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ANTICONVULSANT ACTIONS OF THE PUTATIVE γ-AMINOBUTYRIC ACID (GABA)-MIMETIC, ETHYLENEDIAMINE

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1 Ethylenediamine, 31.6–1000 mg/kg intraperitoneally, inhibited the convulsive effects of pentylenetetrazol, 100 mg/kg (i.p.) in mice.

2 Ethylenediamine, 100–1000 mg/kg (i.p.) increased the convulsion threshold to the intravenous infusion of three convulsants in the order pentylenetetrazol > bicuculline > strychnine.

3 The benzodiazepine antagonist Ro15-1788, 10 mg/kg (i.p.), significantly inhibited the anticonvulsant action of diazepam, 50 μg/kg, but not ethylenediamine, 1000 mg/kg.

4 These results clearly indicate that ethylenediamine has anticonvulsant properties and are consistent with the hypothesis that ethylenediamine is a γ-aminobutyric acid (GABA)-mimetic.

Introduction

Ethylenediamine (1, 2-diaminoethane, EDA) is a powerful neuronal depressant whose actions *in vivo* and *in vitro* can be selectively blocked by the γ-aminobutyric acid (GABA) antagonist, bicuculline (Forster, Lloyd, Morgan, Parker, Perkins & Stone, 1981; Perkins, Bowery, Hill & Stone, 1981; Bokisch, Bold, Perkins, Roberts, Stone & Walker, 1982) or picrotoxin (Blaxter & Cottrell, 1982).

Preliminary *in vitro* binding studies with a rat brain synaptosomal preparation indicate that EDA can displace specifically bound [^{3}H]-GABA (Bowery, Hill, Hudson, Perkins & Stone, 1982). EDA can also be actively taken up by rat brain slices and released by potassium in a calcium-dependent manner (Lloyd, Perkins, Gaitonde & Stone, 1982a) and has been reported to be useful as a specific releasing agent of GABA in rat brain slices (Lloyd, Perkins & Stone, 1982b). EDA thus appears to activate GABA-ergic mechanisms by both direct and indirect means.

The GABA antagonist, bicuculline, is a potent convulsant (Naik, Guidotti & Costa, 1976; Worms, Depoortere & Lloyd, 1979; Unnerstall & Pizzi, 1981) and GABA agonists have been reported to have marked anticonvulsant actions (Naik *et al.*, 1976; Worms *et al.*, 1979; Unnerstall & Pizzi, 1981). GABA-mimetics such as EDA might therefore also be expected to have anticonvulsant actions.

Diazepam is a potent anticonvulsant (Haefely, Kulcsar, Möhler, Pieri, Polo & Schaffner, 1975) with a largely obscure mode of action (Iversen, 1980; Tallman, Paul, Skolnick & Gallager, 1980) although much credence has been given to the view that diazepam acts by enhancing the effectiveness of GABA in the brain (Iversen, 1978; 1980; Tallman *et al.*, 1980). The finding that EDA can modulate *in vitro* benzodiazepine receptors in rat brain synaptosomal preparations (Morgan, Perkins & Stone, 1982; Morgan & Stone, 1982) is consistent with a GABA-mimetic action of EDA discussed above since GABA and other GABA agonists can also modulate benzodiazepine receptors (Briley & Langer, 1978; Wastek, Speth, Reisine & Yamamura, 1978; Martin & Candy, 1978; Tallman, Thomas & Gallager, 1978). However, the finding of *in vitro* modulation of benzodiazepine receptors by EDA also raises the possibility that EDA may have anticonvulsant actions by virtue of a modulatory action on benzodiazepine receptors.

In view of the above considerations it was decided to examine a possible anticonvulsant action of EDA and to assess to what extent this may reflect an action at GABA and/or benzodiazepine receptors. First we investigated a potential anticonvulsant action of EDA against pentylenetetrazol (PTZ) seizures (Lewin & Esplin, 1961; Costa, Guidotti, Mao & Suria, 1975; Nicoll & Padjen, 1976; Pellman & Wilson, 1977), and compared the action of EDA with that of diazepam in the presence of the benzodiazepine antagonist Ro15-1788 (Hunkeler, Möhler, Pieri, Polc, Bonetti, Cumin, Schaffner & Haefely, 1981). Ro15-1788 has been shown to block anticonvulsant actions mediated via benzodiazepine receptors (Hunkeler *et al.*, 1981; Nutt, Cowen & Little, 1982). The action of EDA was subsequently tested against several other convulsants having different mechanisms of action.

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Methods

Animals and experimental design

Naive male T.O. mice weighing between 28 to 43 g were used. All experiments were conducted according to a double blind experimental design with the exception of infusion studies which were blind with respect to EDA administration only, but not with respect to the infusion of convulsants.

Experiments with injected pentylenetetrazol

In injection experiments animals were treated with ethylenediamine dihydrochloride (EDA) dissolved in saline (0.9% w/v NaCl solution); diazepam dissolved in propylene glycol; Ro15-1788 (ethyl-8-fluoro-5, 6-dihydro-5-methyl-6-oxo-4H-imidazol (1, 5-a)-(1, 4) benzodiazepine-3-carboxylate) dissolved in saline (containing 2 drops of Tween 40 per 10 ml) or the appropriate vehicle as described, then challenged with pentylenetetrazol dissolved in saline. Details of the dosage schedules used are given in legends to Figures 1 and 2. All injected drugs and vehicles were administered through a 1 ml syringe and a 25 gauge × 5/8 in needle in a drug volume of 0.15 to 0.18 ml according to weight of animals. After PTZ administration animals were observed for a maximum of 30 min, whereupon the number of animals displaying seizures and fatalities were noted.

Experiments with infused convulsants

In infusion experiments animals were pretreated with EDA or vehicle as described above. Convulsants (either PTZ (10 mg/ml) dissolved in saline; strychnine sulphate (1 mg/ml) dissolved in saline: or (+)-bicuculline (0.1 mg/ml) dissolved in saline adjusted to pH 3 with 1M HCl) were infused into a lateral tail vein through a 27 gauge × 3/8 in butterfly infusion set from a 10 ml syringe mounted on a Saga 341 constant infusion pump. (+)-Bicuculline was used within 30 min of being dissolved as this compound rapidly undergoes major changes in aqueous solution (Olsen, Ban, Miller & Johnston, 1975). The latency between starting infusion and the onset of convulsions was measured and from this measurement, the infusion rate (0.84 ml/min), the weight of the animals, and the concentration of the convulsant, the amount of convulsant required to elicit convulsions (convulsion threshold) was calculated.

EDA, PTZ and (+)-bicuculline were obtained from Sigma, U.S.A.; diazepam and Ro15-1788 were gifts from Roche Switzerland.

Results

Behavioural effects

Animals treated with doses of EDA above 10 mg/kg appeared to be sedated in a dose-dependent manner, but remained responsive to tactile stimulation and retained the righting reflex. High doses of EDA (100 to 1000 mg/kg) also induced diarrhoea.

Antagonism of pentylenetetrazol convulsions by ethylenediamine

EDA antagonized the convulsive action of PTZ when given by the same route (i.p.): this was apparent both as a prevention of PTZ-induced convulsions, and in preventing PTZ-induced mortality (Figure 1).

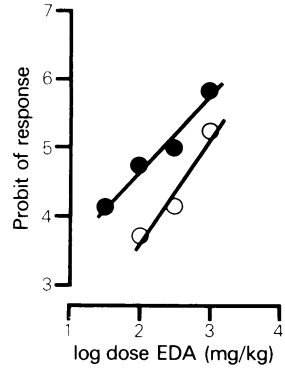

Figure 1 The antagonistic action of ethylenediamine (EDA) on pentylenetetrazol (PTZ)-induced convulsions. Animals were pretreated with EDA (1 to 1000 mg/kg i.p.) or saline vehicle 30 min before being given a dose of PTZ (100 mg/kg i.p.) which was both a convulsant and lethal dose to all saline vehicle-treated animals ($n = 10$). Log dose-probit curves demonstrate the protective action of EDA in (○) preventing PTZ-induced convulsions ($ED_{50} = 840$ mg/kg) and (●) preventing PTZ-induced mortality ($ED_{50} = 200$ mg/kg). Each data point represents the protection afforded to 10 mice. Probit values are unadjusted.

Anticonvulsant effects of ethylenediamine against infused convulsants

EDA was found to increase significantly the seizure threshold to intravenously infused PTZ, bicuculline and strychnine (Figure 2). EDA appeared to be more effective against seizures induced by PTZ than against seizures induced by strychnine. Thus after pretreating animals with a dose of EDA of 1000 mg/kg (i.p.) the percentage increase in seizure

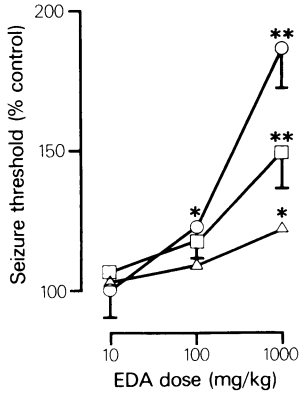

Figure 2 Comparison of the anticonvulsant action of ethylenediamine (EDA) against three infused convulsants. Animals were pretreated with EDA (10 to 1000 mg/kg) or saline vehicle (both in a volume of 0.15 to 0.18 ml i.p.) 30 min before being infused (0.84 ml/min) with either pentylenetetrazol (PTZ, 10 mg/ml, ○), (+)-bicuculline (0.1 mg/ml, □), or strychnine (1 mg/ml, △) into a lateral tail vein. From the latency between initiating the infusion and the onset of convulsions the amount of convulsant required to elicit convulsions (seizure threshold) was determined as described in the Methods. Control values of seizure threshold were 31.9 ± 2.5 (6) mg/kg PTZ; 0.50 ± 0.03 (8) mg/kg bicuculline and 2.96 ± 0.23 (6) strychnine (mean ± s.e.mean's with n in parentheses). Each data point in the figure represents the mean of determinations from 4 to 8 animals with s.e.mean's shown where greater than 5%. * = $P < 0.05$, ** = $P < 0.005$ (unpaired t test; n = 4 to 8).

threshold to PTZ was significantly greater than the percentage increase to strychnine ($P < 0.01$, unpaired t test). The percentage increase to bicuculline, however, was not significantly different ($P > 0.05$, unpaired t test) from the percentage increase to either PTZ or strychnine.

Effect of the benzodiazepine antagonist, Ro15-1788

The specific benzodiazepine receptor antagonist, Ro15-1788, significantly ($P < 0.01$, Chi squared test) reduced the anticonvulsant action of diazepam, but did not reduce the action of EDA (Table 1).

Discussion

The results demonstrate that EDA is an effective anticonvulsant drug. Its profile of action against the convulsants tested is consistent with the possibility that EDA is a GABA-mimetic (Forster *et al.*, 1981; Perkins *et al.*, 1981; Bokisch *et al.*, 1982; Lloyd *et al.*, 1982b), although the present results clearly do not reveal the site of action of EDA. Bearing in mind the postulate that EDA may be a GABA-mimetic it is also of interest that the behavioural effects of EDA are reminiscent of behavioural effects reported for the GABA agonist, muscimol, by Unnerstall & Pizzi (1981).

EDA is able to potentiate [^{3}H]-diazepam binding to rat cerebral synaptosomal membranes (Morgan, Perkins & Stone, 1982; Morgan & Stone, 1982), and the anticonvulsant action of benzodiazepines has been attributed at least in part to an enhancement of GABA-ergic transmission (Braestrup & Nielsen, 1980). However, the lack of effect of the benzodiazepine antagonist, Ro15-1788, on the action of EDA precludes the possibility that benzodiazepine receptor modulation plays a major role in the anticonvulsant action of EDA.

With regard to the effectiveness of EDA in increasing the seizure threshold to PTZ, bicuculline, and strychnine, it is interesting to note that similar results have been observed with the GABA agonist, muscimol (Naik *et al.*, 1976). It should be noted, however, that several authors have reported that

Table 1 The effect of Ro15-1788 on the anticonvulsant action of diazepam and ethylenediamine (EDA) against pentylenetetrazol (PTZ)-induced convulsions

	% protected		
Treatment	*Vehicle* (Tween/saline i.p.)	Ro15-1788 (10 mg/kg i.p.)	*Probability* of *difference*
Vehicle	0% (10)	0% (10)	NS
Diazepam 50 μg kg^{-1}	37% (19)	5% (20)	$P < 0.01$
EDA 1000 mg kg^{-1}	50% (18)	56% (18)	NS

Animals were pretreated with EDA (1000 mg/kg i.p.) or saline vehicle 60 min before injection of PTZ (100 mg/kg i.p.) and 30 min before injection of diazepam (50 μg/kg i.p.) or propylene glycol vehicle. Ro15-1788 (10 mg/kg i.p.) or saline (containing 2 drops of tween 40 per 10 ml) vehicle was given 15 min after diazepam treatment and 15 min before PTZ administration. Data represent the percentage of animals which did not convulse with numbers of animals tested in parentheses. Probability of difference determined by Chi squared test, NS = not significant.

muscimol is a more effective anticonvulsant against strychnine-induced seizures than against either PTZ- or bicuculline-induced seizures (Worms *et al.*, 1979; Unnerstall & Pizzi, 1981), and it has been suggested that it may even be a proconvulsant against PTZ- or bicuculline-induced convulsions (Unnerstall & Pizzi, 1981). This reported variability in the effects of muscimol, which seems to depend on dose, route and species may result from its rapid metabolism *in vivo* (Maggi & Enna, 1979; Baraldi, Grandison & Guidotti, 1979; Unnerstall & Pizzi, 1981). EDA may therefore be a more useful compound for studying central GABA-ergic mechanisms after peripheral administration.

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References

BARALDI, M., GRANDISON, L. & GUIDOTTI, A. (1979). Distribution and metabolism of muscimol in the brain and other tissues in the rat. *Neuropharmac.*, **18,** 57–62.

BLAXTER, T.J. & COTTRELL, G.A. (1982). Responses of hippocampal pyrimidal cells to GABA and ethylenediamine. *J. Physiol.*, (in press).

BOKISCH, A.J., BOLD, J.M., PERKINS, M.N., ROBERTS, C.J., STONE, T.W. & WALKER, R.J. (1982). Actions of ethylenediamine on Limulus and Helix central neurons and on rat cerebellar and sympathetic ganglion neurons. *Br. J. Pharmac.*, **76,** 297P.

BOWERY, N.G., HILL, D.R., HUDSON, A.L., PERKINS, M.N. & STONE, T.W. (1982). GABA receptor binding in Physiological salt solution. *Br. J. Pharmac.*, **76,** 47P.

BRAESTRUP, C. & NIELSEN, M. (1980). Searching for endogenous benzodiazepine receptor ligands. *Trends Pharmac. Sci.*, **1,** 424–427.

BRILEY, M.S. & LANGER, S.Z. (1978). Influence of GABA receptor agonists and antagonists on the binding of ^{3}H-diazepam to the benzodiazepine receptor, *Eur. J. Pharmac.*, **52,** 129–132.

COSTA, E., GUIDOTTI, A., MAO, C.C. & SURIA, A. (1975). New concepts on the mechanism of action of benzodiazepines. *Life Sci.*, **17,** 167–185.

FORSTER, P., LLOYD, H.G.E., MORGAN, P.F., PARKER, M., PERKINS, M.N. & STONE, T.W. (1981). Ethylenediamine acts upon GABA receptors and uptake sites. *Br. J. Pharmac.*, **74,** 274P.

HAEFELY, W., KULCSAR, A., MÖHLER, H., PIERI, L., POLC, P. & SCHAFFNER, R. (1975). Possible involvement of GABA in the central actions of benzodiazepines. *Mechanism of Action of benzodiazepines*, Vol. 14. ed. Costa, E. & Greengard, P. pp. 131–151. New York: Raven Press.

HUNKELER, W., MÖHLER, H., PIERI, L., POLC, P., BONETTI, E.P., CUMIN, R., SCHAFFNER, R. & HAEFELY, W. (1981). Selective antagonists of benzodiazepines. *Nature*, **290,** 514–516.

IVERSEN, L.L. (1978). GABA and benzodiazepine receptors. *Nature*, **275,** 477–478.

IVERSEN, L.L. (1980). The present state of tranquility. *Nature*, **285,** 285–286.

LEWIN, J. & ESPLIN, J. (1961). Analysis of the spinal excitatory action of petylenetetrazol. *J. Pharmac. exp. Ther.*, **132,** 245–250.

LLOYD, H.G.E., PERKINS, M.N., GAITONDE, M.K. & STONE, T.W. (1982a). Uptake and calcium-dependent release of Ethylenediamine (1, 2-diaminoethane) by rat brain slices. *J. Neurochem.*, **38,** 1118–1122.

LLOYD, H.G.E., PERKINS, M.N. & STONE, T.W. (1982b). Ethylenediamine as a specific releasing agent of γ-aminobutyric and in rat striatial slices. *J. Neurochem.*, **38,** 1168–1169.

MAGGI, A. & ENNA, S.J. (1979). Characteristic of muscimol accumulation in mouse brain after systemic administration. *Neuropharmac.*, **18,** 361–366.

MARTIN, I.L. & CANDY, J.M. (1978). Facilitation of benzodiazepine binding by sodium chloride and GABA. *Neuropharmac.*, **17,** 993–998.

MORGAN, P.F., PERKINS, M.N. & STONE, T.W. (1982). Ethylenediamine potentiates *In Vitro* ^{3}H-diazepam binding to rat cerebral cortex. *Br. J. Pharmac.*, **75,** 95P.

MORGAN, P.F. & STONE, T.W. (1982). Ethylenediamine and GABA potentiation of ^{3}H-diazepam binding to benzodiazepine receptors in rat cerebral cortex. *J. Neurochem.* (in press).

NAIK, S.R., GUIDOTTI, A. & COSTA, E. (1976). Central GABA receptor agonists: Comparison of muscimol and baclofen. *Neuropharmac.*, **15,** 479–84.

NICOLL, R.A. & PADJEN, A. (1976). Pentylenetetrazol: an antagonist of GABA at primary afferents of the isolated frog spinal cord. *Neuropharmac.*, **15,** 69–71.

NUTT, D.J., COWEN, P.J. & LITTLE, H.J. (1982). Unusual interactions of benzodiazepine receptor antagonists. *Nature*, **295,** 436–438.

OLSEN, R.W., BAN, M., MILLER, J. & JOHNSTON, G.A.R. (1975). Chemical instability of the GABA antagonist bicuculline under physiological conditions. *Brain Res.*, **98,** 383–388.

PELLMAN, T.C. & WILSON, W.A. (1977). Synaptic mechanism of pentylenetetrazole: selectivity for chloride conductance. *Science*, **197,** 912–914.

PERKINS, M.N., BOWERY, N.G., HILL, D.R. & STONE, T.W. (1981). Neuronal responses to ethylenediamine: Preferential blockade by bicuculline. *Neurosci. Lett.*, **23,** 325–328.

TALLMAN, J.F., THOMAS, J.W. & GALLAGHER, D.W. (1978). GABA-ergic modulation of benzodiazepine binding site sensitivity. *Nature*, **274,** 383–385.

TALLMANN, J.F., PAUL, S.M., SKOLNICK, P. & GALLAGHER, D.W. (1980). Receptors for the age of anxiety: Pharmacology of the benzodiazepines. *Science*, **207,** 274–281.

UNNERSTALL, J.R. & PIZZI, W.J. (1981). Muscimol and

γ-hydroxybutyrate: Similar interactions with convulsant agents. *Life Sci.*, **29,** 337–344.

WASTEK, G.W., SPETH, R.C., REISINE, T.D. & YAMAMURA, H.I. (1979). The effect of γ-aminobutyric acid on ^{3}H-flunitrazepam binding in the rat brain. *Eur. J. Pharmac.*, **50,** 445–447.

WORMS, P., DEPOORTERE, H. & LLOYD, K.G. (1979). Neuropharmacological spectrum of muscimol. *Life Sci.*, **25,** 607–614.

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