Br. J. Pharmac. (1984), **82**, 101–105

Inhibition of calcium spikes and transmitter release by γ-aminobutyric acid in the guinea-pig myenteric plexus

E. Cherubini & R. A. North

Neuropharmacology Laboratory, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA. 02139, U.S.A.

1 The effect of γ-aminobutyric acid (GABA) (1 μM–1 mM) on synaptic transmission in isolated myenteric ganglia of guinea-pig ileum was investigated with intracellular recording techniques.

2 GABA (up to 1 mM) had no effect on the resting membrane potential and membrane conductance of S neurones.

3 GABA reduced the amplitude of the fast excitatory postsynaptic potential (e.p.s.p.) without changing the amplitude of the nicotinic response to ionophoretic application of acetylcholine (ACh). This effect was mimicked by baclofen (10–100 μM) and was not blocked by bicuculline (10 μM). The preparation did not become desensitized during prolonged GABA applications.

4 Cholinergic and non-cholinergic slow e.p.s.ps evoked by single or repetitive presynaptic nerve stimulation were reduced in amplitude by GABA. GABA did not depress muscarinic responses to ionophoretic application of ACh.

5 GABA reduced the duration of the action potential in AH neurones in concentrations that did not affect the membrane potential or conductance. The effect was very marked when electrodes were filled with CsCl, and tetrodotoxin was in the superfusing solution. This effect was also mimicked by baclofen, was insensitive to bicuculline and was not reduced with repeated application or GABA.

6 It is concluded that GABA inhibits release of ACh and the transmitter mediating the slow e.p.s.p. This effect may result from inhibition of an inward calcium current.

Introduction

An important action of γ-aminobutyric acid (GABA) in the nervous system is the inhibition of transmitter release. It has recently been proposed that this may result from a direct reduction by GABA of calcium currents entering the cell (Kato & Kuba, 1980; Dunlap & Fischbach, 1981). This action of GABA can be distinguished from the chloride conductance increase by several criteria, including its insensitivity to bicuculline (Dunlap, 1981; Désarmenien *et al.*, 1982).

In the previous paper we described a bicuculline-sensitive, rapidly desensitizing chloride activation by GABA in AH neurones of the guinea-pig myenteric plexus. This appeared to be mediated by $GABA_A$ type receptors. It was also observed that superfusion with GABA induced a membrane depolarization even in the presence of bicuculline, which required extracellular calcium ions. This depolarization was mimicked by baclofen, suggesting that it may involve $GABA_B$ receptors (see Bowery *et al.*, 1983). The possibility was considered that this depolarization may result from suppression of a constant inward calcium current occurring at a nonsomatic location. We therefore examined the effects of GABA on two calcium-dependent events in myenteric neurones, synaptic transmission between neurones and the calcium spike of AH neurones.

Methods

Intracellular recordings were made from neurones of the myenteric plexus of the guinea-pig ileum. The techniques used have been described by Nishi & North (1973) and in the preceding paper (Cherubini & North, 1984). GABA and other drugs were applied to neurones by superfusion. Acetylcholine (ACh) was applied by ionophoresis (Morita *et al.*, 1982). Synaptic potentials were evoked by focal stimulation of the surface of the ganglion or an

interconnecting strand, using a glass microelectrode (tip diameter 10–20 μm) containing Krebs solution. Drugs used were GABA (Sigma), ACh chloride (Sigma), bicuculline methiodide (Pierce), β-*p*-chlorophenyl-GABA (baclofen), atropine sulphate (Merck), hyoscine hydrochloride (Sigma) and tetrodotoxin (Sigma).

Results

GABA depresses the fast e.p.s.p.

GABA (1 μM–1 mM) was applied by superfusion to 52 S neurones. In only 5 cells it caused a small membrane depolarization (< 10 mV) and conductance increase; the remaining cells showed no change in potential or input resistance. The amplitude of the fast e.p.s.p. was depressed by GABA in 43 cells in a concentration-dependent manner (Figure 1): 100 μM GABA caused the peak amplitude to fall to 51.5 ± 3.2% (mean ± s.e.mean, $n = 18$)) of its control value. The time course of the response to GABA was often prolonged: a typical application of GABA lasting for 5 min caused a depression of the e.p.s.p. which became maximal during the first 3–5 min of superfusion and which washed out slowly over the next 10–15 min. Baclofen (10 μM–1 mM) mimicked the action of GABA. During prolonged superfusions (up to 10 min) with either GABA or baclofen (1 mM), the fast e.p.s.p. remained depressed throughout the period of superfusion; no desensitization was apparent. The depression of the fast e.p.s.p. by GABA was not altered by the concurrent presence of bicuculline (10–30 μM). GABA (up to 1 mM) did not change the amplitude or time course of the nicotinic depolarizations induced by ionophoretic application of ACh onto S neurones.

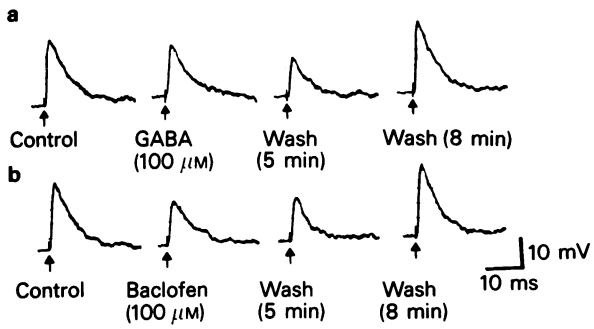

Figure 1 Depression of the fast e.p.s.p. by GABA and baclofen. The fast e.p.s.p. was evoked by a single presynaptic shock (at the arrow). During superfusion with GABA (100 μM) in (a) or baclofen (100 μM) in (b) the fast e.p.s.p. was reduced and this effect reversed in about 8 min.

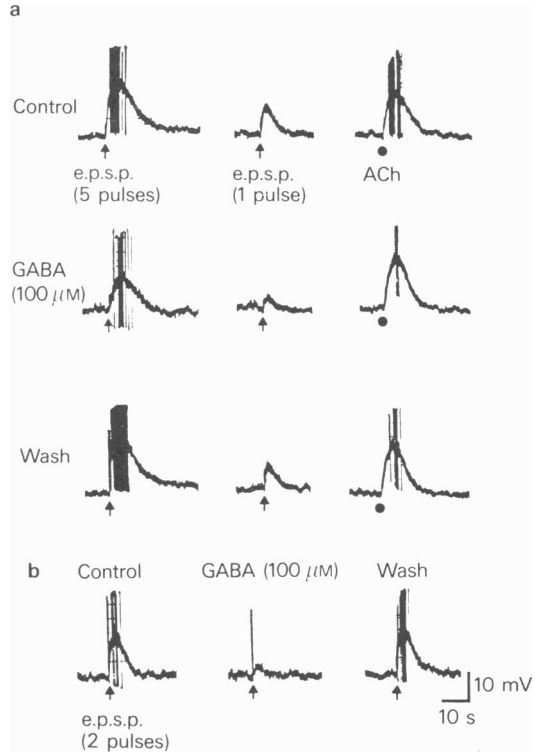

Figure 2 The depression of slow e.p.s.p. by GABA. (a) Superfusion with GABA (100 μM) depressed both the slow e.p.s.p. evoked by single shock nerve stimulation (middle) or by 5 shocks at 10 Hz (left). The muscarinic slow depolarization induced by application of acetylcholine (ACh, right) was not affected. ACh was applied by ionophoresis (20 nA, 10 ms). (b) After addition of hyoscine (300 nM) 2 shocks at 10 Hz evoked a noncholinergic slow e.p.s.p. This was greatly depressed by GABA superfusion (100 μM).

GABA depresses the slow e.p.s.p.

Slow e.p.s.ps in S neurones were sometimes evoked by a single pulse stimulus applied to the presynaptic nerves (North & Tokimasa, 1982). These slow e.p.s.ps were due to ACh acting on muscarinic receptors, since they were reversibly abolished by hyoscine. GABA (10 μM–1 mM) reversibly depressed the single shock slow e.p.s.p. (Figure 2).

In both S and AH neurones, slow e.p.s.ps were also evoked by repeated pulse stimuli (typically 10 pulses at 10 Hz). This slow e.p.s.p. is due to release of a noncholinergic transmitter which is probably substance P (Johnson *et al.*, 1981). GABA reduced the amplitude and duration of this slow e.p.s.p. (Figure 2). In the presence of hyoscine, GABA exerted the same effect. Thus one may expect that GABA in-

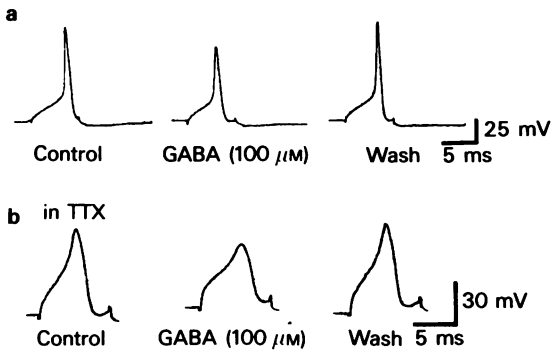

Figure 3 GABA depresses the directly evoked action potentials. (a) Single oscilloscope traces of direct evoked action potentials. Superfusion with GABA (100 μM) reversibly reduced the amplitude of the action potential. (b) In another neurone, perfusion of GABA (100 μM) reversibly reduced the amplitude of the tetrodotoxin (TTX)-resistant spike. TTX (600 nM) present.

hibits the release of both ACh and the noncholinergic transmitter which mediates the slow e.p.s.p. GABA (up to 1 mM) did not change the amplitude or the time course of the muscarinic depolarization induced by ionophoretic application of ACh onto S or AH neurones (Figure 2). The depression of the slow e.p.s.p. by GABA was not blocked by prior superfusion with bicuculline (10 μM).

GABA depresses calcium action potentials

AH neurones in the myenteric plexus show action potentials in the presence of tetrodotoxin (TTX) which are due to inward movement of calcium ions (Nishi & North, 1973; North, 1982). They were reversibly depressed by GABA (5–100 μM) (Figure 3). This effect of GABA was particularly marked when the action potential was prolonged by inhibiting spike repolarization. In 16 neurones, recording electrodes were filled with CsCl (1–2 M) and the effect of GABA was studied after the impalement had been continued for almost 30 min and until the action potential duration reached a steady value (usually 80–500 ms). These prolonged action potentials were reversibly blocked by cobalt (1–2 mM) or by changing to a calcium-free, high (10 mM) magnesium solution. Superfusion with GABA dramatically reduced the duration of these calcium spikes, sometimes eliminating them altogether (Figure 4). This action of GABA occurred whether or not the membrane potential or conductance was changed. Action potentials were always studied after returning the membrane potential to its control level, in those cells which were depolarized by GABA. The effect was dose-dependent (5 μM–100 μM), had a rapid

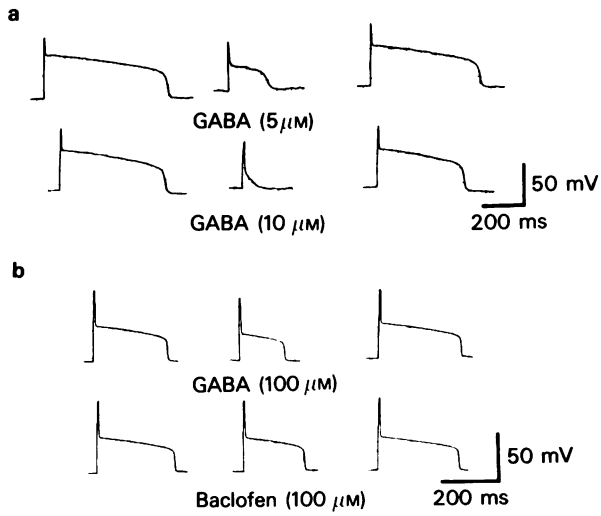

Figure 4 The depression of the Ca spikes by GABA and baclofen. (a) Superfusion with GABA (5 and 10 μM) reduced in a dose-dependent way the amplitude and duration of the direct evoked action potentials. Single oscilloscope traces of directly evoked action potentials recorded with CsCl filled electrode and in the presence of tetrodotoxin (TTX, 600 nM). The traces on the left represent the controls, those on the right, the recovery after washing out GABA. No changes in membrane potential or resistance were observed during GABA superfusion. (b) In another neurone, superfusion with GABA (100 μM) and baclofen (100 μM) reduced the duration of the direct evoked action potentials. Single oscilloscope traces of directly evoked action potentials recorded with CsCl-filled electrodes in presence of TTX (600 nM). The traces on the left are the controls, and on the right the recoveries after washing out GABA and baclofen. Both GABA and baclofen caused a 5–6 mV depolarization; spikes shown were evoked at the resting membrane potential.

onset (within 1–3 min) and reversed on washing over 8–10 min. Baclofen mimicked this action of GABA, although slightly higher concentrations were required (Figure 4). The effects of GABA and baclofen were not antagonized by the concomitant presence of bicuculline (10–30 μM). Prolonged perfusion with GABA (100 μM) (up to 8 min) caused a depression of the calcium spike which persisted throughout the application.

Discussion

GABA depressed the release of ACh and noncholinergic transmitter (probably substance P) from myenteric nerves, by an action which is bicuculline-insensitive, non-desensitizing and mimicked by bac-

lofen. A similar depression of ACh release which did not desensitize and which was picrotoxin-insensitive was described by Kato & Kuba (1980) in bullfrog ganglion cells, and also observed by Adams & Brown (1975) in rat superior cervical ganglion. Many more studies with a variety of agonists and antagonists would be required to characterize the receptor involved in this action of GABA; from the results to date it seems likely to be similar to that described as $GABA_B$ by Bowery *et al.* (1981). It is interesting that this effect is bicuculline-insensitive, because this contrasts with the bicuculline-sensitive activation of chloride conductance in myenteric and other neurones (see Cherubini & North, 1984).

Mechanism of inhibition of transmitter release

There are basically three ways in which GABA might reduce transmitter release in the present experimental circumstances. First, fibre excitability may be reduced leading to the excitation of a small number of fibres; one action which would be expected to reduce fibre excitability would be a chloride conductance increase. Second, propagation of action potentials may be blocked between the point of stimulation and the site of transmitter release. Such an effect accompanies the potassium conductance increase produced by enkephalin (Morita & North, 1981) and might be expected to result from a large increase in chloride conductance. However, the chloride conductance increases observed in AH cells were rapidly desensitizing and bicuculline-sensitive, whereas the depression of the e.p.s.ps was not. A third mechanism is a block of inward calcium current at the transmitter release site. Such an action was observed in the soma of AH cells, and was non-desensitizing and bicuculline insensitive. A similar effect occurring at release sites may underlie the depression of synaptic potentials. This action of GABA is similar to that first described by Dunlap (1981) in immature chick dorsal root ganglion cells.

In the previous paper, we described a depolarization by GABA of the membrane of AH neurones, which was also mimicked by baclofen, not blocked by bicuculline, and which did not desensitize rapidly. This depolarization was not associated with the large increase in conductance which characterized the chloride activation. A proportion of AH neurones appear to have an inward calcium current at the resting potential, which may hold open a membrane potassium conductance (Grafe *et al.*, 1980). GABA might reduce such a resting calcium current in a manner similar to manganese (Grafe *et al.*, 1980), and the resulting closure of calcium-dependent potassium channels may contribute to the depolarization of AH cells.

GABA actions in enteric nervous system

Experiments which measure the tension developed by the longitudinal muscle of the guinea-pig ileum have shown that GABA both excites cholinergic neurones and inhibits the release of ACh from them, whether this occurs spontaneously or when it is evoked by electric field stimulation (Giotti *et al.*, 1983; Krantis & Kerr, 1981). The first action is designated as resulting from activation of $GABA_A$ receptors, the second has the characteristics of a $GABA_B$ response (Ong & Kerr, 1983; Giotti *et al.*, 1983). Our experiments indicate that GABA reduces the release of ACh at synapses between neurones, in addition to release of ACh which reaches the muscle. Many other substances have similar effects, and the neurones which release ACh at the two sites may well be identical (see North, 1982). If the reduced calcium spike of the AH cell underlies the inhibition of transmitter release, one might conclude that some AH neurones make nicotinic synapses onto S neurones, while others make noncholinergic synapses onto both S and AH cells, and yet others liberate ACh which reaches the muscle. Other evidence for such connections has been reviewed (North, 1982).

Whether GABA normally plays any role in peristaltic movements of the intestine cannot be addressed by the present findings. Krantis & Kerr (1981) found that bicuculline slowed the rate of pellet movement through the large intestine, and concluded that GABA may interact with $GABA_A$ sites during peristalsis. However, the present electrophysiological studies show that the only action of GABA which can be blocked by bicuculline is the rapidly desensitizing activation of chloride conductance. Synaptic potentials due to chloride channel opening have not been described in myenteric neurones. The other action of GABA, the inhibition of transmitter release and block of calcium spikes, occurs at similar or lower concentrations than chloride channel activation. Selective antagonists of these effects of GABA will be most helpful in elucidating this action of GABA in the control of gastrointestinal motility.

Supported by U.S. Department of Health and Human Services grant NS18111 (NIH) and DA03160 (ADAMHA).

References

ADAMS, P.R. & BROWN, D.A. (1975). Actions of γ-aminobutyric acid on sympathetic ganglion cells. *J. Physiol.*, **250,** 85–120.

BOWERY, N.G., HILL, D.R. & HUDSON, A.L. (1983). Characteristics of $GABA_B$ receptor binding sites on rat whole brain synaptic membranes. *Br. J. Pharmac.*, **78,** 191–206.

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.

CHERUBINI, E. & NORTH, R.A. (1984). Actions of γ-aminobutyric acid on neurones of guinea-pig myenteric plexus. *Br. J. Pharmac.*, **82,** 93–100.

DESARMENIEN, M., FELTZ, P., LOEFFLER, J.P., OCCHIPINTI, G. & SANTANGELO, F. (1982). Multiple GABA-receptors on A δ and C primary afferent neurones in the adult rat. *Br. J. Pharmac.*, **76,** 289P.

DUNLAP, K. (1981). Two types of γ-aminobutyric acid receptor on embryonic sensory neurones. *Br. J. Pharmac.*, **74,** 579–585.

DUNLAP, K. & FISCHBACH, G.D. (1981). Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones *J. Physiol.*, **317,** 519–535.

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.

GRAFE, P., MAYER, C.J. & WOOD, J.D. (1980). Synaptic modulation of calcium-dependent potassium conductance in myenteric neurones in the guinea-pig. *J. Physiol.*, **305,** 235–248.

KATO, E. & KUBA, K. (1980). Inhibition of transmitter release in bullfrog sympathetic ganglia induced by γ-aminobutyric acid. *J. Physiol.*, **298,** 271–283.

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

JOHNSON, S.M., KATAYAMA, Y., MORITA, K. & NORTH, R.A. (1981). Mediators of slow synaptic potentials in the myenteric plexus of the guinea-pig ileum. *J. Physiol.*, **320,** 175–186.

MORITA, K. & NORTH, R.A. (1981). Opiates and enkephalin reduce the excitability of neuronal processes. *Neuroscience*, **6,** 1943–1951.

MORITA, K., NORTH, R.A. & TOKIMASA, T. (1982). Muscarinic agonists inactivate potassium conductance of guinea-pig myenteric neurones. *J. Physiol.*, **333,** 125–139.

NISHI, S. & NORTH, R.A. (1973). Intracellular recording from the myenteric plexus of the guinea-pig ileum. *J. Physiol.*, **231,** 471–491.

NORTH, R.A. (1982). Electrophysiology of the enteric nervous system. *Neuroscience*, **7,** 315–325.

NORTH, R.A. & TOKIMASA, T. (1982). Muscarinic synaptic potentials in guinea-pig myenteric plexus neurones. *J. Physiol.*, **333,** 151–156.

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

(Received August 8, 1983.
Revised November 29, 1983.)