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Interactions of carbamazepine with benzodiazepine receptors

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Little is known of the molecular mechanisms of anticonvulsant action of carbamazepine (CBZ), although its effectiveness in the therapy of partial epilepsies and grand mal seizures is widely recognized (Suria & Killam 1980). Recently we have demonstrated that this anticonvulsant, at therapeutic concentrations, may bind to adenosine receptors in the brain, while not directly influencing the binding of radioligands to several neurotransmitter (GABA, glutamate, muscarinic cholinergic or β-adrenergic) receptors (Skerritt et al 1982c). Since many purines have diazepam-like sedative, muscle relaxant and anticonvulsant activity, and are moderately potent inhibitors of benzodiazepine binding to brain membranes (Davies et al 1980; Skerritt et al 1982a,b) we examined the effects of CBZ upon benzodiazepine receptors in-vivo and in-vitro. CBZ was found to be a competitive inhibitor of the binding of a variety of benzodiazepine ligands to rat brain membranes and its anticonvulsant action against leptazol (metrazol, pentylenetetrazol)-induced convulsions in mice could be partially reversed by the benzodiazepine antagonist Ro15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5a],[1,4]-benzodiazepine-3-carboxylate).

Washed synaptosomal membranes from whole rat brain were prepared and benzodiazepine binding assays performed as described by Skerritt et al (1982d). In Tris-HCl buffer, pH 7·4 at 2 °C, CBZ inhibited [^{3}H]diazepam binding in a concentration-dependent manner. Half-maximal inhibition of [^{3}H]diazepam binding occurred at 129 ± 4 μM carbazepine (mean ± s.e.m. of 3 experiments). Inhibition of [^{3}H]diazepam by CBZ appeared to be competitive, with 100 μM CBZ decreasing binding affinity without alteration in the maximal binding site density for [^{3}H]diazepam (Control K_D = 12·5 ± 1·9 nM, B_{max} = 1·23 ± 0·07 pmol mg^{-1}; 100 μM CBZ K_D = 30·3 ± 5·9 nM*, B_{max} = 1·46 ± 0·07 pmol mg^{-1}; means ± s.e.m. calculated by linear regression analysis of Scatchard plots of ligand bound versus the ratio of bound to free ligand; *P <0·05 Student's t-test, 3 experiments). Less potent inhibition of diazepam binding by CBZ under different assay conditions has been reported by Olsen & Leeb-Lundberg (1981). At 50 μM, corresponding to the upper therapeutic range of CBZ levels in serum and brain tissue (Morselli et al 1977), CBZ inhibited [^{3}H]diazepam binding by 31 ± 1% (mean ± s.e.m. of 3 experiments). Several groups have demonstrated that doses of diazepam and other benzodiazepines, producing only a 30% or lower occupancy of benzodiazepine receptors, are sufficient to block seizures induced by picrotoxin or leptazol (Duka et al 1979; Paul et al 1979).

Although CBZ and diazepam have differing anticonvulsant spectra, both are relatively potent antagonists of tonic hindlimb extension induced in rodents by leptazol (Desmedt et al 1976). The imidazodiazepine, Ro15-1788, is a specific benzodiazepine receptor antagonist, having been shown to block the major electrophysiological, biochemical and anticonvulsant actions of the benzodiazepines (Hunkeler et al 1981). The compound, however, lacks convulsant or proconvulsant (against leptazol) activity, and as such is useful in elucidating whether centrally acting drugs may exert behaviourally relevant actions on central benzodiazepine receptors.

Convulsions were induced in QS strain male mice (17–23 g) by intraperitoneal injection of leptazol (120 mg kg^{-1}). Moderately large doses of Ro15–1788 (10 mg kg^{-1}) caused a slight reduction (of borderline statistical significance) in the percentage of mice with seizures, following challenge with leptazol 15 min later. Other workers have also observed weak 'partial agonist' activity of Ro15–1788 (Nutt et al 1982). Ro15–1788 (10 mg^{-1} kg, i.p.) given 15 min before leptazol, reversed the anticonvulsant action of diazepam pretreat-

Table 1. Effects of Ro15-1788 (10 mg kg^{-1}) on protection by anticonvulsants from tonic seizures in mice induced by leptazol (120 ml kg^{-1}). Mice were injected intraperitoneally with anticonvulsant drugs 60 min before challenge with leptazol (120 ml kg^{-1} i.p.). Vehicle or Ro15-1788 (10 ml kg^{-1} p.) pretreatments were given 15 min before leptazol injection. The vehicle used throughout for drugs was 0·9% NaCl, containing 2% Tween 80 to solubilize diazepam, Ro15-1788 and CBZ. Mice were scored by a 'blind' observer for the presence or absence of tonic hindlimb extensor seizures in the 15 min after convulsant challenge.

Anticonvulsant	Pretreatment	No of mice	% Mice with tonic hindlimb extension	P*
-	Vehicle	50	94	
	Ro15-1788	12	78	0.062
Diazepam	Vehicle	15	0	
(5mg kg^{-1})	Ro15–1788	15	67	0.00010
Carbamazepine	Vehicle	36	14	
(20mg kg^{-1})	Ro15-1788	36	42	0.0067
Phenobarbitone	Vehicle	12	8	
(15mg kg^{-1})	Ro15-1788	12	17	0.39
Trimethadione	Vehicle	12	8	
(400mg kg^{-1})	Ro15-1788	12	0	0.50
Sodium Valproate	Vehicle	12	8	
(4000mg kg^{-1})	Ro15-1788	12	0	0.50

* Value for difference from vehicle-pretreated animals in each group. Statistical analyses performed using the Fisher exact probability test.

* Correspondence.

Table 2. Inhibition by carbamazepine of binding of various ligands to benzodiazepine (Bz) and purine receptors. Data shown are from 3 or 4 experiments performed in quadruplicate on separate rat brain homogenates. Inhibition constant (K_i) values were calculated using the equation: $K_i = IC50/(1 + L/K_D)$, where L is the concentration of the ligand and K_D its apparent dissociation constant.

	K_i value (μM)
[^{3}H]Diazepam (nonselective Bz agonist)	122 ± 5
(+)-[^{3}H]3-Methylclonazepam (receptor selective Bz agonist	105 ± 13
[^{3}H]Phenylisopropyladenosine (A_1 purine agonist)	44 ± 5*
[^{3}H]Ro15-1788 (receptor selective Bz antagonist)	176 ± 19
[^{3}H]Ro5-4864- ('acceptor' selective Bz ligand)	34 ± 4
	IC50 value (μM)
[^{3}H]Ro15-1788 (−GABA)	290 ± 9
(+ 100 μM GABA)	282 ± 8

The kinetic parameters of binding of the ligands used were as follows:

[^{3}H]Diazepam K_D = 12·5 ± 1·9 nM, B_{max} = 1233 ± 74 fmol^{-1}mg.

[^{3}H]Methylclonazepam K_D = 1·40 ± 0.10 nM, B_{max} = 927 ± 35 fmol^{-1}mg.

[^{3}H]Ro15-1788 K_D = 0·84 ± 0·04 nM, B_{max} = 1350 ± fmol mg^{-1}.

[^{3}H]Ro5-4864 K_D = 1·85 ± 0·11 nM, B_{max} = 262 ± 9 fmol mg^{-1}.

[^{3}H]Phenylisopropyladenosine K_D = 3·10 ± 0.32 nM, B_{max} = 583 ± 35 fmol mg^{-1}.

* Skerritt et al 1982c.

ment (5 mg kg^{-1}; 60 min before leptazol with tonic extensor seizures appearing in 67% of Ro15–1788–treated mice (Table 1). The benzodiazepine antagonist also significantly, but to a lesser extent, decreased protection from tonic seizures in mice pretreated with CBZ, reducing the percentage of mice protected by the anticonvulsant from 86 to 58%. The anticonvulsant efficacy of phenobarbitone (15 mg kg^{-1}, i.p.), trimethadione (400 mg kg^{-1} i.p.) and sodium valproate (400 mg^{-1} i.p.) were unaltered by Ro15-1788. These anticonvulsants had no significant effect on [^{3}H]diazepam binding at concentrations up to 400 μM.

Further binding studies were performed in order to characterize the interactions of CBZ with various benzodiazepine receptor subclasses. Like the purines, the potency of CBZ as an inhibitor of binding of the benzodiazepine antagonist [^{3}H]Ro15-1788 was lower than that for 'agonist' (+)-[^{3}H]3-methylclonazepam or [^{3}H]diazepam (Table 2) binding. CBZ affinity for benzodiazepine binding is unaltered by 100 μM GABA, while the potencies of anticonvulsant benzodiazepines such as clonazepam, diazepam and nitrazepam are increased by GABA (Skerritt et al 1982b). Like CBZ, purine affinities are also not increased by GABA. CBZ interacted with both (a) 'receptor' sites thought to mediate the major actions of the benzodiazepines in the brain, and labelled by [^{3}H]Ro15-1788 and [^{3}H]methylclonazepam, and (b) 'acceptor' sites found in brain and periphery and labelled by [^{3}H]Ro5-4864. Although substantial labelling of 'acceptor' sites would occur at serum concentrations of CBZ, the functional relevance of this finding is at present unclear. Ro5-4864 differs significantly in its pharmacological profile from other clinically useful benzodiazepines, although it has been reported to have have anxiolytic activity in man (Zbinden & Randall 1967). While purines selectively interact with benzodiazepine 'receptor sites', some, but not all, anticonvulsant benzodiazepines have been shown to label both 'receptor' and 'acceptor' sites in brain (Marangos et al 1982; Skerritt et al 1982a).

In conclusion, these data suggest that an action of CBZ on brain benzodiazepine receptors may contribute, in addition to an action on purine receptors, to the therapeutic or side effects of this anticonvulsant.

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REFERENCES

Davies, L. P., Cook, A. F., Poonian, M., Taylor, K. M. (1980) Life Sci. 26: 1089–1095

Desmedt, L. K. C., Niemegeers, C. J. E., Lewi, P. J., Janssen, P. A. J. (1976) Arzeim. Forsch. 26: 1592–1602

Duka, T. Hollt, V., Herz, A. (1979) Brain Res 179: 147–156

Hunkeler, W., Mohler, H., Pieri, L., Polc, P., Bonetti, E. P., Cumin, R., Schaffner, R., Haefely, W. (1981) Nature (London) 290: 514–516

Marangos, P. J., Patel, J., Boulenger, J-P, Clark-Rosenberg, R. (1982) Mol. Pharmacol. 22: 26–32

Morselli, P. L. Baruzzi, A., Gerna, M., Bossi, L., Porta, M. (1977) Br. J. Clin. Pharmacol. 4: 535–540

Nutt, D. J., Cowen, P. J., Little, H. J. (1982) Nature (London) 295: 436–438

Olsen, R. W., Leeb-Lundberg, F. (1981) in: Morselli, P. L., Lloyd, K. G., Loscher, W., Meldrum, B., Reynolds, E. H. (eds) Neurotransmitters Seizures and Epilepsy. Raven Press, New York. pp 151–163

Paul S. M., Syapin, P. J., Paugh, B. A., Moncada, V., Skolnick, P. (1979) Nature (London) 221: 688–9

Skerritt, J. H., Chen Chow, S., Johnston, G. A. R., Davies, L. P. (1982a) Neurosci. Lett. 34: 63–68

Skerritt, J. H., Davies, L. P., Chen Chow, S., Johnston, G. A. R. (1982b) Ibid. 32: 169–174

Skerritt, J. H., Davies L. P., Johnston, G. A. R. (1982c) Eur. J. Pharmacol. 82: 195–197

Skerritt, J. H., Willow, M., Johnston, G. A. R. (1982d) Neurosci. Lett. 29: 63–66

Suria, A., Killam, E. K. (1980) in: Glaser, G. H., Penry, J. K., Woodbury, D. M. (eds) Antiepileptic Drugs: Mechanism of Action. Raven Press, New York, pp 563–575

Zbinden, G., Randall, L. O. (1967) Adv. Pharmacol. 5: 213–291