Br. J. Pharmacol. (1989), **97**, 1225–1231

Modulation of non-adrenergic, non-cholinergic neural bronchoconstriction in guinea-pig airways via $GABA_B$-receptors

M.G. Belvisi, M. Ichinose & [1]P.J. Barnes

Department of Thoracic Medicine, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY

1 Evidence suggests that γ-aminobutyric acid (GABA) and its receptors are present in the peripheral nervous system. We have now investigated the effect of GABA and related substances on non-adrenergic, non-cholinergic (NANC) neurally-evoked bronchoconstriction in the anaesthetised guinea-pig.

2 Bilateral vagal stimulation (5 V, 5 ms, 3 or 5 Hz) for 30 s, after propranolol (1 mg kg^{-1} i.v.) and atropine (1 mg kg^{-1} i.v.) evoked a NANC bronchoconstrictor response manifest as a mean tracheal pressure rise of 21.9 ± 1.04 cmH_2O (n = 70). The bronchoconstrictor response was reproducible for any given animal.

3 GABA (10 μg–10 mg kg^{-1} i.v.) did not alter basal tracheal pressure but reduced the NANC bronchoconstrictor response to vagal stimulation in a dose-dependent manner (ED_{50} = 186 μg kg^{-1} with a maximal inhibition of 74 ± 3.4% at 10 mg kg^{-1}). Neither the opioid antagonist naloxone (1 mg kg^{-1} i.v.) nor the α-adrenoceptor antagonist phentolamine (2.5 mg kg^{-1} i.v.) had any significant effect on the inhibitory response produced by GABA (500 μg kg^{-1}).

4 GABA-induced inhibition was not antagonised by the $GABA_A$-antagonist bicuculline (2 mg kg^{-1} i.v.).

5 The $GABA_B$-agonist baclofen (10 μg–3 mg kg^{-1} i.v.) caused a dose-dependent inhibition of the NANC response (ED_{50} = 100 μg kg^{-1} with a maximal inhibition of 35.5 ± 2.8% at 3 mg kg^{-1}). The $GABA_A$-agonist, 4,5,6,7-tetrahydroisoxazolo[5,4-C] pyridin-3-ol (THIP), also inhibited the NANC bronchoconstrictor response. However, the dose of THIP required for this effect was high (3 mg kg^{-1}) and the effect (< 10% inhibition) was small.

6 Substance P (SP; 5 μg kg^{-1} or 25 μg kg^{-1}), produced a bronchoconstrictor response equivalent to that produced by NANC vagal stimulation. This response was significantly increased by injection of GABA. Baclofen had no significant effect on responses evoked by exogenous SP.

7 We conclude that GABA inhibits the release of transmitter from NANC nerves via an action at $GABA_B$ receptors and that GABA might play a role in the regulation of neurogenic responses in the airways.

Introduction

γ-Aminobutyric acid (GABA) is a well-characterised inhibitory neurotransmitter in the mammalian central nervous system (Curtis & Johnston, 1974). Recent evidence suggests, however, that GABA and its receptors are present in peripheral neurones (Giotti *et al.*, 1983; Tanaka, 1985; Santicioli *et al.*, 1986). In guinea-pig trachea GABA decreases the contractile response of airway smooth muscle to cholinergic nerve stimulation possibly by inhibiting the evoked release of acetylcholine (Tamaoki *et al.*, 1987).

Non-adrenergic, non-cholinergic (NANC) bronchoconstriction evoked by vagal stimulation *in vivo* may be due to the release from sensory nerves of neuropeptides such as substance P (SP) and neurokinin A (Lundberg *et al.*, 1983). We have now studied the effect of GABA on the NANC bronchoconstrictor response evoked by vagal stimulation to evaluate whether GABA has an inhibitory action on sensory neurones.

[1] Author for correspondence.

At least two subtypes of GABA receptor have been identified; bicuculline-sensitive and bicuculline-insensitive receptors, which are termed $GABA_A$- and $GABA_B$-receptors, respectively (Bowery *et al.*, 1981; Kaplita *et al.*, 1982). We therefore also investigated the GABA receptor subtype involved by use of selective agonists and an antagonist.

Methods

Measurements

Male Dunkin-Hartley outbred guinea-pigs (Charles River U.K. Ltd., Kent) of 310–470 g weight were housed in a temperature-controlled (21°C) room with food and water freely available. They were anaesthetised with an intraperitoneal injection of urethane (8 ml kg^{-1} of a 25% solution w/v in saline) and placed on a heated blanket (Homeothermic system, Harvard Apparatus Ltd., Edenbridge, Kent) which maintained body temperature at 37°C. The left jugular vein was cannulated for the injection of drugs. The left carotid artery was cannulated for monitoring blood pressure, via an indwelling Portex cannula, filled with heparin-saline (10 u ml^{-1}), linked to a pressure transducer (Druck blood pressure transducer, Druck Ltd., Groby, Leicestershire) and connected to a recorder (Lectromed Multitrace 2, Ormed Ltd., Welwyn Garden City, Hertfordshire). The trachea was cannulated and the animal ventilated with a small animal constant volume respiration pump (Bioscience U.K., Sheerness, Kent), operating at 60 strokes min^{-1} of 1 ml laboratory air per 100 g body weight. Overflow pressure was measured by a modification of the Konzett and Rossler procedure (Konzett & Rossler, 1940) by a differential pressure transducer (Farnell Electronic Components Ltd., Leeds). Both cervical vagus nerves were carefully dissected free, sectioned (to avoid stimulating the CNS) and their peripheral ends placed on platinum electrodes. A Fenton Lewis double-channel stimulator was used to stimulate the nerves using pulses of 5 ms duration 5 V strength for 30 s. The frequency used was either 3 Hz or 5 Hz depending on the size of the NANC bronchoconstrictor response obtained. Only one of these two pulse frequencies was used in a given animal and each animal served as its own control.

Experimental protocol

Atropine (1 mg kg^{-1}) and propranolol (1 mg kg^{-1}) were administered intravenously (i.v.) and the vagi were stimulated after 30 min to give a NANC bronchoconstrictor response as previously described (Belvisi *et al.*, 1988). In some experiments phentolamine (2.5 mg kg^{-1}) was also injected 30 min before vagal stimulation. Vagal stimulation was repeated again 10 min later or when the overflow pressure had returned to its baseline value. Hyperinflation of the lungs was used to return the pressure to control values after a bronchoconstriction. After two identical responses had been elicited the effects of GABA and various GABA receptor agonists were investigated. GABA (10 μg–10 mg kg^{-1}), baclofen (10 μg–3 mg kg^{-1}) or 4,5,6,7-tetrahydroisoxazolo[5,4-c]-pyridin-3-ol (THIP, 3 mg kg^{-1}) were administered i.v. 1 min before vagal stimulation. The time course of the action of GABA (500 μg kg^{-1}) was also studied. Several doses of GABA could be examined in one animal as GABA had a very short duration of action. The action of GABA was also examined in the presence of bicuculline (2 mg kg^{-1}), a $GABA_A$-receptor antagonist, and naloxone (1 mg kg^{-1}), an opioid receptor antagonist, each antagonist being given 10 min before the injection of GABA. In other experiments bicuculline was injected alone to determine whether it had any effect *per se* on NANC bronchoconstriction at these stimulation parameters.

The effects of GABA (500 μg kg^{-1}) and baclofen (2 mg kg^{-1}) on bronchoconstrictor responses to exogenous substance P (SP, 5–25 μg kg^{-1}) (which produced a constrictor response equivalent to that produced by NANC stimulation) were also evaluated. Two bronchoconstrictor responses to SP (5 or 25 μg kg^{-1}) were elicited. A period of 30 min was allowed between successive injections of SP. The effect of GABA on the bronchoconstrictor response evoked by SP was investigated by injecting GABA 1 min before the administration of SP.

Drugs

Drugs and chemicals were obtained from the following sources: urethane, substance P, γ-amino-n-butyric acid, (−)-bicuculline methiodide (Sigma Chemical Co., Poole, Dorset), atropine sulphate BP (Phoenix Pharmaceuticals Ltd., Oxford), propranolol hydrochloride (Imperial Chemical Industries, Cheshire), (±)-baclofen, 4,5,6,7-tetrahydroisoxazolo[5,4,-c]pyridin-3-ol hydrochloride (Research Biochemicals Inc., MA, U.S.A.), naloxone hydrochloride (Du Pont U.K., Hertfordshire), phentolamine mesylate BP (Ciba Laboratories, West Sussex), heparin injection BP (CP Pharmaceuticals Ltd., Wrexham). All drugs were made up in 0.9% saline on the day of experimentation.

Statistical analyses

Mean arterial blood pressure (BP) was calculated from recorded traces as: diastolic pressure + 0.33

(systolic pressure – diastolic pressure). Results are presented as means ± s.e.mean. Differences between means were analysed by Student's t test for paired data. A probability level of $P < 0.05$ was considered statistically significant. The dose of drug required to produce 50% of the maximal inhibition of the NANC bronchoconstrictor response evoked by vagal stimulation (ED_{50}) was read directly from curves constructed using % maximal responses as a function of the dose of GABA or baclofen.

Results

Effect of vagal stimulation

Thirty minutes after atropine and propranolol (each 1 mg kg^{-1}), vagal stimulation produced a significant bronchoconstrictor response that was reproducible within any given animal. The NANC bronchoconstrictor response was manifest as an increase in airways pressure in the range 8–52 cmH_2O. The mean amplitude (±s.e.mean) of the response was 21.9 ± 1.04 cmH_2O, $n = 70$.

Effect of GABA on NANC bronchoconstrictor response

All drugs were dissolved in saline but i.v. saline had no effect on NANC bronchoconstrictor responses. GABA, when injected 1 min before stimulation, caused a dose-dependent decrease in the NANC bronchoconstrictor response evoked by vagal stimulation. The ED_{50} for GABA at inhibiting this response was 186 μg kg^{-1} with a maximum inhibition of 74 ± 3.4% at 10 mg kg^{-1} (Figure 1). The inhibitory effect of GABA was assessed 1, 10 and 20 min after its injection. The inhibitory effect of GABA (500 μg kg^{-1} i.v.) had virtually disappeared by 10 min (Figure 2) and the inhibitory effects of larger doses of GABA followed a similar time course.

The effect of GABA before and after bicuculline was studied in the same animal so that paired data were obtained. The $GABA_A$-antagonist bicuculline (2 mg kg^{-1} i.v.), when injected 10 min before GABA, increased the inhibitory effect of GABA (100 μg kg^{-1}) on the NANC response from 38.3 ± 3.1% to 53.6 ± 6.8% ($n = 5$) ($P < 0.05$). In these experiments the inhibitory effects of bicuculline and GABA in combination were somewhat persistent. The NANC bronchoconstrictor response remained inhibited even at 10 min after the injection of GABA. Bicuculline alone (100, 500 μg kg^{-1} and 2 mg kg^{-1}) also inhibited the NANC bronchoconstrictor response in a persistent manner. Indeed the inhibitory effects of bicuculline increased over a time period up to 30 min after its injection (Figure 3). Phentolamine (2.5 mg kg^{-1}) (Boschetto *et al.*, 1989)

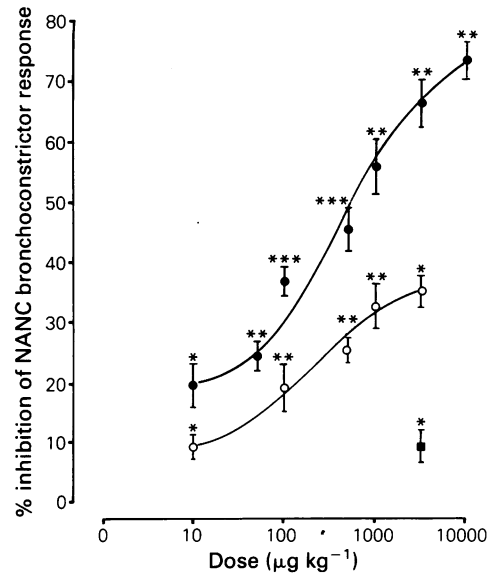

Figure 1 The non-adrenergic, non-cholinergic (NANC) bronchoconstrictor response evoked by vagal stimulation: its inhibition by intravenous GABA (●), baclofen (○) and 4,5,6,7-tetrahydroisoxazolo[5,4,-c] pyridin-3-ol (THIP) (■). Data are means of 4–11 animals. Vertical lines show s.e.mean. Significance of inhibition: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

($n = 4$), injected 30 min before stimulation, did not affect the inhibitory response produced by GABA (500 μg kg^{-1}) nor did naloxone (1 mg kg^{-1}) (Belvisi *et al.*, 1988) ($n = 4$), injected 10 min before GABA (Figure 4).

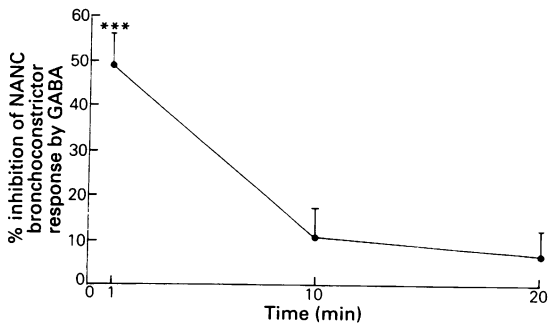

Figure 2 Time course of inhibition of the NANC bronchoconstrictor response by GABA (500 μg kg^{-1}). Bilateral vagal stimulation was carried out at 3 different time points (1, 10 and 20 min) after administration of GABA. Data are means of the same six animals at each time point. Vertical lines show s.e.mean. Significance of inhibition: ***$P < 0.001$.

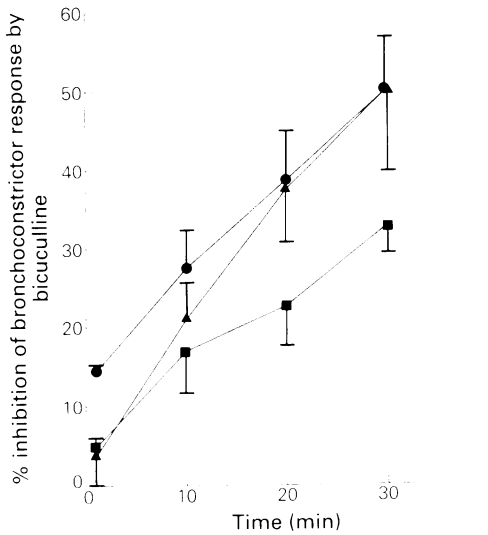

Figure 3 Time course of inhibition of the NANC bronchoconstrictor response by bicuculline 100 μg (■), 500 μg (▲) and 2 mg kg^{-1} (●). Bilateral vagal stimulation was carried out at 4 different time points (1, 10, 20 and 30 min) after administration of bicuculline. Data are means of 3–4 animals at each dose at each time point. Vertical lines show s.e.mean.

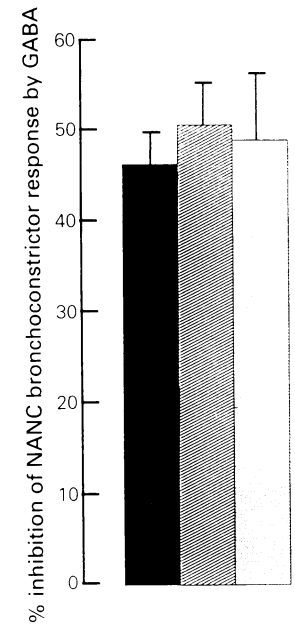

Figure 4 The effect of phentolamine (2.5 mg kg^{-1}) and naloxone (1 mg kg^{-1}) on the inhibitory effect of GABA (500 μg kg^{-1}) on the NANC bronchoconstrictor responses evoked by vagal stimulation. Solid column: inhibitory effect of GABA on NANC bronchoconstrictor response ($n = 8$); hatched column: inhibitory effect of GABA on NANC bronchoconstrictor response in the presence of phentolamine (2.5 mg kg^{-1}) ($n = 4$); stippled column: inhibitory effect of GABA on NANC response in the presence of naloxone (1 mg kg^{-1}) ($n = 4$). Data are presented as means and bars show s.e.mean.

Effect of selective GABA receptor agonists

The $GABA_B$-agonist baclofen, when injected 1 min before stimulation, caused a dose-dependent decrease in the NANC bronchoconstrictor response elicited by vagal stimulation. The ED_{50} for baclofen was 100 μg kg^{-1} with a maximum inhibition of 35 ± 2.8% at 3 mg kg^{-1} (Figure 1). However, the $GABA_A$-selective agonist THIP only inhibited the NANC response at 3 mg kg^{-1} (9.7 ± 2.6%, $n = 6$, $P < 0.05$) (Figure 1).

Effect on substance P responses

Responses to SP were elicited following the injection of atropine and propranolol (each 1 mg kg^{-1}). Doses of SP (5 or 25 μg kg^{-1}) were selected which produced responses equivalent to those evoked by vagal stimulation in each animal.

Increases in tracheal pressure evoked by exogenous SP (5 or 25 μg kg^{-1}) were enhanced by GABA (500 μg kg^{-1}) from 25.8 ± 4.4 cmH_2O to 34 ± 4.7 cmH_2O ($n = 5$, $P < 0.05$) (Figure 5). This dose of GABA inhibited the NANC response by 45.8 ± 3.7%. However, baclofen at a dose (2 mg kg^{-1}) which inhibited the NANC response by approximately 34.5%, did not significantly alter the response to exogenous SP (25 μg kg^{-1}) (Figure 5).

Bicuculline (2 mg kg^{-1}), given alone 10 min before SP (25 μg kg^{-1}) increased the response evoked by SP from 26 ± 2.4 cmH_2O to 32 ± 2.4 cmH_2O ($n = 4$, $P < 0.05$).

Effect of GABA and baclofen on blood pressure

The mean arterial blood pressure (BP) recorded from the left carotid artery before the injection of GABA or the test substance was 40.2 ± 0.96 mmHg ($n = 66$). Flushing the artery, to prevent blood clotting in the indwelling cannula, with heparin-saline before experimentation and i.v. saline had no significant or consistent effect on BP. GABA transiently but significantly decreased the mean arterial BP at every dose used (see Table 1). However, in all but one case the BP was not significantly different from baseline at the time of vagal stimulation. Baclofen (10 μg kg^{-1} to 3 mg kg^{-1}) had a similar effect on BP whereas THIP (3 mg kg^{-1}) had no significant effect.

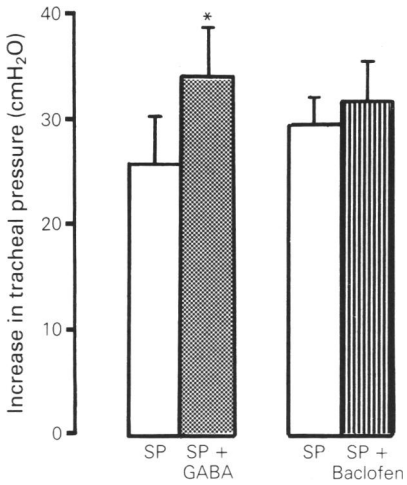

Figure 5 The effect of GABA and baclofen on bronchoconstriction evoked by exogenous substance P (SP). Open columns: SP (5–25 $\mu g\,kg^{-1}$) alone; stippled column: SP (5 $\mu g\,kg^{-1}$) after GABA (500 $\mu g\,kg^{-1}$); lined column: SP (5–25 $\mu g\,kg^{-1}$) after baclofen (2 $mg\,kg^{-1}$). Data are presented as means of 5 or 6 animals. Vertical lines show s.e.mean. *$P < 0.05$.

Discussion

Our study shows that GABA reversibly decreases the neurally-mediated bronchoconstrictor response evoked by stimulation of the peripheral portion of the sectioned vagus in anaesthetised guinea-pigs. This excitatory response resulting from vagal nerve stimulation in the presence of atropine and propranolol is non-adrenergic and non-cholinergic and probably due to the release of neuropeptides, such as SP and neurokinin A, from sensory nerve endings (Andersson & Grundstrom, 1983; Lundberg *et al.*, 1983).

In order to establish whether the inhibitory effect of GABA is due to an effect on neuronal terminals, on tachykinin receptors on airway smooth muscle, or on excitation-contraction coupling in smooth muscle, we compared the effect of GABA on the bronchoconstrictor response to vagal stimulation with its effect on the bronchoconstrictor response to SP. GABA inhibited the NANC response but had no inhibitory effect on the contractile response to SP and even increased the tracheal pressure rise evoked by exogenous SP. This implies that GABA-induced inhibition is not related to an alteration of smooth muscle function but probably represents a prejunctional mechanism, such as inhibition of tachykinin release from sensory nerve endings.

GABA-induced inhibition could be accomplished by enhancing the release of noradrenaline since noradrenaline has been shown to inhibit neural NANC bronchoconstrictor responses in the guinea-pig (Grundstrom *et al.*, 1984). However, the involvement of GABA-induced prejunctional inhibitory α-adrenoceptor activation is unlikely as phentolamine was without effect on the inhibitory action of GABA. GABA transiently decreases blood pressure and this may result in the release of catecholamines which could act on β-adrenoceptors in the airway to inhibit bronchoconstriction. This is probably not the case as the animals had been pretreated with the β-adrenoceptor antagonist, propranolol. The lowering of the blood pressure itself could induce an inhibition of the NANC response to vagal stimulation. However, 1 min after stimulation, which was when GABA was injected, blood pressure had returned to normal, before the effects of nerve stimulation were

Table 1 Effect of GABA and baclofen on mean arterial blood pressure (BP)

Dose GABA ($\mu g\,kg^{-1}$)	n	*Decrease in BP*[a]	*Decrease in BP at stimulation time*[b]
50	5	7.04 ± 2.6*	1.9 ± 1.2 (NS)
100	11	6.9 ± 1.1***	0.6 ± 0.54 (NS)
500	9	13.4 ± 1.2***	2.7 ± 1.8 (NS)
1000	4	14.1 ± 3.1**	7.3 ± 4.4 (NS)
3000	5	10.8 ± 2.9*	7.7 ± 2.2*
Dose baclofen ($\mu g\,kg^{-1}$)			
10	5	15.3 ± 1.6***	1.1 ± 1.5 (NS)
100	5	5.7 ± 1.6*	2.3 ± 1.2 (NS)
500	4	11.5 ± 3.3*	7.8 ± 3.4 (NS)
1000	4	6.2 ± 6.0 (NS)	7.7 ± 3.7 (NS)
3000	3	9.1 ± 2.8 (NS)	7.3 ± 4.1 (NS)

Data are presented as means ± s.e.mean.
[a] Measured at the peak drug effect.
[b] BP 1 min after GABA at the onset of vagal stimulation.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS = not significant.

studied. In the CNS GABA has been shown to potentiate the release of methionine enkephalin (Sawynok & Labella, 1981; Bourgoin *et al.*, 1985). Opioids of the μ and possibly δ type inhibit release of transmitter from NANC nerves by stimulating opioid receptors (Frossard & Barnes, 1987; Belvisi *et al.*, 1988). However, the ineffectiveness of naloxone suggests that this is not the mode of action of GABA in guinea-pig lung.

The GABA receptor antagonist bicuculline failed to prevent the inhibitory effect of GABA, but paradoxically enhanced the effect of GABA. However, we also found that bicuculline alone inhibited NANC responses in an irreversible manner. It is possible that bicuculline has a toxic effect on sensory nerves as it did not inhibit the responses to exogenous SP. The $GABA_B$-agonist, baclofen, has a dose-related and reversible inhibitory effect on NANC bronchoconstrictor responses. Baclofen was more potent than GABA but did not achieve the same maximum inhibitory effect. An explanation for this relative lack of efficacy is the fact that baclofen is stereoselective. (−)-Baclofen is more potent than the racemic mixture of (±)-baclofen used in our study (Giotti *et al.*, 1983). However, for this explanation to stand (+)-baclofen must be an antagonist or a partial agonist. In fact the (+)- and (−)-isomers of baclofen have been shown to have opposite effects in other systems. (−)-Baclofen (4 μM) inhibits release of preloaded [^{3}H]-D-aspartate from slices of rat cerebral cortex, whereas (+)-baclofen was inactive under these conditions and at 10 μM weakly augmented D-aspartate release (Johnston *et al.*, 1980). THIP, a $GABA_A$-agonist, was only weakly effective (<10% inhibition) at a high dose (3 mg kg^{-1}). All this evidence suggests the involvement of the $GABA_B$-type receptor (Bowery *et al.*, 1981). Confirmation of the involvement of a $GABA_B$-receptor might be obtained with the use of a specific $GABA_B$-antagonist. The only selective antagonist currently available, phaclofen, is not suitable for *in vivo* studies because of its very low potency (Kerr *et al.*, 1987).

In conclusion, the action of GABA in the lung appears to be exerted through a modulatory action on NANC nerves. One site of action is probably the bicuculline-insensitive receptor ($GABA_B$), which may be located on sensory nerve terminals, as GABA and baclofen inhibit the NANC bronchoconstrictor response but THIP is weakly effective. Tamaoki *et al.* (1987) have demonstrated that GABA decreases the contractile response of airway smooth muscle to cholinergic nerve stimulation by inhibiting the evoked release of acetylcholine. However, these authors have suggested that this is a bicuculline-sensitive effect and therefore mediated by $GABA_A$-receptors. Hence, the receptor involved remains to be identified because baclofen, a $GABA_B$-agonist, was found to be equipotent with GABA and muscimol, a $GABA_A$-agonist, was found to be less potent. In the present study GABA enhanced the response to exogenous SP but baclofen was unable to do this. This suggests that a similar system operates here as has been observed in the ileum (Giotti *et al.*, 1983). That is GABA as well as inhibiting SP release also enhances its activity, but via $GABA_A$-receptors.

GABA and its synthetic enzyme glutamic acid decarboxylase appear to be present in the peripheral autonomic nervous system, suggesting that GABA may function as a peripheral neurotransmitter or neuromodulator (Jessen *et al.*, 1979). GABAergic nerves have been found in the myenteric plexus of the gut (Jessen *et al.*, 1986) and since the innervation of the respiratory tract has a common embryological origin with that in the gastrointestinal tract, it seems likely that GABAergic nerves may also be present in the airways. If so this suggests that GABA may have a neuromodulatory role on neurogenic bronchoconstriction. Sensory nerves may play an important role in the neurogenic inflammation in the airways and may be involved in the airway inflammation of asthma (Barnes, 1986). Regulation of the release of sensory neuropeptides from nerves may therefore reduce neurogenic inflammation, as has been previously demonstrated for opioids both *in vivo* (Belvisi *et al.*, 1988) and *in vitro* (Frossard & Barnes, 1987). We have now shown that GABA acting via peripheral $GABA_B$-receptors may have a similar effect and this could suggest a novel approach in the chemotherapy of bronchial asthma.

Supported by a grant from Fisons Pharmaceuticals.

References

ANDERSSON, R.G.G. & GRUNDSTROM, N. (1983). The excitatory non-cholinergic non-adrenergic nervous system of the guinea-pig airways. *Eur. J. Respir. Dis.*, **64**, 141–157.

BARNES, P.J. (1986). Asthma as an axon reflex. *Lancet*, **i**, 242–245.

BELVISI, M.G., CHUNG, K.F., JACKSON, D.M. & BARNES, P.J. (1988). Opioid modulation of non-cholinergic neural bronchoconstriction in guinea-pig *in vivo*. *Br. J. Pharmacol.*, **95**, 413–418.

BOSCHETTO, P., ROBERTS, N.M., ROGERS, D.F. & BARNES, P.J. (1989). Effect of antiasthma drugs on microvascular

leakage in guinea-pig airways. *Am. Rev. Respir. Dis.*, **139**, 416–421.

BOURGOIN, S., ARTAUD, F., CESSELIN, F., GLOWINSKI, J. & HAMON, M. (1985). Local and remote effects of intracaudate administration of GABA-related drugs on met-enkephalin release in basal ganglia. *Brain Res.*, **361**, 361–362.

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 nerve terminals. *Eur. J. Pharmacol.*, **71**, 53–70.

CURTIS, D.R. & JOHNSTON, G.A.R. (1974). Amino acid transmitters in the mammalian central nervous system. *Rev. Physiol. Biochem. Exp. Pharmacol.*, **69**, 97–188.

FROSSARD, N. & BARNES, P.J. (1987). U-opioid receptors modulate non-cholinergic constrictor nerves in guinea-pig airways. *Eur. J. Pharmacol.*, **141**, 519–522.

GRUNDSTROM, N., ANDERSSON, R.G.G. & WIKBERG, J.E.S. (1984). Inhibition of the excitatory non-adrenergic non-cholinergic neurotransmission in the guinea-pig tracheo-bronchial tree mediated by alpha$_2$- adrenoceptors. *Acta Pharmacol. Toxicol.*, **54**, 8–14.

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

JESSEN, K.R., HILLS, J.M. & SAFFREY, M.J. (1986). Immunohistochemical demonstration of GABAergic neurons in the enteric nervous system. *J. Neurosci.*, **6**, 1628.

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

JOHNSTON, G.A.R., HAILSTONE, M.H. & FREEMAN, C.G. (1980). Baclofen: stereoselective inhibition of excitant amino acid release. *J. Pharm. Pharmacol.*, **32**, 230–231.

KAPLITA, P.V., WATERS, D.H. & TRIGGLE, D.J. (1982). GABA action on guinea-pig ileal myenteric plexus. *Eur. J. Pharmacol.*, **79**, 43–51.

KERR, D.I.B., ONG, J., PRAGER, R.H., GYNTHER, B.D. & CURTIS, D.R. (1987). Phaclofen: a peripheral and central baclofen antagonist. *Brain Res.*, **405**, 150–154.

KONZETT, H. & ROSSLER, R. (1940). Versuchsanordnung zu untersuchungen an der bronchialmuskulatur. *Arch. Exp. Path. Pharmak.*, **195**, 71–74.

LUNDBERG, J.M., SARIA, A., BRODIN, E., ROSELL, S. & FOLKERS, K. (1983). A substance P antagonist inhibits vagally induced increase in vascular permeability and bronchial smooth muscle contraction in guinea-pig. *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 1120–1124.

SANTICIOLI, P., MAGGI, C.A. & MELI, A. (1986). The post ganglionic excitatory innervation of the mouse urinary bladder and its modulation by prejunctional $GABA_B$ receptors. *J. Auton. Pharmacol.*, **6**, 53–66.

SAWYNOK, J. & LABELLA, F.S. (1981). GABA and baclofen potentiate the K^+ evoked release of methionine-enkephalin from rat striatal slices. *Eur. J. Pharmacol.*, **70**, 103–110.

TAMAOKI, J., GRAF, P.D. & NADEL, J.A. (1987). Effect of γ-aminobutyric acid on neurally mediated contraction of guinea-pig trachealis smooth muscle. *J. Pharmacol. Exp. Ther.*, **243**, 86–90.

TANAKA, C. (1985). γ-aminobutyric acid in peripheral tissues. *Life Sci.*, **37**, 2221–2235.

(Received June 20, 1988
Revised March 10, 1989
Accepted March 29, 1989)