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Short communication

ENHANCEMENT OF IN VITRO BINDING AND SOME OF THE PHARMACOLOGICAL PROPERTIES OF DIAZEPAM BY A NOVEL ANTHELMINTIC AGENT, AVERMECTIN B_{1a}

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A novel macrocyclic lactone disaccharide anthelmintic agent, Avermectin B_{1a} (AVM) has been found to cause a concentration-dependent increase in the in vitro binding of ^{3}H-diazepam to rat and mouse brain membranes. The increase in binding is manifested as both an increase in the affinity and number of binding sites for ^{3}H-diazepam. Preliminary in vivo studies demonstrate that AVM can also enhance some of the pharmacological actions of diazepam.

^{3}H-Diazepam binding Anthelmintic Diazepam

1. Introduction

A high affinity, specific binding site for ^{3}H-diazepam has been described in mammalian brain. From correlations of the pharmacological actions of various benzodiazepines and their effects to displace ^{3}H-diazepam binding, this site has been designated as an "anxiolytic receptor" (Squires and Braestrup, 1977; Mohler and Okada, 1977). Several diverse compounds, among the Ni^{2+} (Mackerer and Kochman, 1978), γ-aminobutyric acid (GABA) and muscimol (Tallman et al., 1978; Williams and Risley, 1979) and a novel anxiolytic, SQ 20009 (Williams and Risley, 1979) have been shown to enhance the binding of ^{3}H-diazepam to rat brain membranes. The significance of these findings is not entirely clear although the modulation of ^{3}H-diazepam binding by GABAergic agents appears compatible with the well-known in vivo interactions of benzodiazepines and GABA (Haefley et al., 1975). Additionally, the interactions of SQ 20009 with diazepam binding may have some relevance to the purported anxiolytic actions of this compound (Beer et al., 1972). In the present studies, we have examined the interaction of a novel macrocyclic lactone disaccharide anthelmintic agent, Avermectin B_{1a}* (AVM: Fritz et al., 1979) with ^{3}H-diazepam binding and with some of the pharmacological actions of diazepam.

* Avermectin B_{1a} = (2aE,4E,5'S,6S,6'R,7S,8E,11R,13R,15S,17aR,20R,20aR,20bS)-6'-[(R)-sec-butyl]-7-[[2,6-dideoxy-4-O-(2,6-dideoxy-3-O-methyl-α-L-arabino-hexopyranosyl)-3-O-methyl-α-L-arabino-hexopyranosyl]oxy]-5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5',6,8,19-tetramethyl-spiro-[11,15-methano-2H,13H,17H-furo-[4,3,2-pq][2,6]benzodioxyacyclo-octadecin-13,2'-[2H]pyran]-17-one.

2. Materials and methods

2.1. ^{3}H-diazepam binding

Binding of ^{3}H-diazepam to crude synaptic membranes prepared from rat brain was measured by a modification of previously published methods (Squires and Braestrup, 1977; Mohler and Okada, 1977). The binding assay routinely consisted of triplicate tubes to

which were added: ^{3}H-diazepam (NEN; specific activity 39.1 Ci/mmol) to a final concentration of 1.5 nM; 1 ml of P_2 protein suspension (1 mg/ml) and 50 mM Tris HCl pH 7.4 to a final volume of 2 ml. Experimental compounds when used were diluted in 50 mM Tris HCl pH 7.4 and added to the incubation mixture in a volume of 0.1 ml to the final concentrations indicated. The reaction was initiated by the addition of membrane protein to tubes maintained at 4°C in an ice water bath and continued for 15 min at which time equilibrium was reached, and terminated by filtration under vacuum through Whatman GF/B filters. The filters were washed twice with 5 ml of ice-cold 50 mM Tris HCl pH 7.4, transferred to scintillation vials and 10 ml of Aquasol 2 (NEN) added. After shaking at room temperature for 30 min, radioactivity was measured in a Packard Model 3255 Scintillation Spectrometer at an efficiency of 38%. Specific binding was determined as the difference in the radioactivity bound in the absence and presence of 1 μM unlabelled diazepam and was approximately 90% of the total radioactivity bound. Scatchard analysis of the changes in ^{3}H-diazepam binding was determined over a concentration range of 0.625—10 nM.

2.2. Behavioral studies

Carworth, CF_1, female mice weighing 18—24 g were used in all studies. "Muscle power" was assessed through the use of a fixed wire mesh platform attached to a calibrated linear differential transformer which was connected electrically to a Servo pen recorder. Mice placed on the platform and restrained by the tail exerted considerable force in attempting to grip the mesh when pulled across it by the tail. This force was recorded as spaces of pen deflection. Locomotor activity was assessed with "donut" shaped photocell chambers consisting of a circular runway with a central light source and six photocells equally spaced around the outer perimeter of the runway. Counts resulting from interruption of the light beams were recorded on Sodeco counters. Animals were placed (one mouse per chamber) in a particular chamber on a randomized basis, and treated and control animals were tested concurrently.

3. Results

3.1. ^{3}H-diazepam binding

AVM caused a concentration-dependent increase in the binding of ^{3}H-diazepam to rat brain membranes (table 1) with a near maximal effect observed at 10 μM. Examination of the effects of AVM on ^{3}H-diazepam binding by kinetic analysis showed that the increase in binding was reflected as an increase in both the affinity (decrease in K_D) and number of binding sites (table 1). The effects of AVM on ^{3}H-diazepam binding to mouse brain membranes yielded similar data to that observed in rats (data not presented).

TABLE 1

AVM enhancement of ^{3}H-diazepam binding to rat brain membranes.

(A) Concentration—responses of AVM to enhance ^{3}H-diazepam binding

AVM concentration (μM)	% Increase in binding (mean ± S.D.; n = 4)
0.1	19 ± 9
1	44 ± 7
10	65 ± 3

(B) The effect of 500 nM AVM on affinity and number of ^{3}H-diazepam binding sites

	Control (mean + S.D.; n = 4)	+500 nM AVM (mean ± S.D.; n = 4)	% Change (significance)
K_D	4.56 ± 0.46	3.65 ± 0.32	−20 ($P < 0.02$)
n (pmoles/mg protein)	1.46 ± 0.07	1.64 ± 0.06	+12 ($P < 0.01$)

To the extent tested the action of AVM on ^{3}H-diazepam binding appeared specific insofar as it exhibited no activity at concentrations up to 50 μM on the binding of ^{3}H-WB 4101 (α-adrenergic receptor), ^{3}H-GABA (GABA receptor), ^{3}H-spiroperidol (dopamine receptor), ^{3}H-quinuclidinyl benzilate (muscarinic cholinergic receptor), ^{3}H-kainic acid (glutamate receptor) and ^{3}H-LSD (serotonin receptor).

3.2. Behavioral studies

Pretreatment with AVM (5 mg/kg i.p.) enhanced the reduction in "muscle power" induced by diazepam (table 2). This dose of AVM did not alter the muscle relaxant properties of cyclobenzaprine assessed by this test procedure. Furthermore, diazepam caused a dose-related decrease in spontaneous motor activity which was enhanced at all dose levels by pretreatment (30 min prior to receiving diazepam) with 5 mg/kg i.p. of AVM (data not shown). This dose of AVM did not significantly affect motor activity by itself.

TABLE 2

Enhancement by AVM of the decrease in 'muscle power' induced by diazepam [1].

Pretreatment [2] (mg/kg i.p.)		Treatment [3] (mg/kg i.p.)	Units of pen deflection [4] (X ± S.E.M.)
Methocel	+	Methocel	9.4 ± 0.2
Methocel	+	Diazepam (5)	7.5 ± 0.4 [5]
Methocel	+	Diazepam (10)	7.1 ± 0.4 [5]
Methocel	+	Diazepam (20)	6.3 ± 0.5 [5]
AVM (5)	+	Diazepam (5)	5.3 ± 0.5 [6]
AVM (5)	+	Diazepam (10)	4.8 ± 0.4 [6]
AVM (5)	+	Diazepam (20)	4.0 ± 0.4 [6]
AVM (5)	+	Methocel	9.0 ± 0.2

[1] N = 20 mice/treatment.
[2] 30 min before treatment.
[3] 30 min before test.
[4] 10 units of pen deflection = 100 g.
[5] Significantly different ($P < 0.05$) from methocel + methocel (Duncan's multiple range test).
[6] Significantly different ($P < 0.05$) from methocel + corresponding doses of diazepam (Duncan's multiple range test).

4. Discussion

AVM enhances the binding of ^{3}H-diazepam to rat brain membranes by increasing both the affinity and number of binding sites for the benzodiazepine. To our knowledge this is the first substance shown to increase the number of binding sites available to the radioligand, Ni^{2+}, GABA, muscimol and SQ 20009 producing their effects solely via a change in the affinity of the binding site. To the extent tested, this effect of AVM appears specific to the diazepam receptor. Furthermore, the observed in vivo enhancement by AVM of diazepam induced decreases in motor activity and muscle power would appear to indicate a degree of pharmacological relevance for the enhancement of binding observed in vitro. Clearly, further studies are required to determine the specificity of the pharmacological interaction between AVM and diazepam in vivo. Furthermore, while it is possible that this interaction may have relevance to the novel anthelmintic actions of this macrolide and its reported effects on chloride channels in invertebrates (Fritz et al., 1979), the lack of diazepam binding sites in invertebrates (Neilsen et al., 1978) would question such an extrapolation. It may be noted, however, that a relationship between the diazepam receptor and chloride anion channels in mammalian brain membranes has been inferred (Costa et al., 1979).

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References

Beer, B., M. Chasin, D.E. Clody, J.R. Vogel and Z.P. Horovitz, 1972, Cyclic adenosine monophosphate

phosphodiesterase in brain: Effect on anxiety, Science 176, 428.

Costa, T., D. Rodbard and C.B. Pert, 1979, Is the benzodiazepine receptor coupled to a chloride anion channel?, Nature London 277, 315.

Fritz, L.C., C.C. Wang and A. Gorio, 1979, Avermectin B_{1a} blocks inhibitory and excitatory postsynaptic potentials at the lobster neuromuscular junction, Proc. Nat. Acad. Sci. U.S.A. (in press).

Haefley, W., A. Kulscar, H. Mohler, L. Pieri, P. Polc and R. Schaffner, 1975, Possible involvement of GABA in the central actions of benzodiazepines, Advan. Biochem. Psychopharmacol. 14, 131.

Mackerer, C.R. and R.L. Kochman, 1978, Effects of cations and anions on the binding of ^{3}H-diazepam to rat brain, Proc. Soc. Exptl. Biol. Med. 158, 393.

Mohler, H. and T. Okada, 1977, Properties of ^{3}H-diazepam binding to benzodiazepine receptor in the rat cerebral cortex, Life Sci. 20, 2102.

Neilsen, M., C. Braestrup and R.F. Squires, 1978, Evidence for a late evolutionary appearance of brain-specific benzodiazepine receptors: an investigation of 18 vertebrate and 5 invertebrate species, Brain Res. 141, 342.

Squires, R.F. and C. Braestrup, 1977, Benzodiazepine receptors in rat brain, Nature London 266, 732.

Tallman, J.F., J.W. Thomas and D.W. Gallager, 1978, GABAergic modulation of benzodiazepine binding site sensitivity, Nature London 274, 383.

Williams, M. and E.A. Risley, 1979, Enhancement of the binding of ^{3}H-diazepam to rat brain membranes in vitro by SQ 20009, a novel anxiolytic, γ-aminobutyric acid (GABA) and muscimol, Life Sci. 24 (in press).