Br. J. Pharmac. (1984), **83**, 169–177

Evidence that ethylenediamine acts in the isolated ileum of the guinea-pig by releasing endogenous GABA

D.I.B. Kerr & Jennifer Ong

Department of Physiology, University of Adelaide, Adelaide, South Australia 5000, Australia

1 Ethylenediamine (EDA) released [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) in a dose-dependent manner from the isolated preloaded ileum of the guinea-pig maintained in Krebs-bicarbonate solution (pH 7.4, 37°C), in the presence of β-alanine and amino-oxyacetic acid (AOAA) to prevent GABA uptake into glial cells and catabolism. This release was reversibly prevented by 3-mercaptopropionic acid (3-MPA), also in a dose-dependent manner.

2 In the isolated ileal preparations of the guinea-pig maintained in Krebs-bicarbonate solution, EDA induced a dose-dependent transient, cholinergic contractile response ($GABA_A$-receptor-mediated effect), followed by an 'after-relaxation' ($GABA_B$-receptor-mediated effect). EDA also induced a transient contraction superimposed on repetitive twitch responses to electrical transmural stimulation of the cholinergic neurones, followed by a depression of the twitch contractions.

3 This $GABA_A$-receptor-mediated contraction was antagonized by bicuculline methochloride and picrotoxinin, whilst the $GABA_B$-receptor-mediated 'after-relaxation', and depression of cholinergic twitch contractions, was susceptible to antagonism by δ-aminovaleric acid. The pA_2 value for bicuculline methochloride antagonism of EDA was estimated to be 5.8, identical with that for GABA.

4 3-Mercaptopropionic acid also prevented these pharmacological actions induced by EDA without affecting responses to GABA, 3-aminopropranesulphonic acid, muscimol, baclofen or the twitch responses to transmural stimulation.

5 It is concluded that EDA releases both [^{3}H]-GABA and endogenous GABA in the guinea-pig ileum, thus providing further evidence that GABA is a transmitter in the enteric nervous system.

Introduction

It has been suggested that γ-aminobutyric acid (GABA) is a neurotransmitter in the enteric nervous system (Jessen *et al.*, 1979; 1983; Miki *et al.*, 1983), where GABA induces $GABA_A$- and $GABA_B$-receptor-mediated responses in the isolated ileum of the guinea-pig (Bowery *et al.*, 1981; Giotti *et al.*, 1983; Ong & Kerr, 1983), and is evidently involved in the control of intestinal motility (Krantis & Kerr, 1981c; Ong & Kerr, 1983). In both the central and peripheral nervous systems, ethylenediamine (EDA) has a direct action in mimicking closely bicuculline-sensitive responses to GABA (Perkins *et al.*, 1981), and induces the release of GABA from brain slices (Forster *et al.*, 1981; Davies *et al.*, 1983). In the guinea-pig ileum, EDA elicits both $GABA_A$-and $GABA_B$-receptor-mediated responses, which has been suggested to result from the release of endogenous GABA from neurones of the myenteric plexus (Kerr & Ong, 1982) rather than by EDA having a direct action at these GABA-receptor sites. In order to confirm that the actions of EDA are indeed due to the release of endogenous GABA in the guinea-pig ileum, we have now employed 3-mercaptopropionic acid (3-MPA) which prevents GABA release from brain cortical slices (Fan *et al.*, 1981), and here show that 3-MPA not only prevents the EDA-induced release of [^{3}H]-GABA from the preloaded myenteric plexus of the guinea-pig ileum, but also prevents EDA-induced GABAergic responses in the ileum without affecting those elicited by GABA or its analogues.

Methods

[^{3}H]-GABA release by ethylenediamine (EDA)

Guinea-pigs of either sex, weighing between 200–400g, were stunned by a blow on the head and bled. Segments of the ileum, 3–4 cm in length, taken 2–3 cm from the ileo-caecal valve, were quickly removed and emptied of their contents. The tissues were incubated for 10–20 min in aerated Krebs solution (95% O_2 and 5% CO_2, pH 7.4, 37°C) of the following composition (mM): Na^+ 151.0, K^+ 4.6, Mg^{2+} 2.8, Cl^- 134.9, HCO_3^- 24.9, $H_2PO_4^-$ 1.3, SO_4^{2-} 0.6, glucose 7.7. The tissues were then transferred to 5 ml Krebs solution, containing [^{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 metabolism of [^{3}H]-GABA and to prevent its uptake into glial-cells (Krantis & Kerr, 1981b). The tissues were then removed, blotted to remove excess incubating medium and suspended in glass perfusion chambers. After washing the tissues repeatedly over an equilibration period of 60 min to establish a basal efflux of [^{3}H]-GABA, 2 ml fractions of the superfusate were collected over 2 min periods. EDA at varying doses was then added to the medium and 2 ml samples again collected. In experiments where 3-MPA was used, it was added to the superfusate at least 5 min before a dose of EDA was added.

Radioactivity was determined with liquid scintillation spectrometry, and has been expressed as d.p.m. after correction for quenching by EDA and 3-MPA. All experimental procedures were run in duplicate and were repeated at least twice, with at least 6 tissues being used from a minimum of 3 animals. Statistical analysis using Student's *t* test for paired and unpaired samples was done to assess the significance of differences between the means of samples.

Guinea-pig isolated ileal preparations

Segments of guinea-pig isolated ileum, removed as described above, were mounted vertically in a 10 ml organ bath containing normal Krebs-bicarbonate solution. Mechanical activity of the longitudinal muscle of the tissue was recorded isometrically at a resting tension of 1 g, using a Grass Model FTO3 force transducer coupled to a Grass polygraph recor-

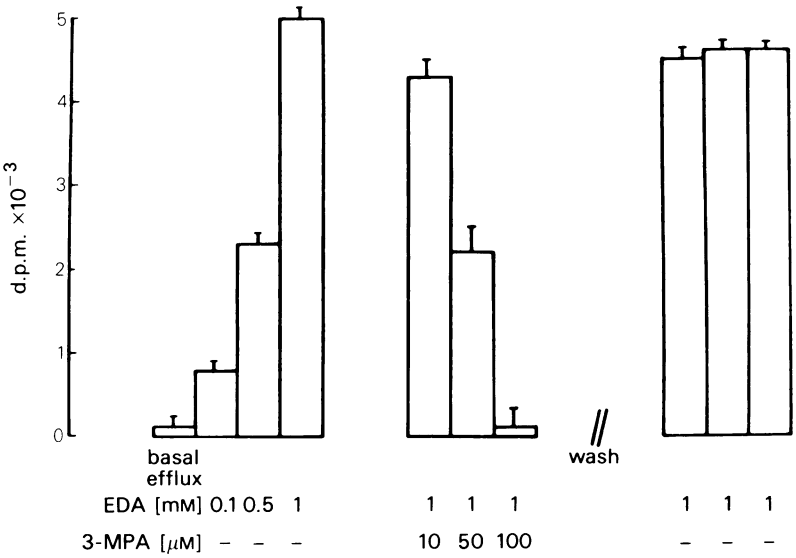

Figure 1 Dose-dependent release of [^{3}H]-GABA by ethylenediamine (EDA) in the preloaded isolated ileum of the guinea-pig. Segments of ileum were incubated for 20 min in Krebs-bicarbonate solution (pH 7.4, 37°C) containing [^{3}H]-GABA (10 nM; 66 Ci mmol^{-1}), amino-oxyacetic acid (0.1 mM) and β-alanine (1 mM). EDA was present for 2 min in the perfusion chamber before 2 ml samples were collected and radioactivity determined using liquid scintillation spectrometry, the results being expressed as d.p.m. 3-Mercaptopropionic acid (3-MPA) added to the chamber for 5 min prevented the release of [^{3}H]-GABA by EDA, also in a dose-dependent manner; after washing out the 3-MPA from the tissues, as indicated, [^{3}H]-GABA was released by EDA as before. Amino-oxyacetic acid and β-alanine were present throughout the experiment. Each column represents the mean of at least 10 values, with the vertical line showing s.e.mean.

der. Effects of drug treatments were examined on the resting tissue or on electrically evoked contractions of the tissue stimulated by a pair of parallel platinum electrodes positioned around the tissue segment in the bath. Single pulses (duration 0.1 ms, supramaximal voltage) resulting in a single twitch contractile response, or repetitive twitch contractions (frequency 0.1 Hz), were delivered from a Grass S48 stimulator to give transmural stimulation of cholinergic intrinsic neurones.

Tissues were allowed to equilibrate for 60 min in the organ bath before any drug application or electrical stimulation. Drugs were applied within 15–20 min intervals, depending on the recovery of the tissue responses to baseline, and they remained in contact with the tissue for 1–2 min or more, depending on the experiment, before wash-out. All antagonists were added at least 5–10 min before agonists were tested, and drug volumes used were never more than 1% of the bath volume. Student's t test for paired and unpaired samples was used to assess the significance ($P < 0.05$) of differences between mean values of the dose-response effects.

Chemicals

2,3-[^{3}H]-GABA was obtained from Radiochemical Centre, Amersham; GABA, 3-aminopropanesulphonic acid, ethylenediamine dihydrochloride (EDA), amino-oxyacetic acid, β-alanine, atropine sulphate, tetrodotoxin (TTX), picrotoxinin (dissolved in 1:9 absolute alcohol and distilled water) δ-aminovaleric acid and muscimol were from Sigma; baclofen (Ciba-Geigy); bicuculline methochloride was from Pierce, and 3-mercaptopropionic acid (3-MPA) was from Koch-Light.

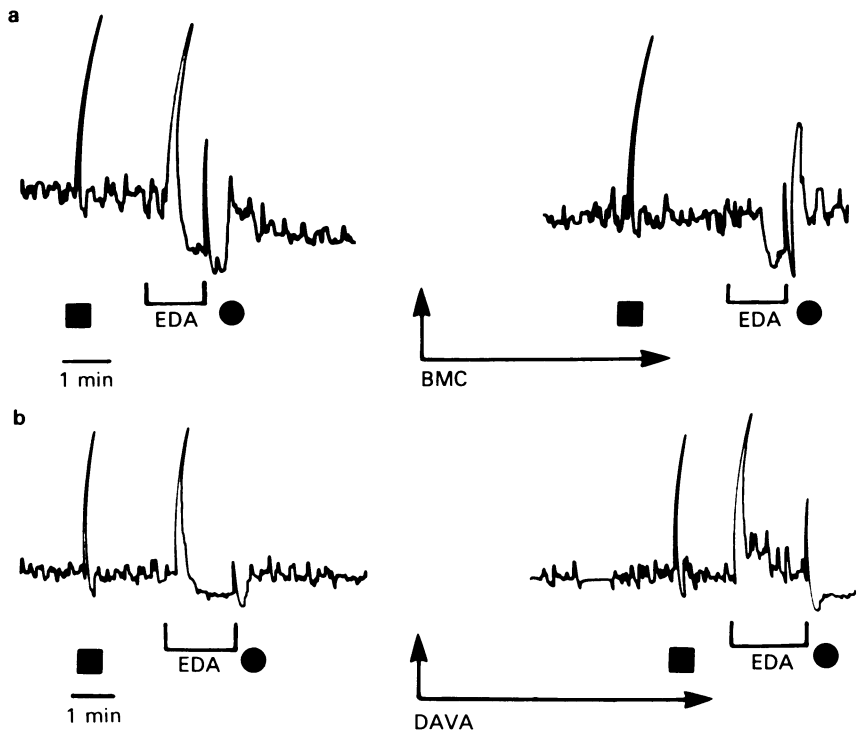

Figure 2 Isolated ileal preparations of the guinea-pig were set up in an organ bath containing Krebs-bicarbonate solution (pH 7.4, 37°C). Ethylenediamine (EDA, 1 mM) elicited a transient longitudinal contractile response followed by an 'after-relaxation'. (a) Bicuculline methochloride (BMC) (10 μM) antagonized the contractile response induced by EDA without affecting the 'after-relaxation' or the response to transmural stimulation (TS:0.1 ms duration, supramaximal voltage) as indicated by (■). (b) δ-Aminovaleric acid (DAVA) (500 μM) antagonized the 'after-relaxation' induced by EDA without affecting the initial contractile component or the response to TS. All antagonists were left in the bath for 10 min before EDA was applied. (●) Indicates tissue wash-out after each drug application. The number of experiments performed in each case was at least 6.

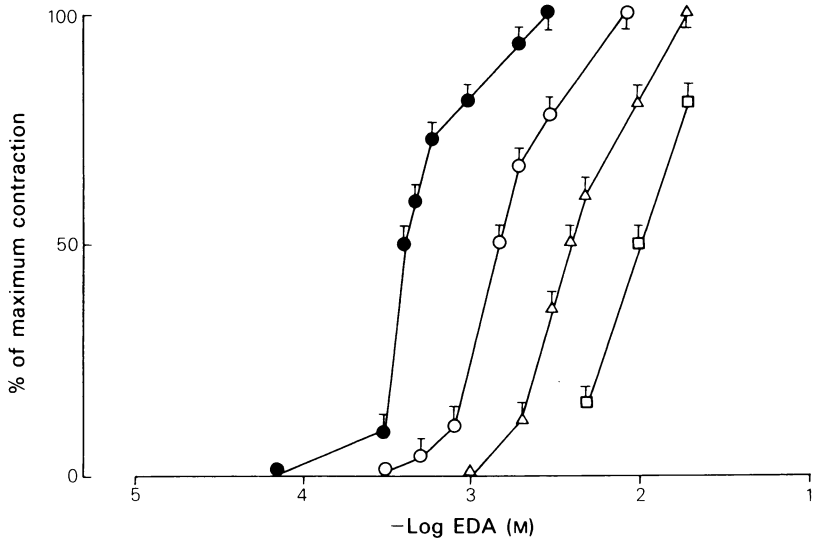

Figure 3 Dose-response curve of the longitudinal contractile response induced by (●) ethylenediamine (EDA) in the isolated ileum of the guinea-pig, maintained in Krebs-bicarbonate solution. In the presence of different concentrations of bicuculline methochloride 5 μM (○); 10 μM (△); 50 μM (□), the curve was shifted to the right in a parallel fashion; estimated pA_2 value = 5.8. The results are expressed as a mean of the percentage of the maximal contraction induced by EDA, with each point representing the mean and vertical lines s.e.mean ($n = 8$).

Results

Release of [^{3}H]-GABA from preloaded ileum by ethylenediamine (EDA)

Within one hour of loading the tissue with [^{3}H]-GABA in the presence of amino-oxyacetic acid (0.1 mM) and β-alanine (1 mM), and subsequent washing in normal Krebs solution, the basal or resting efflux of tritium from the tissues had declined to a low, steady level. As seen in Figure 1, addition of EDA to the superfusing medium induced a dose-dependent release of [^{3}H]-GABA. When 3-MPA (mM) was added to the superfusate for 5 min before

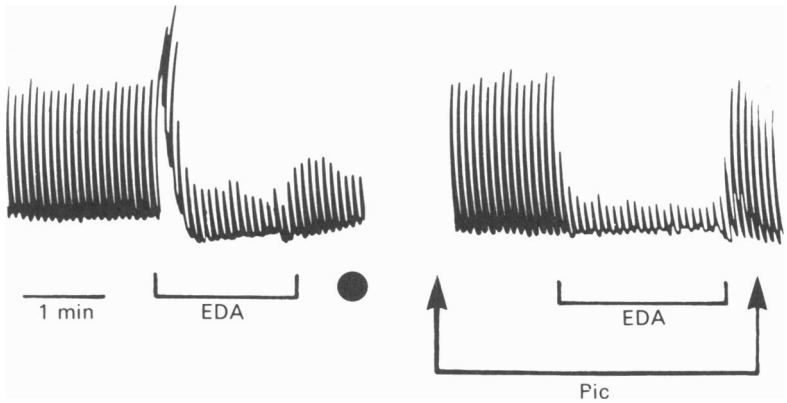

Figure 4 Repetitive twitch contractions (0.1 Hz, supramaximal voltage) of the guinea-pig ileum were elicited by electrical stimulation of the cholinergic neurones. Ethylenediamine (EDA, 1 mM) caused a transient superimposed contractile response followed by a depression of the twitch contractions. Picrotoxinin (Pic) (10 μM) antagonized the superimposed contractile response without affecting the depression of the twitch contractions by EDA. Pic was left in the bath for at least 5 min before EDA was applied to the bath. The number of experiments performed was 8, and (●) indicates tissue wash-out.

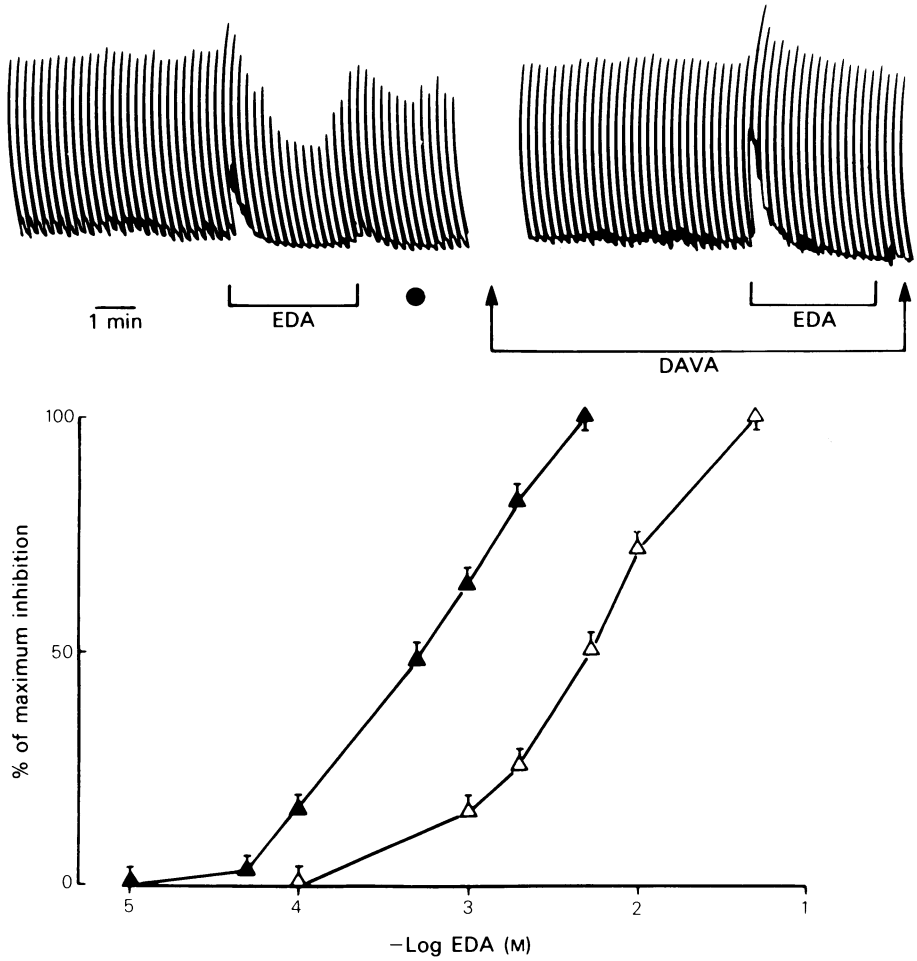

Figure 5 Using the electrically stimulated isolated segments of the guinea-pig ileum (0.1 Hz, 0.5 ms, supramaximal voltage), ethylenediamine (EDA, 1 mM) elicited a contractile response superimposed on the repetitive twitch contractions, followed by a depression of their amplitude. This depression was antagonized by δ-aminovaleric acid (DAVA, 500 μM) without affecting the superimposed contractile response. The dose-response curve for the depression of the twitch contractions by EDA in the isolated ileum of the guinea-pig was shifted to the right in a parallel manner by δ-aminovaleric acid (500 μM). (▲) Control inhibition of responses by EDA, (△) inhibition in the presence of δ-aminovaleric acid. Results are expressed as a percentage of the maximum inhibition of the twitch contractions induced by EDA. Each point is the mean and vertical lines show s.e.mean of at least 6 values obtained with 6 tissues using a minimum of 3 animals. In all experiments, δ-aminovaleric acid was left in the bath for 5 min before a test dose of EDA was applied. (●) Indicates tissue wash-out after drug application.

the addition of EDA, this release of [^{3}H]-GABA was prevented in a dose-dependent manner. After washing out the 3-MPA with normal Krebs for 10 min, the EDA-induced release of [^{3}H]-GABA was again observed.

The effects of ethylenediamine (EDA) on isolated ileal preparations

In all subsequent experiments, GABA was not applied to the bath before the addition of EDA. A transient dose-dependent contractile response was induced by EDA (1 mM) and this was followed by a relaxation ('after-relaxation') in the unstimulated ileum (Figure 2). These contractions were cholinergic and neurogenic in origin, being abolished by atropine (0.1 μM) and TTX (0.1 μM). The contractile response was also sensitive to bicuculline methochloride (10 μM), a specific $GABA_A$-receptor antagonist that has no effect on responses to ACh or transmural stimulation of cholinergic neurones (Krantis & Kerr, 1981a), but the 'after-relaxation' was unaf-

fected by bicuculline (Figure 2a). The dose-response curve for the contraction elicited by EDA was displaced to the right in a parallel fashion by bicuculline (Figure 3), with a pA_2 value of 5.8. Picrotoxinin (10 μM) also antagonized the EDA-induced contractile response, without affecting the 'after-relaxation', but the $GABA_B$-receptor antagonist δ-aminovaleric acid (500 μM) prevented the 'after-relaxation' phase of the ileal response to EDA without affecting the initial contractile component or the response to the transmural stimulation (Figure 2b).

The effects of ethylenediamine (EDA) on repetitive twitch contractions of the ileum

When repetitive twitch contractions of the ileum were challenged with EDA at varying concentrations, both $GABA_A$- and $GABA_B$-receptor-

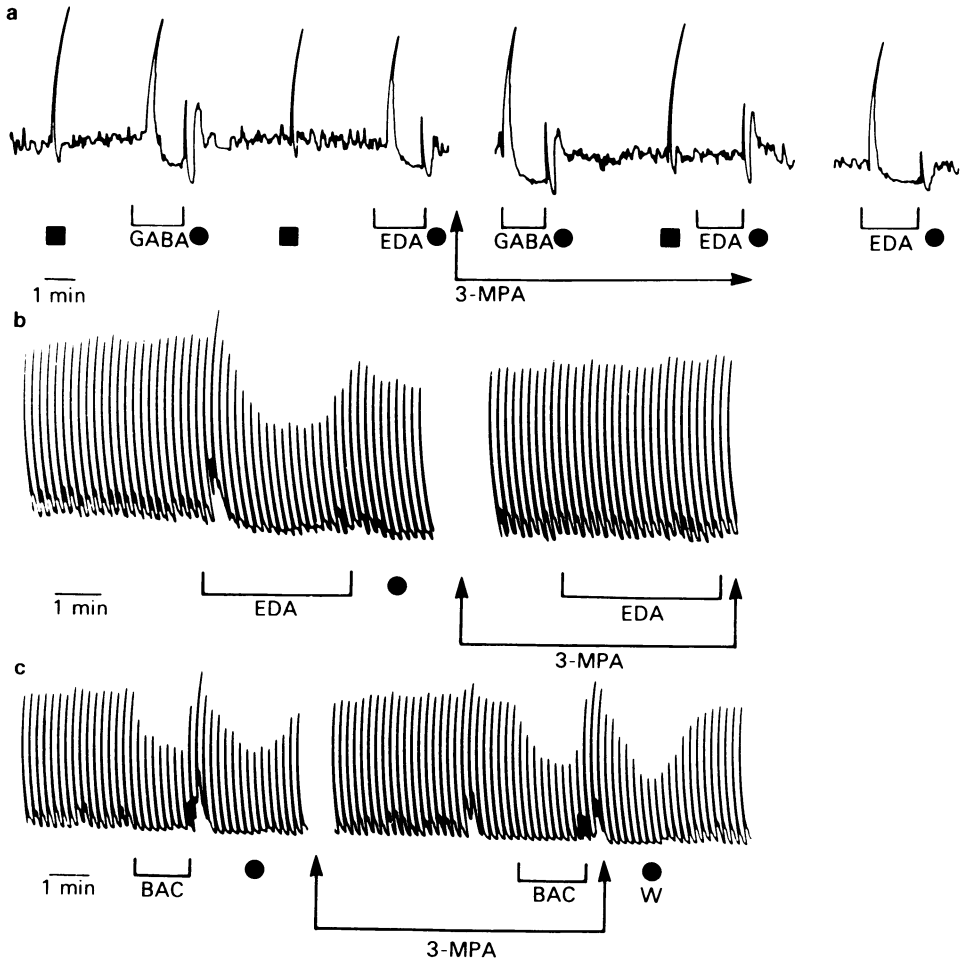

Figure 6 Effects of 3-mercaptopropionic acid (3-MPA) on responses induced by GABA, baclofen (BAC) and ethylenediamine (EDA) in the isolated ileum of the guinea-pig maintained in Krebs-bicarbonate solution: (a) Both GABA (50 μM) and EDA (1 mM) induced a transient contractile response followed by a prolonged 'after-relaxation' in the unstimulated ileum. 3-MPA (1 mM), applied by divided doses at least 10 min before transmural stimulation or any drug application, prevented the EDA-induced response without affecting responses to GABA or to transmural stimulation of cholinergic neurones (0.1 ms duration). Upon wash-out of 3-MPA (●), the EDA-induced response recovered within 5–10 min. The number of experiments performed was 6. (b) Using repetitive twitch contractions of the isolated ileum (0.1 Hz), 3-MPA (1 mM) prevented the EDA (1 mM)-induced responses (superimposed contractile response followed by a depression of the twitch contractions), $n = 5$. (c) 3-MPA (1 mM) did not affect the bactofen (50 μM)-induced depression of twitch contractions in the same isolated ileal preparations, $n = 6$. (●) Indicates tissue wash-out in each experiment.

mediated actions were observed. There was first a $GABA_A$-receptor-mediated contraction superimposed on the twitch contractions, which was followed by a delayed depression of the twitch responses ($GABA_B$-receptor-mediated). The superimposed contraction was antagonized selectively by bicuculline (10 μM) or picrotoxinin (10 μM), whilst the delayed depressant action was unaffected by these antagonists (Figure 4). Conversely, the dose-dependent depressive effect of EDA on twitch contractions was antagonized by δ-aminovaleric acid (500 μM) but the superimposed contraction was not inhibited. This antagonism by δ-aminovaleric acid caused a parallel displacement, to the right, of the dose-response curve for the EDA-induced depression of electrically elicited twitch contractions, indicating a competitive antagonism (Figure 5).

Prevention of the effects of ethylenediamine (EDA) in the ileum by 3-mercaptopropionic acid (3-MPA)

Both the $GABA_A$- and $GABA_B$-receptor-mediated actions induced by EDA in the ileum were prevented by 3-MPA (Figure 6a). 3-MPA was added in divided doses to a total of 1 mM over 3–5 min, then left in the bath for a further 5 min before responses to EDA, GABA, 3-aminopropanesulphonic acid, muscimol or baclofen were elicited. Only the responses to EDA were specifically prevented by 3-MPA (Figure 6a), its action being dose-dependent with a threshold concentration around 50 μM. It was necessary to increase the doses of 3-MPA gradually to a final concentration of 1 mM, since a single application at 1 mM caused non-specific depressive effects on single twitch response to transmural stimulation of the ileum. The inhibitory effects of 3-MPA on EDA-induced responses declined after it had been in the bath for 15–20 min, but could be reinstated by additional doses, which suggests that 3-MPA was rendered inactive in the bath rather than the tissue becoming desensitized to it. Both the $GABA_A$- and $GABA_B$-receptor-mediated actions of EDA on the ileum returned after washing 3-MPA from the bath.

Using repetitive twitch responses to transmural stimulation in the ileum, 3-MPA (50–500 μM) had no depressive effect on the twitch contractions but prevented in a dose-dependent manner the responses to EDA. As shown in Figure 6b, 3-MPA (1 mM) blocked the $GABA_B$-receptor-mediated depression of the twitch contractions induced by EDA (1 mM) without affecting the $GABA_B$-receptor-mediated inhibition induced by baclofen (50 μM) (Figure 6c).

Discussion

EDA releases [^{14}C]-GABA from preloaded brain slices (Lloyd *et al.*, 1982), and has now been shown to release [^{3}H]-GABA from preloaded guinea-pig ileal preparations. The latter release was blocked by 3-MPA which both inhibits GABA synthesis (Lamar, 1970; Karlsson *et al.*, 1974) and prevents GABA release through a mechanism that is rapid in onset (Fan *et al.*, 1981). In the present experiments not only was [^{3}H]-GABA release prevented by 3-MPA but it also prevented the $GABA_A$- and $GABA_B$-receptor-mediated responses induced by EDA, without affecting responses of the ileum to GABA, muscimol, 3-aminopropranesulphonic acid, baclofen or transmural stimulation of cholinergic neurones. Hence, we conclude that 3-MPA also prevents an EDA-elicited release of endogenous GABA from neurones of the enteric nervous system, without affecting cholinergic neurotransmission. This suggests that EDA acts by releasing endogenous GABA rather than by acting as a GABA-mimetic as has been reported by Perkins *et al.*, (1981). Here, the released endogenous GABA resulted in $GABA_A$-receptor-mediated contractile responses in the ileum which were sensitive to bicuculline and picrotoxinin as with exogenous GABA (Krantis & Kerr, 1981a). The released GABA also activated $GABA_B$-receptors, leading to an 'after-relaxation' and a depression of cholinergic responses to transmural stimulation through a bicuculline-insensitive and Cl^--independent mechanism, presumably by a reduction in transmitter output (Bowery *et.*, 1981; Kleinrok & Kilbinger, 1983). δ-Aminovaleric acid, a $GABA_B$-receptor antagonist (Muhyaddin *et al.*, 1982), competitively antagonized this $GABA_B$-action induced by EDA, as shown by the parallel shift of the dose-response curves. Although EDA is described as being almost equipotent with GABA in depressing neuronal firing, and to be more sensitive than GABA to antagonism by bicuculline in brain cortical slices (Perkins *et al.*, 1981), in contrast, it was less potent (approximately 100 fold) than GABA in the guinea-pig ileum, with a pA_2 value (5.8) for bicuculline-induced antagonism identical to that found for exogenous GABA (Krantis & Kerr, 1981a), which also argues against a direct agonist effect of EDA in the ileum. The GABA released by EDA must have been of endogenous origin since particular care was taken not to pre-expose the intestine to any exogenous GABA before testing the effects of EDA.

Levels of GABA can be manipulated in the central nervous system by using 3-MPA or thiosemicarbazide as inhibitors of GABA synthesis (Meldrum, 1982), which suggests that one could use these agents in order to investigate the effects of altered GABA metabolism in the intestine. But in this system, 3-MPA evidently acts by preventing GABA release rather than by inhibiting its synthesis, since the duration of action of 3-MPA was short, with a rapid onset

and similarly rapid recovery after washout. However, we have already found that thiosemicarbazide will abolish EDA-induced GABAergic responses in the guinea-pig ileum by blocking GABA synthesis (Kerr, *et al.*, 1983). Thus, alteration of endogenous GABA levels in the intestine, through varying its metabolism will affect EDA actions, as does preventing GABA release with 3-MPA, again emphasising that EDA acts indirectly in the ileum by releasing endogenous GABA.

Recently much evidence has accumulated in favour of GABA being a neurotransmitter in the enteric nervous system. Most importantly, GABA and its metabolic enzymes are present in the myenteric plexus of the entire intestine (Jessen *et al.*, 1979; Miki *et al.*, 1983). The plexus also contains high affinity uptake sites, characteristic of GABAergic neurones, that can be demonstrated by autoradiography after labelling with [^{3}H]-GABA (Jessen *et al.*, 1979; Krantis & Kerr, 1981b). Following such high affinity uptake [^{3}H]-GABA is released from neurones of the plexus by electrical stimulation, the release being TTX-sensitive and Ca^{2+}-dependent (Taniyama *et al.*, 1982; Jessen *et al.*, 1983; Kerr & Krantis, 1983). In addition, exogenously applied GABA stimulates both $GABA_A$- and $GABA_B$-receptors to elicit responses in the isolated intestine, and these responses are sensitive to antagonists for the respective receptors (Krantis & Kerr, 1981a; Kaplita *et al.*, 1982; Ong & Kerr, 1983). These antagonists, or desensitization to GABA, also markedly reduce peristaltic activity as measured by the rate of faecal-pellet propulsion in the isolated colon. (Krantis & Kerr, 1981c; Ong & Kerr, 1983). All the above evidence is reinforced by the present results showing that EDA releases endogenous GABA, which then elicits the same spectrum of pharmacological responses with the same sensitivity to antagonists as does exogenously applied GABA in the isolated ileum. These most recent findings fulfil one of the criteria proposed by Iversen (1979), that a substance may be considered a transmitter if an exogenously applied agent has an identical pharmacological action to the endogenous substance and its action is similarly antagonized by the appropriate antagonists, and thus support the conclusion that GABA is a transmitter in the myenteric plexus of the intestine.

References

BOWERY, N.G., DOBLE, A., HILL, D.R., HUDSON, A.L., SHAW, J.S., TURNBULL, M.J. & WARRINGTON, R. (1981). Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. *Eur. J. Pharmac.*, **71**, 53–70.

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.

GIOTTI, A., LUZZI, S., SPAGNESI, S. & ZILLETTI, L. (1983). $GABA_A$ and $GABA_B$ receptor-mediated effects in guinea-pig ileum. *Br. J. Pharmac.*, **78**, 469–478.

IVERSEN, L.L. (1979). Putative neurotransmitters; criteria for establishing a neurotransmitter. *Neurosci. Res. Prog. Bull.*, **17**, 406.

JESSEN, K.R., HILLS, J.M., DENNISON, M.E. & MIRSKY, R. (1983). γ-Aminobutyrate as an autonomic neurotransmitter: release and uptake of [^{3}H] γ-aminobutyrate in guinea pig large intestine and cultured enteric neurons using physiological methods and electron microscopic autoradiography. *Neuroscience*, **10**, 1427–1442.

JESSEN, K.R., MIRSKY, R., DENNISON, M.E. & BURNSTOCK, G. (1979). GABA may be a neurotransmitter in the vertebrate peripheral nervous system. *Nature*, **281**, 71–74.

KAPLITA, P.V., WATERS, D.H. & TRIGGLE, D.J. (1982). γ-Aminobutyric acid action in guinea-pig ileal myenteric plexus. *Eur. J. Pharmac.*, **79**, 43–51.

KARLSSON, A., FONNUM, F., MALTHE-SØRENSSEN, D. & STORM-MATHISEN, J. (1974). Effect of the convulsive agent 3-mercaptopropionic acid on the levels of GABA, other amino acids and glutamate decarboxylase in different regions of the rat brain. *Biochem. Pharmac.*, **23**, 3053–3061.

KERR, D.I.B. & KRANTIS, A. (1983). Uptake and stimulus-evoked release of [^{3}H]-γ-aminobutyric acid by myenteric nerves of guinea-pig intestine. *Br. J. Pharmac.*, **78**, 271–276.

KERR, D.I.B., KRANTIS, A. & ONG, J. (1983). GABA is a transmitter in the enteric nervous system. *Internat. Union Physiol. Sci. 29th Congress Proc.*, **15**, 453.

KERR, D.I.B. & ONG, J. (1982). GABA actions in guinea-pig ileum through release of endogenous GABA by ethylenediamine. *Clin. exp. Pharmac. Physiol.*, **9**, 390P.

KRANTIS, A. & KERR, D.I.B. (1981a). GABA induced excitatory responses in the guinea-pig small intestine are antagonized by bicuculline, picrotoxinin and chloride ion blockers. *Naunyn-Schmiedebergs Arch. Pharmac.*, **317**, 257–261.

KRANTIS, A. & KERR, D.I.B. (1981b). Autoradiographic localization of [^{3}H]-gamma-aminobutyric acid in the myenteric plexus of the guinea-pig small intestine. *Neurosci. Lett.*, **23**, 263–268.

KRANTIS, A. & KERR, D.I.B. (1981c). The effect of GABA antagonism on propulsive activity of the guinea-pig large intestine. *Eur. J. Pharmac.*, **76**, 111–114.

KLEINROK, A. & KILBINGER, H. (1983). γ-Aminobutyric

acid and cholinergic transmission in the guinea-pig ileum. *Naunyn-Schmiedebergs Arch. Pharmac.*, **322,** 216–220.

LAMAR, C. Jr. (1970). Mercaptopropionic acid: a convulsant that inhibits glutamate decarboxylase. *J. Neurochem.*, **17,** 165–170.

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

MELDRUM, B. (1982). Pharmacology of GABA. *Clin. Neuropharmac.*, **5,** 293–316.

MIKI, Y., TANIYAMA, K., TANAKA, C. & TOBE, T. (1983). GABA, glutamic acid decarboxylase and GABA transaminase levels in the myenteric plexus in the intestine of humans and other mammals. *J. Neurochem.*, **40,** 861–865.

MUHYADDIN, M., ROBERTS, P.J. & WOODRUFF, G.N. (1982). Presynaptic γ-aminobutyric acid receptors in the rat anococcygeus muscle and their antagonism by 5-aminovaleric acid. *Br. J. Pharmac.*, **77,** 163–168.

ONG, J. & KERR, D.I.B. (1983). $GABA_A$- and $GABA_B$-receptor-mediated modification of intestinal motility. *Eur. J. Pharmac.*, **86,** 9–17.

PERKINS, M.N., BOWERY, N.G., HILL, D.R. & STONE, T.W. (1981). Neuronal responses to ethylenediamine: preferential blockade by bicuculline. *Neurosci. Lett.*, **23,** 325–327.

TANIYAMA, K., KUSUNOKI, M., SAITO, N. & TANAKA, C. (1982). Release of γ-aminobutyric acid from cat colon. *Science*, **217,** 1038–1040.

(*Received January 20, 1984.*
Revised April 3, 1984.)