GABA_A receptor-mediated increase in membrane chloride conductance in rat paratracheal neurones

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1 The actions of γ -aminobutyric acid (GABA) on the intramural neurones of 14–18 day old rats were studied *in situ* by use of intracellular current- and voltage-clamp techniques. The ionic conductance changes and the effects of various GABA-receptor agonists and antagonists on these neurones were also investigated.

2 Prolonged application of GABA either by ionophoresis (10 pC–10 nC) or superfusion (10–100 μ M), evoked a biphasic membrane depolarization in over 90% of all paratracheal neurones studied. Typically, the response consisted of an initial rapid depolarization (18–45 ms) that subsequently faded over a period of 15–25 s to reveal a second smaller depolarization which was maintained for the duration of GABA application. Both components of the evoked response resulted in an increase in membrane conductance and an inward flow of current.

3 The amplitude of the transient inward current, recorded during the initial phase of the response, was linearly related to the membrane potential at which it was elicited and reversed symmetrically at a membrane potential of -32.7 mV. The underlying increase in conductance was largely independent of membrane potential. The equilibrium potential for the sustained inward current was -38.7 mV. Replacement of extracellular chloride with gluconate ions initially enhanced the GABA-evoked inward current. With successive applications of GABA in low chloride, the evoked current and conductance changes declined markedly.

4 Muscimol superfusion (1–10 μ M) or ionophoresis (10 pC–10 nC) mimicked both the initial and late phases of the GABA-induced conductance change and inward current. Baclofen (1–100 μ M) had no effect upon either resting membrane potential or conductance in any of the cells tested.

5 The large transient initial phase of the GABA-evoked inward current and depolarization were potently inhibited by picrotoxin (1–50 μ M), whereas the smaller sustained inward current was largely resistant to picrotoxin.

6 All of the observed actions of GABA and muscimol were antagonized by bicuculline (0.1–10 μ M) in an apparently competitive manner.

7 It is concluded that GABA acts via GABA_A receptors present on the soma of paratracheal neurones to produce an increase in membrane chloride conductance. Prolonged application of GABA results in a decline in the observed current due to a combination of two processes: receptor desensitization and shifts in the chloride equilibrium potential. The possible roles for GABA in neural regulation of airway excitability are discussed.

Introduction

γ -Aminobutyric acid (GABA) is considered to be the principal inhibitory neurotransmitter in the mammalian central nervous system (CNS) (Kelly & Beart, 1975). The first evidence to suggest that there are GABAergic neurones in the vertebrate peripheral nervous system was the discovery that a population of intrinsic neurones in the guinea-pig myenteric plexus possessed a high-affinity uptake system for GABA, and were able to synthesize GABA from glutamic acid (Jessen *et al.*, 1979). Subsequent investigations now indicate a possible neurotransmitter role for GABA in a number of peripheral tissues (for review see Erdo & Bowery, 1986). In the periphery, as in the CNS, both GABA_A and GABA_B receptor subtypes are present (Dunlap, 1981; Cherubini & North, 1984a,b). Peripheral GABA_A receptors are similar to those in the CNS, in that they have an integral chloride channel and are antagonized by bicuculline and/or picrotoxin (DeGroat, 1970; Adams & Brown, 1975; Bormann *et al.*, 1987). In contrast, peripheral GABA_B receptors appear to differ from those found in the CNS, in that they are almost exclusively linked to calcium channels (Dunlap & Fischbach, 1981; Cherubini & North, 1984b; Deisz & Lux, 1985; Holz *et al.*, 1986), whereas central

GABA_B receptor activation involves an increase in potassium conductance (Newberry & Nicoll, 1984; Gähwiler & Brown, 1985). The main function of many of the GABA receptors, especially peripheral GABA_B receptors, appears to be the modulation of release of other neurotransmitters such as acetylcholine, substance P and noradrenaline (Adams & Brown, 1975; Kato & Kuba, 1980; Cherubini & North, 1984b).

In the airways, GABA has been implicated in the regulation of tracheal smooth muscle tone through both central and peripheral mechanisms (Haxhui *et al.*, 1986; Tamaoki *et al.*, 1987). Results suggest that GABA decreases the contractile response of airway smooth muscle to cholinergic nerve stimulation by inhibiting the release of acetylcholine from postganglionic parasympathetic nerves in the guinea-pig trachea. This effect is thought to be mediated by chloride-dependent, bicuculline-sensitive receptors present on the postganglionic neurones (Tamaoki *et al.*, 1987). Furthermore, in an animal model of asthma, GABA and the GABA_B agonist (–)-baclofen have been found to protect against anaphylactic bronchospasm as well as spasm induced by aerosols of histamine and prostaglandins (Luzzi *et al.*, 1987). More recently, GABA has also been shown to inhibit bronchoconstriction, via the action of GABA_B receptors that are thought to be present on sensory nerve terminals (Belvisi *et al.*, 1989).

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We have recently developed a preparation of rat trachea that permits us to visualize and record from intramural paratracheal neurones present in the trachealis muscle *in situ*. In a previous study we examined the basic electrophysiological characteristics of these cells and found that they were made up of a population of neurones showing two extreme types of firing behaviour (Allen & Burnstock, 1990). The majority of cells (65–75%) discharged short high-frequency bursts of action potentials at regular intervals in response to prolonged stimulation; these bursts of firing were followed by a slow calcium-dependent after-hyperpolarization. A further 10–15% of cells showed no burst firing activity, but fired tonically at low frequencies for the duration of current stimulation; the remaining cells displayed characteristics in between these two extremes. In this paper, we describe the effects of GABA and related substances upon these cells and discuss the possible implications of these actions on airway function.

Methods

All experiments were performed on 14–18 day old Sprague-Dawley rat pups. Animals were stunned then killed by cervical dislocation. The trachea between the base of the larynx and the bifurcation of the left and right bronchi was removed, cut midline along the length of its ventral surface and pinned out onto a small block of Sylgard (Dow Corning). The Sylgard block bearing the trachea was pre-bonded to a glass slide which formed the base of a Perspex recording chamber. The assembled chamber was then clamped to the stage of a Zeiss Ergoval microscope equipped with modulation contrast optics (Hoffman, 1977) giving 320 and 640 fold magnification. The preparation was perfused at a rate of 6 ml min^{-1} with Krebs solution maintained at $33\text{--}34^\circ\text{C}$ by a remote thermostatically-controlled heating coil. The Krebs solution was of the following composition (mM): NaCl 117, KCl 4.7, MgCl_2 1.2, NaH_2PO_4 1.2, CaCl_2 2.5, NaHCO_3 25 and glucose 11, gassed with 95% O_2 /5% CO_2 .

Impalements were made by electrodes with d.c. resistances between 90 and 130 M Ω , containing 2 M potassium citrate solution (pH 7.2). Before impalement, electrode resistance and offset potentials were nulled to allow estimations of input resistance and membrane potential to be made during the recording. In order to aid the placement of electrodes and impalement of neurones, the final 3–6 mm of the electrode were bent through an angle of 60° before being filled. Single electrode current- and voltage-clamp recordings were made by an amplifier with a 30% current passing duty cycle by use of sampling rates of between 3 and 5 kHz (Axoclamp-2A).

Ionophoretic application of drugs was made from micro-electrodes containing 0.1–0.5 M solutions (pH 3–4); the tips of the pipettes being placed within 1–5 μm of the cell soma. The recording and ionophoretic electrodes were connected to separate input stages of a recording system with active bridge and constant current circuits (Axoclamp-2A).

Fractional increases in conductance during drug responses were calculated as $(R/R^1) - 1$ where R is the input resistance at resting membrane potential and R^1 the input resistance at a given point during the response. Predicted equilibrium potentials were calculated from the observed current and conductance changes from the equation: $V_h - E_r = 1/(G^1 - G) \times I$ where V_h is the membrane potential at which the cell is held, E_r is the equilibrium potential for the response, G is the resting membrane conductance, G^1 is the membrane conductance at a given time (t) during the response and I the amplitude of the induced current at time (t).

Data were either stored on tape for future analysis (Racal store 4DS) or displayed using Tektronix storage oscilloscope (model D13) and a Gould pressure ink recorder (model 2200S).

Ionic substitution

Low chloride-containing solutions were made by directly sub-

stituting sodium gluconate for sodium chloride in the Krebs solution. Low calcium-containing solutions were made by direct substitution for magnesium.

Drugs

GABA, bicuculline methiodide, muscimol, picrotoxin, tetrodotoxin (Sigma), cadmium chloride (Fisons), baclofen (CIBA) and phaclofen (Tocris Neuramin) were used.

Results

Application of GABA, either by superfusion (1–100 μM) or ionophoresis (0.5 M, pH 4.0, 2–100 nA) produced a membrane depolarization in 156 out of a total of 171 (approximately 90%) rat paratracheal neurones studied. Typically, GABA evoked a two-component response, that consisted of a large initial depolarization which rapidly faded to reveal a second, smaller depolarization which was maintained for the duration of GABA application (see Figure 1a(i), b and c(i)). Both the initial transient and the slow sustained responses to GABA appeared to result from the direct action of GABA upon the impaled cell, since the responses were unaffected by superfusion with either calcium-free or tetrodotoxin-containing (0.3–1 μM) solutions.

The currents underlying the GABA-induced depolarizations were investigated under voltage-clamp. Brief ionophoretic application of GABA for a period of 1–100 ms (10 pc–10 nC) produced a large increase in membrane conductance that resulted in a transient inward flow of current (see Figure 1a(ii)). Under optimal conditions, where there was no connective tissue between the ionophoretic electrode and the impaled cell, the latency of response was between 18 and 45 ms. The magnitude of the inward current was dependent upon the total amount of ionophoretic charge ejected (see Figure 1a(ii)) and became larger at more negative potentials (see later). Prolonged application of GABA, either by superfusion or by ionophoresis (2–5 nA) for periods of up to 2 min, induced a similar rapid, transient inward flow of current, which subsequently declined over a period of 15–25 s to reveal a second smaller, sustained component to the inward current. This sustained inward current was also associated with an increase in membrane conductance and showed little or no rundown during prolonged GABA application (see Figure 1c(ii)). At the end of the period of stimulation, membrane conductance and current returned to control values within 10–20 s.

Conductance changes

Fast transient component For any given cell studied under current-clamp, the greater the amplitude of the fast depolarization, the larger the underlying conductance increase. However, as the total ionophoretic charge ejected was increased, the amplitude of response became maximal whereas the peak conductance continued to rise (see Figure 1a(i)). When ionophoretic currents sufficient to evoke a maximal depolarization were applied, the absolute amplitude of the response was almost entirely dependent upon the membrane potential of the cell. At any given membrane potential, the maximum size of the evoked depolarization occurred when the amplitude of the response became large enough to cause the cell to depolarize to a potential of approximately -35 mV . This potential was found to correspond quite closely to the equilibrium/reversal potential for the response (see later).

Examination of the observed increase in conductance under voltage-clamp revealed that the magnitude of the GABA-evoked current was directly related to the observed increase in conductance. The peak fractional increase in conductance (see Methods) varied considerably between cells, and ranged between 1.05 and 12.8 (mean 4.51 ± 0.336 , $n = 83$). For any given cell, the amplitude of the inward current was dependent upon membrane potential (see later). However, the fractional

Figure 1 The effects of brief and prolonged GABA application upon rat paratracheal neurones. (a, i) Rapid depolarizations induced by brief (100 ms) ionophoretic applications of GABA, the total charge ejected in each case is indicated in nC below the responses. Downward deflections are the membrane voltage responses to constant hyperpolarizing current pulses (100 pA, 50 ms) used to monitor membrane resistance changes. (a, ii) Membrane currents for the same cell to similar GABA ionophoretic currents recorded under voltage-clamp. Downward deflections are membrane currents in response to -10 mV (50 ms duration) voltage steps. Membrane/holding potential in (a, i) and (a, ii) was -52 mV. (b) The effect of prolonged superfusion of GABA ($100 \mu\text{M}$) for the period indicated by the bar. The recording was made under current-clamp (membrane potential -60 mV), downward deflections are membrane voltage responses to constant current pulses (100 pA/50 ms). (c) The effects of prolonged ionophoretic application of GABA (8 nA) for the period indicated by the bar (1.5 min). At the onset of GABA ionophoresis there was a rapid depolarization, this response then subsided to reveal a sustained depolarization which persisted for the duration of GABA ionophoresis (see c, i). Downward deflections are the membrane voltage responses to passing brief (50 ms/80 pA) constant intrasomal current pulses to monitor input resistance. (c, ii) The effects of prolonged GABA ionophoresis upon membrane current in the same cell as in (c, i). Downward deflections are the membrane current in the same cell as in (c, i). Downward deflections are the membrane currents in response to -5 mV, 50 ms duration voltage commands. Resting/holding potential in (c, i and ii) was -50 mV.

increase in conductance underlying the response was largely independent of the membrane potential at which it was evoked (see Figure 2).

Slow sustained component The conductance change and the resulting sustained inward current flow during prolonged

application of GABA were considerably smaller than those seen during the initial transient response. The amplitude of the inward current became larger with increasing concentrations of GABA and was generally maximal in the presence of GABA at concentrations greater than $100 \mu\text{M}$. Over the normal range of resting membrane potentials (-50 to -65 mV), the maximum amplitude of the sustained inward current rarely exceeded 150 pA (mean 107 ± 6.7 pA, $n = 19$). When the response was evoked by prolonged ionophoretic application of GABA, the amplitude of the sustained inward current reached a maximum even with very low ejection currents (1–3 nA) and could rarely be increased by further raising the current. However, in the presence of agents which acted to inhibit the initial transient inward current, raising the ionophoretic ejection current increased the rate of rise to this plateau, maximum value of the sustained inward current. It is worth noting that care was needed when applying prolonged ionophoretic currents. If excessively high currents were applied (> 10 nA), then the prolonged ionophoretic current itself could induce a sustained inward current flow similar to the GABA-induced current. In order to remove this source of error, the ionophoretic current was adjusted to a level where all of the inward current was antagonized by superfusion with bicuculline (see later). The observed increase in conductance during the slow response varied between cells, with the mean fractional increase in conductance ranging between 0.15 and 1.78 (mean 0.846 ± 0.061 , $n = 42$).

Ionic- and voltage-dependence

Transient inward current The voltage-dependence of the fast GABA-evoked inward current was investigated in the presence of tetrodotoxin ($0.3\text{--}1 \mu\text{M}$; see Figure 2a). The amplitude of the inward current was linearly related to the membrane potential at which it was elicited (see Figure 2b). When potassium citrate-filled electrodes were used in the recording, the mean null/reversal potential for the response was $-32.7 \text{ mV} \pm 1.66$ ($n = 8$; see Figure 2). For membrane potentials between -20 and -80 mV, both the time-course and the peak conductance change underlying the depolarization were largely independent of membrane potential (see Figure 2a). At strongly depolarized potentials (< -20 mV), outward rectification of the response was observed in some cells. However, this phenomenon was not studied in detail as it was difficult to maintain impalements at such depolarized potentials. Altering the extracellular chloride concentration from 129.1 to 12.1 mM transiently increased the amplitude of the fast GABA-evoked current by approximately 150–180%. However, multiple exposures to GABA, in the presence of low chloride-containing solutions, results in a pronounced decline in both the inward current and the observed conductance increase, as a result of a fall in intracellular chloride concentration. No initial reversal in the polarity of the GABA-evoked response was observed at the start of low chloride superfusion. On returning to normal chloride-containing solutions, both the inward current and the conductance increase slowly returned to their control values over a period of 5–10 min.

Slow sustained inward current In order to determine the equilibrium potential for the sustained GABA-evoked response, current-voltage relationships were constructed before and during GABA ionophoresis (see Figure 3a). The point of intersection of the curves gave a mean equilibrium/null potential for the response of -38.7 ± 1.56 mV ($n = 12$; see Figure 3b). This value agrees well with the predicted equilibrium potential of -39.2 ± 1.1 mV ($n = 15$) calculated from the observed increase in conductance (see Methods).

GABA analogues

Muscimol, applied either by superfusion or ionophoretically, mimicked all the actions of GABA upon paratracheal neu-

Figure 2 The voltage-dependence of the rapid transient GABA induced inward current. (a) Records from a single paratracheal neurone held at different membrane potentials. Downward deflections are the membrane current responses to hyperpolarizing voltage commands (10 mV, 50 ms) used to monitor changes in membrane conductance. Arrows beneath the records indicate the point at which GABA was applied (5 nA/100 ms). Note that the underlying fractional increase in membrane conductance was largely independent of the holding potential (V_h) at which the response was elicited. (b) The amplitude of the transient GABA-induced inward current as a function of holding potential. Line is least squares fit to raw data. The current was linearly related to membrane potential (correlation coefficient, $r = 0.998$) and reversed symmetrically at a membrane potential of -34.1 mV to become an outward current.

rones. When applied briefly by ionophoresis, it induced a similar rapid depolarization and an increase in conductance. Under voltage-clamp this could be seen to result from a transient inward flow of current (see Figure 4a). When muscimol was applied for prolonged periods, the initial current subsided in a similar way to the GABA-induced response to reveal a non-inactivating component to the inward current (see Figure 4b and c). The GABA_B receptor agonist β -*p*-chlorophenyl-GABA (baclofen) at concentrations up to $100 \mu\text{M}$ had no effect upon either the resting membrane potential or conductance in any of the cells tested ($n = 11$).

Antagonist studies

Both the fast transient depolarization evoked by brief application of GABA and the initial transient depolarization/inward current seen at the start of prolonged GABA ionophoresis were reversibly inhibited by picrotoxin (1 – $50 \mu\text{M}$) in a dose-related manner (see Figure 5a). However, picrotoxin at concentrations up to $500 \mu\text{M}$ was never observed to antagonize the sustained depolarization/inward current evoked by either superfusion or prolonged ionophoretic application of GABA or muscimol (see Figure 5b).

Bicuculline (0.1 – $10 \mu\text{M}$) reversibly inhibited the fast GABA-evoked depolarization. Approximate dose-response curves were constructed by briefly applying GABA with increasing ionophoretic currents (see Figure 6a). Bicuculline produced a parallel shift to the right in the dose-response curve for GABA (see Figure 6b). The GABA-induced sustained depolarization/inward current was also potently antagonized by bicuculline. However, the sensitivity of this response to inhibition by bicuculline was slightly lower than that observed for the transient component and required concentrations of bicuculline of between 10 and $50 \mu\text{M}$ to antagonize fully the actions of GABA (Figure 7).

Phaclofen at concentrations up to 1 mM did not antagonize either of the GABA- or muscimol-induced inward currents in any of the cells tested ($n = 5$).

Other agents

Superfusion with nipecotic acid (1 – $10 \mu\text{M}$; $n = 5$), a GABA uptake blocker, or inhibition of electrogenic pumping with ouabain (1 – $100 \mu\text{M}$; $n = 4$) were never observed to alter either the amplitude or the time-course of the two components of the GABA-induced inward currents. Superfusion with either calcium-free, high magnesium-containing solutions, or blockade of calcium entry with CdCl_2 ($100 \mu\text{M}$), did not reduce the observed amplitude or the underlying increase in conductance during either phase of the GABA-induced response.

Discussion

The findings of the present study showed that GABA exerted a powerful and direct effect upon a large proportion of the parasympathetic intramural neurones of the rat trachea. This effect was mediated via bicuculline-sensitive receptors and resulted in a large increase in resting chloride conductance. In a typical cell, held near its resting membrane potential, application of GABA produced a biphasic response consisting of an initial, transient depolarization, which rapidly declined to reveal a smaller, sustained depolarization. The maximum amplitude of the initial depolarization under these conditions was usually between 15 and 25 mV and reflected the level of the chloride equilibrium potential, which was approximately 20 mV positive to the resting membrane potential in these cells. Substituting extracellular chloride ions for a less permeant anion, such as gluconate, produced a transient increase in the amplitude of the GABA-induced depolarization and inward current. However, with repeated exposure to GABA in the presence of low extracellular chloride, both the current and the underlying increase in conductance declined markedly. This rundown of the GABA-evoked response in low

Figure 3 The voltage-dependence of the sustained GABA-induced inward current in a paratracheal neurone examined under voltage-clamp. (a) Under control conditions with the cell held at a membrane potential of -50 mV (the resting membrane potential for this cell), the cell was subjected to a series of 500 ms duration positive and negative voltage commands of increasing amplitude (5 mV increments). GABA was then applied ionophoretically (ejection current 5 nA) for the period indicated by the bar. This evoked the characteristic transient inward current which subsequently declined to reveal the sustained inward current. During the plateau phase of the sustained current, the cell was subjected to a second series of voltage commands identical to those applied under control conditions. At the end of this procedure the ionophoretic ejection current was switched off and the current returned to control levels. (b) The current-voltage relationships for the cell shown in (a) under control conditions and in the presence of GABA. The point of intersection of the two curves gives a value for the equilibrium potential (E_s) for sustained current response of approximately -38 mV.

chloride-containing solutions can best be explained by considering the underlying changes in the electrochemical gradient for chloride ions (see Adams & Brown, 1975). Initially, superfusion with low-chloride-containing solutions would be expected to increase the net outward electrochemical driving force on chloride ions by shifting E_{Cl} to more positive potentials. This would result in a large increase in the outward flow of chloride ions from the cell during the GABA-evoked response, and account for the large increase in the observed depolarization. Repeated application of GABA would, however, reduce the intracellular concentration of chloride ions causing a large negative shift in E_{Cl} , which would lead to a decline in the amplitude of subsequent responses. This reduction in the concentration of intracellular chloride ions, together with the low extracellular chloride concentration, would also explain the observed reduction in the underlying conductance that would be predicted to occur as a result of a

Figure 4 The actions of muscimol, a GABA_A receptor agonist, upon rat paratracheal neurones. (a) Brief (100 ms) ionophoretic application of muscimol using increasing ejection currents (total ionophoretic charge ejected is given in nC below each record). Downward deflections are membrane current responses to -10 mV voltage steps used to monitor changes in membrane conductance. Holding potential for the cell shown in (a) was -62 mV. (b) Prolonged ionophoretic application of muscimol, for the period indicated by the bar (7 nA/1.5 min), produced a biphasic inward current similar to that produced by GABA (downward deflections are current responses to -10 mV voltage steps as in a). Holding potential -54 mV. (c) Superfusion with muscimol (10 μ M) for the period indicated by the bar mimicked the effect of prolonged ionophoretic application, but the latency and rate of rise of the initial transient current were considerably slower. Downward deflections are current responses to 50 ms duration -10 mV voltage steps. Holding potential -53 mV.

deficiency in the total number of charge-carrying chloride ions. Interestingly, the voltage-dependence of the transient GABA-induced chloride current in rat paratracheal neurones displayed very little outward rectification over the membrane potential range -20 to -80 mV. This is contrary to what has been obtained in other central and peripheral neurones (Gray & Johnston, 1985; Ashwood *et al.*, 1987; Bormann *et al.*, 1987; Peters *et al.*, 1989) and also what might be expected from the asymmetrical distribution of chloride ions across the membrane. Outward rectification of the GABA response in paratracheal neurones was sometimes observed at very strongly depolarized potentials, but in the present study this phenomenon was not explored in detail as it was difficult to maintain impalements at such depolarized levels.

In addition to the initial transient response, all paratracheal neurones displayed a second, sustained depolarization which persisted for the duration of GABA application. Biphasic responses to prolonged application of GABA have been described in a variety of different cells (Deschenes *et al.*, 1976; Mayer *et al.*, 1981; Cherubini & North, 1984a; Yasui *et al.*, 1985). Superficially, the second depolarizing phase in many of these cells was similar to the sustained response seen in rat paratracheal neurones. However, in enteric AH type cells for example, the slow GABA response was mimicked by baclofen, had a low affinity for bicuculline and was attributed to activation of GABA_B receptors (Cherubini & North, 1984a). In contrast, in rat paratracheal neurones, baclofen was never observed to produce membrane depolarization, whereas

Figure 5 Inhibitory action of picrotoxin (PTX) upon the GABA-evoked response in a rat paratracheal neurone. (a) The fast GABA-evoked depolarization. Upper panel shows control depolarizations to GABA produced by increasing the total ionophoretic charge ejected (all pulses 50 ms duration; the amount of charge ejected in each case is indicated in nC below each response). In the presence of picrotoxin (10 and $50 \mu\text{M}$; middle panels), the amplitude of the evoked depolarizations was reduced in a dose-related manner. Resting membrane potential -54 mV. (b) The rapid transient and sustained inward currents evoked by prolonged ionophoretic application of GABA (8 nA/1.5 min). In the presence of picrotoxin ($50 \mu\text{M}$), the initial rapid transient component of the response was almost totally abolished, whilst the slow sustained current and associated increase in conductance remained largely unaffected. Downward deflections are membrane currents in response to 10 mV/50 ms voltage steps. Holding potential -68 mV.

Figure 6 Antagonism by bicuculline of the transient depolarization in response to ionophoretic application of GABA. (a) Graded depolarizations were evoked by increasing the total ionophoretic charge ejected (all applications were for 100 ms, total charge ejected was increased from 1 to 10 nC as indicated). Upper panel, control depolarizations and associated increases in membrane conductance. Middle and lower panels show responses from the same cell to a similar series of ionophoretic applications of GABA in the presence of bicuculline, 1 and $10 \mu\text{M}$ respectively. Resting membrane potential was -54 mV. (b) Dose-response curves constructed from an experiment similar to the one shown in (a). Bicuculline at increasing concentrations produced a concentration-dependent shift in the dose-response curve to GABA.

muscimol mimicked all the actions of exogenously applied GABA. This finding, together with the selective inhibition of all of the observed actions of GABA and muscimol by bicuculline, indicated that the sustained as well as the transient responses in rat paratracheal neurones, were mediated by GABA_A receptors.

Multi-component, GABA_A receptor-mediated chloride currents have been described in dorsal root ganglion neurones, for example (Akaike *et al.*, 1987). In these cells, the sustained inward current was similar to the one described here in rat paratracheal neurones, in that it was largely insensitive to picrotoxin (Yasui *et al.*, 1985). The equilibrium potential for the sustained inward current in paratracheal neurones was close to that of the transient component, indicating that, as in the dorsal root ganglion, it may also result from an increase in chloride conductance. The slightly more negative equilibrium potential of the sustained response probably reflected a small shift in E_{Cl} , as a result of the large efflux of chloride ions that occurred during the initial transient, response. Similar shifts of the GABA reversal potential (E_{GABA}) between the initial and late phases of the GABA-evoked response have been described in rat and cat ganglion cells (Adams & Brown, 1975; Mayer *et al.*, 1983).

In paratracheal neurones, the time-dependent decline of the GABA-evoked current, which occurred during continuous application of GABA, was similar to that described in, for example, hippocampal and dorsal root ganglion neurones. In these cells, the initial and late phases of the GABA current

have been shown to result from a combination of two processes; receptor desensitization and shifts in E_{GABA} (Yasui *et al.*, 1985; Huguenard & Alger, 1986; Akaike *et al.*, 1987). It seems likely that similar mechanisms underlie the biphasic inward current in rat paratracheal neurones.

Given the powerful effect that GABA exerted upon rat paratracheal neurones, it is obviously important to determine if there is any endogenous GABA within the airways. GABA has been localized in a population of neurones in the enteric nervous system (Jessen *et al.*, 1986) and it is possible, given the shared embryological origin of the gut and trachea, that there are also intrinsic GABAergic neurones in the airways. Alternatively, GABA may be released from extrinsic nerves to act upon the intramural ganglia. Another possible source of GABA could be blood, since levels of circulating GABA, at concentrations near to those required to depress ganglionic transmission, have been demonstrated in the bullfrog and cat (Crowcroft *et al.*, 1967; Kato *et al.*, 1980). In addition, it has been shown that glial cells in mammalian sympathetic ganglia are capable of taking up GABA from solutions containing concentrations of GABA as low as $1 \mu\text{M}$ (Bowery & Brown, 1972; Iversen & Kelly, 1975). Therefore, glial cells could regulate the extracellular GABA concentration and thereby modulate the excitability of neurones; it has been proposed that this type of mechanism may occur in sympathetic ganglia (Brown & Galvan, 1977).

If the excitability of paratracheal neurones were in some way modulated by either circulating or locally released GABA, then this could have wide-ranging consequences for airway function. It has previously been shown that rat paratracheal neurones display a high level of subthreshold, spontaneous synaptic activity (Allen & Burnstock, 1990). High levels of subthreshold synaptic activity have also been observed in cat tracheal ganglia *in vivo*, and it has been suggested that synchronization of multiple inputs are required to elicit action potential discharge in these cells (Mitchell *et al.*, 1987). One of the observed actions of GABA in the present study was a large increase in resting membrane conductance. This would act as a shunt across the membrane and result in a decrease in the size of the depolarization produced by individual synaptic events. Under such conditions, the overall intensity of vagal input required to elicit action potential discharge would be greatly increased by GABA and may explain the observed GABA-evoked reduction in acetylcholine release from post-ganglionic parasympathetic nerves in the guinea-pig trachea (Tamaoki *et al.*, 1987). Conversely, a fall in levels of GABA acting at the ganglion level, or a reduction in the sensitivity of these cells to GABA, could increase the excitability of the ganglion cells and partially explain the hyperreactivity of tracheo-bronchial smooth muscle observed in conditions such as asthma.

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Figure 7 Antagonism of the transient and sustained inward currents by bicuculline in a rat paratracheal neurone voltage-clamped at -54 mV . (a) Control response to prolonged GABA ionophoresis ($8 \text{ nA}/1.5 \text{ min}$). (b) In the presence of $10 \mu\text{M}$ bicuculline, there was a pronounced decrease in the conductance change and inward current flowing at the start of GABA application, and a smaller reduction in the size of the sustained inward current. (c) In the presence of $50 \mu\text{M}$ bicuculline, both the initial transient and the slow sustained GABA-evoked currents were almost totally abolished. (d) Wash. Downward deflections are the induced membrane currents resulting from 10 mV negative commands (50 ms duration) used to monitor changes in membrane conductance.

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