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Bicarbonate-dependence of responses to ethylenediamine in the guinea-pig isolated ileum: involvement of ethylenediamine-monocarbamate

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1 γ-Aminobutyric-acid (GABA)-mimetic responses were induced by ethylenediamine (EDA) in the isolated ileum of the guinea-pig maintained in bicarbonate buffered Krebs-Henseleit (KBC) solution, pH 7.4, 37°C, the responses consisting of a contraction followed by a relaxation. There were no such responses to EDA in bicarbonate-free phosphate buffered (KPO) or HEPES buffered (KHO) Krebs solution, gassed with 100% O_2, pH 7.4, 37°C, yet the ileum responded to GABA in bicarbonate-free Krebs solution.

2 Similar GABA-mimetic responses were induced by EDA in the isolated ileum maintained in bicarbonate-free KPO or KHO modified Krebs solution, gassed with O_2, if HCO_3^- (5 mM) was first added immediately before the test dose of EDA (0.1–1 mM), the threshold [HCO_3^-] being 2 mM for EDA-induced responses in these preparations. However, ileal GABA-mimetic responses were induced in bicarbonate-free KPO or KHO solutions by EDA that had been pretreated with carbon dioxide, where the final [HCO_3^-] in the bath did not exceed 25 μM.

3 Ethylenediamine monocarbamate (synthetic EDAC) released [^{3}H]-GABA from preloaded segments of ileum maintained in bicarbonate-free KPO or KHO solution containing amino-oxyacetic acid and β-alanine, the release being sensitive to 3-mercaptopropionic acid which prevents GABA release. EDA itself did not evoke any such release in the absence of bicarbonate, but released [^{3}H]-GABA from segments maintained in KBC solution.

4 GABA-mimetic responses were induced by EDAC in the isolated ileum maintained in bicarbonate-free KPO solution, as was a δ-aminovalerate-sensitive depression of ileal twitch responses elicited by transmural stimulation, all of which were also sensitive to 3-mercaptopropionic acid.

5 It is concluded that GABA-mimetic responses to EDA in the isolated ileum of the guinea-pig, maintained in normal Krebs bicarbonate medium, result from the release of endogenous GABA by ethylenediamine monocarbamate formed through the rapid reaction of EDA with the carbon dioxide of bicarbonate buffered Krebs solution. Furthermore, in the ileum, HCO_3^- ions *per se* are not necessary for this GABA-releasing property of EDA if the latter is first converted to the monocarbamate, since synthetic ethylenediamine monocarbamate elicits ileal GABA-mimetic responses in the total absence of bicarbonate.

Introduction

Ethylenediamine (EDA) interacts with γ-aminobutyric acid (GABA) transport systems and releases GABA from brain slices (Forster *et al.*, 1981; Lloyd *et al.*, 1982a,b; Davies *et al.*, 1983), as well as showing GABA-mimetic properties at $GABA_A$-receptor sites where it displaces GABA and increases benzodiazepine binding (Forster *et al.*, 1981; Davies *et al.*, 1982; Perkins & Stone, 1982). The structural requirements for activity at GABA-receptors (Allan & Johnston, 1983; Krogsgaard-Larsen *et al.*, 1983) make these actions rather unexpected in such a diamine, and, whilst it has been proposed that EDA acts as a GABA agonist by virtue of its paired amine groups (Morgan & Stone, 1982; Perkins & Stone, 1982; Stone & Perkins, 1984), there is some evidence that the bicarbonate ion of Krebs-Henseleit (bicarbonate buffered) solution (KBC) is in some way required for the expression of GABA-mimetic properties by EDA in

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isolated preparations, with the suggestion that EDA monocarbamate (EDAC) is formed under such conditions (Curtis & Malik, 1984). Thus, virtually no uptake of EDA occurs in the absence of bicarbonate (Davies *et al.*, 1983). EDA is more active in receptor binding studies if bicarbonate is present (Bowery *et al.*, 1982), and the pharmacological responses to EDA, mediated through GABA receptors, require physiological concentrations of bicarbonate in the medium (Hill, 1985). Although all this may merely indicate that HCO_3^- ions are somehow necessary for these EDA actions, since bicarbonate directly influences the affinity of GABA receptors (Kurioka *et al.*, 1981), it is also possible that EDA must, instead, first undergo conversion to some directly GABA-mimetic compound in the presence of the bicarbonate buffer of KBC, the most likely product being the monocarbamate (EDAC) that readily forms from EDA in the presence of carbon dioxide (Jensen & Christensen, 1955; Frahn & Mills, 1964). The latter possibility is strongly supported by the observation (Curtis & Malik, 1984) that EDAC, prepared in aqueous solution from EDA by treatment with carbon dioxide, is a GABA-mimetic with a potency and speed of action comparable with that of GABA itself, in contrast to the parent diamine which is less potent and slower acting on dorsal horn cells of the cat spinal cord.

More recently, in the guinea-pig isolated ileum maintained in KBC solution, we have shown that EDA releases endogenous GABA which then induces both $GABA_A$- and $GABA_B$-receptor-mediated actions: a bicuculline-sensitive, Cl^- -dependent contraction of the intestine, and a bicuculline-insensitive relaxation or depression of transmurally elicited twitch contractions (Kerr & Ong, 1984). In view of the evident importance of bicarbonate ions for other GABA related EDA actions, we have now investigated the bicarbonate-dependence of EDA-induced responses in the isolated ileum, and here report that EDA is only effective in eliciting ileal GABA-mimetic responses, through the release of GABA, if converted to the monocarbamate or used in the presence of bicarbonate buffer from which the carbamate may be formed, this release being prevented by 3-mercaptopropionic acid (Fan *et al.*, 1981).

Methods

Guinea-pig isolated intestinal preparations

Segments of guinea-pig isolated distal ileum were mounted vertically in 10 ml organ baths containing Krebs-Henseleit bicarbonate solution (KBC). The longitudinal muscle activity of each tissue was recorded isometrically at a resting tension of 10 mN, by means of a Grass Model FT03 force transducer coupled to a Grass polygraph recorder. Tissues were allowed to equilibrate for 60 min in the organ baths before any drug treatments. Volumes of drugs used were never more than 1% of the bath volume. Where bicarbonate-free Krebs solutions were used, the tissues were allowed to equilibrate for a further 10–15 min before addition of drugs.

[^{3}H]-GABA release by ethylenediamine (EDA) and its monocarbamate (EDAC)

Guinea-pigs of either sex, weighing between 200–400 g, were stunned by a blow on the head and bled. Segments of the distal ileum, 3–4 cm in length, taken 2–3 cm from the ileo-caecal valve, were quickly removed, and, after being emptied of their contents, equilibrated for 10–20 min in aerated Krebs-Henseleit bicarbonate solution (95% O_2 and 5% CO_2, pH 7.4, 37°C). The tissues were loaded with [^{3}H]-GABA in separate vessels containing this warmed Krebs-bicarbonate solution (5 ml), with [^{3}H]-GABA (10 nM) (66 Ci mmol^{-1}), amino-oxyacetic acid (0.1 mM), and β-alanine (1 mM) for 20 min at 37°C. Amino-oxyacetic acid and β-alanine were subsequently present throughout each experiment to minimize [^{3}H]-GABA metabolism and to prevent [^{3}H]-GABA uptake into glial cells (Kerr & Ong, 1984). The tissues were then removed, blotted to remove excess incubating medium, and transferred to a glass chamber for the efflux studies, where they were washed at 2 min intervals over an equilibration period of 60 min. The basal efflux of [^{3}H]-GABA was established by collecting 2 ml fractions of the superfusate over 2 min periods. Drugs were then added to the medium and 2 ml samples again collected. 3-Mercaptopropionic acid (3-MPA), when used, was added to the superfusate at least 5 min before a dose of EDA or the monocarbamate EDAC. When bicarbonate-free Krebs solution was used, the superfusate was changed to phosphate or HEPES buffered Krebs solution after basal efflux had been reached; 2 ml fractions were then again collected to re-establish the basal efflux, after which various doses of EDA or EDAC were added to the superfusate in the presence or absence of 3-MPA, and further 2 ml samples collected. Radioactivity was determined with liquid scintillation spectrometry (Beckman LS2800), and expressed as d.p.m. after correction for quenching by all drugs used. All experimental protocols were performed in duplicate and repeated at least twice, with at least 6 tissues from a minimum of 3 animals. Statistical analysis by Student's *t* test for paired and unpaired samples was used to assess the significance of difference between the means of samples.

Bicarbonate-buffered and bicarbonate-free media

Bicarbonate-buffered Krebs-Henseleit medium

(KBC) contained (mM): Na^+ 151, K^+ 4.6, Ca^{2+} 2.5, Mg^{2+} 0.6, Cl^- 134.9, HCO_3^- 24.9, $H_2PO_4^-$ 1.3, SO_4^{2-} 0.6, glucose 7.7 (gassed with 95% O_2, 5% CO_2; pH 7.4, 37°C): phosphate buffered bicarbonate-free medium (KPO) contained (mM): Na^+ 151, K^+ 4.6, Ca^{2+} 1.3, Mg^{2+} 0.6, Cl^- 142, SO_4^{2-} 0.6, $H_2PO_4^-$/HPO_4^{2-} 14 (pH 7.4), gassed with 100% O_2; HEPES-buffered, bicarbonate-free medium (KHO) contained (mM): Na^+ 151, K^+ 4.6, Ca^{2+} 1.6, Mg^{2+} 0.6, Cl^- 157.2, SO_4^{2-} 0.6, $H_2PO_4^-$ 1.3, HEPES 5 (pH 7.4), gassed with 100% O_2.

Sources of chemicals

β-Alanine (BALA), γ-aminobutyric acid (GABA), amino-oxyacetic acid, δ-aminovaleric acid (DAVA), atropine sulphate, ethylenediamine dihydrochloride (EDA) (Sigma); 2, 3[^{3}H]-GABA (Radiochemical Centre, Amersham); bicuculline methochloride (BMC) (Pierce); 3-mercaptopropionic acid (3-MPA) (Koch-Light). Ethylenediaminine monocarbamate, $H_2N.CH_2.CH_2.NH.COOH$. (EDAC) was synthesized (D.I.B.K.) by the method of Katchalski *et al.* (1951), by passing dry CO_2 through ethylenediamine base dissolved in methyl alcohol at −20°C, confirmed by n.m.r. and molecular composition.

Results

Bicarbonate-dependence of ethylenediamine-induced ileal responses

As previously shown (Kerr & Ong, 1984), comparable concentration-dependent responses to both GABA and EDA were elicited in the guinea-pig isolated ileum maintained in normal bicarbonate-buffered Krebs (KBC) solution, containing 25 mM HCO_3^- and gassed with 5% Carbogen (95% O_2, 5% CO_2), the responses consisting of a contraction followed by an 'after-relaxation'. But there was no response to EDA (0.3–3 mM) in modified Krebs solutions containing no bicarbonate (phosphate buffered, KPO, or HEPES buffered, KHO, gassed with 100% O_2), yet responses to GABA (10–100 μM) persisted in the absence of bicarbonate. However, in KPO or KHO solution, responses to EDA (0.3–1 mM) occurred if bicarbonate (2–5 mM) was added to the bath immediately before EDA (Figure 1). The critical concentration of added HCO_3^- was 2 mM, although generally 5 mM HCO_3^- has been used since this yielded more consistent results, the HCO_3^- content being altered by the addition of various proportions of normal KBC solution containing 25 mM HCO_3^- and gassed with 5% Carbogen. In the presence of HCO_3^- (2–5 mM), contractile responses induced by EDA were sensitive to BMC (10 μM) and atropine (0.7 μM), whilst the

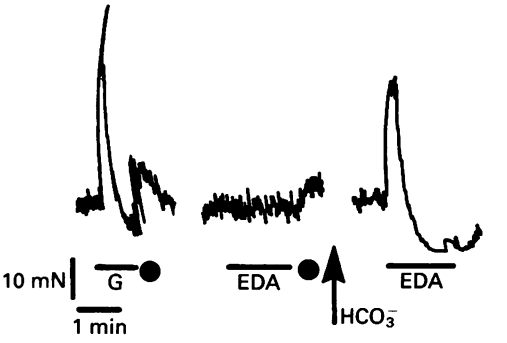

Figure 1 Effect of bicarbonate on GABA-mimetic responses to ethylenediamine in the guinea-pig isolated ileum maintained in bicarbonate-free Krebs solution buffered with phosphate and gassed with 100% O_2. In the absence of bicarbonate, the ileum responded to GABA (10 μM) but not to ethylenediamine (EDA, 1 mM). On adding 5 mM HCO_3^- (arrow), EDA (1 mM) elicited a response comparable with the previous control response to GABA; (●) indicates repeated washout.

'after-relaxation' was antagonized by DAVA (0.1 mM). Responses to EDA were also prevented by 3-MPA (0.5 mM) which prevents GABA release (Kerr & Ong, 1984).

In order to test if this minimal [HCO_3^-] was in itself responsible for thus rendering EDA effective in eliciting GABA-mimetic responses, EDA (1 M) was pretreated with 5% Carbogen for 30 min, with or without 25 mM HCO_3^-, and subsequently applied to ileal tissues maintained in KPO or KHO solution. Although there would then be no more than 25 μM HCO_3^- finally present in the KPO or KHO solution, this pretreated EDA (0.3–1 mM) still elicited responses identical to those with EDA in KBC, suggesting the possibility that the EDA had been converted to some GABA analogue capable of releasing endogenous GABA from the myenteric plexus in the virtual absence of HCO_3^-. For this reason the experiments were repeated in the total absence of bicarbonate but using synthetic ethylenediamine monocarbamate (EDAC) which is the most likely product, and the ability of EDAC to release [^{3}H]-GABA was also tested.

[^{3}H]-GABA efflux induced by synthetic EDAC in the bicarbonate-free medium

In normal KBC solution containing amino-oxyacetic acid (AOAA, 0.1 mM) and β-alanine (1 mM), both EDA (1 mM) and synthetic EDAC (1 mM) induced a comparable release of [^{3}H]-GABA from preloaded isolated ileum segments. This release was reduced by 3-mercaptopropionic acid (3-MPA, 1 mM), with a res-

toration of [^{3}H]-GABA release after washing out the 3-MPA. EDAC (1 mM) also induced a 3-MPA-sensitive release of [^{3}H]-GABA in bicarbonate-free KPO containing AOAA (0.1 mM) and β-alanine (1 mM), gassed with 100% O_2, whereas EDA (1 mM) itself did not induce any such release under these conditions with HCO_3^- absent (Table 1); there was no significant difference in the [^{3}H]-GABA release induced by EDAC in normal KBC, with bicarbonate present, as compared with the release in KPO without bicarbonate.

GABA-mimetic responses induced in the ileum by synthetic EDAC

In the ileum maintained in bicarbonate-free KPO or KHO, the addition of synthetic EDAC (0.3–3 mM) induced concentration-dependent GABA-mimetic responses, a neurogenic, cholinergic contraction followed by an 'after-relaxation'. Just as with GABA, such contractile responses were antagonized by bicuculline methochloride (BMC, 10 μM) as seen in Figure 2a, or picrotoxinin (10 μM), whilst the 'after-relaxation' was antagonized by δ-aminovaleric acid (DAVA, 0.5 mM). These responses induced by EDAC (1 mM), in either KBC or KPO solution, were prevented by 3-MPA (0.5 mM) which blocks GABA release (Figure 2b). EDAC was similarly effective in KBC solution gassed with carbogen.

In bicarbonate-free KPO solution, using repetitive twitch contractions elicited by transmural stimulation of the ileal segment (1 ms pulse duration, 0.1 Hz, supramaximal voltage), EDAC (1 mM) induced a depression of the twitch height (Figure 2c). This depressive action of EDAC was antagonized by DAVA (0.5 mM), a weak antagonist at $GABA_B$ sites (Kerr & Ong, 1984). Such EDAC-induced depression of ileal twitch responses was also attenuated, or prevented by 3-MPA (0.5 mM) applied 2–5 min before EDAC.

Discussion

In the guinea-pig isolated ileum maintained in KBC bicarbonate buffered solution, EDA exerts its GABA-mimetic actions through releasing endogenous GABA from the myenteric plexus (Kerr & Ong, 1984). It appears that EDA must first be converted to EDAC, the monocarbamate of EDA, for such release to occur. In keeping with this, synthetic EDAC caused a release of [^{3}H]-GABA from preloaded ileal segments, and induced GABA-mimetic actions, when the KBC was replaced with a bicarbonate-free solution (KPO or KHO, gassed with 100% O_2), but the parent EDA was itself ineffective in the absence of bicarbonate. However, responses to EDA promptly appeared if bicarbonate solution, as KBC previously gassed with carbon dioxide, was first added to the bath containing KPO or KHO. It was only necessary to add a

Table 1 Release of [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) from preloaded isolated ileum of the guinea-pig by ethylenediamine and its monocarbamate: effect of bicarbonate-free solution, and of 3-mercaptopropionic acid (3-MPA).

	Ethylenediamine (1 mM)	*Ethylenediamine-monocarbamate (1 mM)*
	d.p.m. × 10^{-3}	
KBC	4.8 ± 0.3	4.9 ± 0.1
KBC + 3-MPA 5 × 10^{4}M	2.1 ± 0.5	2.5 ± 0.3
KBC after wash	4.3 ± 0.2	4.1 ± 0.2
KPO	0.5 ± 0.1	5.0 ± 0.2
KPO + 3-MPA 5 × 10^{-4}M	—	2.3 ± 0.1
KPO after wash	—	4.2 ± 0.4

Results are expressed as d.p.m. × 10^{-3} ± s.e.mean ($n = 6$ for all treatments). KBC = Krebs-Henseleit bicarbonate buffered solution gassed with 95% O_2, 5% CO_2; KPO = modified bicarbonate-free Krebs-Henseleit phosphate buffered solution, gassed with 100% O_2. All solutions contained β-alanine (1 mM) and amino-oxyacetic acid (0.1 mM) to inhibit glial uptake and the metabolism of [^{3}H]-GABA. The release of [^{3}H]-GABA by ethylenediamine (EDA) was virtually absent, barely above basal release, in bicarbonate-free KPO solution relative to that in KBC containing bicarbonate, but the monocarbamate (EDAC) was equi-effective in releasing [^{3}H]-GABA when the ilea were superfused either with KBC or KPO. The release with both EDA and EDAC was significantly inhibited ($P < 0.05$) by (3-MPA) which prevents GABA release, with resumption of release following washout of 3-MPA.

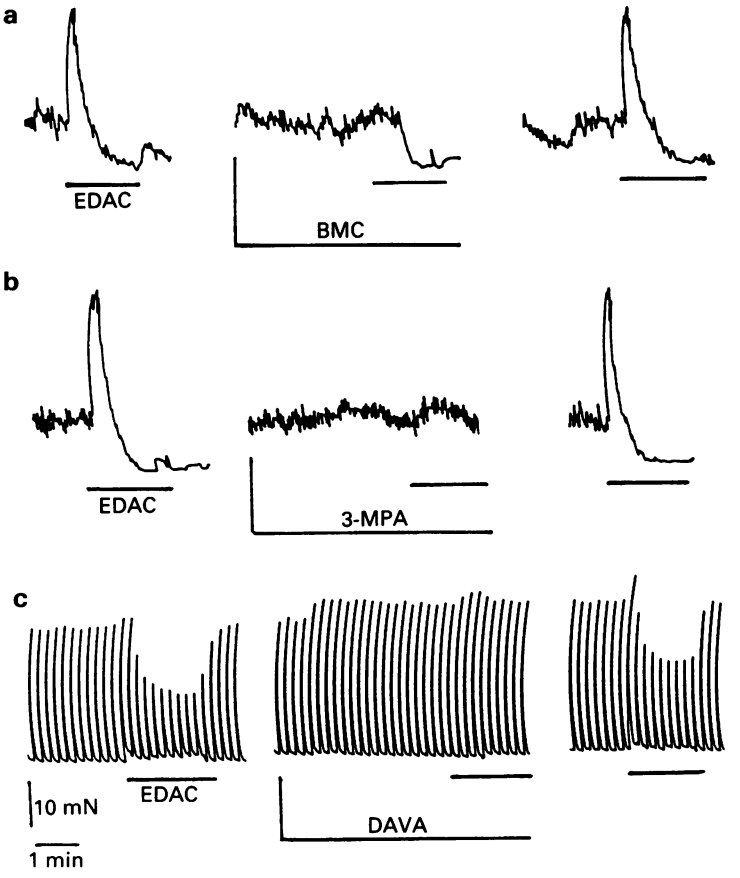

Figure 2 GABA-mimetic responses to ethylenediamine-monocarbamate (EDAC) in the guinea-pig isolated ileum maintained in bicarbonate-free Krebs-solution buffered with phosphate and gassed with 100% O_2. (a) In the absence of bicarbonate, EDAC (1 mM) elicited a $GABA_A$-receptor-mediated contractile response, sensitive to bicuculline methochloride (BMC, 10 μM), followed by a BMC-insensitive $GABA_B$-receptor-mediated relaxation. In (b) both components of the response to EDAC (1 mM) were prevented by 3-mercaptopropionic acid (3-MPA, 0.5 mM) which prevents GABA release from neurones, whilst in (c) the EDAC (1 mM)-induced depression of ileal repetitive twitch responses (0.1 Hz, supramaximal stimulation) was antagonized by δ-aminovaleric acid (DAVA, 0.5 mM), a weak $GABA_B$-receptor antagonist. Control responses to EDAC were re-established following washout of these drugs with bicarbonate-free medium.

minimum of 2–5 mM bicarbonate-carbon dioxide buffer to bring about ileal GABA-mimetic responses to EDA in KPO of KHO medium. Alternatively, EDA became active in the bicarbonate-free solutions, if added from an aqueous stock solution already gassed with carbon dioxide, as was done by Curtis & Malik (1984). Hence it is unlikely that the HCO_3^- ion, as such, in some way rendered GABA-containing myenteric neurones sensitive to the GABA-releasing property of EDA, since HCO_3^- was present only in low concentrations ($<25\,\mu M$) in the latter experiments. Instead, all the available chemical evidence indicates that EDAC, and not EDA, would be the active material releasing GABA from the myenteric neurones, since EDA would be rapidly and largely converted to EDAC by the carbon dioxide present in the solutions (Jensen & Christensen, 1955; Frahn & Mills, 1964). Furthermore, the synthetic EDAC used here was prepared by the method of Katchalski *et al.* (1951), through the reaction of EDA-base with carbon dioxide in the anhydrous state; addition of solutions of this EDAC to ileal preparations maintained in bicarbonate-free KPO or KHO medium would thus not involve any added HCO_3^-, yet GABA-mimetic responses were still induced by EDAC and [^{3}H]-GABA was released from preloaded

ileal segments. From this we conclude that EDAC, formed from EDA, is the active material releasing GABA from neurones of the myenteric plexus to elicit these responses, and that this release is independent of HCO_3^- ions.

Although the exact mechanism whereby EDAC exerts this GABA-releasing effect is at present unclear, there is the possibility that EDAC may counter-exchange for endogenous GABA in neurones of the myenteric plexus. This could, perhaps, occur by virtue of charge neutralization on the parent EDA molecule, due to the reaction of carbon dioxide with one amine group on EDA to yield EDAC, an N-carboxylic acid that is an analogue of GABA, so allowing the 'bicarbonate-dependent' uptake of EDA which is in part sensitive to inhibitors of GABA transport (Davies *et al.*, 1983).

Because EDAC acts in this manner, by releasing GABA from GABAergic neurones of the myenteric plexus rather than by directly stimulating GABA receptors, it seems desirable to investigate the possibility that a similar release of GABA from central neurones (Lloyd *et al.*, 1982a; Blaxter & Cottrell, 1985) might also be partly responsible for the GABA-mimetic properties of EDA, or EDAC, in the central nervous system. Furthermore, the present results show the ease with which EDAC is formed from EDA in the presence of carbon dioxide; indeed, EDAC can even be formed from EDA with atmospheric carbon dioxide (Frahn & Mills, 1964), which suggests that this conversion would probably occur in any experiments on EDA actions in the presence of bicarbonate buffer, either *in vivo* or *in vitro*. However, the bicarbonate ion itself may modify neuronal properties, possibly rendering neurones sensitive to EDA, since changes in neuronal behaviour, and GABA-receptor affinity, have been seen in the presence of bicarbonate-carbon dioxide buffer (Brown & Berman, 1970; Kurioka *et al.*, 1981; Fukuda, 1984;). Nevertheless, the present results emphasise that EDAC, formed by carbon dioxide from the parent diamine, is probably responsible for the GABA-releasing properties of EDA, and its consequent GABA-mimetic actions in the guinea-pig isolated ileum maintained in Krebs-bicarbonate solution.

References

ALLAN, R.D. & JOHNSTON, G.A.R. (1983). Synthetic analogs for the study of GABA as a neurotransmitter. *Medicinal Res. Revs*, **3**, 91–118.

BLAXTER, T.J. & COTTRELL, G.A. (1985). Actions of GABA and ethylene-diamine on CA1 pyramidal neurones of the rat hippocampus. *Q.J. exp. Physiol.*, **70**, 75–93.

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.

BROWN, A.M. & BERMAN, P.R. (1970). Mechanism of excitation of *Aplysia* neurones by carbon dioxide. *J. gen. Physiol.*, **56**, 543–558.

CURTIS, D.R. & MALIK, R. (1984). Ethylenediamine: a GABA-mimetic? *Trends Pharmac. Sci.*, **5**, 458.

DAVIES, L.P., HAMBLEY, J.W. & JOHNSTON, G.A.R. (1982). Ethylenediamine as a GABA agonist: Enhancement of diazepam binding and interaction with GABA receptors and uptake sites. *Neurosci. Lett.*, **29**, 57–61.

DAVIES, L.P., DREW, C.A., CHEN CHOW, S., SKERRITT, J.H. & JOHNSTON, G.A.R. (1983). Relationships between ethylenediamine and GABA transport systems in rat brain slices. *Neurochem Int.*, **5**, 57–64.

FAN, S.G., WUSTEMAN, M. & IVERSEN, L.L. (1981). 3-Mercaptopropionic acid inhibits GABA release from rat brain slices *in vitro*. *Brain Res.*, **229**, 371-377.

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.

FRAHN, J.L. & MILLS, J.A. (1964). Paper ionophoresis of amino compounds. Formation of carbamates, and related reactions. *Aust. J. Chem.*, **17**, 256–273.

FUKUDA, Y. (1984). Cholinergic synaptic activation due to HCO_3^- in the superior cervical ganglion of rat. *Eur. J. Physiol.*, **402**, 94–99.

HILL, D.R. (1985). The influence of bicarbonate ions on the GABA-mimetic activity of ethylenediamine. *Neuropharmacology*, **24**, 147–155.

JENSEN, A. & CHRISTENSEN, R. (1955). Studies on Carbamates XI. The carbamate of ethylenediamine. *Acta. chem. scand.*, **9**, 486–492.

KATCHALSKI, E., BERLINER-KLIBANSKI, C. & BERGER, A. (1951). The chemical structure of some diamine carbamates. *J. Am. Chem. Soc.*, **73**, 1829–1831.

KERR, D.I.B. & ONG, J. (1984). Evidence that ethylenediamine acts in the isolated ileum of the guinea-pig by releasing endogenous GABA. *Br. J. Pharmac.*, **83**, 169–177.

KROGSGAARD-LARSEN, P., JACOBSEN, P. & FALCH, E. (1983). Structure-activity requirements of the GABA receptor. In *The GABA Receptors*. ed. Enna, S.J. pp. 149–176. New Jersey: Humana Press.

KURIOKA, S., KIMURA, Y. & MATSUDA, M. (1981). Effects of sodium and bicarbonate ions on gamma-aminobutyric acid receptor-binding in synaptic membranes of rat brain. *J. Neurochem.*, **37**, 418–421.

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

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

MORGAN, P.F. & STONE, T.W. (1982). Ethylenediamine and GABA potentiation of [^{3}H] diazepam binding to ben-

zodiazepine receptors in rat cerebral cortex. *J. Neurochem.*, **39**, 1446–1451.

PERKINS, M.N. & STONE, T.W. (1982). Comparison of the effects of ethylenediamine analogues and γ-aminobutyric acid on cortical and pallidal neurones. *Br. J. Pharmac.*, **75**, 93–99.

STONE, T.W. & PERKINS, M.N. (1984). Ethylenediamine as a GABA-mimetic. *Trends Pharmac. Sci.*, **5**, 241–243.

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