β-CCE showed opposite actions to BDZs in that it significantly ($P < 0.01$) decreased the depolarization produced by GABA, 38.8 μM (results, expressed as in Fig. 1, were 85±3% at 15 min, 78±5% at 30 min, 85±6% at 45 min and 83±7% at 60 min). This effect was antagonized by Ro 15-1788 at the same concentration which blocked the effect of chlordiazepoxide (3.34 μM).

It thus seems that in two experimental systems in which specific BDZ activity may be assessed, β-CCE has the opposite effect to BDZs. Ro 15-1788 seems to be a selective and potent antagonist of both compounds in both systems. Investigations have failed to demonstrate specific binding of either β-CCE[1] or Ro 15-1788 (ref. 8) to CNS sites other than the BDZ receptor. In view of the selective and high-affinity BDZ receptor binding of β-CCE and Ro 15-1788, and the parallels between their binding and pharmacological activity, it is likely that the pharmacological effects observed are due to interaction at the BDZ receptor site.

We suggest three possible explanations for these findings. First, the partial agonist effect of Ro 15-1788 may be sufficient to account for its reversal of the actions of β-CCE. This seems unlikely because the concentrations of Ro 15-1788 that antagonized β-CCE had no agonist action when tested alone. However, it is possible that in the presence of β-CCE the BDZ receptor is altered so that Ro 15-1788 has a greater agonist effect.

The second explanation is that the functional interaction of ligands with the BDZ receptor may be unusual in that specific high-affinity ligands may produce opposite pharmacological effects and yet have a common antagonist. The possible existence of this type of receptor interaction has been predicted from receptor theory (see, for example, ref. 20). The allosteric models of receptor behaviour suggest that receptors exist in equilibrium between two states (which may be termed 'active' and 'inactive') and that this equilibrium is altered by ligand binding. If we apply this theory to our findings, BDZs would be considered to be agonists, binding to the active state, whereas Ro 15-1788 would be a conventional antagonist, binding to both forms without altering the equilibrium. If β-CCE bound preferentially to the receptors in the inactive state it would shift the equilibrium in the opposite direction. This form of antagonism has been predicted but not so far demonstrated.

A third explanation derives from results of ligand binding studies suggesting the existence of subclasses of BDZ receptors. These have identical affinities for the BDZs, including Ro 15-1788 (ref. 8), but differ in their affinities for the β-carbolines[2,21]. The pharmacological actions of the BDZs and β-CCE may thus be exerted through different subpopulations of BDZ receptors, and it is conceivable that the unusual interactions we have described represent a functional consequence of this differential binding.

We thank Dr B. Jones, Glaxo, and Dr Ian Martin, MRC Neurochemical Pharmacology Unit, Cambridge, for β-CCE, Dr W. Haefely, Roche, for Ro 15-1788, Synthelabo (Paris) for progabide, Professors Sir William Paton and D. G. Grahame-Smith and Dr A. R. Green for helpful advice and criticism and Mr C. Howard for technical assistance.

Received 26 August; accepted 16 December 1981.

1. Braestrup, C., Nielsen, M. & Olsen, C. E. *Proc. natn. Acad. Sci. U.S.A.* **77,** 2288–2292 (1980).
2. Nielsen, M. & Braestrup, C. *Nature* **286,** 606–607 (1980).
3. Braestrup, C. & Nielsen, M. *Trends pharmac. Sci.* **1,** 424–427 (1980).
4. Tenen, S. & Hirsch, J. D. *Nature* **288,** 609–610 (1980).
5. Oakley, N. R. & Jones, B. J. *Eur. J. Pharmac.* **68,** 381–382 (1980).
6. Mitchell, R. & Martin, I. *Eur. J. Pharmac.* **68,** 513–514 (1980).
7. Cowen, P. J., Green, A. R., Nutt, D. J. & Martin, I. *Nature* **290,** 54–55 (1981).
8. Möhler, A., Burkard, W. P., Keller, A. H., Richards, J. G. & Haefely, W. *J. Neurochem.* **37,** 714–722 (1981).
9. Hunkeler, W. *et al. Nature* **290,** 514–516 (1981).
10. Darrogh, A. *et al. Lancet* **ii,** 8–10 (1981).
11. Randall, L. O. & Kappell, B. in *The Benzodiazepines* (eds Garattini, S., Mussini, E. & Randall, L. O.) (Raven, New York, 1973).
12. Bowery, N. G. & Dray, A. *Br. J. Pharmac.* **63,** 197–215 (1978).
13. Nutt, D. J., Cowen, P. J. & Green, A. R. *Neuropharmacology* **19,** 1017–1023 (1980); *Eur. J. Pharmac.* **71,** 287–295 (1981).
14. Costa, E., Guidotti, A., Mao, C. C. & Suria, A. *Life Sci.* **17,** 167–176 (1975).
15. Olsen, R. W. *J. Neurochem.* **37,** 1–13 (1981).
16. Kerwin, R. W., Olpe, H-R. & Schmutz, M. *Br. J. Pharmac.* **7,** 545–551 (1980).
17. Lloyd, K. G. *et al. Adv. Biochem. Pharmac.* **26,** 199–206 (1981).
18. Adams, P. R. & Brown, D. A. *J. Physiol., Lond.* **250,** 85–120 (1975).
19. Bowery, N. G. & Brown, D. A. *Br. J. Pharmac.* **50,** 205–218 (1974).
20. Colquhoun, D. in *Drug Receptors* (ed. Rang, H. P.) 149–182 (Macmillan, London, 1975).
21. Ehlert, F. J., Roeske, W. R. & Yamamura, H. I. *Life Sci.* **29,** 235–248 (1981).
22. Brown, D. A. & Marsh, S. *J. Physiol., Lond.* **246,** 24–25P (1974).

Errata

In the *Nature Directory of Biologicals 1982*, on page vi James Manley of Columbia University is incorrectly listed as Michael Manley under the heading 'Scientific Advisory Committees'.

In the letter 'Tree remains in a North York Moors peat profile' by I. G. Simmons & J. B. Innes, *Nature* **294,** 76–77 (1981), Figs 1 and 2 are transposed. Thus Fig. 1 is the pollen diagram shown on page 78.

In the article 'Magma chamber profiles from the Bay of Islands ophiolite complex' by J. F. Casey & J. A. Karson, *Nature* **292,** 295–301 (1981), NAM in Fig. 1 legend stands for North Arm Mountain. The received date for the article should read 29 September 1980.

Corrigendum

In the letter 'Mouse IgG3 antibodies are highly protective against infection with *Streptococcus pneumoniae*' by D. E. Briles *et al.*, *Nature* **294,** 88–90 (1981), the dose of *S. pneumoniae* is given as 100 CFU in Table 2 and 150 in the text. This should read 100 CFU throughout.